Emerging Epidemics
Management and Control

Emerging Epidemics

Management and Control

Prakash S. Bisen and Ruchika Raghuvanshi

Defense Research Development Establishment
Defense Research Development Organization
Ministry of Defense, Government of India
Gwalior, India

WILEY Blackwell

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:

Bisen, Prakash S.
Emerging epidemics : management and control / Prakash S. Bisen and Ruchika Raghuvanshi.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-118-39323-9 (cloth : alk. paper) – ISBN 978-1-118-39325-3 (epub) – ISBN 978-1-118-39326-0 (epdf) – ISBN 978-1-118-39327-7 – ISBN 978-1-118-39328-4 (emobi)
I. Raghuvanshi, Ruchika. II. Title.
[DNLM: 1. Communicable Diseases, Emerging–prevention & control. 2. Epidemics–prevention & control. 3. Communicable Disease Control–standards. 4. Hazardous Substances–standards. WA 110]
RA651
614.4–dc23

2013006644

Printed in Malaysia

10 9 8 7 6 5 4 3 2 1

We would like to dedicate this work to our mentor Professor G. P. Agarwal, Founder Professor of Microbiology and Former Dean, Faculty of Life Sciences, R. D. University, Jabalpur, M. P., India. He is the pioneer to start Life Science teaching and research in this region with modest resources. The institution has now full facilities in teaching and research in various fields of life sciences under one roof with financial support from several national and international funding agencies.

Contents

Preface

Emerging diseases continue to make news headlines as they have done in years past. They are the moving targets. It is widely expected that emerging disease will continue to affect people because populations grow and increasingly penetrate native environments. An *epidemic* is an outbreak of a contagious disease that spreads rapidly and widely in a particular geographical area. A disease can, however, occur both as an epidemic or an *endemic*, depending on the expected occurrence and the relative number of cases. Emerging infectious diseases result from newly identified and previously unknown infections, which cause local or international public health problems. Re-emerging infectious diseases are the result of the reappearance of, and an increase in, the number of infections from a disease that is known but that had formerly caused so few infections it had no longer been considered a public health risk. This book details the connecting link between basic and advances in infection. Each chapter promises a thorough account of previous epidemics, the recent outbreaks, and the latest advances, which highlight the challenges in the field.

Emerging Epidemics: Management and Control includes 24 chapters, which include the basic knowledge of a particular disease, followed by its management and control. This book is particularly useful for graduate and postgraduate medical and paramedical students because of its inclusion of recent information regarding each epidemic disease. A unique feature of the book is the current information of various infectious diseases with references to tuberculosis, plague, dengue, Japanese encephalitis, chikungunya, West Nile fever, *Hantavirus* disease, and influenza.

The book begins with a prologue distinguishing between epidemics, emerging epidemics, and re-emerging epidemics. The second and third chapters deal with the fundamentals of epidemics and the various diseases resulting from natural disasters. The fourth chapter provides a complete assessment of biosafety risk and management. Further chapters deal with the bacterial, viral, and parasitic emerging diseases with particular reference to their control and management.

The book also deals with the current biological warfare and bioterrorism threat to humanity, with a clear discrimination between biowarfare and bioterrorism, which undermine and destruct economic progress and stability. Additionally, there is a separate chapter on antimicrobial drug resistance, which poses a threat to physicians and the health-care workers who treat patients during an outbreak. Finally, the book addresses the various conventional and new and potential techniques of mosquito control and the other vectors of diseases because these are the potential carriers and agents involved in the transmission of various infectious diseases. The book is comprehensive in scope and can be applied at many levels of bioscience learning, including undergraduates studying bioscience or medical fields.

We have taken the utmost care to include all relevant information about concepts discussed; however, there are chances that some pertinent information was missed, and we encourage and request all readers to send us comments and suggestion for improvement of this book.

Prakash S. Bisen and Ruchika Raghuvanshi

Acknowledgment

We would like to express our sincere thanks to Dr. G. S. Davar and Mr. Puneet Davar, Tropolite Foods Pvt. Ltd., Gwalior, India; Mr. R. S. Rathore, Mr. Anil Singh and Mrs. Richa Verma, Vikrant Institute of Technology and Management, Gwalior, India; and to Dr. S. J. S. Flora, Additional Director, Defense Research Development Establishment, Defense Research Development Organization, Gwalior, India for extending their invaluable advice and moral support.

We express a debt of gratitude to Prof. G. B. K. S. Prasad, Chairman, School of Studies in Biochemistry, Jiwaji University, Gwalior; Dr. Ram P. Tiwari and Dr. Anubhav Jain, Diagnostic Division, RFCL Limited (formerly Ranbaxy Fine Chemicals Limited), Avantor Performance Materials, New Delhi, India; Professor (Dr.) B. R. Shrivastav, Department of Surgery, Professor Neelima Chandel, Department of Biochemistry, Dr. Shweta Sahai, Department of Medicine, Dr. Bharat Jain, Department of Microbiology, from G. R. Medical College, Gwalior, India; Professor (Dr.) V. K. Ramnani, Department of Microbiology and Immunology, Gandhi Medical College, Bhopal, M. P., India; Dr. Zakir Khan Viral Neuro-Immunology Unit, Department of Virology, Institut Pasteur, Paris, France; Mr. Bhagwan S. Sanodiya, Mr. Gulab S. Thakur, Mr. Rakesh K. Baghel, and Mr. Rohit Sharma, Tropilite Foods Pvt. Ltd., Gwalior, for their valuable assistance in preparation of this book.

Our sincere thanks to Mr. Devendra Singh, Mr. Avinash Dubey, and Mr. Rahul Jha for all computational work for preparing the book in the presentable form. The cover page of the book was designed by courtesy of Mr. Avinash Dubey.

Thanks are also due to Anna Ehler and Mindy Okura-Marszycki at John Wiley & Sons Inc., Hoboken, New Jersey, for their full support in publishing this book in time with patience and interest.

Finally, we thank our families for their constant support, cooperation and understanding. We are grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, for the award of Emeritus Scientist to Professor P. S. Bisen.

Prakash S. Bisen and Ruchika Raghuvanshi

Emerging Epidemics
Management and Control

Chapter 1
Prologue

Introduction

Epidemiology is considered the basic science of public health or a quantitative basic science built on a working knowledge of probability, statistics, and sound research methods. It is a tool for public health action to promote and protect the health of people based on science, causal reasoning, and a dose of practical common sense. The method of causal reasoning is based on developing and testing hypotheses pertaining to the occurrence and prevention of morbidity and mortality (Figure 1.1). Hence, the essential role of epidemiology is to improve the health of populations by involving both science and public health practice. The term *applied epidemiology* is sometimes used to describe the application or practice of epidemiology to address public health issues.

The word *epidemiology* originated from the Greek words *epi*, meaning "on or upon"; *demos*, meaning "people"; and *logos*, meaning "the study of." For epidemiology, many definitions have been proposed, but the correct definition based on the underlying principles and the public health spirit is "the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems (Last, 1988). Epidemiology actually arose from Hippocrates's observation more than two thousand years ago that environmental factors influence the occurrence of disease. However, it was not until the 19th century that the distribution of disease in specific human population groups was measured to any large extent. Comparing rates of disease in subgroups of the human population became common practice in the late 19th and early 20th centuries. This approach was initially applied to the control of communicable diseases, but it proved to be a useful way of linking environmental conditions or agents to specific diseases.

In the second half of the 20th century, these methods were applied to chronic noncommunicable diseases such as heart disease and cancer, especially in middle- and high-income countries. Epidemiology in its modern form is a relatively new discipline (Beaglehole and

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

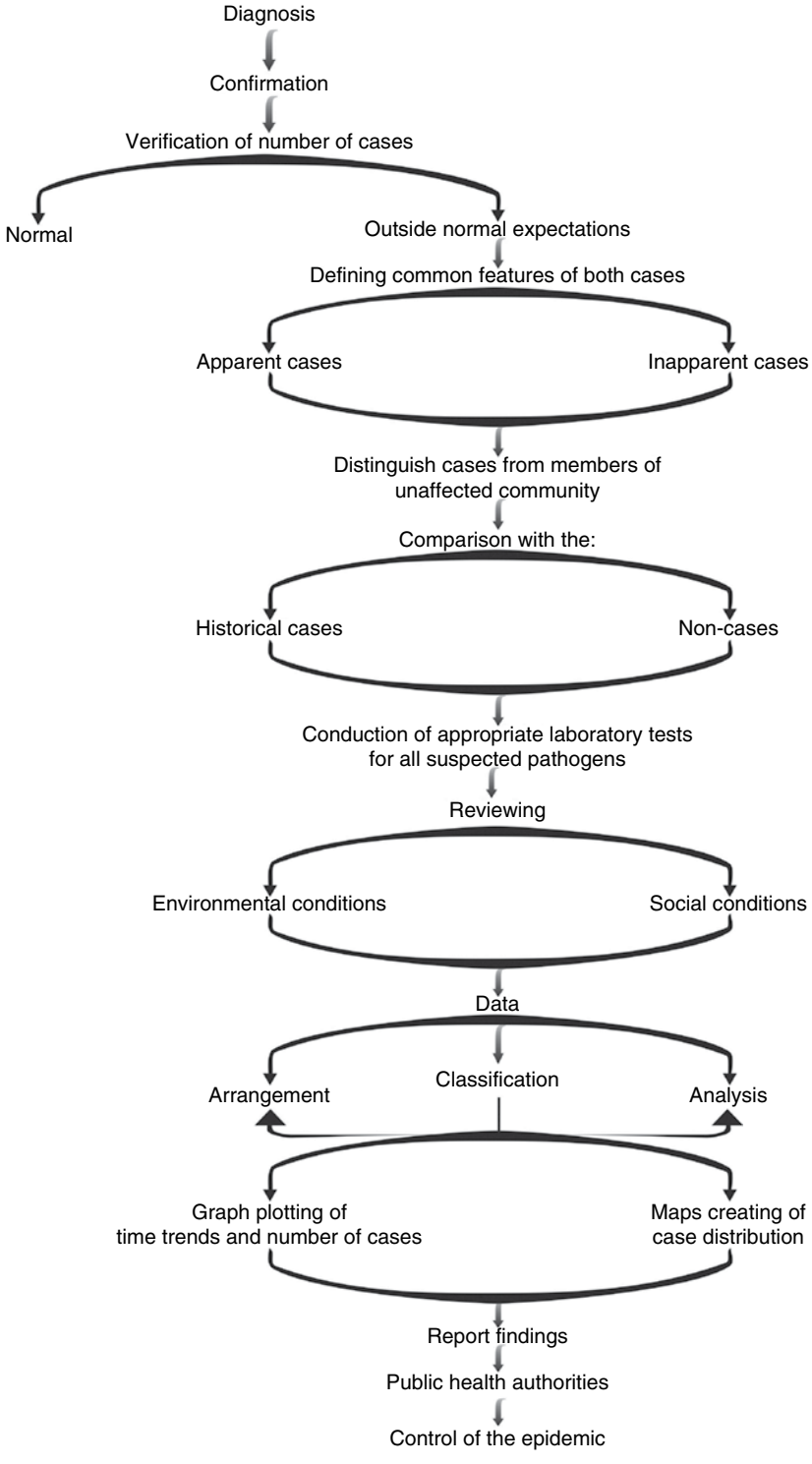

Figure 1.1. Steps to investigate an epidemic.

Bonita, 2004) and uses quantitative methods to study diseases in human populations to inform prevention and control efforts. For example, Richard Doll and Andrew Hill (1954) studied the relationship between tobacco use and lung cancer, beginning in the 1950s; their work was preceded by experimental studies on the carcinogenicity of tobacco tars and by clinical observations linking tobacco use and other possible factors to lung cancer.

Epidemiologists are concerned not only with death, illness, and disability, but also with more positive health status and, most importantly, with the means to improve health. The term *disease* is actually an uncomfortable feeling that encompasses all unfavorable health changes, including injuries and mental health. Epidemiology is concerned with the frequency and pattern of health events in a population. Frequency includes not only the number of such events in a population, but also the rate or risk of disease in the population. The patterns by which epidemics spread through groups of people is determined, not just by the properties of the pathogen carrying it, including its contagiousness, the length of its infectious period, and its severity, but also by network structures within the population it is affecting. The social network within a population recording who knows whom determines a lot about how the disease is likely to spread from one person to another. A focus of an epidemiological study is the population defined in geographical or other terms; for example, a specific group of hospital patients or factory workers could be the unit of study. A common population used in epidemiology is one selected from a specific area or country at a specific time.

Although epidemiologists and physicians in clinical practice are both concerned with disease and control of disease, they differ greatly in how they view the patient. Clinicians are concerned with the health of an individual; epidemiologists are concerned with the collective health of the people in a community or other area. While examining a patient with diarrheal disease, for example, the clinician and the epidemiologist have different responsibilities. Although both are interested in establishing the correct diagnosis, the clinician usually focuses on treating and caring for the individual, whereas the epidemiologist focuses on the exposure (action or source that caused the illness), the number of other persons who may have been similarly exposed, and the potential for further spread in the community, and interventions to prevent additional cases or recurrences.

Epidemic sweating sickness recurred several times in medieval Europe, but it has vanished since. The Black Death or plague that struck Europe in 1347 killed between one-third and one-half of the people in many cities and towns, arresting the advance of civilization for several generations. Some epidemic diseases, such as the plague, smallpox, typhus, and influenza, have persisted throughout recorded history. Smallpox was eradicated worldwide by 1980. Cholera appeared along the world's major trade routes in several devastating epidemics beginning in the 18th century, and it still causes massive epidemics, most recently in South America in early 1990s.

In the final quarter, of the 20th century more than 30 new infectious pathogens were identified. Many of these have caused deadly localized epidemics (e.g., *Ebola* virus, *Hantavirus*, and other viral hemorrhagic fevers), and some have spread worldwide; HIV/AIDS being the foremost among these. Since its first recognition in 1981, HIV has affected almost 40 million people and killed more than 10 million, making it the most lethal and dangerous pandemic, second only to the Black Death. Other new and emerging infections that have caused epidemics include Legionnaire's disease, Lyme disease, newly identified hepatitis viruses that spread in epidemic form through contaminated blood and blood products used in transfusion services, and several bacterial and viral diseases affecting the gastrointestinal tract.

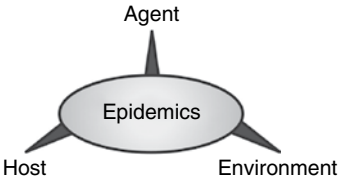

Figure 1.2. An epidemiological triad.

In an epidemiological investigation, three attributes should be considered; the host (the affected individuals), the agent (the cause of the condition), and the environment (Figure 1.2) along with their physical, biological, social, behavioral, and cultural factors. Investigating an epidemic can be as exciting as detective fiction, and such investigations (both real and fictional) have yielded many best-selling books and movies. The Epidemic Intelligence Service (EIS) of the US Centers for Disease Control and Prevention (CDC) has an illustrious record of successfully investigating and controlling epidemics, including some great public health importance.

While studying a disease outbreak, epidemiologists depend on clinical physicians and laboratory scientists for the proper diagnosis of individual patients, and at the same time, epidemiologists contribute to physicians' understanding of the clinical picture and natural history of disease. For example, in late 1989, three patients in New Mexico were diagnosed as having myalgias (severe muscle pains in the chest or abdomen) and unexplained eosinophilia (an increase in the number of eosinophils). Their physician could not identify the cause of their symptoms or put a name to the disorder. Epidemiologists began looking for other cases with similar symptoms, and within weeks had found enough additional cases of the eosinophilia-myalgia syndrome to describe the illness, its complications, and its rate of mortality. Similarly, epidemiologists have documented the course of HIV infection, from the initial exposure to the development of a wide variety of clinical syndromes that include AIDS. They have also documented numerous conditions that are associated with cigarette smoking from pulmonary and heart disease to lung and cervical cancer.

A basic task of a health department is counting cases to measure and describe morbidity. When physicians diagnose a case of a reportable disease they send a report of the case to their local health department. These reports are legally required to contain information on time (when the case occurred), place (where the patient lived), and person (the age, race, and sex of the patient). The health department combines the reports and summarizes the information by time, place, and person. From these summaries, the health department determines the extent and patterns of disease occurrence in the area and identifies clusters or outbreaks of disease. A simple count of cases, however, does not provide all the information required by the health department. To compare the occurrence of a disease at different locations or during different times, a health department converts the case counts into rates, which relate the number of cases to the size of the population where they occurred. Rates are useful in many ways. With rates, the health department can identify groups in the community with an elevated risk of disease. These so-called *high-risk groups* can be further assessed and targeted for special intervention; the groups can be studied to identify risk factors that are related to the occurrence of disease. Individuals can use knowledge of these risk factors to guide their decisions about behaviors that influence health.

Causative Factors

Although analytic epidemiology used to search for causes of disease, this is not a straightforward matter. First, not all associations between exposures and disease are causal relations. In addition, the accepted models of disease causation require the precise interaction of factors and conditions before the occurrence of disease. Finally, the concept itself continues to be debated as a philosophical matter in the scientific literature. Nonetheless, the following models and guidelines provide a framework for considering causation at a practical level. An increase in the factor leads to an increase in disease; similarly reduction in the factor leads to a reduction in disease. If the disease always results from the factor, then it is termed as the causative factor *sufficient*. For example, exposure to *Mycobacterium tuberculosis* is necessary for tuberculosis to develop, but it is not sufficient because not everyone infected develops disease. On the other hand, exposure to a large inoculum of rabies virus is a sufficient cause in a susceptible person because clinical rabies and death will almost inevitably occur. A variety of models of disease causation has been proposed. Models are purposely simplified representations. In this instance, the purpose of the model is to facilitate the understanding of nature, which is complex. Two of these models are discussed herein.

The Epidemiologic Triangle or Triad: Agent, Host, and Environment

It is the traditional model of infectious disease causation. It has three components: an external agent, a susceptible host, and an environment that brings the host and agent together (*see* Figure1.2). In this model, the environment influences the agent, the host, and the route of transmission of the agent from a source to the host.

Agent Factors

Agent refers to an infectious microorganism, such as virus, bacteria, fungi, protozoa, and helminth, etc. Generally, these agents are necessary, but not always sufficient, to cause a disease. As epidemiology has been applied to noninfectious conditions, the concept of agent in this model has been broadened to include chemical and physical causes of disease. These include chemical contaminants, such as the l-tryptophan contaminant responsible for eosinophilia-myalgia syndrome, and physical forces, such as repetitive mechanical forces associated with carpal tunnel syndrome. This model does not work well for some noninfectious diseases because it is not always clear whether a particular factor should be classified as an agent or as an environmental factor.

Host Factors

These are intrinsic factors that influence an individual's exposure and susceptibility toward a causative agent. These include age, race, sex, behaviors (e.g., smoking, drug abuse, lifestyle, sexual practices and contraception, eating habits), nutritional and immunologic status, genetic composition, and socioeconomic status, etc.

Environmental Factors

These are extrinsic factors, which affect the agent and provide the opportunity for exposure. Generally, environmental factors include physical factors such as geology,

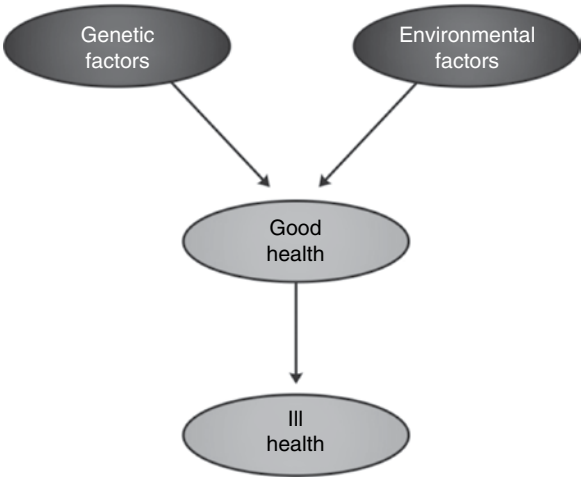

Figure 1.3. Disease causation.

climate, and physical surroundings (e.g., a nursing home, hospital); biologic factors such as insects that transmit the agent; and socioeconomic factors such as crowding, sanitation, and the availability of health services.

Agent, host, and environmental factors interrelate in a variety of complex ways to produce disease in humans. Their balance and interactions are different for different diseases. All the three components and their interactions should be analyzed to find practical and effective prevention and control measures of epidemics.

An Interaction of Genetic and Environmental Factors

Although some diseases are caused solely by genetic factors, most of them result from an interaction between genetic and environmental factors (Figure 1.3). Diabetes, for example, has both genetic and environmental components. Any biological, chemical, physical, psychological, economic, or cultural factors can affect health. Personal behaviors affect this interplay, and epidemiology is used to study their influence and the effects of preventive interventions through health promotion. Epidemiology is often used to describe the health status of population groups. Knowledge of the disease burden in populations is essential for health authorities.

Disease rates change over time. Some of these changes occur regularly and can be easily predicted. For example, the seasonal increase of influenza (flu) cases with the onset of cold weather is a pattern that is familiar to everyone. This helps health departments to effectively design their flu shot campaigns. Other disease rates make unpredictable changes. By examining events that precede a disease rate increase or decrease, causes may be identified and appropriate actions taken to control or prevent further occurrence of the disease. Some epidemics are transferred from one host to another (Table 1.1), whereas some are unique to a specific host.

Causation is an essential concept in the practice of epidemiology, but a single cause may not always be sufficient for an epidemic to occur. For example, tobacco smoking is a cause of lung cancer, but it is not a sufficient cause. First, the term *smoking* is too

Table 1.1. Epidemics that are transferred from one host to another.

Host-to-Host Epidemics			
Disease	Causative Agent	Infection Sources	Reservoirs
Diphtheria	*Corynebacterium diphtheriae* (B)	Human cases and carriers; infected food and fomites	Humans
Hantavirus pulmonary syndrome	*Hantavirus* (V)	Inhalation of contaminated fecal material	Rodents
Meningococcal meningitis	*Neisseria meningitidis* (B)	Human cases and carriers	Humans
Pneumococcal pneumonia	*Streptococcus pneumoniae* (B)	Human carriers	Humans
Tuberculosis	*Mycobacterium tuberculosis* (B)	Sputum from human cases; contaminated milk	Humans, cattle
Whooping cough	*Bordetella pertussis* (B)	Human cases	Humans
German measles	*Rubella* virus (V)	Human cases	Humans
Influenza	*Influenza* virus (V)	Human cases	Humans, animals
Measles	Measles virus (V)	Human cases	Humans
HIV disease	HIV (V)	Infected body fluids, blood, semen, etc.	Humans
Chlamydiasis	*Chlamydia trachomatis* (B)	Urethral, vaginal, and anal secretions	Humans
Gonorrhea	*Neisseria gonorrhoeae* (B)	Urethral and vaginal secretions	Humans
Syphilis	*Treponema pallidum* (B)	Infected exudates or blood	Humans
Trichomoniasis	*Trichomonas vaginalis* (P)	Urethral, vaginal, prostate secretions	Humans

B, bacteria; P, protozoa; V, virus.

imprecise to be used in a causal description. One must specify the type of smoke (e.g., cigarette, cigar, pipe), whether it is filtered or unfiltered, the manner and frequency of inhalation, and the onset and duration of smoking. More importantly, smoking, even defined explicitly, will not cause cancer in everyone. Apparently, there are some people who, by virtue of their genetic makeup or previous experience, are susceptible to the effects of smoking and others who are not. These susceptibility factors are other components in the various causal mechanisms through which smoking causes lung cancer.

Infectious disease at a fairly constant level is described as being *endemic*. Endemic disease may rise to a higher level, but if it remains at this new plateau it is not described as an epidemic. Usually, however, rises are followed by a fall. This occurs for a number of reasons: Those most likely to be infected have died, or the survivors have built up a resistance to the disease; among those infected, many have a natural resistance; the densest populations have already been decimated. Some infectious diseases do not provoke strong immune reactions, and hence it is difficult to produce vaccines: examples are cholera and modern bubonic plague. The vocabulary employed is descriptive but not analytical. Nearly all terms come from Greek, through Latin and French into English. Several are built around the Greek word, *demos*, from which *demography* is derived; examples are

endemic (among people), epidemic (through or over people), and pandemic (among all people). There are many infectious diseases, which may become epidemic: measles, whooping cough, chicken pox, scarlet fever, influenza, smallpox, cholera, influenza, and poliomyelitis. In the following section, three examples are given that can be called pandemics because they infected many people over broad areas and were associated with high mortality. These are:

1. The plague (in various forms)
2. Spanish influenza
3. HIV/AIDS

The plague pandemics were once thought to be identical, but this is now doubtful (Herlihy 1997; Cohn 1997, 2002, 2003). Oriental bubonic plague emerged from Southern China in 1894 and attacked Hong Kong, then Bombay, and other places including Sydney. It is not yet extinct. In Hong Kong, it was shown to be caused by a bacillus (*Yersinia pestis*), and in Mumbai (Maharashtra, India) it was proved that this was transmitted by a rat flea. Because it was characterized by buboes, or pronounced swellings of the lymph glands in the groin, it has long been assumed that Oriental Bubonic Plague was identical with the Black Death and the Great Plague. So it was believed that by studying this pandemic modern science could unlock the secrets of these previous pandemics.

Spanish influenza (usually just Spanish flu) acquired its name because Spain was the first heavily infected European country, but it almost certainly originated elsewhere, possibly in Asia. Its pathogen could not be identified at the time, but efforts are still being made to secure DNA for identification from its long-dead victims. The epidemic burst on Europe in 1918 and spread to the whole world; a spread partly facilitated by troops returning home from World War I.

The symptoms of AIDS as a new disease began to be noticed in the United States in 1979, and official confirmation of its existence and of deaths arising from it came in 1981 and later in the same year in Europe. It was not until 1983 that the pathogen (a retrovirus) was identified, and it was 1985 before a test for its presence was available. The disease has certain unusual characteristics. It kills by suppressing the immune system, thus permitting death from pneumonia, tuberculosis, and other causes. Its average period from infection to death was 10 years and to major symptoms almost as long, compared with a few days in the cases of Black Death or Spanish Influenza. Treatment with antiretrovirals can now lengthen that period, although perhaps not indefinitely. Until the development of antiretroviral treatment, case mortality was 100 percent, which may still be the level of premature death.

Unlike Spanish influenza, and probably Black Death, HIV is not transmitted from person to person through the breath, but through blood in sexual intercourse, shared use of intravenous drug needles, during birth, or in maternal breastfeeding. Where heterosexual intercourse is an important means of infection, as in sub-Saharan Africa or the Caribbean, more females than males may be infected, but where homosexual transmission predominates as in North America, Western Europe, and Australasia, only 20 to 25 percent of those infected are females. By the year 2000, HIV/AIDS had killed 20 million people (15 million of them in sub-Saharan Africa) and had infected 40 million others, condemning the great majority to premature death. Everywhere commercial sex workers have unusually high levels of infection and are themselves a serious source of infection. Table 1.2 details the various death numbers resulting from the top seven infectious diseases of the world.

Table 1.2. Top seven infectious diseases in the world by death number.

Name of Infectious Disease	Number of Deaths
Respiratory infections	4,259,000
Diarrheal diseases	2,163,000
HIV/AIDS	2,040,000
Tuberculosis	1,464,000
Malaria	889,000
Childhood infections	847,000
Tropical diseases	152,000

International Federation of Red Cross and Red Crescent Societies. 2009. The epidemic divide. Accessed March 25, 2013, at http://www.ifrc.org/Global/Publications/Health/170800-Epidemic-Report-EN-LR.pdf.

Salient Features

Epidemiology played a crucial role in identifying the cause and in the control of what was one of the first reported epidemics of disease caused by environmental pollution. The first cases were thought to be infectious meningitis. However, it was observed that 121 patients with the disease mostly resided close to Minamata Bay. A survey of affected and unaffected people showed that the victims were almost exclusively members of families whose main occupation was fishing and whose diet consisted mainly of fish. On the other hand, people visiting these families and family members who ate small amounts of fish did not suffer from the disease. It was therefore concluded that something in the fish had caused the poisoning and that the disease was not communicable or genetically determined (McCurry, 2006). All epidemics usually follow some common characteristic features:

1. An unexpected number of cases of a particular disease occur at a particular point of time affecting large segment of the population.
2. Generally confined to a definite population or geographical area and hence geographic patterns provide important sources of clues about the causes of diseases.
3. Usually have a common source of infection. For containment of epidemics, it is important to identify the source of infection so that the appropriate measures can be adopted to eliminate the common source of infection to prevent further spread of epidemic.
4. An epidemic generally tends to follow a pattern and repeat periodically when the conditions are favorable again.
5. The way an epidemic presents it in the community depends on the distribution and characteristics of people living in that area, their social pattern, their cultural behavior, and the various environmental factors.

Emerging Epidemics

Epidemiology has been a major contributor to the success of the disease control efforts of the past century, culminating in such signal triumphs as the global eradication of smallpox and the eradication of polio from the Western Hemisphere. However, in recent

years, partly because these very successes led to a pervasive optimism about infectious diseases in the future, there has been a waning interest in infectious disease epidemiology even though infectious diseases remain the leading cause of death worldwide and an important cause of death in the United States (Lederberg et al., 1992; McGinnis and Foege, 1993).

AIDS, like many of the plagues of the past, falls into the category of emerging infections (seemingly new diseases that appear suddenly and unexpectedly). Emerging infections can be defined as those that either have newly appeared in a population or that are rapidly increasing their incidence or expanding their geographic range (Morse, 1991). Other recent examples include hanta virus pulmonary syndrome, Lyme disease, hemorrhagic colitis, and hemolytic uremic syndrome (resulting from a food-borne infection caused by certain strains of *Escherichia coli*), and Ebola hemorrhagic fever in Africa (Satcher, 1995). Past scourges can also recur and are referred to as *re-emerging diseases*, which are often conventionally understood and well-recognized public health threats that have increased or reappeared because previously active public health measures have lapsed or sanitary infrastructure has deteriorated. Figure 1.4 illustrates various emerging and re-emerging epidemic diseases that struck the world in the past 30 years. The researchers stressed the need for at-risk countries to act quickly for the expansion of surveillance to these epidemics and access to testing, prevention, and treatment services for persons at risk.

So many factors participate in the emergence of epidemics, and modern life conditions and human behavioral changes make these factors more prevalent, giving reason to expect increased occurrence of emerging diseases. Sources of epidemics vary depending on the causative agent (Table 1.3) and the environmental conditions of a particular geographical area. Historically, "new" diseases had appeared and spread as by-products of exploration, trade, or warfare, when the movement of people, animals, or goods brought geographically isolated infections to new grounds (McNeill, 1976). In the 19th century, steamships carried cholera to Europe and Africa. Today, trucks, freighters, and airplanes have largely replaced caravans and steamships, allowing even richer opportunities for infections to emerge and spread efficiently. Speed of travel and global reach of infections are borne out by studies modeling the spread of influenza epidemics (Longini et al., 1986) and of HIV (Flahault and Valleron, 1992), as well as by the actual progress of known epidemics.

Other factors are also allowing emerging infections to appear at increasing rates and could facilitate wider and more rapid spread. In many parts of the world, economic conditions are encouraging the mass movement of workers from rural areas to cities. It has been estimated that, largely as a result of this migration, by the year 2025, 65 percent of the world population, including 61 percent of the population in developing regions, will live in cities. The phenomenon of rural to urban migration can allow infections arising in isolated rural areas, which may once have remained unrecognized and localized, to reach larger populations, with the city serving an amplifying function. An infection can become further disseminated when other migrants return home with an infection acquired in the city, a pattern now being observed with HIV in Asia.

Epidemics such as AIDS demonstrate the interplay of complex ecological, social, and behavioral factors. As of 2009, an estimated 33.3 million people worldwide had HIV, according to the latest United Nations data, and 22.5 million of those live in sub-Saharan Africa. There is little published data on the Middle East and North African regions, and Ghina Mumtaz, who led the study with colleague Laith Abu-Raddad, said this had been

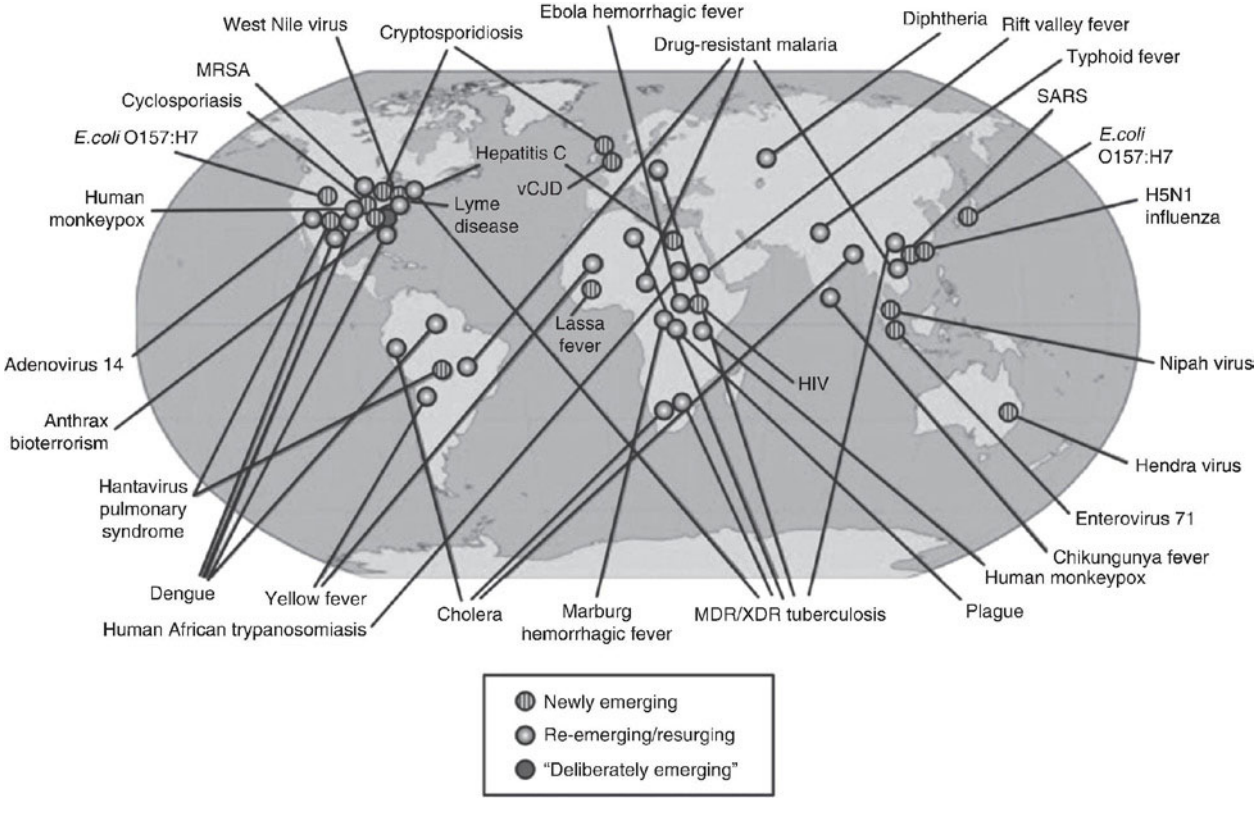

Figure 1.4. Newly emerging, re-emerging/resurging, and "deliberately emerging" diseases. Selected emerging diseases of public-health importance in the past 30 years (1977–2007).

Table 1.3. Sources of some common epidemic diseases.

Disease	Causative Agent	Source of Infection	Reservoirs
Anthrax	*Bacillus anthracis* (B)	Milk or meat from infected animals	Cattle, swine, goats, sheep, horses
Bacillary dysentery	*Shigella dysenteriae* (B)	Fecal contamination of food and water	Humans
Botulism	*Clostridium botulinum* (B)	Soil-contaminated food	Soil
Brucellosis	*Brucella melitensis* (B)	Milk or meat from infected animals	Cattle, swine, goats, sheep, horses
Cholera	*Vibrio cholerae* (B)	Fecal contamination of food and water	Humans
Giardiasis	*Giardia* spp. (P)	Fecal contamination of water	Wild mammals
Hepatitis	Hepatitis A, B, C, D, E (V)	Infected humans	Humans
Paratyphoid	*Salmonella paratyphi* (B)	Fecal contamination of food and water	Humans
Typhoid fever	*Salmonella typhi* (B)	Fecal contamination of food and water	Humans

B, bacteria; P, protozoa; V, virus.

driving misconceptions that there is no reliable information at all (Kelland, 2011). Controlling the infections of tomorrow will increasingly require understanding and assessing the roles of each of these factors in disease and designing appropriate strategies that take them into account. Epidemiology must integrate approaches to studying the ecological, social, and behavioral context of disease into its own core subject matter. There have been epidemiologic traditions that have incorporated strong ecological underpinnings (Pavlovskii, 1996), and there are opportunities now to develop an integrated epidemiology that will further develop these approaches (Koopman, 1995; Susser, 1995). Human behavior, which is often a key factor in appearance and spread of infections, must also, be included in the equation. Many of the difficulties in dealing with the AIDS pandemic have stemmed precisely from the inability to deal effectively with these social factors that have allowed the virus to spread.

Being prepared for emerging infections will require strengthened surveillance and response capacity (Henderson, 1993). Numerous expert analyses have called for the development of an effective global early warning and response system (Berkelman et al., 1994) while noting the perilous and fragmented state of present systems. In all of this, epidemiology must play a central role. However, epidemiologists trained in infectious disease surveillance and control remain in short supply, with limited attention to the subject in both medical and public health curricula. There is an equally critical need to integrate epidemiologic and laboratory surveillance functions. The availability of powerful new tools makes this an opportune time. Biotechnology continues to expand the power of laboratory-based surveillance and increases the feasibility of field applications worldwide. New analytic tools, including geographic modeling and electronic data collection and analysis, have similarly expanded capabilities that can be harnessed on the epidemiologic side, making it possible for the epidemiologist and laboratorian to share information.

The next essential step is to integrate these elements at the front line, by fostering settings for infectious disease surveillance in which the specialties work closely together and learn to share data and vocabularies. At the same time, it is critically important to improve communication and collaboration between clinicians and clinical microbiologists, who are often the first to recognize emerging and re-emerging infections, and public health professionals with expertise in epidemiology and microbiology.

Molecular epidemiology is one promising product of the interaction between epidemiology and the laboratory (Moore, 1992). As indicated by recent progress in applying molecular approaches to ecology (Berry et al., 1992), this interaction can even mutually reinforce the priority of integrating ecological, social, and behavioral sciences into epidemiology. Even with the appropriate structures in place, however, there are serious problems that must be overcome. In addition to trained personnel, now in short supply, a career path, requiring stable funding for these efforts, must also be assured. The recent responses to outbreaks of plague in India and Ebola hemorrhagic fever in Zaire demonstrated how thin the resources are, both in terms of trained personnel and laboratory capacity. The international community was able to mount an adequate ad hoc response only by stretching available capabilities to the limit. In the case of plague, for example, the CDC had the only functioning World Health Organization Collaborating Center for plague, and this center was staffed by only one full-time scientist. Assembling a team to assist with the international investigation in India required the involvement of epidemiologists, microbiologists, and experts in rodent and vector control from different parts of the organization. Effective surveillance will also require capturing the clinical data and recognizing the unusual. Ironically, although insurance companies have devised systems for collecting and sharing medical information for claims processing, there is no consistent way to access medical records electronically for medical or public health purposes (Dick and Steen, 1991). Therefore, clinicians and clinical microbiologists play a critically important role in recognizing and reporting such unusual diseases and syndromes.

The 20th-century revolution in health and the consequent demographic transition lead inexorably to major changes in the pattern of disease. This epidemiological transition results in a major shift, in causes of death and disability from infectious diseases to noncommunicable diseases. Health policy makers in the early decades of the 21st century will thus need to address a double burden of disease: first, the emerging epidemics of noncommunicable diseases and injuries, which are becoming more prevalent in industrialized and developing countries alike, and second, some major infectious diseases which survived the 20th century part of the unfinished health agenda.

Whether an emerging microorganism develops into a public health threat, depends on factors related to the microorganism and its environment, or the infected human and his or her environment. Such factors include ease of transmission between animals and people and among people, potential for spread beyond the immediate outbreak site, severity of illness, availability of effective tools to prevent and control the outbreak, and ability to treat the disease. Some of the new agents detected in the past 25 years are now genuine public health problems on a local, regional, or global scale.

The populations of developing countries, and particularly the disadvantaged groups within these countries, remain in the early stages of the epidemiological transition, where infectious diseases are still the major cause of death. Immunization programs have yielded the most significant changes in child health in the last few decades. Although some vaccines represent the most cost-effective public health intervention of all, the world does not use them enough. At least 2 million children still die each year

from diseases for which vaccines are available at low cost. Similarly, for diarrheal disease, there exists a simple, inexpensive, and effective intervention: the oral rehydration therapy. Diarrheal diseases and pneumonia together account for a high proportion of deaths of children in developing countries. In several developing countries, therefore, diarrheal disease control programs have been merged with a simplified approach, promoted by the World Health Organization (WHO), to detect acute respiratory infections (primarily pneumonia).

Despite the extraordinary advances of the 20th century, a significant component of the burden of illness globally still remains attributable to infectious diseases, undernutrition and complications of childbirth. These conditions are primarily concentrated in the poorest countries, and within those countries they disproportionately afflict populations that are living in poverty. Those living in absolute poverty, compared with those who are not poor, are estimated to have five times higher probability of death between birth and the age of 5 years, and a 2.5 times higher probability of death between the ages of 15 and 59 years.

Emerging infectious diseases result from newly identified and previously unknown infections, which cause public health problems, either locally or internationally. A recent example of an emerging disease is the new variant of Creutzfeldt-Jakob disease, which was first described in the United Kingdom, in 1996. The agent is considered to be the same as that causing bovine spongiform encephalitis, a disease which emerged in the 1980s and affected thousands of cattle in the United Kingdom and some other European countries. Following are examples of some agents causing emerging diseases.

Bacterial Agents

- *Legionella pneumophilia*: The detection of the bacterium in 1977 explained an outbreak of severe pneumonia in a convention center in the United States in 1976, and it has since been associated with outbreaks linked to poorly maintained air-conditioning systems.
- *Escherichia coli* O157:H7: Detected in 1982, this bacterium is typically transmitted through contaminated food and has caused outbreaks of hemolytic uremic syndrome in North America, Europe, and Japan. A widespread outbreak in Japan in 1996 caused more than six thousand cases among school children. During a single outbreak in Scotland in 1996, 496 people fell ill, of those 16 died.
- *Borrelia burgdorferi*: Detected in the United States in 1982 and identified as the cause of Lyme disease, this bacterium is now known to be endemic in North America and Europe and is transmitted to humans by ticks.
- *Vibrio cholerae* O139: First detected in 1992 in India, this bacterium has since been reported in seven countries in Asia. The emergence of a new serotype permits the organism to continue to spread and cause disease even in populations protected by antibodies generated in response to previous exposure to other serotypes of the same organism.

Viral Agents

- Ebola virus: The first outbreaks occurred in 1976, and the virion was discovered in 1977. Indigenous cases have been confirmed in four countries in Africa (Côte d'Ivoire, Democratic Republic of Congo, Gabon, and Sudan). Through June 1997, 1054 cases had been reported to WHO, 754 of which proved fatal. Monkeys infected with an

Asian strain of Ebola (Ebola-Reston) were the cause, but it does not appear to cause illness in humans.

- HIV: It was first isolated in 1983. By the beginning of June 1998, the number of HIV-positive cases reported to WHO by national authorities since the beginning of the epidemic was close to 1.9 million. However, it is estimated that since the start of the epidemic, 30.6 million people worldwide have become HIV infected and nearly 12 million have died from AIDS or AIDS-related diseases.
- Hepatitis C: Identified in 1989, this virus is now known to be the most common cause of post-transfusion hepatitis worldwide, with approximately 90 percent of cases in Japan, the United States, and Western Europe. Up to 3 percent of the world population is estimated to be infected, among which 170 million are chronic carriers at risk of developing liver cirrhosis or liver cancer.
- *Sin nombre* (i.e., an unnamed) virus was isolated from cases of a local outbreak of a highly fatal respiratory disease in the southern United States in 1993. It has subsequently been diagnosed in sporadic cases across the country and in Canada and several South American countries.
- Influenza A (H5N1) virus: Influenza virus is a well-known pathogen in birds and has been isolated from human subjects for the first time in 1997. The emergence of human influenza A (H5N1) initially followed a possible scenario of the expected next influenza pandemic, but in the event, the virus transmitted poorly, and the spread of the virus appeared to have been contained in 1997.

Re-Emerging Epidemics

As stated previously, the re-emerging infectious diseases are the result of the reappearance of, and an increase in, the number of infections from a disease that is known, but that had formerly caused so few infections that it had no longer been considered a public health risk.

Bacterial Diseases

- Cholera: Cholera has been reintroduced into countries and continents where it had previously disappeared and where it can spread because water and sanitation systems have deteriorated and food safety measures are not adequate. In 1991, the seventh cholera pandemic reached the Americas, a place where cholera had not been registered for a century. In that year, more than 390,000 cases were notified in more than 10 South American countries, which altogether accounted for two-thirds of the number of cases in the world. In 1997, cholera outbreaks chiefly affected Eastern Africa, and although the overall numbers have declined since 1991, there were still more than 147,000 cases reported globally in 1997. In 1998, the epidemic spread over eastern and southern Africa and new outbreaks occurred in South America.
- Diphtheria: Diphtheria re-emerged in the Russian federation and some other republics of the former Soviet Union in 1994 and culminated in 1995 with more than fifty thousand cases reported. The re-emergence was linked to a dramatic decline in the immunization programs following the disruption of health services during the unsettled times immediately after the break-up of the Soviet Union.

- Meningococcal meningitis: Meningococcal meningitis occurs worldwide but devastating, large-scale epidemics have mainly been in the dry sub-Saharan regions of Africa, designated the "African meningitis belt." Since the mid-1990s, epidemics in this area have been on an unprecedented scale, and epidemic meningitis has also emerged in countries south of the meningitis belt.

Viral Diseases

- Dengue fever: Dengue fever has spread in many parts of Southeast Asia since the 1950s and re-emerged in the Americas in the 1990s following deterioration in active mosquito control and spread of the vector into urban areas. An infection with dengue virus, dengue hemorrhagic fever (DHF), is common in Asia, but it has also been reported in 24 countries including central and Southern America.
- Rift Valley fever (RVF): It is a zoonotic disease typically affecting sheep and cattle in Africa transmitted by mosquitoes among animals and to humans. The disease in humans is typified by fever and myalgia, but in some cases, progresses to retinitis, encephalitis, or hemorrhage. Following abnormally heavy rainfall in Kenya and Somalia in late 1997 and early 1998, RVF occurred over vast areas, producing disease in livestock and causing hemorrhagic fever and death among the human population.
- Yellow fever (YF): YF is an example of a disease for which an effective vaccine exists, but because it is not widely used in many areas, at-risk epidemics continue to occur. The threat of YF is present in 33 countries, including Africa and eight in South America. YF is typically a disease of the tropical forest areas where the virus survives in monkeys. Humans bring it back to their villages, and if a suitable mosquito vector is present, the disease will spread quickly and kill a large proportion of the susceptible population.

Antimicrobial Resistance

Another emerging public health issue is the rapidly growing number of pathogens becoming resistant to an increasing range of antibiotics. In many regions, the low-cost, first-choice antibiotics have lost their power to clear infections of *E. coli*, *Neisseria gonorrhoeae*, *Pneumococcus*, *Shigella*, *Staphylococcus aureus*, which increases the cost and length of treatment of many common diseases including epidemic diarrheal diseases, gonorrhea, pneumonia, and otitis. Further problems stem from the use of antimicrobial substances in food animal production. This view of antimicrobial resistance predicts that it could be reduced by delaying the emergence of resistance genes or by retarding their dissemination after they emerge.

The global spread of microbial resistance is a predominant reason why infectious diseases have not been conquered. It is commonly expressed that physician misuse of antibiotics is the cause of antibiotic resistance in microbes and that, if physicians could be convinced to use antibiotics responsibly, the war against microbes could be won. Unfortunately, this belief is a fallacy that reflects an alarming lack of respect for the incredible power of microbes.

It is obvious that microbes do not need human help in creating antibiotic resistance. On the other hand, what human beings can do is affect the rate of spread of bacterial resistance by applying selective pressure via exposure to thousands of metric tons of

antibiotics we have used in patients and livestock over the past half century (Palumbi, 2001). Methods to control unnecessary use of antibiotics include appropriate regulations on use of antibiotics in agriculture (including elimination of use of antibiotics to promote growth of food animals), restriction of antibiotic use to pathogen-specific agents, and limits on the common practice of using antibacterial agents for viral infections Clearly, it is desirable to use antibiotics only when appropriate, to try to limit selective pressure that increases the frequency of resistance. Nevertheless, the distinction between causality of microbial resistance and the rate of spread of resistance must be recognized if a true solution to the problem of antibiotic resistance is to be created. If the misuse of antibiotics causes drug resistance, the solution would be to strictly use antibiotics only when truly indicated and forever defeat microbial resistance.

Antimicrobial effectiveness is a precious, limited resource. Therefore, preserving antibiotic effectiveness can be viewed similar to society's responses to overconsumption and depletion of other precious, limited resources, such as oil and other energy sources, clean water and air, and forests (Laxminarayan et al., 2007). Society has tried to protect this resource against depletion through antimicrobial stewardship, including the placement of appropriate restrictions on antibiotic use and through infection control. Unfortunately, society has not acted to promote antibiotic restoration (i.e., the development of new antibiotics), and antibiotic restrictions have the unintended, negative consequence of further destabilizing an already fragile market situation for antibiotic research and development.

Another problem is that many of the antimicrobial agents no longer kill many strains of bacteria. Those resistant strains make proteins that nullify the effects of the agents in various ways. Each such protein is expressed by a gene that the strain's susceptible ancestors lacked. The rise of the prevalence of such resistance genes in the bacterial populations that always cover and sometimes infect is the problem. It can be seen as two processes: emergence and dissemination. The diversity of resistance genes, the intricacy of the networks of bacterial populations that disseminate them, and the specificity with which the use of antimicrobial agents build those networks and drive that dissemination make the control of resistance a complex and unending task.

The importance of host-to-host transmission of resistant strains makes infection control skills central to the management of resistance. These skills may be strengthened by establishing, targeting, and providing quality assurance for infection control procedures. In addition, skilled clinical use of antimicrobials is essential in retarding the spread of resistance and in treating patients infected with resistant bacteria. Proper usage of antimicrobials may be imparted to other clinicians, for example, by consultation and by collaborating with those clinicians to work out guidelines for usage of antimicrobials. The skills needed to monitor and manage resistance completely are unlikely to be found in one person and are probably not fully available or integrated in many places now. Thus, implementation may often require organization or reorganization of a team, acquisition of new skills, and new management of information. Existing infection control teams may have many of the skills, but assuming accountability for managing antimicrobial resistance might be the occasion for their augmentation.

Several factors contribute to the emergence and re-emergence of infectious diseases, but most can be linked with the increasing number of people living and moving in the world; rapid and intense international travel; overcrowding in cities with poor sanitation; substantially increased international trade in food, mass distribution of food and unhygienic food preparation practices; increased exposure of humans to disease vectors and

reservoirs in nature; and alteration of the environment and climatic changes, which have a direct impact on the composition and size of the population of insect vectors and animal reservoirs. Other factors include a deteriorating public health infrastructure that is unable to cope with the needs of the population. Travel has always been a vehicle to spread disease across the world, which needs monitoring and management at the country level because countries may differ greatly in their practices, policies, and problems, and at the global level because resistance genes and strains travel between countries. The most important monitoring and management, however, appear to be that done at the local level at each medical center. The movement of resistance genes and strains through the bacterial populations of a community, a hospital, an intensive care unit, or even a single patient may be more complex than the movement of a strain of influenza virus through humans worldwide. National or regional reference laboratories may test selected pathogens referred from medical centers, but local laboratories supporting the centers generate most of the information. Results of antimicrobial susceptibility tests need to be scrutinized, interpreted, and screened locally for accuracy before they can be used to project national or global trends.

Public Health Implications

Epidemiology is a tool that is essential for carrying out four fundamental functions: public health surveillance, disease investigation, analytical studies, and program evaluation. Although an active epidemiology unit will do other things as well, these are the key areas through which epidemiology contributes to the promotion of the public's health. A health department systematically collects, analyzes, interprets, and disseminates health data on an ongoing basis (Thacker and Berkelman, 1988). Public health surveillance, which has been called "information for action" (Orenstein and Bernier, 1990), is how a health department takes the pulse of its community. By knowing the ongoing pattern of disease occurrence and disease potential, a health department can effectively and efficiently investigate, prevent, and control disease in the community.

At the local level, the most common source of surveillance data is reports of disease cases received from health-care providers, who are required to report patients with certain "reportable" diseases, such as cholera, measles, or syphilis. In addition, surveillance data may come from laboratory reports, surveys, disease registries, death certificates, and public health program data such as immunization coverage. It may also come from investigations by the health department of cases or clusters of cases reported to it. Most health departments use simple surveillance systems. They monitor individual morbidity and mortality case reports, record a limited amount of information on each case, and look for patterns by time, place, and person. Unfortunately, with some reportable diseases, a health department may receive reports of only 10 to 25 percent of the cases that actually occur (Marier, 1977). Nevertheless, health departments have found that even a simple surveillance system can be invaluable in detecting problems and guiding public health action. The principal epidemiologist of a large county health department has said "surveillance is the practicing epidemiologist's primary occupation; it pervades and keynotes all his activities" (Peterson, 1970).

For some diseases, the most appropriate intervention may be directed at controlling or eliminating the agent at its source. In the hospital setting, patients may be treated or isolated, with appropriate enteric, respiratory, and universal precautions and the like for

different exit pathways. In the community, soil may be decontaminated or covered to prevent escape of the agent.

Recent epidemiological and modeling studies have attempted to provide explanatory theories for the mechanisms of multiple outbreaks of an infectious pathogen capable of establishing an epidemic (Alexander et al., 2009). Spontaneous behavioral changes (e.g., a change in the number of contacts as a result of modified behavior of susceptible individuals) have been shown to affect the course of infection events and produce subsequent outbreaks in an epidemic episode (Poletti et al., 2009). This has been further investigated through modeling "concerned awareness" of individuals that may result in contagion dynamics of fear and disease (Epstein et al., 2008), and the implementation of public health control measures (e.g., social distancing) that may interfere with the individuals' contact patterns during the epidemic (Caley et al., 2008). Coinfection has also been suggested as a possible explanation for multiple infection outbreaks as a result of increased transmissibility in co-infected individuals and non-synchronicity in the time course of the two co-circulating infections. Other possible mechanisms include transient postinfection immunity and evolutionary changes that may occur in the characteristics of the infectious pathogens.

According to the CDC (2009), control measures available to individuals are simple and include the following: avoiding hand contact with nose and mouth after contact with diverse individuals, regular hand-washing, covering the mouth when coughing and sneezing, staying away from public places when infected to protect others from infection, avoiding overcrowded places, seeking prompt medical attention when suspect symptoms appear, avoiding nonessential travel to areas hit by the epidemic and heeding health agencies' cautions and warnings on the infection, where available. In the home, household surfaces can also be disinfected with a disinfectant or household bleach.

Control measures for adoption by government agencies and corporate bodies include: declaring the real infection rates within the country because countries are known to hide infection figures (Chew, 2007); provision of modern and effective equipment for diagnosis; ensuring effective screening of immigrants and emigrants at borders and where necessary, quarantining to contain infection spread; establishing of surveillance center to monitor and report infections; providing widely available and affordable antiflu medications such as Tamiflu® and Relenza®; and provision of vaccinations.

Effective public health measures regarding containment and management of emergencies, including information dissemination, are at best slow acting and limited largely to the urban center. This leaves the larger proportion of the rural population at ignorant risk of infections to which they lack basic information on preventive measures. Compounding this is apathy to warnings and cautions, when given, from health authorities by the largely illiterate populace.

Since 1992, alarm over emerging and re-emerging diseases has resulted in a number of national and international initiatives to restore and improve surveillance and control of communicable diseases. In 1995, a resolution of the World Health Assembly (WHA) urged all member states to strengthen surveillance for infectious diseases to promptly detect re-emerging diseases and identify new infectious diseases. This resolution led to WHO's establishment of the Division of Emerging and other Communicable Diseases Surveillance and Control (EMC), whose mission is to strengthen national and international capacity in the surveillance and control of communicable diseases, including those that representing new, emerging, and re-emerging public health problems.

References

Alexander ME, Dietrich SM, Hua Y, Moghadas SM. 2009. A comparative evaluation of modelling strategies for the effect of treatment and host interactions on the spread of drug resistance. *J Theor Biol* 259:253–263.

Beaglehole R, Bonita R. 2004. *Public Health at the Crossroads: Achievements and Prospects*, 2nd ed. Cambridge: Cambridge University Press.

Berkelman RL, Bryan RT, Osterholm MT, LeDuc JW, Hughes JM, et al. 1194. Infectious disease surveillance: A crumbling foundation. *Science* 264(5157):368–370.

Berry RJ, Crawford TJ, Hewitt GM. 1992. *Genes in Ecology*. Oxford: Blackwell Scientific.

Caley P, Philip DJ, McCracken K. 2008. Quantifying social distancing arising from pandemic influenza. *J R Soc Interface* 5:631–639.

Centers for Disease Control and Prevention (CDC). 2009. What to do if you get sick: 2009 H1N1 and seasonal flu. Accessed March 25, 2013, at http://www.cdc.gov/h1n1flu/sick.htm.

Chew SK. 2007. SARS: How a global epidemic was stopped. *Bull World Health Org* 85:324–324.

Cohn SK. 1997. Introduction. In: Herlihy D, ed. *The Black Death and the Transformation of the West*, pp. 1–15. Cambridge, Mass: Harvard University Press.

Cohn SK. 2002. *The Black Death Transformed: Disease Culture in Early Renaissance Europe*. London: Edward Arnold.

Cohn SK. 2003. Black Death. In: Demeny P, McNicoll G, eds. *Encyclopedia of Population*, pp. 98–101. New York: Macmillan.

Dick RS, Steen EB. 1991. *The Computer-Based Patient Record: An Essential Technology for Health Care*. Washington, DC: National Academy Press.

Doll R, Hill AB. 1954. The mortality of doctors in relation to their smoking habits; A preliminary report. *Br Med J* 1(4877):1451–1455.

Epstein JM, Parker J, Cummings D, Hammond RA. 2008. Coupled contagion dynamics of fear and disease: Mathematical and computational explorations. *PLoS ONE* 3:e3955.

Flahault A, Valleron AJ. 1992. A method for assessing the global spread of HIV-1 infection based on air travel. *Math Popul Stud* 3:161–171.

Henderson DA. 1993. Surveillance systems and intergovernmental cooperation. In: Morse SS, ed. *Emerging Viruses*, pp. 283–289. New York: Oxford University Press.

Herlihy D. 1997. *The Black Death and the Transformation of the West*. Cambridge, Mass: Harvard University Press.

International Federation of Red Cross and Red Crescent Societies. 2009. The epidemic divide. Accessed March 25, 2013, at http://www.ifrc.org/Global/Publications/Health/170800-Epidemic-Report-EN-LR.pdf.

Kelland K. 2011. HIV epidemics emerging in Middle East, North Africa: Study. Accessed February 11, 2013, at http://www.reuters.com/article/2011/08/02/us-aids-mideast-idUSTRE77165O20110802.

Koopman J. 1995. Brazilian congress highlights new directions for epidemiologic methodology. *Epidemiol Monitor* 16:3–8.

Last JM. 1988. *Dictionary of Epidemiology*, 2nd ed. New York: Oxford University Press.

Laxminarayan R, Malani A, Howard D, Smith DL. 2007. Extending the cure: Policy responses to the growing threat of antibiotic resistance. Washington DC: RFF. Accessed February 11, 2013, at http://www.rff.org/rff/Documents/ETC_fullreport.pdf.

Lederberg J, Shope RE, Oaks SC Jr., eds. 1992. *Emerging Infections: Microbial Threats to Health in the United States*. Washington, DC: National Academy Press.

Longini IM Jr, Fine PE, Thacker SB. 1986. Predicting the global spread of new infectious agents. *Am J Epidemiol* 123:383–391.

Marier R. 1977. The reporting of communicable diseases. *Am J Epidemiol* 105:587–590.

McCurry J. 2006. Japan remembers Minamata. *Lancet* 367:99–100.

McGinnis JM, Foege WH. 1993. Actual causes of death in the United States. *JAMA* 270: 2207–2212.
McNeill WH. 1976. *Plagues and peoples*. Garden City, NY: Anchor Press.
Moore PS. 1992. Meningococcal meningitis in sub-Saharan Africa: A model for the epidemic process. *Clin Infect Dis* 14: 515–525.
Morse SS. 1991. Emerging viruses: Defining the rules for viral traffic. *Perspect Biol Med* 34:387–409.
Orenstein WA, Bernier RH. 1990. Surveillance: Information for action. *Pediatr Clin North Am* 37:709–734.
Palumbi SR. 2001. Humans as the world's greatest evolutionary force. *Science* 293:1786–1790.
Pavlovskii EN. 1996. *Natural Nidality of Transmissible Diseases, with Special Reference to the Landscape Epidemiology of Zooanthroponoses*. Urbana: University of Illinois Press.
Peterson DR. 1970. The practice of epidemiology. In: Fox JP, Hall CE, Elveback LR, eds. *Epidemiology: Man and Disease*, pp. 315–332. New York: Macmillan.
Poletti P, Caprile B, Ajelli M, Pugliese A, Merler S. 2009. Spontaneous behavioural changes in response to epidemics. *J Theor Biol* 260:31–40.
Satcher D. 1995. Emerging infections: Getting ahead of the curve. *Emerg Infect Dis* l:l–6.
Susser discusses "ecologism" as new paradigm. 1995. *Epidemiol Monitor* 16:1,7,13.
Thacker SB, Berkelman RL. 1988. Public health surveillance in the United States. *Epidemiol Rev* 10:164–190.

Chapter 2
Epidemics Fundamentals

Introduction

Ancient people observed the incidental association of conspicuous natural events with outbreaks of epidemics and presumed that divine or supernatural forces were responsible for outbreaks. Smallpox (or "Mahamari") epidemics in India were attributed to divine wrath. In Europe, increased incidence of rabies was observed at the time of rising of Sirius ("dog star"). In Egypt, outbreaks of gastrointestinal diseases that occurred as a result of flooding of the Nile were attributed to "fury of the Gods." In 1717, Lancisi explained the origin of epidemics using the "miasma theory," which was based on the assumption that when air was of "bad quality" (precisely undefined state), the persons breathing that air would become ill. In contagious diseases, the miasma was believed to pass on from affected individuals to susceptible individuals. Malaria (Latin *Mal* = bad) was attributed to miasma (Last, 1983). The predominance of cases of malarial fever in marshy areas led to the concept "bad air" causes malaria. Apart from these there are so many potential epidemics that impose a great threat to humanity. In response, the World Health Organization (WHO) ensures the international coordination of epidemic knowledge, particularly for diseases of international public health importance or when countries lack the capacity to respond to an epidemic themselves. These responses can vary from investigating the cause of an epidemic, to verifying and disseminating information, and to providing needed equipment and laboratory supplies.

Definitions

- **Epidemic**: (Greek. *Epi* = upon; *demos* = people) is "an unusual or unexpected occurrence of a disease or a health-related condition in a region or a population, which is clearly in excess of the expected occurrence." The expected (or usual) occurrence may vary from region to region (Bissell, et al., n.d.).

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

- **Endemic**: (Greek. *en* = within; *demos* = people) is the constant presence of a disease or a health-related condition in a population in a particular geographical area, which is not imported from outside the area.
- **Pandemic**: (Greek. *pan* = all; *demos* = people) is an epidemic occurring over a wide area and usually affecting a large proportion of the world population.
- **Elimination (of a disease)**: Elimination refers to the "termination of transmission of a disease from a large geographical region" (Dowdle, 1999). The term *elimination* is equivalent to "regional eradication." Because diseases are not restricted to national boundaries, this definition is arbitrary. It can be thought of as a step ahead of control, progressing toward the eradication of a disease. The last reported case of guinea worm disease in India was in the year 1996. Measles has been eliminated from many developed countries. Andaman and Nicobar Islands (India), Australia, Britain, Cyprus, Finland, Gibraltar, Iceland, Ireland, Japan, New Zealand, Sweden, and Taiwan (China) are rabies free. Seas and oceans appear to form a natural barrier against the spread of rabies.
- **Eradication (of a disease)**: This is the "termination of all transmission of a disease by the extermination of the infectious agent through surveillance and containment" (Dowdle, 1999). It denotes an irreversible, enduring, extermination of the infectious agent, without the possibility of its persistence in humans, animals, or the environment. Smallpox is the sole example of an eradicated disease. WHO declared global eradication of smallpox on May 8, 1980.
- **Surveillance**: refers to "continuous scrutiny (or vigil) over the factors in the agent, host and environment, and the occurrence, frequency and distribution of diseases and health-related events in the community" (Manitoba Health, n.d.)
- **Reservoir of infection**: is "any person, animal, arthropod, plant, soil, or a combination of these, in which an infectious agent normally lives and reproduces itself in such a manner that it can be transmitted to a susceptible host." It has also been defined as "an ecological niche in which an infectious agent persists by a cycle of transmission or reproduction or both." Thus, a reservoir is a "natural habitat (animate or inanimate), in which an infectious agent metabolizes and replicates" (Last, 1983).
- **Agent**: A physical, chemical or biological factor, whose presence (e.g., pathogen), excessive presence (e.g., hypervitaminosis), or relative absence (e.g., hypovitaminosis) is responsible for the occurrence of a disease.
- **Clinical algorithm** (synonym: clinical protocol): is an explicit description of steps to be taken in patient care in specified circumstances. The algorithmic approach uses branching logic and all pertinent data to arrive at decisions that yield maximum benefit and minimum risk.
- **Natural history (of a disease)**: Many diseases have well-defined stages, such as stage of pathogenesis, presymptomatic stage, and symptomatic stage. Early detection and intervention may alter the natural disease process.

Declaring an Epidemic

A disease can occur both as an epidemic or an endemic, depending on the relative number of cases. For example, consider a hypothetical locality with a population of 100,000 where the monthly incidence of gastrointestinal diseases in July and December is about 900 and 130, respectively. The occurrence of 1,400 cases of gastrointestinal diseases in July of a given year would be considered as an epidemic, whereas the occurrence of 150

cases in December would constitute an endemic. On the other hand, the occurrence of a single case of a communicable disease, which is absent for a long period from the given area or population, may be labeled as an epidemic. For an example, smallpox is one of the two infectious diseases to have been globally eradicated; the other is rinderpest, which was declared eradicated in 2011 (Tognotti, 2010; The Mail and Guardian, 2011). The expected occurrence of any of these diseases is zero. Thus, the occurrence of even a single case of smallpox or rinderpest anywhere in the world will be clearly in excess of expected occurrence and will be considered an epidemic. Likewise, the first case of a disease, not previously known in that region, may be considered as an epidemic. The expected occurrence of yellow fever in India is zero. Hence, the occurrence of even a single case of yellow fever anywhere in India would be considered as an epidemic.

It is difficult to label a disease as an epidemic in a region where it is normally endemic. If the number of cases were more than two standard deviations from the mean number of cases (mean endemic frequency), the disease would be labeled an "epidemic" (Last, 1983).

Control of a Disease

According to Centers for Disease Control and Prevention (CDC; 1999), control refers to the "ongoing operations aimed at reducing the prevalence of a disease to a level where it is not a public health problem." This definition implies that interventional measures are used to achieve the reduction in prevalence to a level at which the disease is not a public health problem, however, transmission of the disease may continue. The level of prevalence at which control is considered as achieved is fixed arbitrarily and varies for each disease. The parameters for control of a disease would depend on various factors such as availability of resources for control operations, level of knowledge and technology, and sociopolitical conditions. During an epidemic, the emphasis is on the control of the epidemic because many diseases cannot be eliminated because of the carrier state of the disease (a state in which individuals harbor the pathogen, without manifesting clinical symptoms), unknown modes of transmission, vector resistance, and resistance of the causative organism.

Types of Epidemics

An epidemic outbreak usually follows a pattern based on environmental conditions and host factors (distribution, behavior, and sociodemographic characteristics). The epidemic tends to recur if these conditions remain persist. Hence for the prevention and control of epidemics, it is essential to know the types of epidemics and conditions that allow them to occur.

Point-Source Epidemics (Single-Exposure Epidemics)

A point-source outbreak is a common source outbreak in which the exposure period is relatively brief, and all cases occur within one incubation period. In these epidemics, the affected population is exposed to the causative agent at only one point of time. When the exposure is brief and simultaneous, all the vulnerable persons are usually affected during the incubation period of the disease. The time after which half of the cases will occur is called the *median incubation period*. The epidemic curve shows only one spurt, which rises

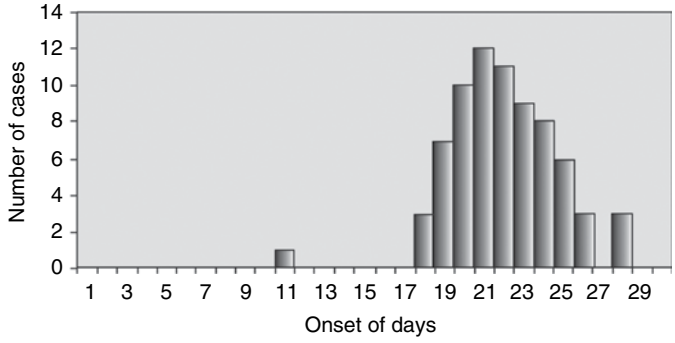

Figure 2.1. Point-source epidemic curve.

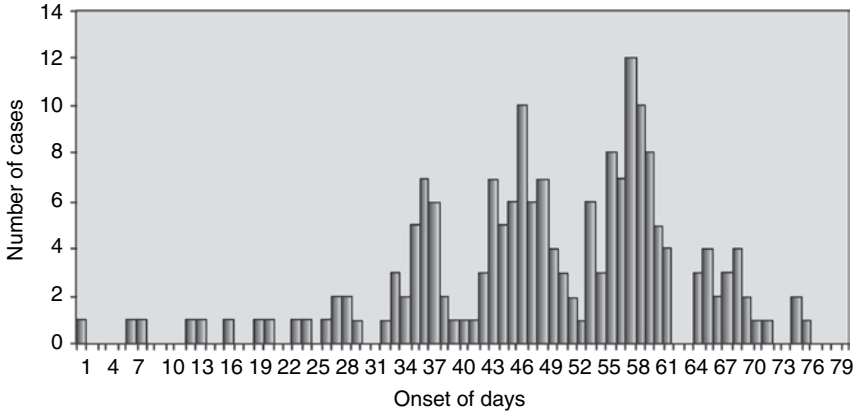

Figure 2.2. Propagated epidemic curve.

steeply and declines equally abruptly (Figure 2.1). In *explosive epidemics*, the epidemic curve shows "time-clustering" of cases during a narrow time interval. Examples include epidemics of food poisoning, accidents such as the Bhopal gas disaster in India and the Chernobyl nuclear accident in the former Soviet Union, which was the worst nuclear power plant accident in history (Last, 1983; Black, 2011).

Propagated Epidemics (Continuous- or Multiple-Exposure Epidemics)

A propagated outbreak is one that is spread from person to person. Because of this, propagated epidemics may last longer than common-source epidemics and may lead to multiple waves of infection, if secondary and tertiary cases occur. In these outbreaks, the affected population is exposed to the causative agent continuously. Such an epidemic continues, with a radial spread, until the source of infection is removed or controlled. The epidemic curve shows multiple spurts, which rise and fall (Figure 2.2), depending on the number of persons affected (secondary cases). The fall of the curve is slow, and it ends when the vulnerable population is either depleted or protected by interventional measures. A propagated epidemic of a disease with a short incubation period and high secondary

Table 2.1. Differences between point-source and propagated epidemics.

Features	Point-Source Epidemics	Propagated Epidemics
Type of exposure	Exposure to causative agent is simultaneous and from a common source	Exposure to causative agent is not simultaneous, but through a chain of successive transmission
Clinical manifestations	Clinical manifestations occur in all affected persons within the range of a single incubation period	Clinical manifestations occur in affected persons within the range of a number of incubation periods
Control	Usually controlled by intervention at the source of epidemic	Usually controlled by interruption of chain of transmission
Epidemic curve	Epidemic curve exhibits single, rapid spurt; there are no secondary curves*	Epidemic curve exhibits gradual rise and multiple spurts†
Eradication	The epidemic ends with cessation of exposure to source or with initiation of control measures	The epidemic ends when the susceptible population is depleted or protected by control measures

*Epidemics of cholera may start as point-source epidemics and continue as propagated epidemics.
†Epidemic curve of diseases with short incubation periods (such as cholera) may show a rapid rise and rapid fall.

attack rate (a highly infectious disease) will exhibit a rapidly rising and falling epidemic curve similar to that of a point-source epidemic. The speed of spread of the epidemic depends on host behavior, population density (which also determines the opportunity for contact), herd immunity, and secondary attack rate (Last, 1983).

Propagated epidemics are infectious in origin; examples include epidemics resulting from contamination of food by food handlers (such as typhoid carriers), gastroenteritis or cholera resulting from contamination of food or drinking water, sexually transmitted diseases, conjunctivitis, air-borne diseases, and vector-transmitted diseases such as malaria. Table 2.1 shows the cardinal differences between point-source and propagated epidemics.

Seasonal Cyclicity of Epidemics

Seasonal cyclicity refers to the annual cycling of incidence on a seasonal basis. Many infectious diseases exhibit increased incidence during certain seasons because environmental conditions favor transmission of the disease. A few health-related events also recur cyclically as a result of absurd human behavior; for example road accidents show an increased incidence during the New Year because of drunken driving. Demographic phenomena (marriages, births) also exhibit seasonal cyclicity (Last, 1983). Examples include:

- Monsoon: road accidents, water-borne and housefly-borne diseases (gastroenteritis, poliomyelitis), malaria, and snakebites
- Winter: air-borne diseases (upper respiratory tract infection, meningococcal meningitis)
- Early spring: measles and chickenpox
- Spring: asthma and hay fever
- Summer-Stroke and gastroenteritis.

Secular Cyclicity of Epidemics

Secular cyclicity refers to the long-term (more than 1 year) cycling of incidence of diseases. Epidemics of infectious diseases usually follow a pattern and repeat periodically when conditions are conducive for disease transmission, and there is an increase in the number of compromised patients (such as nonimmune children) in a relatively stable population. Epidemics of measles are known to occur in a cycle of 2 to 3 years, whereas hepatitis A has a higher incidence, every 7 years. Creation of herd immunity in the population (through natural infection or immunization) tends to break this cycle, preventing the disease from occurring as an epidemic (Last, 1983). Prior knowledge of cyclicity of epidemics can help in timely initiation of control measures.

Epidemics of Noncommunicable Diseases

Because of altered human lifestyle, the incidence of health-related events, such as accidents, and that of noncommunicable diseases, such as cancer, coronary heart diseases, diabetes mellitus, and mental/psychosomatic diseases, have shown a progressively increasing trend. Timely interventions (mainly comprising risk assessment and behavior modification) can control this epidemic (Last, 1983).

Epidemiological Triad

As described in the previous chapter, an epidemiological triad represents the interaction between an agent, host, and environment or place within a specific time dimension (Figure 2.3); these three epidemiological factors are responsible for determining the

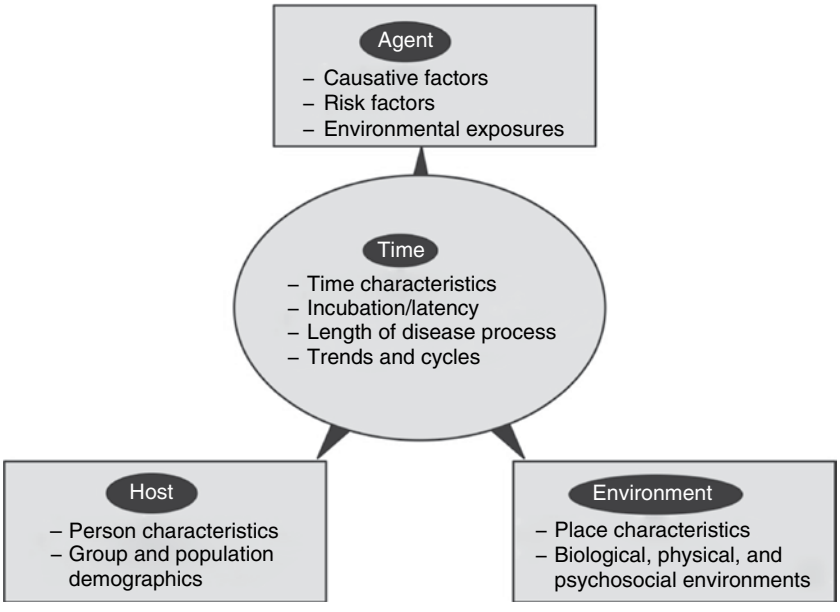

Figure 2.3. An epidemiological triad.
Source: Miller RE. 2002. *Epidemiology for Health Promotion and Disease Prevention Professionals*. New York: The Haworth Press.

occurrence, distribution, and frequency of a communicable or noncommunicable disease or a health-related condition such as accidents (Miller, 2002).

The agent is a necessary factor, which has to be present for morbidity, although it may not inevitably lead to disease. To perpetuate a disease, it requires the combination of sufficient factors, which include a host that might be an individual or group of susceptible individuals and an environment.

Agent Factors

- Physical: heat, cold, light, noise, vibration, or radiation.
- Chemical: endogenous (uremia, ketone bodies, bilirubin) and exogenous (pollution, food additives, food adulterants, pesticide and fertilizer residues in foods, extrinsic toxins in foods).
- Biological: microorganisms (protozoa, bacteria, viruses), vectors, or rodents.
- Nutritional: excess or deficiency of nutrients, intrinsic toxins in foods.
- Social: poverty, lifestyle, or addictions.

For noncommunicable diseases, such as hypertension, diabetes mellitus, and obesity, the agent factor cannot be clearly identified, and the etiology is discussed in terms of risk factors. Multiple risk factors that may cause a disease have to be directly or indirectly related to this triad. These risk factors may be:

- Causative: tobacco smoking, and lung cancer
- Contributory: lack of exercise and ischemic heart disease
- Predictive: maternal illiteracy and protein energy malnutrition in children
- Additive: tobacco smoking and occupational exposure to dust
- Synergistic: tobacco smoking and hypertension

Host Factors

Host factors may be non-modifiable (not amenable to alteration) or modifiable (amenable to alteration by the action of the host). Nonmodifiable host factors may be demographic (age, sex, ethnic, racial) and biological (genetic, immunity). The modifiable host factors are socioeconomic, educational (poverty, illiteracy), and lifestyle (food habits, clothing, housing, recreation, occupation).

Environmental Factors

- Physical: air, water, soil, terrain, lighting, ventilation, noise, or vibration
- Chemical: pollutants or pesticide contamination of the food chain
- Biological: microorganisms (protozoa, bacteria, viruses), vectors, or rodents
- Psychosocial and Cultural: fads, beliefs, peer pressure, traditions, or taboos. These are responsible for drug abuse, gender discrimination, promiscuity, and juvenile delinquency.

All the three factors (agent, host, and environment) of an epidemiological triad are not the watertight compartments. For example, food may be the agent factor for overnutrition,

undernutrition, and specific deficiency diseases. It is the host factor when the nutritional status of the individual is considered. Food becomes an environmental factor when it is a vehicle for food poisoning or carcinogens.

In combination, these three factors determine the onset and distribution of disease in individuals and groups. A disease will occur only if the situation is favorable in relation to these three factors. Reduction or elimination of any one factor leads to reduction or elimination of the other factors. Thus, the concept of an epidemiological triad provides multiple approaches for prevention and control of diseases.

Forecasting an Epidemic

The manifestation of an epidemic in a given community depends on social and demographic characteristics of the host population, cultural behavior and lifestyle, environmental factors, and genetic changes in the causative agent; thus an epidemic may not always exhibit its distinguishing features and may present with unusual manifestations. Accurate data is essential for forecasting an epidemic. The data sources for forecasting epidemics are given in Figure 2.4.

Along with the analysis of data, it is important to identify environmental conditions that are favorable for outbreaks. A hot summer may lead to shortage of drinking water, and thus, an epidemiologist can anticipate an epidemic of water-borne diseases resulting from consumption of contaminated water. Similarly, one can forecast the likelihood of water-borne and mosquito-borne diseases in cases of heavy monsoons because of contamination of drinking water and water logging.

Surveillance Data

Surveillance is the French word for "watching over." It is the monitoring of the behavior, activities, or other changing information, usually of people for the purpose of influencing, managing, directing, or protecting (Lyon, 2007). Disease surveillance is monitoring the progress of a disease in a community. Surveillance data represents an important data source for forecasting an epidemic.

Active Surveillance

Active surveillance is an active search for detecting diseases that are not recorded under routine system of reporting (passive surveillance). This includes the sampling

Data sources

Surveillance data	Data from health care system
• Active surveillance • Passive surveillance • Sentinel centers • Epidemiological investigations • Sample surveys	• Data from public health system • Disease notification by private medical practitioners • Lay reporting by spotters

Figure 2.4. Data sources for forecasting epidemics.

of clinically normal samples of the population, which are useful in the surveillance of diseases in which subclinical cases and carriers predominate. Recognition cards, showing the typical symptoms of a particular disease, have been used for active case detection of guinea worm disease, poliomyelitis, and neonatal tetanus. Active surveillance is carried out for malaria, diarrheal diseases, acute respiratory infections, and diseases prevented by vaccine. Health-care personnel, school teachers, and community leaders report easily recognizable conditions such as acute flaccid paralysis and diseases such as malaria, measles, and tuberculosis. Although time consuming and costly, active surveillance remains one of the most effective techniques in forecasting an epidemic.

Passive Surveillance

Passive surveillance is the examination of only clinically affected cases of specified diseases in the population. Passive surveillance is a cost-effective and uncomplicated technique for surveillance of disease and early forecasting of epidemics. Routinely collected data from various levels of the public health care system are reported in a standard format to the health authorities. Examples of diseases monitored nationwide are tuberculosis, poliomyelitis, diphtheria, pertussis, tetanus, measles, diarrheal diseases, cholera, malaria, dengue fever, hemorrhagic fevers, viral encephalitis, syphilis, gonococcal infections, meningococcal infection, influenza, enteric fever, chicken pox, and viral hepatitis.

Data from Sentinel Centers

Sentinel centers provide reliable information on selected diseases so that immediate action can be initiated. Criteria for selecting a sentinel center include high patient load in the institution, availability of accurate diagnostic facilities, and a reliable recording and reporting system. Immediate intervention is required if any abnormal increase in number of cases is reported as compared to the corresponding period in the previous years. Seasonal pattern of diseases can also be clearly established from data provided by sentinel centers. A disadvantage of sentinel surveillance is that sentinel centers provide selective data on patients attending these centers. This data cannot be extrapolated to indicate a trend of a disease in a locality.

Data from Epidemiological Investigations

Epidemiological investigations are useful in identifying cases, their age and sex-distribution; determining the geographical distribution of cases (using spot maps); incubation period and duration of an epidemic (from epidemic curve); determining the mode of transmission; and deciding the mode of intervention to contain the spread of an epidemic.

Sample Surveys

A sample survey is an active method of surveillance that is also used to assess outcome of interventional measures. Its disadvantages include its high cost, need for trained highly skilled personnel, and difficulty in conducting the survey.

Data from Health-Care System

Data from Public Health-Care System

Routine reports are collected on a monthly basis from all levels of the health-care system in the public sector and are compiled at district and state levels.

Notification

Notification of diseases by private medical practitioners also helps in early detection of outbreaks. Unfortunately, this system is still inadequate in many countries of the world, including India. Moreover, the list of diseases that need to be reported has interstate variations.

Lay Reporting by Spotters

Spotters are laypersons from the local community (school teachers and community leaders), who help in early detection of outbreaks. These spotters can report easily recognizable diseases such as diarrhea, malaria, measles, and chicken pox with reasonable accuracy.

Forecasting Techniques

Trends are analyzed by tabulating data on incidence and prevalence of morbidity and mortality and age and sex distribution on a monthly basis (for the corresponding months of the previous 3 years) and on an annual basis (for at least 3 years). The data should be depicted graphically and examined for seasonal and cyclic variation. Spot maps should also be used to detect geographical clustering of cases.

Contingency Plan

Ideally, the contingency plan should fit into the administrative structure of the state and should also be integrated into the existing plan for disaster preparedness. This involves preparation of inventory of the existing and required resources and a plan for mobilization of these resources.

Manpower

Various categories of manpower, from all possible sources, are to be listed and later trained. They should be available for mobilization at short notice. It is essential to prepare a list (containing names, addresses, contact numbers) of medical specialists, medical practitioners, laboratory technicians, and paramedical workers working in the governmental, nongovernmental and voluntary, and private sectors. For epidemics of zoonotic diseases, the services of veterinary doctors may be required. These trained personnel should be oriented about their role in the event of an epidemic. Volunteers are indispensable during emergencies. A list (containing names, addresses, and contact numbers) of all possible local volunteers, socially active individuals, and community leaders should be prepared with the help of voluntary and philanthropic organizations, and these persons should also be trained about their role in the event of an epidemic.

Money

A list of all the sources from where funds may be available in the event of an epidemic should be prepared so that the persons in charge of managing the epidemic know the exact amount of available money. Heads of expenditure are to be listed. Voluntary and philanthropic organizations can donate money, and socially active individuals can help in getting donations. Their names, addresses, and contact numbers should also be listed.

Materials

A list of all equipment (for laboratory investigations, resuscitation, clinical management, and vaccination) and drugs (including vaccines) that would be necessary for managing common epidemics should be prepared. Organizations (pharmaceutical manufacturers and suppliers; voluntary and philanthropic institutions) that may be willing to supply or assist in procuring vaccines, medicines, and equipment during an emergency should be identified. All the voluntary agencies, schools, community centers, and recreation facilities that are willing to provide accommodation and food for volunteers and other personnel during an emergency should also be listed.

Transport

Transport is required for movement of health-care personnel, volunteers, and for sending patients to referral hospitals. A list of all the available ambulances and vehicles such as buses, trucks, and pick-up vans that can be used as ambulances during an emergency should be compiled. All the organizations and individuals, who would be willing to provide vehicles (by type of vehicle) for transporting volunteers and other personnel during an emergency, should be enlisted.

Hospitals and Health Facilities

Locations of all existing hospitals and health facilities (government, municipal, private) are to be listed with the available number of beds and facilities, such as isolation ward, intensive care unit, and ambulance. The names, addresses, and contact numbers of managerial and specialist staff is also to be recorded. Schools, community centers, and recreation facilities can also be converted into temporary hospitals and outpatient facilities.

Media

During an outbreak, it is necessary to disseminate correct information quickly and efficiently, using all available media. Along with health messages on prevention and control measures, it is essential to give a correct daily update on the epidemic situation. For this purpose, arrangements are required with local radio stations, cable television operators, and local newspapers for delivering health messages. As a result of current technological advances, it is also possible to send text messages to a large number of people through cell phones.

Laboratory Support

A list of the essential routine and special laboratory investigations that are required to be carried out for various infectious diseases and the laboratories (at local, regional, and national levels) that carry out these tests should be prepared. For carrying out special investigations, a referral system should be developed and should also mention the method of transporting samples to the laboratory.

Investigation of Epidemics

Verification of Initial Reports

The initial report of an outbreak may be obtained from active, passive, and sentinel surveillance reports; reports from health facilities; reports in the media; and reports from the community itself. All initial reports, including rumors, must be investigated thoroughly. Information should be collected from all possible sources and verified. A retrospective study should be carried out in all health facilities, using records of patients for the past few months. Emphasis should be given to old cases in which the clinical manifestations were similar to that in the present outbreak and cases with unconfirmed diagnosis. An investigating team should visit the area from where the cases have been reported. On confirming the outbreak, steps should be initiated immediately for investigation and management. Investigation of an epidemic should go on simultaneously with control operations. Investigation into the etiology of the epidemic will help in preventing its recurrence. Various aspects to be probed include person distribution (age, sex, occupation, and other host factors); place distribution (geographical areas affected); possible etiology for the outbreak; and measures for containing the epidemic.

Steps for Case Definition

In the absence of standard case definitions, a provisional case definition should be prepared before an attempt to investigate an epidemic. The case definition includes usual and rare manifestations of the disease and criteria for deciding a "suspect," "probable," and "confirmed" case.

- **Suspect case**: The clinical manifestations are similar to that of the disease under investigation, but laboratory evidence of infection is lacking.
- **Probable case**: The clinical manifestations are similar to that of the disease under investigation, and the laboratory reports are suggestive of recent infection, but the reports do not provide conclusive evidence of infection.
- **Confirmed case**: The laboratory reports establish recent infection.

Formulating a Working Hypothesis

The working hypothesis should be based on the manifestations of the disease and its mode of transmission. This working hypothesis is subject to further modification or alteration based on the details ascertained during the investigation.

Field Investigation and Case Finding

Investigating teams should be sent at the earliest available time to the area where the outbreak is reported. The size of the investigating teams will depend on the extent of the affected area, geographical factors such as terrain and accessibility, and density of population in the affected area.

Laboratory Investigations

Laboratory examinations are essential for the diagnosis of a confirmed case. If laboratory facilities are lacking in a particular area, the specimens should be sent to other regional or national-level laboratories for the confirmation of diagnosis. Once the diagnosis is confirmed by laboratory reports in a few cases, it is not necessary to wait for laboratory confirmation for other cases. For containing the spread of the epidemic, suspect and probable cases should also be treated as confirmed cases.

A case investigation form is to be prepared for the investigation, and all investigators in the field should use this form. If it is not feasible to devise a common case investigation form that can be used for all types of epidemics, the reporting form should include:

1. Serial number, name of the investigator, and the date of investigation.
2. Personal particulars of the patient: full name, complete address with local landmarks, age, sex, occupation, and place of work.
3. Current history: Source of drinking water at home and place of work (piped water supply, public well (bore or dug), private well (bore or dug), river or lake/pond.). History of disinfection of drinking water and details of meals or snacks consumed at home and place of work is also recorded.
4. History of the past 6 months: places visited; history of food and water consumption (type of food, source of drinking water and place of consumption), vaccination or injections taken, history of contact with similar cases, and history of similar illness in the family.
5. Clinical history and examination: date of onset of symptoms, symptoms (anorexia, diarrhea, vomiting, aversion to tobacco/alcohol, high-colored urine, jaundice), signs (hepatomegaly, icterus), and details of the treatment given.
6. Laboratory investigations: This includes:
   (a) Clinical investigations: bile salts and pigments in urine, liver function tests, serum electrolytes, Australia antigen
   (b) Epidemiological investigations: chemical and bacteriological tests for drinking water and sewage
7. Field investigations:
   (a) Community profile: geographical location, terrain, social classes, endemic diseases in the area, and history of outbreaks with dates
   (b) Sources of water supply: sources that were actually visited and verified
   (c) Disinfection of water: person responsible for disinfection; date of disinfection; disease vectors in the area such as houseflies, rodents, and cockroaches; hygiene in eating houses; storm water drains: underground or surface and fecal contamination of surface drains; human excreta disposal: sewerage system, septic tank, service-type, open-air defecation; irrigation: type of irrigation or sewage farming in the area.

Detecting Source or Reservoir of Infection

Sources of infection may be cases or carriers. The reservoir of infection may be animate or inanimate. After finding out the range of incubation periods (from the dates of onset of the disease in identified cases), the source of infection should be sought in the time span between the minimum and maximum incubation periods.

Contact Tracing

Contact tracing refers to detection of cases or carriers among persons who come in any type of contact with the patient (members of the patient's household, health-care personnel, sexual partners of the patient, etc.). Contact tracing yields information on the mode of transmission of the disease.

Health-care personnel may be affected if the disease is transmitted by aerosols (air-borne droplets) or by contact with the patient's body fluids. All contacts should be clinically monitored and given chemoprophylaxis or immunoprophylaxis, where available. In highly infective diseases, contacts can be classified, based on the risk of getting infected:

- Close contact: A person who has had occasional face-to-face contact with the patient, has shared the same meal or the same room, has handled the patient's belongings, or has given personal care without personal protective equipment (masks, gloves) during the period of communicability of the disease.
- Possible or Casual contact: A person who does not fulfill the preceding criteria but may have been exposed in identical circumstances in the next bed, in the same hospital ward, or at the workplace, or in public transport.

Labeling and Information

Specimens are to be clearly labeled with the following information:

- Name of the patient and his or her registration number
- Name of the patient's mother and father (for identification)
- Age, sex, and complete residential address of the patient
- Name and address of the institution sending the sample
- Date of onset of illness
- Date of hospitalization
- Date of collection of sample
- Brief clinical history, examination findings, and provisional diagnosis
- Results of clinical laboratory investigations.

Storage and Transportation of Specimens

All samples and tissues should be stored and transported in appropriate media at the recommended temperatures. The laboratory that handles highly infectious pathogens must have high levels of biosafety. All the samples should be properly transported to the regional and public health laboratories.

Data Analysis

The disease causing the epidemic can be provisionally ascertained by recording the signs and symptoms up to the period of convalescence. It is possible to determine the incidence rate (the number of new cases per thousand population), the case fatality rate (the number of deaths among the cases divided by the total number of cases), and the probability of continued spread of the epidemic by analyzing the epidemiological data. The data are subjected to statistical analysis to confirm the observed mode of transmission. Data analysis helps in testing the working hypothesis. This can be further followed by preparation of spot maps and epidemic curve.

Spot Map

A spot map shows the geographic location of people with a specific attribute (Last, 1983). In epidemiology, spot maps are used to depict the geographical distribution of a disease or health condition. Spot maps are needed because geographical clustering of cases may not be reflected in morbidity and mortality statistics. The location of health-related events, such as cases and deaths, is depicted on a map. The map may be local, regional, national, or global, depending on the health-related event being studied. Spot maps provide information that is easy to grasp and available at a glance. The spot map depicts the progress of an epidemic: places affected at a given time, linear or centrifugal spread, and spread along water bodies. Clustering of cases gives clue about shared risk factor or common source of infection.

Epidemic Curve

An epidemic curve is a graph showing the distribution of cases by the time of onset (Last, 1983). The shape of the curve (rapid rise and fall; slow rise and gradual decline), number of spurts (single or multiple), and pattern of time-clustering reveal the type of the epidemic. It may reveal a relationship between incidence and various parameters (related to the host and outcome of interventional measures).

Management of Epidemics

All categories of personnel working in the public health sector and private medical practitioners should immediately notify any occurrence of diseases to local government or municipal health authorities. While notifying, the following information, should be provided:

- Name and address of the notifying health-care provider
- Name, age, sex, and address of the suspected case
- Date of onset of symptoms
- Dates and types of exposure to other affected persons
- Types of specimens collected for laboratory diagnosis
- Current status of the patient(s): whether cured, improving, worsened, or dead at the time of notification.

The number of new cases (incidence rate) is high for water-borne and air-borne diseases, and this may overburden the health care system. The response to such

outbreaks would need the mobilization of personnel, materials, and temporary health-care facilities (that should be identified in the contingency plan). If necessary, patients are to be hospitalized in an isolation ward, and procedures for concurrent disinfection carried out depending on the infectivity of disease. Routine health programs such as immunization should be continued in the event of an epidemic (Pan American Health Organization, 1985).

Control of Epidemics

The chain of disease transmission may be broken by interventions at the level of reservoir or source of infection, route of transmission, and susceptible population.

Control of Reservoir or Source of Infection

Control of Animal Reservoir or Source

Zoonotic diseases, such as brucellosis, rabies, and anthrax, can be controlled by screening animals and animal handlers for infection; treating infected animals and humans; destroying infected animals; avoiding close contact with animals; licensing, restraint, and vaccination of pets; and meat inspection and meat hygiene at slaughter houses.

Control of Human Reservoir or Source

To control human reservoir, case detection should be performed. Active case detection is undertaken for the early detection of infectious diseases to curb their spread to susceptible, whereas passive case detection is done at health-care facilities.

Isolation

Infected persons or domestic animals should be separated from others, for the period of communicability of the disease, to prevent direct or indirect transmission of the infectious agent. A patient may be isolated in the isolation ward of a general hospital or in a specialized isolation hospital. Home isolation may also be considered, provided preventive measures are undertaken. Home isolation is acceptable because it does not involve additional costs for the patient and also prevents nosocomial infections. The duration of isolation is disease specific because it should cover the entire period of communicability of the disease.

Quarantine

Quarantine is the limitation of freedom of movement of asymptomatic individuals or domestic animals suspected to have been exposed to a particular disease for the longest known incubation period of the disease to prevent contact with those not similarly exposed. Modified quarantine is a selective partial limitation of freedom of movement designed to meet particular situations. Exclusion of children from school is an example. International travelers (coming from an area, which is endemic for an internationally quarantinable disease) are compulsorily quarantined near international airports for a specified period if they do not have a valid vaccination certificates under the International Health Regulations formulated by WHO.

Health Education

In the event of an epidemic, the entire community should be educated to modify behavior so that the health of the susceptible in the population is safeguarded. In the event of an epidemic, all diagnosed patients should also be advised about protective measures to prevent the spread of the disease. For example, in diseases such as enteric fever, patients continue to excrete pathogens during convalescence. Because it is not feasible to detect all such carriers in a locality by laboratory tests, all convalescing patients should be advised to take simple precautions and maintain good personal hygiene.

Chemotherapy and Chemoprophylaxis

Effective chemotherapy for the recommended duration will render the patient non-infectious. Chemoprophylaxis may be recommended for carriers and patients contacts to reduce the environmental load of pathogens. These contacts should also be observed for early symptoms and signs of the disease.

Legal Aspects

Many governments have enacted laws that enumerate a list of diseases that should be compulsorily notified to the local health authority.

Interrupting Transmission

The transmission of endemic diseases can be interrupted by employing following measures:

- Safe and healthy housing: Poor housing and overcrowding are associated with increased risk of developing infestations such as skin diseases (resulting from increased physical contact), respiratory diseases (as a result of droplet infection), and diseases transmitted by vectors. Ventilation helps in diluting and removing the air-borne pathogens.
- Good housekeeping: Poor domestic and peri-domestic hygiene is responsible for providing hiding places for disease vectors such as arthropods and rodents. Dust suppression (by wet mopping of floors) can help in preventing transmission of air-borne infections.
- Disinfection and sterilization: Drinking water should be disinfected during epidemics of water-borne diseases. Disinfestation is the "destruction of ectoparasites and their ova by physical or chemical methods" (Montreal Protocol on substances that deplete the ozone layer, 1994). The method of disinfection will vary according to the mode of transmission of the disease.
- Disinfestation and vector control: This also includes the destruction of rodents, bed-bugs, and fleas.
- Nursing techniques: Barrier nursing is also useful to interrupt the transmission. It refers to the use of physical or chemical barriers by all categories of hospital personnel and is not restricted to nursing staff. Physical barriers include gowns, gloves, and other personal protective equipment (PPE), whereas washing hands with disinfectant constitutes a chemical barrier. The objective of barrier nursing is to prevent the spread of pathogens through the intermediary of hospital staff. Health-care providers with cuts, injuries, or infectious diseases should not be involved in patient care.

While imparting mouth-to-mouth resuscitation, the risk of transmission of HIV is low. However, it is safe to use a barrier; placing a gauze piece on the patient's mouth is advocated (Gangakhedkar, 1999). The procedures for effective barrier nursing include:

1. Repeated hand washing after attending to each patient
2. Concurrent and terminal disinfection
3. Using PPE
4. Establishing multidisciplinary hospital infection control committee
5. Periodic supervision of disinfection
6. Microbiological surveillance

- Barrier nursing is indicated in high-risk areas such as infectious disease wards and hospitals, neonatal wards, premature baby units, intensive care units, postoperative wards, and burn wards.
- Avoiding physical contact: An individual should avoid physical contact with the source of infection. For example, in areas that are endemic for hookworm infestation, using footwear would help in avoiding physical contact with the source of infection (i.e., the soil). Likewise, using insect repellents, mosquito nets, and screening of houses will prevent mosquito bites.
- Food and milk hygiene: Taking precautionary measures during food handling, storage, cooking, and serving of food can prevent the transmission of many food- and milk-borne diseases. Care should be taken to prevent contamination of milk after it is pasteurized or boiled.

Protecting Susceptible Population

The susceptible population should be protected by the socioeconomic development of a community, which leads to improved nutritional status. Nosocomial infections should be prevented by disinfection and sterilization of the hospital compound, isolating patients, and minimizing the number of visitors. Further immunoprophylaxis (prevention using active and passive immunizing agents as vaccines and antibody preparations) and chemoprophylaxis (use of chemotherapeutic agents in preinfection stage or in the incubation period to prevent the establishment of the pathogens) should be recommended depending on the type of infectious agent, severity of infection, and the immune state of the host. Chemoprophylaxis will be an effective measure only if the susceptible groups for the disease are easily identifiable, satisfactory compliance can be ensured, the risk of adverse effects and drug resistance is low, and the intervention is cost effective. It is difficult to distinguish between mass drug administration (also called "blanket chemotherapy") and chemoprophylaxis in public health practice. This method is useful for covering short-term risk in an easily identifiable small group of persons with a high risk of developing a disease.

Principles of Planning Emergency Services

Policies and Protocols

The objectives of emergency care are to render effective emergency medical and surgical care, to act as a receiving area for immediate clinical care and evaluation of casualties

during man-made or natural disasters, medico-legal conditions, psychiatric emergencies, etc., and to disseminate information about the emergency situation and casualties to the relatives, community members and the mass media.

- Policy decisions: While taking policy decisions on emergency care, guidelines for handling medico-legal and contagious patients; the financial policy such as hospital charges, if any; the scope of services to be provided; and the procedure for transfer/referral to other hospitals are to be pre-decided. Procedural delays are to be minimized. Patients are usually kept in an observation room for a maximum of 8 to 12 hours. Acute conditions requiring observation include renal colic, chest pain/discomfort, bronchial asthma, and hypersensitivity reactions.
- Protocol for treatment: Written protocols should be prepared for clinical decision making and treatment. Its objective is to ensure maximum efficiency and uniformity with minimum wastage of time. Algorithms (step-by-step procedures) may be prepared for specific conditions, such as injuries, fractures, burns, and infectious diseases. These protocols and algorithms are to be prepared by a committee of experts in related faculties. Copies of these protocols must be displayed in emergency or casualty department.
- Policy on record keeping: Records (clinical notes, treatment given, referrals, copy of written instructions given to patients before discharge from hospital) should be kept because the emergency or casualty department is prone to legal liabilities. It is necessary to develop norms for storage, retrieval, and transfer of records.
- Staffing: Policy for securing services of all categories of staff is to be pre-decided. Written job description for each category of staff; round the clock duty roster for each category of staff (including telephone numbers of doctors and consultants); and alternative arrangement in case of absenteeism of staff is to be displayed.
- Chain of command: The chain of command is to be decided beforehand and is to be conveyed to all categories of personnel. Usually, hospital services are controlled by three chains of command (administrative, medical, and nursing), but during emergencies, personnel from multiple organizations and multiple disciplines (medical and nursing personnel, home guards, fire brigade) may be deployed.

Classification of Emergency Facilities

The US National Academy of Sciences has classified emergency facilities into four types (Table 2.2). Scope of services to be provided at each type of facility is to be pre-decided while planning emergency services.

Types of Basic Infrastructure

The types of infrastructure for an emergency facility include:

- Core type: Beds are located around a central nursing station. The patients will enter and exit cubicles through a peripheral corridor.
- Arena type: Basically a core type but without the peripheral corridor. The patients will enter and exit cubicles through the central arena.
- Corridor type: Many variations of this type are possible.

Table 2.2. Types of emergency facilities.

Feature	Type I	Type II	Type III	Type IV
Type of facility	Major	Basic	Stand-by	Referral
Staff	Specialists on round-the-clock duty, medical officers, and trained nurses	Medical officers on round-the-clock duty, specialists on call, and trained nurses	Emergency nurse and medical officer on call	Only emergency nurse OR auxiliary nurse on duty for first-aid purposes only
Example	Teaching hospital	Sub-district or district hospital	Primary health center	Sub-center

National Institute of Health and Family Welfare (NIHFW). 1993. Facilitator's guide: Immunization handbook for medical officers. Accessed March 25, 2013, at http://nihfw.org/pdf/NCHRC-Publications/ImmuniFacGui.pdf.

Basic Physical Infrastructure

This should have a provision of a wide doorway with ramp for entry of stretchers, trolleys, and wheelchairs; provision for a parking space for vehicles such as ambulances and police vans; space to be provided for police checkpoint adjacent to emergency or casualty department; waiting area for visitors or relatives to be provided with public address system, public phones, drinking water, toilets, and seating facilities; isolation room for patients with contagious diseases; and sound-proof security room for noisy and disturbed or mentally ill patients.

Coordination

- **Intra-institutional**: coordination between pathology, microbiology, and biochemistry laboratories, radiography, electrocardiography, central sterile supplies department, maintenance and engineering, operation theater, labor room, pharmacy, stores, mortuary, super-specialty departments, and hospital administration. Only minimum and urgent investigations are to be ordered by the emergency or casualty department.
- **Interinstitutional**: coordination with related services in the locality and facilitates sharing facilities and help in immediate supplies during emergencies. These include other hospitals involved in health-care delivery including private medical practitioners, ambulance, hearse, blood banks, laboratories, and chemists.
- **Intersectorial**: coordination with the police department, home guards, press, and the electronic media. Written guidelines for reporting to mass media are to be specified. Only one responsible senior official is to be assigned to inform the mass media and community members. This arrangement should be known to all categories of staff.

Mobilization of Resources

Resources (manpower, materials, and money) are best mobilized by establishing an emergency committee comprising the emergency medical officer, specialists, nursing staff, and hospital administration. Steps in resource mobilization include assessing existing procedures for emergencies; preparing a list of existing manpower and inventory of

existing materials and equipment in the institution and other institutions in the locality; assessing additional resources required; and identifying resource persons in the locality and networking with them.

Quality of Emergency Services

In most of the developing countries with a shortage of skilled health-care personnel and a large population, emergency departments may also provide basic health care to patients in need. This fact should be kept in mind while evaluating the quality of care. The parameters for evaluating quality include:

- mobilization of resources
- use of manpower
- team work
- maintenance of equipment
- availability of supplies
- quality of supervision
- **Concurrent review**: This includes assessment of patient care at the time of service delivery by observation and appraisal of client satisfaction by interviewing patients (feedback).
- **Retrospective review**: This is also called a *medical audit* and involves a systematic retrospective analysis of medical records. Retrospective review reveals conformity with pre-existing norms for emergency patient care and whether patient care was of acceptable quality and timely. It also helps in continuing improvement of quality of care, provides comparative assessment of patient care at present and in the past, and enables interventions for improving patient care strategies in future.

References

Bissell RA, Lopez R, Burkeholder-Allen K. n.d. Post-disaster infectious disease management. Accessed March 25, 2013, at http://mediccom.org/public/tadmat/training/NDMS/inf_disease.pdf.

Black R. 2011. Fukushima: As bad as Chernobyl? Bbc.co.uk. Accessed February 12, 2013, at http://www.bbc.co.uk/news/science-environment-13048916.

Centers for Disease Control and Prevention (CDC). 1999. Achievements in public health, 1900–1999: Control of infectious diseases. *MMWR Weekly* 48(29):621–629.

Dowdle WR. 1999. The principles of disease elimination and eradication. *MMWR* 48(SU01):23–27.

Gangakhedkar RR. 1999. Universal precautions in hospital settings. *AIDS Research and Review* 2:146–150.

Lancisi GM. 1717. *De noxiis paludum effluvius eorumque remediis*. Rome: J. M. Salvioni.

Last JM. 1983. *A Dictionary of Epidemiology*. Oxford: Oxford University Press.

Lyon D. 2007. *Surveillance Studies: An Overview*. Cambridge: England: Polity Press.

Manitoba Health, Public Health, Communicable Disease Control Unit. n.d. Communicable disease surveillance. Communicable Disease Management Protocol Manual. Accessed March 25, 2013, at http://www.gov.mb.ca/health/publichealth/cdc/protocol/surveillance.pdf.

Miller RE. 2002. *Epidemiology for Health Promotion and Disease Prevention Professionals*. New York: The Haworth Press.

Montreal Protocol on substances that deplete the ozone layer. 1994. 1994 Report of the Methyl Bromide Technical Options Committee. Accessed March 25, 2013, at http://ozone.unep.org/teap/Reports/MBTOC/MBTOC94.pdf.

National Institute of Health and Family Welfare (NIHFW). 1993. Facilitator's guide: Immunization handbook for medical officers. Accessed March 25, 2013, at http://nihfw.org/pdf/NCHRC-Publications/ImmuniFacGui.pdf.

Pan American Health Organization (PAHO). 1985. *The Earthquake in Mexico*. Emergency Preparedness and Disaster Relief Coordination Program. Disaster Reports Series No. 3. Washington, DC: PAHO.

The Mail and Guardian. 2011. The world is free of rinderpest. Accessed February 12, 2013, at http://mg.co.za/article/2011-05-25-the-world-is-free-of-rinderpest/.

Tognotti E. 2010. The eradication of smallpox, a success story for modern medicine and public health: What lessons for the future? *J Infect Dev Ctries* 4:264–266.

Chapter 3
Disasters and Epidemics

Fundamentals

According to Noji 1997, "a disaster is a result of a vast ecological breakdown in the relation between humans and their environment, a serious or sudden event on such a scale that the stricken community needs extraordinary efforts to cope with it, often with outside help or international aid" (p. 4). These disasters may be natural disasters, transportation disasters, or disasters resulting of terrorism or technological events, and they more commonly follow an epidemic or pandemic. This chapter only deals with the epidemics following natural disasters. Some disasters such as floods, drought, earthquakes, tornadoes, tsunamis, volcanic eruptions, and landslides may be followed by a wide range of epidemic diseases, which is particularly a result of an overestimation of the capacity for disease spread from dead bodies (Toole, 1997). After a natural disaster, the communicable diseases primarily spread as a result of population displacement and overcrowding with a breakdown in infrastructure. Various factors contribute to the severity of a disaster including human vulnerability resulting from poverty and social inequality, environmental degradation, and rapid population growth, especially among the poor.

These natural disasters are catastrophic events with atmospheric, geologic, and hydrologic origins, which can have rapid or slow onset and serious health, social, and economic consequences. They have killed millions of people over the last 20 years, impacting the lives of at least one billion more people and resulting in enormous economic damages. In the decade from 1994 to 2004, there were approximately one million thunderstorms, 100,000 floods, tens of thousands of landslides, earthquakes, wildfires, and tornadoes, and several thousand hurricanes, tropical cyclones, tsunamis, and volcanoes (Noji, 2005). They disproportionately affected developing countries because of the lack of resources, infrastructure, and disaster-preparedness systems (Watson et al., 2007).

Natural disasters comprise three phases: an impact, postimpact, and recovery phases (Peters, 2011). The impact phase starts from 0 to 4 days and involves the extrication of

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Table 3.1. Risk of communicable diseases after disasters, by mode of transmission.

Disaster Type	Person-to-Person	Water-Borne	Food-Borne
Volcano	Medium	Medium	Medium
Earthquake	Medium	Medium	Medium
Hurricane	Medium	High	Medium
Tornado	Low	Low	Low
Heat wave	Low	Low	Low
Cold wave	Low	Low	Low
Flood	Medium	High	Medium
Famine	High	High	Medium
Air pollution	Low	Low	Low
Industrial accident	Low	Low	Low
Fire	Low	Low	Low
Radiation	Low	Low	Low
Civil war or refugees	High	High	High

Adapted from Noji EK. 1997. The nature of disasters. In: Noji EK, ed., *The Public Health Consequences of Disaster*, pp. 21–36. New York: Oxford University Press.

victims and treatment of immediate soft-tissue infections. Hypothermia, heat, illness, and dehydration are characteristic features of this impact phase. The postimpact phase starts from 4 days to 4 weeks post-disaster and involves air-borne, food-borne, water-borne and vector-borne diseases, such as cholera, bacterial dysentery, cryptosporidiosis, rotavirus infection, norovirus infection, salmonellosis, typhoid and paratyphoid, giardiasis, hepatitis A and E, and leptospirosis. This phase also includes some communicable respiratory infections as viral (e.g., influenza, respiratory syncytial virus [RSV], adenoviruses), bacterial (e.g., *Streptococcus pneumoniae*, *Diphtheria pertussis*, *Mycobacterium tuberculosis*, *clostridium tetani*, *Legionella* sp., *Mycoplasma pneumonia*, and *Neisseria meningitides*), and diseases transmitted via the respiratory route (e.g., measles, varicella). The recovery phase begins after 4 weeks and involves diseases with long incubation periods (leishmaniasis and leptospirosis), vector-borne diseases (malaria, western/Saint Louis encephalitis, dengue, yellow fever, and West Nile virus), and chronic diseases (cardiac disease, hypertension, diabetes, and asthma) (Peters, 2011). Table 3.1 shows risk severity of communicable diseases after various forms of disasters depending on the modes of transmission.

During the recovery phase, already existing infectious diseases in the community (endemic) as well as newly imported diseases may lead to an outbreak of epidemic. There are so many infectious diseases reported occurring due to the displacement of the population following natural disasters (Table 3.2).

Public health emphasizes the prevention of these communicable diseases and the promotion of health. Disaster preparedness and accurate response are other significant areas in the field of public health. Disasters pose threats to the general public through increases in injury, death, and changes in infrastructure. The public health response to disaster includes assessments of the impact in the community, surveillance for disease, addressing sanitary health concerns, and providing accurate and timely information to

Table 3.2. Infectious diseases outbreak following natural disaster from 2000 to 2011.

Country	Disaster Event	Year(s)	Infectious Disease Outbreak Following the Natural Disaster	Reference
United States	Tornado	2011	Cutaneous mucormycosis	CDC, 2011[a]
Japan	Earthquake	2011	Diarrhea (norovirus), influenza	AMDA, 2011[b]
Haiti	Earthquake	2010	Cholera	WHO, 2010[c]
Côte d' Ivory	Flood	2010	Dengue	IRIN, 2010[d]
Brazil	Flood	2008	Dengue	WHO, 2008[e]
United States	Hurricane Katrina	2005	Diarrhea, TB	CDC, 2003[f], 2005[g]
Pakistan	Earthquake	2005	Diarrhea, hepatitis E, measles, meningitis, tetanus	WHO, 2005a[h], 2006[i]
Dominican Republic	Flood	2004	Malaria	WHO, 2004[j]
Bangladesh	Flood	2004	Diarrhea	Qadri et al., 2005[k]
Indonesia	Tsunami	2004	Diarrhea, hepatitis A and E, ARI, measles, meningitis, tetanus	Brennan and Kimba, 2005[l]; WHO, 2005b[m]
United States	Hurricane (Allison)	2001	Diarrhea	Waring et al., 2002[n]
Taiwan	Typhoon (Nali)	2001	Leptospirosis	Yang et al., 2005[o]
China	Typhoon (Nali)	2001	Leptospirosis	Yang et al., 2005[o]
El Salvador	Earthquake	2001	Diarrhea, ARI	Woersching and Snyder, 2004[p]
Thailand	Flood	2000	Leptospirosis	WHO, 2004[j]
Mozambique	flood	2000	Diarrhea	Kondo et al., 2002[q]
India	Flood	2000	Leptospirosis	Karande et al., 2003[r]

[a] Center for Disease Control and Prevention (CDC). 2011. Fatal fungal soft-tissue infections after a tornado—Joplin, Missouri. *MMWR Morb Mortal Wkly Rep* 60:992.
[b] Association of Medical Doctors of Asia (AMDA). 2011. Emergency Relief Activities for the Great East-Japan Earthquake and Tsunami:11 March–20 April 2011. Accessed February 12, 2013, at http://www.amdainternational.com/english/activity_report/tohoku1.pdf.
[c] World Health Organization (WHO). 2010. Haiti: Response to the cholera outbreak. Accessed February 12, 2013, at www.who.int/hac/crises/hti/highlights/october2010/en/index.html.
[d] IRIN. 2010. Humanitarian news and analysis: Cote d' Ivoire: Yellow Fever, Dengue kill at least three. Accessed February 12, 2013, at www.irinnews.org/report.aspx?ReportID=90022.
[e] World Health Organization (WHO). 2008. Dengue/Dengue hemorrhagic fever in Brazil. Accessed February 12, 2013, at www.who.int/csr/don/2008_04_10/en/index.html.
[f] Center for Disease Control and Prevention (CDC). 2003. Treatment of tuberculosis: American Thoracic Society, DCD, and Infectious Diseases Society of America. *MMWR Morb Mortal Wkly Rep* 52 (RR11):1–77.
[g] Center for Diseases Control and Prevention (CDC). 2005. Norovirus outbreak among evacuees from Hurricane Katrina—Houston, Texas. *MMWR Morb Mortal Wkly Rep* 54: 1016–1018.
[h] World Health Organization (WHO). 2005a. Acute water diarrhea outbreaks. *Wkly Morb Mortal Rep* 1:6.
[i] World Health Organization (WHO). 2006. Acute jaundice syndrome. *Wkly Morb Mortal Rep* 23:8.
[j] World Health Organization (WHO). 2004. Flooding and communicable diseases fact sheet. Accessed February 12, 2013, at www.who.int/hac/techguidance/ems/flood_cds/en/.

(*Continued*)

the public. Mental health professionals can also play an important role in responding to disaster in the community.

Epidemiology detects patterns of various occurrences in populations. Common patterns of morbidity and mortality may be apparent during a disastrous event. Epidemiologists particularly focus on predictable patterns and clusters of diseases and injury. The thorough objective of epidemiological investigations during and after disasters are to assess the needs of disaster-affected populations, match available resources to needs, prevent further adverse health effects, implement disease control strategies, evaluate program effectiveness of disaster relief programs, and finally permit better and contingency planning for various types of future disasters (Noji, 2005).

A traumatic event becomes a public tragedy when there is a collective definition of that event as a significant calamity. A public tragedy combines factors of scope, identification, social value of the victims, consequences, duration, causation, intentionality, predictability, preventability, and perception of suffering (Doka, 2003).

Effective response and fulfilling the needs of the disaster-affected population requires an accurate communicable disease risk-assessment program. The efficient use of humanitarian funds depends on implementing priority interventions on the basis of this risk assessment.

A systematic and comprehensive evaluation should identify:

1. Endemic and epidemic diseases particularly common in the affected area
2. Living conditions of the affected population, including number, size, location, and density of settlements
3. Availability of safe water and adequate sanitation facilities
4. Underlying nutritional status and immunization coverage among the population
5. Degree of access to health care and to effective case management.

After each and every disaster, the risk for communicable disease transmission is primarily associated with the size and characteristics of the population displaced, specifically the proximity of safe water and functioning washrooms, the nutritional status of the displaced population, the level of immunity to vaccine-preventable diseases such as measles, and access to health-care services (Noji, 1997). Outbreaks are usually less

(*Continued*)

[k] Qadri F, Khan AI, Furuque ASG, Begum YA, Chowdhuy F, et al. 2005. Enterotoxigenic *Escherichia coli* and *Vibrio cholerae* diarrhea, Bangladesh. *Emerg Infect Dis* 11:1104–1107.

[l] Brennan RJ, Kimba K. 2005. Rapid health assessment in Aceh Java district. Indonesia, following the December 2006 Tsunami. *Emerg Med Australas* 17:341–350.

[m] World Health Organization (WHO). 2005b. Epidemicprone disease surveillance and response after the tsunami in Aceh Province, Indonesia. *Wkly Epidemiol Rec* 80:160–164.

[n] Waring SC, Reynolds KM, D'souza G, Arafat RR. 2002. Rapid assessment of household needs in the Houston area after tropical storm Allison. *Disaster Manag Response* 3–9.

[o] Yang HY, Hsu PY, Pan MJ, Wu MS, Lee CH, et al. 2005. Clinical distinction and evaluation of Leptospirosis in Taiwan—a case control study. *J Nephrol* 118:45–53.

[p] Woersching JC, Snyder AE. 2004. Earthquakes in El-Salvador: A descriptive study of health concerns in a rural community and the clinical implication—part II. *Disaster Manag Response* 2:10–13.

[q] Kondo H, Seo N, Yasuda T, Hasizume M, Koido Y, et al. 2002. Postflood—infectious diseases in Mozambique. *Prehosp Disaster Med* 17:126–133.

[r] Karande S, Bhatt M, Kelkar A, Kulkarni M, De A, et al. 2003. An observational study to detect leptospirosis in Mumbai, India. *Arch Dis Child* 88:1070–1075.

Table 3.3. Common Characteristics of Disaster Response.

Sharing information
Resource management
Warning and evacuation from danger
Search and rescue
Utilization of the mass media
Triage for assigning priorities for treatment and transport of the injured
Patient tracking
Management of volunteers and donations
Organized plan in response to disruption and unexpected problems

Adapted from Landesman L. 2005. *Public Health Management of Disasters: The Practice Guide*. Washington, DC: American Public Health Association.

frequently reported in disaster-affected populations as compared to conflict-affected populations, where two-thirds of deaths may be from communicable diseases (Noji, 2005). Malnutrition increases the risk for death from communicable diseases and is more common in conflict-affected populations, particularly if their displacement is related to long-term conflict (Spiegel, 2005).

Disaster-related deaths are devastatingly caused by the initial traumatic impact of the disastrous event. To fulfill the health needs of the surviving disaster-affected populations, disaster-preparedness plans should be prepared and especially focused on trauma and mass casualty management. The health effects associated with the sudden crowding of large numbers of survivors, often with inadequate access to safe water and sanitation facilities, will require planning for both therapeutic and preventive interventions, such as the rapid delivery of safe water and the provision of rehydration materials, antimicrobial agents, and vaccination materials for measles.

An effective public health disaster response requires an enormous amount of organizational communication and coordination among various agencies. Although each disaster may vary greatly in magnitude, common activities are likely to be present. Landesman (2005) discussed some common characteristics of a public health disaster response and some tasks that are likely to occur (Table 3.3).

In disaster-affected areas, surveillance is fundamental, helps to understand the impact of natural disasters on communicable disease illness and death, and to obtain relevant information regarding surveillance, However, surveillance is frequently challenging. The destruction of already-existing public health infrastructure can eliminate what may have been weak predisaster systems of surveillance and response. Surveillance officers and public health workers associated with the previous outbreaks may be killed or missing, as in Aceh in 2004. Finally, the population displacement following a disaster can distort the census information, making a calculation of rates for comparison difficult. During the emergency phase, the information about health care is often delivered by a wide range of national and international actors, creating coordination challenges. In addition, a lack of predisaster baseline surveillance information leads to difficulties in perfect and accurate differentiation of an epidemic from background endemic disease transmission. Table 3.4 lists 10 essential public health services particularly useful in emergency conditions.

Table 3.4. 10 Essential Public Health Services.

1. Monitor health status to identify community health problems.
2. Diagnose and investigate health problems and health hazards in the community.
3. Inform, educate, and empower people about health issues.
4. Mobilize community partnerships to identify and solve health problems.
5. Develop policies and plans that support individual and community health efforts.
6. Enforce laws and regulations that protect health and ensure safety.
7. Link people to needed personal health services and assure the provision of health care when otherwise unavailable.
8. Assure a competent public health and personal health-care workforce
9. Evaluate effectiveness, accessibility, and quality of personal and population-based health services.
10. Research for new insights and innovative solutions to health problems.

Adapted from Association of Schools of Public Health (ASPH). 2006. What is public health? Accessed February 12, 2013, at http://www.asph.org/document.cfm?page=300.

Contributory Factors

The Federal Emergency Management Agency (FEMA) declared 460 major disasters in 1990 as a result of severe weather events and natural phenomenon. In the United States alone, the government spent about $1 billion on disasters every week. Although the certainty of natural disasters may be unpredictable, the literature suggests other possible contributing factors including climatic cycles of increased weather extremes, increases in population and urbanization with more people living in high-risk areas, economic growth and technological advances, and increases in terrorist threats (Myers and Wee, 2005).

A variety of some other major factors may also contribute to increases in disaster trends; these includes human vulnerability resulting from poverty and social inequality, environmental degradation resulting from poor land use, and rapid population growth, especially among the poor. "The increasingly sophisticated and technical physical infrastructure of human culture is similarly more vulnerable to destruction than were systems of habituation and culture built in past generations. The result is that today the damage from natural and technological disasters tends to be more and more extensive if proper precautions are not taken" (Lundy and Janes, 2003, p. 346).

The best known example of natural disaster is a 1973 flood in West Virginia. On February 26, 1973, the Buffalo Creek flood devastated the homes of 5,000 people of a West Virginia mountain community, killing 125 and leaving 4,000 homeless. Kai Erikson a sociologist studied the effects of the Buffalo Creek flood on the local community members. He discusses in detail the notions of "communality," and how the flood devastated the community as a whole. "In places like Buffalo Creek, the community in general can be described as the locus for activities that are normally regarded as the exclusive property of individuals. It is the community that cushions pain, the community that provides a context for intimacy, the community that represents morality and serves the repository for old traditions" (Erikson, 1976, p. 103). Erikson further says that "[w]hat happened at Buffalo Creek, then, can serve as a reminder that the preservation (or restoration) of communal forms of life must become a lasting concern, not only for those charged with healing the wounds of acute disaster, but for those charged with planning a truly human future" (p. 154).

Another example is a hurricane Katrina, which hits the gulf coast on August 29, 2005. It devastated communities in Mississippi, Alabama, and Louisiana, killing more than

Table 3.5. Disease risk factors following natural disaster.

Population displacement from nonendemic to endemic areas
Overcrowding (close and multiple contacts)
Stagnant water after flood and heavy rains
Insufficient or contaminated water and poor sanitation conditions
High exposure and proliferation to disease vectors
Insufficient nutrient intake or malnutrition
Low vaccination coverage
Injuries

Adapted from Kouadio IK, Aljunid S, Kamigaki T, Hammad K, Oshitani H. 2012. Infectious diseases following natural disasters: prevention and control measures. *Expert Rev. Anti Infect Ther* 10:95–104.

1,300 people and making it the most destructive natural disaster in US history. Communities throughout the Gulf region were disappointed with the ineffective response of the local, state, and federal officials, with the whole nation watching the events unfold on television on a daily basis. Serious flaws in the response were highlighted and brought to the attention of the government. A catastrophic event such as Katrina should serve as an example of the importance of preparedness at local, state, and federal levels to effectively respond to a crisis.

Disasters do not carry diseases or epidemics. Disease risk factors (Table 3.5) increase or exacerbate the aftereffects of the disaster. Natural disasters and communicable infectious diseases continue to be a major threat to the global community and adversely affect the development of a specific country. To overcome this situation every country should follow preparedness measures by implementing a national preparedness plan, by empowering the local community in rescue activities, by training health and outreach health staff in the identification and management of particular diseases, and by creating a stockpile of supplies and equipments for the proper and accurate diagnosis of various pathogenic microbes, treatment facilities for the existing epidemics and ensuring sanitation. Protocols should be established for health information management, and national surveillance systems have to be strengthened. To prevent infectious diseases in disaster situations, the useful measures include education on hygiene and hand washing, provision of adequate quantities of safe water, sanitation facilities, and appropriate shelter to displaced population.

Investigation of Rumors

Rumors are circulating reports having unknown veracity or opinions that cannot be attributed to a discernible source. During and after a natural disaster, rumors frequently circulate requiring their rapid investigation and confirmation by public health officials who are responsible for surveillance and other information activities. There are so many reasons regarding the origin of these rumors such as the disaster can disrupt normal communication modes between affected population and civil officials and other authorities, health workers lacking an appropriate experience usually misdiagnose diseases with

epidemic potential, civil authorities and relief agencies might report incorrect number of casualties elevating fear level in the community, etc. These rumors should be investigated properly and correctly to reassure people that public health officials are implementing appropriate control measures.

The growth of information technology has produced new demands and possibilities for emerging disease surveillance and response. Increasing numbers of outbreak reports must be assessed rapidly so that control efforts can be initiated, and unsubstantiated reports can be identified to protect countries from unnecessary economic damage. The World Health Organization (WHO) has set up a process for timely outbreak verification to convert large amounts of data into accurate information for suitable action.

Globalization always presents new challenges and opportunities in combating infectious diseases that may lead to an epidemic. As a result of the increased international travel and trade, local events acquire international importance. Similarly, the rapid global expansion of telecommunication facilities and broadened access to news media and the Internet has almost changed the way society treats information. Reports of disease outbreaks are more widely disseminated and more easily accessible than ever before. However, the quality of information is no longer controlled and may be provided out of context, often causing unnecessary public anxiety and confusion. Rumors that later prove to be unsubstantiated may lead to inappropriate response, causing disruption in travel and trade and economic loss to affected countries. WHO, speaking for 191 member countries, is uniquely positioned to coordinate infectious disease surveillance and response at the global level. WHO receives reports of disease outbreaks around the world from various sources, although some of these reports are the warnings of genuine epidemics, others may reflect an endemic disease or may be mere rumors.

Rumors come to the attention of public health officials from many different sources, such as politicians, reporters, relief workers, physicians, and disaster victims themselves. Information from the surveillance system should provide the information as to the total number of casualties and the nature of their injuries and the types of endemic diseases.

Usually the investigation of rumor follows the same step-wise approach that is used for investigating a potential outbreak. The rumor investigator should focus on confirming the event existence (confirm the diagnosis) through following questions:

1. Who reported the event?
2. On what basis has the diagnosis been made?
3. Has the event been confirmed by using reliable methods (laboratory confirmation or collaboration by multiple observers?
4. Have officials consulted other independent sources of information (records from hospitals and other relief agencies) or not?

Along with the rapid assessments and establishments of temporary surveillance systems for monitoring morbidity and mortality, public health officials should conduct focused and special investigations to deal with specific problems associated with rumors. These investigations require laboratory support and expertise laboratory personnel. In association with these investigations safe drinking water and appropriate disposal of waste after any disaster, such as an earthquake, hurricane, or flood, should be ensured. When water supplies get contaminated, people must be advised to consume boiled water to prevent different water-borne epidemics.

Currently, most of the reports regarding the outbreaks are received from the media, and field personnel are mainly contacted for assistance with verifying reported events. This approach is subject to information basis, which results from the uneven dispersal and use of modern technology throughout the world. Also, different languages are not equally represented in the news media or addressed by electronic search engines. Although these shortcomings are partly offset by the information received directly from the WHO network, a more active statement should be established with field personnel. Receiving primary information directly from the field will lead to earlier detection of important events and events that escape identification. Although thought to be small, the number of important outbreaks recognized only locally is unknown.

References

Association of Medical Doctors of Asia (AMDA). 2011. Emergency relief activities for the Great East-Japan earthquake and tsunami:11 March–20 April 2011. Accessed February 12, 2013, at http://www.amdainternational.com/english/activity_report/tohoku1.pdf.

Association of Schools of Public Health (ASPH). 2006. What is public health? Accessed February 12, 2013, at http://www.asph.org/document.cfm?page=300.

Brennan RJ, Kimba K. 2006. Rapid health assessment in Aceh Java district. Indonesia, following the December 2006 Tsunami. *Emerg Med Australas* 17:341–350.

Center for Disease Control and Prevention (CDC). 2003. Treatment of tuberculosis: American Thoracic Society, DCD, and Infectious Diseases Society of America. *MMWR Morb Mortal Wkly Rep* 52 (RR11):1–77.

Center for Diseases Control and Prevention (CDC). 2005. Norovirus outbreak among evacuees from Hurricane Katrina—Houston, Texas. *MMWR Morb Mortal Wkly Rep* 54:1016–1018.

Center for Disease Control and Prevention (CDC). 2011. Fatal fungal soft-tissue infections after a tornado—Joplin, Missouri. *MMWR Morb Mortal Wkly Rep* 60:992.

Doka K. 2003.What makes a tragedy public? In: Lattanzi-Licht M, Doka K, eds., *Living with Grief: Coping with Public Tragedy*, pp. 3–13. Washington, DC: Hospice Foundation of America.

Erikson KT. 1976. *Everything in Its Path: Destruction of Community in the Buffalo Creek Flood*. New York: Simon and Schuster.

IRIN. 2010. Humanitarian news and analysis: Cote d' Ivoire: Yellow Fever, Dengue kill at least three. Accessed February 12, 2013, at www.irinnews.org/report.aspx?ReportID=90022.

Karande S, Bhatt M, Kelkar A, Kulkarni M, De A, et al. 2003. An observational study to detect leptospirosis in Mumbai, India. *Arch Dis Child* 88:1070–1075.

Kondo H, Seo N, Yasuda T, Hasizume M, Koido Y, et al. 2002. Postflood—infectious diseases in Mozambique. *Prehosp Disaster Med* 17:126–133.

Kouadio IK, Aljunid S, Kamigaki T, Hammad K, Oshitani H. 2012. Infectious diseases following natural disasters: Prevention and control measures. *Expert Rev Anti Infect Ther* 10:95–104.

Landesman L. 2005. *Public Health Management of Disasters: The Practice Guide*. Washington, DC: American Public Health Association.

Lundy KS, Janes S. 2003. Disasters in the community. In Lundy KS, Janes S, eds., *Essentials of Community-based Nursing*, p 346. Mississauga, Canada: Jones and Bartlett Publishers.

Myers D, Wee D. 2005. *Disaster Mental Health Services: A Primer for Practitioners*. New York: Brunner-Routledge.

Noji E. 2005. Public health issues in disasters. *Critical Care Medicine* 33 Supplement:s29–s33.

Noji EK. 1997. The nature of disasters. In: Noji EK, ed., *The Public Health Consequences of Disaster*, pp. 21–36. New York: Oxford University Press.

Peters RE. 2011. Patterns of disease after natural disasters. San Diego County Medical Society. Accessed February 12, 2013, at http://www.sdcms.org/article/patterns-disease-after-natural-disasters.

Qadri F, Khan AI, Furuque ASG, Begum YA, Chowdhuy F, et al. 2005. Enterotoxigenic *Escherichia coli* and *Vibrio cholerae* diarrhea, Bangladesh. *Emerg Infect Dis* 11:1104–1107.
Spiegel PB. 2005. Differences in world responses to natural disasters and complex emergencies. *JAMA* 293:1915–1918.
Toole MJ. 1997. Communicable diseases and disease control. In: Noji EK, ed., *The Public Health Consequences of Disaster*, pp. 79–100. New York: Oxford University Press.
Waring SC, Reynolds KM, D'souza G, Arafat RR. 2002. Rapid assessment of household needs in the Houston area after tropical storm Allison. *Disaster Manag Response* 3–9.
Watson JT, Gayer M, Connolly MA. 2007. Epidemics after natural disasters. *Emerg Infect Dis* 13(1):1–5.
Woersching JC, Snyder AE. 2004. Earthquakes in El-Salvador: A descriptive study of health concerns in a rural community and the clinical implication—part II. *Disaster Manag Response* 2:10–13.
World Health Organization. 2004. Flooding and communicable diseases fact sheet. Accessed February 12, 2013, at www.who.int/hac/techguidance/ems/flood_cds/en/.
World Health Organization (WHO). 2005a. Acute water diarrhea outbreaks. *Wkly Morb Mortal Rep* 1:6.
World Health Organization (WHO). 2005b. Epidemicprone disease surveillance and response after the tsunami in Aceh Province, Indonesia. *Wkly Epidemiol Rec* 80:160–164.
World Health Organization. 2006. Acute jaundice syndrome. *Wkly Morb Mortal Rep* 23:8.
World Health Organization. 2008. Dengue/Dengue hemorrhagic fever in Brazil. Accessed February 12, 2013, at www.who.int/csr/don/2008_04_10/en/index.html.
World Health Organization. 2010. Haiti: Response to the cholera outbreak. Accessed February 12, 2013, at www.who.int/hac/crises/hti/highlights/october2010/en/index.html.
Yang HY, Hsu PY, Pan MJ, Wu MS, Lee CH, et al. 2005. Clinical distinction and evaluation of Leptospirosis in Taiwan—a case control study. *J Nephrol* 118:45–53.

Chapter 4
Biosafety

Introduction

Biological safety or biosafety is the application of knowledge, techniques, and equipment to prevent personal, laboratory, and environmental exposure to potential infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. Its aim is to reduce or eliminate accidental exposure to, or release of, infectious agents (includes bacteria, fungi, viruses, parasites, and cell cultures). This term is used to describe efforts to minimize the potential risks that may stem from the use of biotechnology or its derived products. With specific reference to genetically modified organisms (GMOs), biosafety aims at "ensuring that the development and use of GMOs and products derived from them do not negatively affect plant, animal and human health, agricultural systems, or the environment" (Sengupta et al., 2001). GMOs are living organisms, which unlike chemicals do not become diluted once introduced into the environment, instead they disperse to new habitats and multiply. Once the GMO is metabolically active, it continuously produces the transgene product (toxin or enzyme), thereby posing a risk to health and the environment.

In biomedicine, laboratory workers apply these tenets to prevent laboratory-acquired infections and the release of pathogenic organisms into the environment. A biohazard is defined as any microorganism (including, but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa), parasite, vector, biological toxin, infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance that is capable of causing the following:

- Death, disease, or other biological malfunction in humans, animals, plants, or other living organisms.
- Deleterious alteration of the environment
- An adverse impact on commerce or trade agreements.

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

The goal of handling these hazardous agents safely can be accomplished through careful integration of accepted microbiological practices, and the containment of potential biohazards.

Synthetic biology should also be related to biological safety because of the potential future creation of man-made unicellular organisms. Some are considering the effect that these organisms will have on biomass already present. Scientists estimate that within the next few decades, organism design will be sophisticated enough to accomplish special tasks such as creating biofuels and lowering the harmful substance levels in the atmosphere (Collins, 2012). Organizations, such as the Canada-based ETC group, proposed that regulations should control the creation of organisms that could potentially harm existing life. They also argue that the development of these organisms will simply shift the consumption of petroleum to the use of biomass to create energy (Ribeiro, 2012).

Combinations of laboratory practices, containment equipment, and special laboratory design are used to achieve different levels of physical containment. (Historically, the designation *P* was used to indicate the level of physical containment, such as P-1 through P-4.) The current terminology is biosafety level or BSL (US Department of Health and Human Services, 2009). The designation BSL is used in the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), which focuses on protecting laboratory employees (Chosewood and Wilson, 2009). BL is another designation for biosafety level, used in Appendix G of the National Institutes of Health (NIH) publication *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* (US Department of Health and Human Services, 2002). However, Appendix G focuses primarily on physical containment involving work with recombinant DNA molecules. There are four levels of biosafety (designated 1 through 4) that define the parameters of containment necessary to protect personnel and the environment. BSL-1 is the least restrictive, whereas BSL-4 requires a special containment or maximum containment laboratory facility. Positive-pressure protective suits (space suit or blue suit) are used solely in BSL-4 laboratory. Biosafety is not possible without proper and extensive training. The principal investigator or laboratory supervisor is responsible for providing or arranging for appropriate training of all personnel within the laboratory to maintain and sustain a safe working environment.

Components

Biosafety procedures are designed mainly to prevent the entry and dissemination of infirmities in an animal production system or to maintain control over diseases existing in the system. The various important components for biological safety follow.

Risk Assessment

Risk assessment is one of the two keys principles of biosafety. This is actually the backbone of biosafety practices, which are used to identify the hazardous characteristics of an infectious organism, the activities that could allow exposure, the likelihood of becoming infected after exposure, and the consequences to the worker of an infection. This risk assessment provides the framework for the selection of appropriate containment (BSL, microbiological practices, safety equipment, and facility

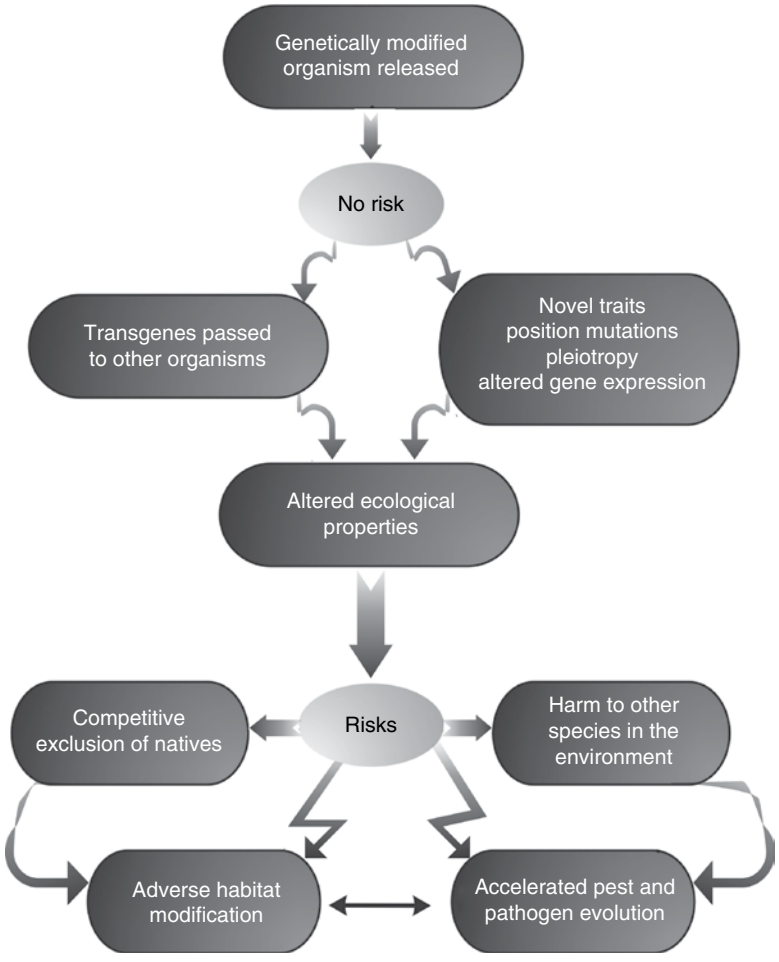

Figure 4.1. Assessment of biosafety risks and management.

safeguards). Although so many tools assist in the risk assessment for a given procedure or experiment, but the most important component is professional judgment. Risk assessments should be performed by the individuals who are familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, animal models that may be used, and the containment equipment and facilities available. The laboratory director or principal investigator is responsible for ensuring that adequate and timely risk assessments are performed (Figure 4.1) and for working closely with the institution's safety committee and biosafety personnel to ensure that appropriate equipment and facilities are available to support the work being considered. Once performed, risk assessments should be reviewed routinely and revised when necessary, taking into consideration the acquisition of new data having a bearing on the degree of risk and other relevant new information from the scientific literature.

Laboratory Safety Management

Most of the biological hazards are associated with laboratory-based practices. To avoid the hazards, universal precautions should be followed, including frequent hand washing, no mouth pipetting, avoid eating and drinking in the lab, and proper disposal of biohazardous or medical waste. These should be coupled with engineering controls such as biosafety cabinets, ventilation systems, closed-top centrifuge rotors, etc. Personal protective equipment (PPE), such as gloves, lab coats, eye protection, face shields or others, must be provided without cost to all individuals who are at risk of occupational exposure to lab-acquired pathogens. PPE is considered appropriate only if it does not permit blood or other potential infectious materials to pass through or reach the individual's clothing, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time.

It is the responsibility of the laboratory director to ensure the development and adoption of a biosafety management plan and a safety manual. Personnel should be advised of special hazards and required to read the safety or operations manual and follow standard practices and procedures, and it is the responsibility of the laboratory supervisor that all personnel should follow practices and procedures. A copy of the safety or operations manual should be available in the laboratory. There should be an arthropod and rodent control program. Appropriate medical evaluation, surveillance, and treatment should be provided for all personnel in case of need, and adequate medical records should be maintained.

Biohazard Guidelines

The scientific community, engaged in infectious disease research, has accepted as unfortunate, but unavoidable, the occasional accidental infection of microbiology laboratory personnel and associated nonlaboratory personnel. Perhaps most of these accidents should not happen if proper precautionary measures are provided and enforced. The last decade, in particular, saw great strides in the development of containment systems and in designing safety equipment to protect the laboratory worker and the exterior environment from contamination by infectious agents. A new science of containment, founded on the concept of continuous agent control through the creation of intelligently designed barrier systems, has emerged. Design of these barriers is based on a rational assessment of risk; the barriers may be created in the form of solid walls, pressure differentials to control movement of air, controlled movement of personnel and materials, or inactivation of the infectious agents themselves. In the maintenance of the barrier systems, one essential factor is that, at all times, the locations of the infectious agents must be known. The Centers for Disease Control and Prevention (CDC) categorizes various diseases in levels of biohazard, level 1 being minimum risk and level 4 being extreme risk. Laboratories and other facilities are categorized as BSL1- to BSL-4 or as P1 through P4; P standing for pathogen or protection.

Biohazard level 1 requires minimal precautions such as gloves and some sort of facial protection. Usually, contaminated materials are left in open (but separately indicated) waste receptacles (Figure 4.2). Decontamination procedures for this level are similar in most respects to modern precautions against everyday viruses (i.e., washing one's hands with antibacterial soap, washing all exposed surfaces of the lab with disinfectants, etc.). Level 2 includes mild human diseases such as salmonella, hepatitis A, B, and C, influenza

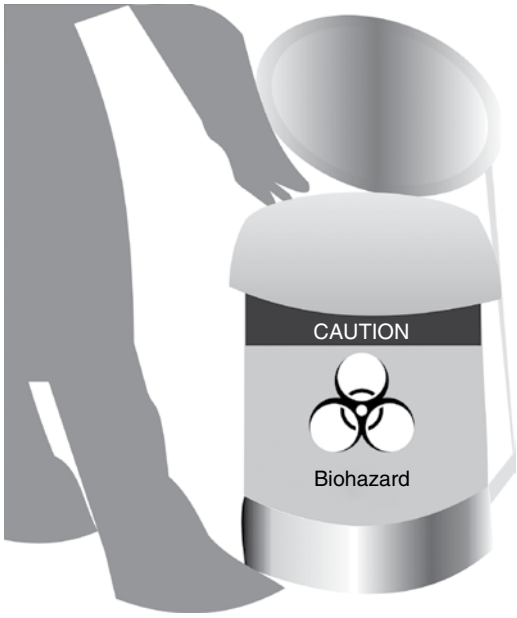

Figure 4.2. Figure presenting the proper disposal of biohazardous wastage.

A, Lyme disease, mumps, measles, scrapie, dengue fever, and HIV. Routine diagnostic work with clinical specimens can be done safely at BSL-2 practices and procedures. BSL-2 (P2) laboratories are also used for research purposes (including co-cultivation, virus replication studies, or manipulations involving concentrated virus) in association with BSL-3 practices and procedures.

Level 3 is useful in handling microbes that can cause severe to fatal disease in humans, but for which vaccines or other treatments exist, such as anthrax, West Nile virus, Venezuelan equine encephalitis, severe acute respiratory syndrome (SARS) virus, tuberculosis, typhus, Rift Valley fever, Rocky Mountain spotted fever, yellow fever, and malaria. Parasites *Plasmodium falciparum*, which causes Malaria, and *Trypanosoma cruzi*, which causes trypanosomiasis, also are included in this category. Level 4 is the most dangerous and includes viruses and bacteria that cause severe to fatal disease in humans and for which vaccines or other treatments are not available.

Laboratory Biosecurity

The laboratory biosafety manual emphasizes the use of good microbiological work practices, appropriate containment equipment, proper facility design, operation and maintenance, and administrative considerations to minimize the risk of worker injury or illness. In following these recommendations, the risk to the environment and surrounding community-at-large is also minimized. Global events in the recent past have highlighted the need to protect laboratories and the materials they contain from being intentionally compromised in ways that may harm people, livestock, agriculture, or the environment.

Before defining laboratory biosecurity needs, it is important to understand the distinction between laboratory biosafety and laboratory biosecurity. *Laboratory biosafety* is the term

used to describe the containment principles, technologies, and practices that are implemented to prevent unintentional exposure to pathogens and toxins or their accidental release. *Laboratory biosecurity* refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens and toxins. Effective biosafety practices are the foundation of laboratory biosecurity activities. Through risk assessments, performed as an integral part of an institution's biosafety program, information is gathered regarding the type of organisms available, their physical location, the personnel who require access to them, and the identification of those responsible for them. This information can be used to assess whether an institution possesses biological materials that are attractive to those who may wish to use them improperly. National standards should be developed that recognize and address the ongoing responsibility of countries and institutions to protect specimens, pathogens, and toxins from misuse.

Biological Laboratory Emergencies

For emergency conditions, a written contingency plan is required for dealing with laboratory and animal facility accidents that work with or stores Risk Group 3 or 4 microorganisms (containment laboratory [BSL-3] and maximum containment laboratory [BSL-4]) involving national or local health authorities. This emergency contingency plan should provide operational procedures for:

- Precautions against natural disasters (e.g., fire, flood, earthquake, and explosion)
- Biohazard risk assessment
- Incident-exposure management and decontamination
- Emergency evacuation of people and animals from the premises
- Emergency medical treatment of exposed and injured persons
- Medical surveillance of exposed persons
- Clinical management of exposed persons
- Epidemiological investigation
- Postincident continuation of operations.

Hand Washing

Hand washing is one of the most effective means of preventing diarrheal diseases, as well as respiratory infections. When it is coupled with safe stool disposal and safe and adequate household water supply, it is even more effective. Hand washing interrupts the transmission of disease agents and can significantly reduce infections (Figure 4.3). Curtis and Cairncross (2003) suggest that hand washing with soap, particularly after contact with feces (post-defecation and after handling a child's stool), can reduce diarrheal incidence by 42 to 47 percent, whereas Rabie and Curtis (2006) suggest that hand washing lowered the risk of respiratory infections ranging from 6 to 44 percent. This remains true even in areas that are highly focally contaminated and have poor sanitation, which is further supported by Barclay (2009). Because hand washing can prevent the transmission of a variety of pathogens, it may be more effective than any single vaccine. Hand washing with soap could be thought of as a do-it-yourself vaccine when promoted on a wide-enough scale.

Figure 4.3. Hand washing to interrupt disease transmission.

While handling biohazardous materials, suitable gloves should be worn; however, this does not replace the need for regular and proper hand washing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals and before leaving the laboratory. In most situations, hand washing using ordinary soap is sufficient to decontaminate hands, but in high-risk situations the use of germicidal soaps is recommended. If proper hand washing is not available, alcohol-based hand sanitizers may be used to decontaminate lightly soiled hands.

Preventing Needlestick Injuries

Needles are an essential part of medical care, but they are potentially hazardous to staff. Awareness of hazards, use of the correct equipment, and a consistent and careful technique are all of great importance in preventing needlestick injuries. Needles that have to be removed from syringes must be dealt with safely. Needle forceps or other suitable aids or devices should be readily available. Up to 40 percent of self-inoculation accidents have been reported to occur while resheathing needles; therefore this must not be done unless there is a safe means available (Jagger et al., 1988; Kennedy, 1988). The high incidence of such injuries led the CDC to opt for a nonsheathing technique but without evidence of its efficacy. Their guidelines were then adopted with minor modifications by the Department of Health and Social Security. These recommendations were based on the assumption that avoiding resheathing should decrease the number of needlestick injuries and consequent problems.

An uncapped needle could project through the walls of substandard containers (still often used even in Britain) or through the top of an overfilled container, causing an injury to someone who cannot know the nature or degree of danger. Data from National Surveillance System for Hospital Health Care Workers (NaSH; Figure 4.4) show that approximately 38 percent of percutaneous injuries occur during use and 42 percent occur

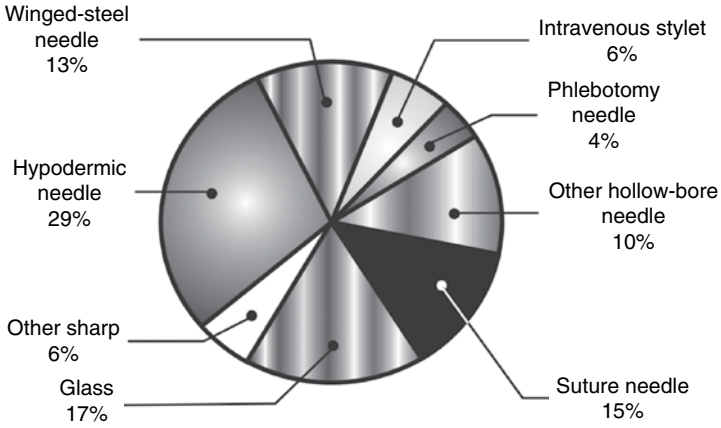

Figure 4.4. Hollow-bore needles and other devices associated with percutaneous injuries in National Surveillance System for Hospital Health Care Workers (NaSH) hospitals, by the percent of total percutaneous injuries ($n = 4{,}951$) from June 1995 to July 1999.
Source: US Department of Health and Human Services. 1999. Public Health Service Centers for Disease Control and Prevention. National Institute for Occupational Safety and Health (NIOSH), DHHS (NIOSH) Publication No. 2000.108. Accessed March 25, 2013 at http://www.cdc.gov/niosh/docs/2000-108/pdfs/2000-108.pdf.

after use and before disposal. A needlestick injury depends partly on the type and design of the device used. For example, needle devices that must be taken apart or manipulated after use (e.g., prefilled cartridge syringes and phlebotomy needle or vacuum tube assemblies) are an obvious hazard and have been associated with increased injury rates (Jagger et al., 1988). In addition, needles attached to a length of flexible tubing (e.g., winged-steel needles and needles attached to intravenous [IV] tubing) are sometimes difficult to place in sharps containers and thus present another injury hazard. Injuries involving needles attached to IV tubing may occur when a health-care worker inserts or withdraws a needle from an IV port or tries to temporarily remove the needlestick hazard by inserting the needle into a drip chamber, IV port or bag, or even bedding. In addition to risks related to device characteristics, needlestick injuries have been related to certain work practices such as recapping, transferring a body fluid between containers, and failure to properly dispose of used needles in puncture-resistant sharps containers. Past studies of needlestick injuries have shown that 10 to 25 percent occurred when recapping a used needle (Krasinski et al., 1987; McCormick and Maki 1981; McCormick et al., 1991; Yassi and McGill, 1991).

Although recapping by hand has been discouraged for some time and is prohibited under the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (29 CFR 1910.1030) unless no alternative exists, 5 percent of needlestick injuries in NaSH hospitals are still related to this practice. Injury may occur when a health-care worker attempts to transfer blood or other body fluids from a syringe to a specimen container (such as a vacuum tube) and misses the target. Needlestick injuries are an important and continuing cause of exposure to serious and fatal diseases among health-care workers. Greater collaborative efforts by all stakeholders are needed to prevent needlestick injuries and the tragic consequences that can result. Such efforts are best

accomplished through a comprehensive program that addresses institutional, behavioural, and device-related factors that contribute to the occurrence of needlestick injuries in health-care workers.

Safe Transport of Biological Material

The packaging and transportation of biological materials is subject to strict state, federal, and international regulations. Individuals involved in the packaging, transportation and shipment of infectious substances must receive training on proper packaging, labeling, and documentation according to the applicable regulations and requirements before shipping such materials. Biological materials transported by laboratory personnel between buildings must be contained in such a way as to prevent their release into the environment. Transportation should follow this procedure:

1. Biological samples must be placed in a primary container or vessel that is a securely closed, leak-proof (or o-ring) tube, vial, or ampoule, which is then placed in an unbreakable, lidded, watertight, secondary container (e.g., Rubbermaid tote or Playmate-type cooler).
2. If outside of the primary container or vessel is suspected of being contaminated, decontaminate before placing in secondary container using a 10-percent bleach solution, a disinfectant approved by the Environmental Protection Agency, or a disinfectant appropriate for the biological material in use.
3. All biohazards must be labeled with the international biohazard symbol on the outside of the secondary container.
4. When transporting liquids in glass vials or containers, place enough absorbent material, such as paper towels, in the space at the top, bottom, and sides between the primary and secondary containers to absorb the entire contents of the primary container(s) in case of breakage or leakage.
5. The outside of the secondary container must be free of any biohazardous material so that the package can be carried safely between buildings without wearing gloves or lab coats.
6. The package must be taken directly to its intended location.
7. If a spill occurs during transport, do not attempt to clean it up without appropriate spill response material and PPE. Keep other persons clear of the spill.

Triple Packaging System

All biological materials must be packaged according to a triple packaging system. The three components of a triple packaging system are:

1. Primary receptacle: The primary receptacle holds the biological material and must be leak proof and watertight. It is packed in the secondary container in a manner that, under normal transportation, they will not break, puncture, or leak their contents into the secondary container. If the primary receptacle is fragile, it must be individually wrapped or separated to prevent contact between multiple primary receptacles.

2. Leak-proof secondary container: The secondary container is a durable, watertight, and leak-proof container that encloses and protects the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary container. If the primary receptacle contains any liquid, the secondary container must contain enough absorbent material to absorb all of the fluid from the primary receptacle(s) in case of breakage.
3. Rigid outer container: The outer container is a rigid and durable container with one side that is at least 10 cm x 10 cm (or 4 inches x 4 inches) that houses the secondary container. The outer package should be properly marked and labeled. It should be able to withstand outside influences such as physical damage while in transit. An itemized list of package contents must be included between the outer and secondary container.

Transport Regulations

Transport of biological and potentially infectious materials is subject to strict national and international regulations. These regulations describe the proper use of packaging materials, as well as other shipping requirements. These regulations are based on the United Nations' (UN) Model Regulations on the Transport of Dangerous Goods. These recommendations are developed by the United Nations Committee of Experts on the Transport of Dangerous Goods (UNCETDG). To become legally binding, the UN Model Regulations have to be introduced into national regulations and international modal regulations by the competent authorities (i.e., the Technical Instructions for the Safe Transport of Dangerous Goods by Air [2002] of the International Civil Aviation Organization (ICAO; 2002) for air transport and the European Agreement concerning the International Carriage of Dangerous Goods by Road [ADR]).

Safe Decontamination of Spills

Composition of a Basic Spill Kit

Microbiological and biomedical research laboratories should prepare and maintain a biological spill kit. A spill kit is an essential safety item for laboratories working with microbiological agents classified at BSL-2 or higher level and for groups working with large volumes (>1 liter) of BSL-1 material. The spill kit should include:

- Concentrated household bleach
- A spray bottle for making 10-percent bleach solutions
- Forceps, autoclavable broom and dust pan, or other mechanical device for handling sharps
- Paper towels or other suitable absorbent
- Biohazard bags for the collection of contaminated spill clean-up items
- Utility gloves and medical examination gloves
- Face protection (eye wear and mask, or full face shield)

Additional personal protective equipment, such as Tyvek jump suits and powered air-purifying respirators (PAPRs), may be required for response to spills in BSL-3 laboratories.

Blood Spills

For blood or other material with a high organic content and low concentration of infectious microorganisms, the following precautions should be followed:

- Wear gloves, eye protection, and a lab coat.
- Absorb blood with paper towels and place in a biohazard bag.
- Using a detergent solution, clean the spill site of all visible blood.
- Spray the spill site with 10-percent household bleach and allow to air dry for 15 minutes. Afterward wipe the area down with disinfectant-soaked paper towels.
- Discard all disposable materials used to decontaminate the spill and any contaminated PPE into a biohazard bag.
- Wash hands with soap and water

Spill in a Biological Safety Cabinet

For spills in a biological safety cabinet, the following precautions should be followed:

- Leave the biological safety cabinet blower on and begin cleanup immediately.
- While wearing PPE (gloves and gown), cover the spill area with paper towels or disinfectant-soaked paper towels.
- If necessary, flood the work surface, the drain pans, and catch basins below the work surface with disinfectant, ensuring that the drain valve is closed before flooding the area. Place a container under the drain valve and drain the disinfectant under the work surface into the container. Wipe the areas under the work surface to remove residual disinfectant.
- Wash hands and exposed skin with soap and water.
- Autoclave all cleanup materials and protective clothing.

Spill of a Biohazardous Radioactive Material

A biohazardous spill involving radioactive material requires some additional emergency procedures considering the type of radionuclide, characteristics of the microorganism, and the volume of the spill.

- Avoid inhaling air-borne material while leaving the room. Notify others to leave. Close the door, and post a warning sign.
- Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag labeled with a radioactive materials label or a radioactive waste container labeled with a biohazard label.
- Wash all exposed skin with soap and water; follow with a three-minute water rinse.
- Inform supervisor and Radiation Safety Office of spill, and monitor all exposed personnel for radiation. If assistance is needed in handling the microorganism, contact the biosafety office.
- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, autoclavable containers, forceps, towel, and sponges), and confirm with radiation safety that it is safe to enter the lab.

- Put on protective clothing (gown, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a respirator that is filtered with high-efficiency particular air (HEPA) instead of a surgical mask.
- Cover the area with disinfectant-soaked towels and carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant because it is diluted by the spill. Allow at least 20 minutes contact time. Never apply bleach solutions on iodinated material because radioiodine gas may be released. Instead, use an alternative disinfectant such as an iodophor or phenolic.
- Handle any sharp objects with forceps. Wipe surrounding areas, where the spill may have splashed, with disinfectant.
- Soak up the disinfectant and spill, and place the biologically decontaminated waste, along with all protective clothing contaminated with radioactive materials, into an approved radioactive waste container and label it according to radiation safety guidelines. Contaminated protective clothing must also be biologically decontaminated prior to disposal as radioactive waste. Do not autoclave the waste unless this action is approved by the radiation safety officer. If waste cannot be autoclaved, add additional disinfectant to ensure biological decontamination of all the materials.
- Wash hands and exposed skin areas with soap and water; monitor personnel and spill area for residual radioactive contamination.
- To discard items contaminated with radioactive materials, place the contaminated item(s) on absorbent paper and spray disinfectant (10% household bleach) on the contaminated areas after 20 minutes wrap the item(s) inside the paper and dispose of as radioactive waste.

Safe Handling of Dead Bodies

All dead bodies are potentially infectious and required standard precautions. Although most organisms in the dead body are unlikely to infect healthy persons, some infectious agents may be transmitted through contact with blood, body fluids, and tissues of the dead body of a person with infectious diseases. To minimize this risk of transmission of known and unsuspected infectious diseases, dead bodies should be handled in a way to reducing workers exposure to blood, body fluids, and tissues. A rational approach should include staff training and education, safe working environment, appropriate work practices, the use of recommended safety devices, and vaccination against hepatitis B. There is a need to maintain the confidentiality of a patient's medical condition even after his or her death. At the same time, there is an obligation to inform personnel who may be at risk of infection through contact with dead bodies so that appropriate measures may be taken to guard against infection. The discrete use of labels such as "danger of infection" on the dead body is considered appropriate. To minimize the distress caused by the sight of dead bodies and the odor produced by their decomposition, it is important to collect and remove corpses to a collection point as quickly as possible.

The occupational risks for pathologists and mortuary staff, who routinely work with dead bodies, are unlikely to be different for persons who are dealing with victims of a natural disaster. However, incidents with a large number of fatalities may require a temporary workforce for the collection, transportation, storage, and disposal of the dead. These workers may include military personnel, rescue workers, volunteers, and others who have little or no experience in handling the deceased.

Figure 4.5. Safe handling of dead bodies.

Table 4.1. Recommendations for handling the dead.

Avoiding cross-contamination of personal item.
Universal precautions for body and body fluids.
Disposal or disinfection of used gloves.
No special arrangements, such as disinfection, with disposal of bodies.
Use of body bags, especially for badly damaged bodies.
New burial areas sited at least 250 m away from drinking water sources and with at least 0.7 m of distance above the saturated zone.
Hepatitis B vaccination

A number of simple measures can be taken to reduce the risk of infection associated with handling dead bodies. Considering that some of the persons doing this work may not have had experience in handling the dead, some basic instruction about the risks and precautions may be required. When handling dead bodies, workers should wear gloves, especially if the bodies are badly damaged. Used gloves should be removed and kept in a suitable bag and disposed of appropriately. Where nondisposable gloves are used, they should be cleaned and disinfected. To avoid cross-contamination, personal items should not be handled while wearing soiled gloves, and a new pair is recommended after each body or group of bodies is handled. Other PPE, such as eyewear, gowns, and masks, are only required when large quantities or splashes of blood are anticipated and are probably not necessary when handling bodies following a natural disaster.

Hands should be washed after handling cadavers and before eating, and all equipment, including clothes, stretchers, and vehicles used for transportation, should be washed carefully with a disinfectant. Body bags will further reduce the risk of infection and are useful for the transport of cadavers that have been badly damaged (Figure 4.5). However, body bags reduce the rate of cooling of the cadaver, thus increasing the rate of decomposition, especially in hot climates (Iserson, 1995). Table 4.1 suggests various recommendations handling the dead bodies.

Personal Protective Equipment

The primary approach in any safety effort is that the hazard to the workers should be controlled by engineering methods including design change, substitution, ventilation, mechanical handling, automation, etc. In situations where it is not possible to introduce any effective engineering methods, the worker is advised to use appropriate types of PPE. Use of PPE is an important and necessary consideration in the development of a safety program. Once the safety professional decides that PPE is to be used by workers, it becomes essential to select the right type of PPE and management should ensure that the worker not only uses it but also maintains it correctly. PPE should provide absolute and full protection against possible hazard, and it is to be so designed and manufactured that it can withstand the hazard against which it is intended to be used. Selection of the right type of PPE requires consideration of the following factors:

1. Nature and severity of the hazard
2. Type of contaminant, its concentration, and location of the contaminated area
3. Expected activity of the worker and duration of work.
4. Comfort of worker when using PPE
5. Operating characteristics and limitations of PPE
6. Ease of maintenance and cleaning conformity to national or international standards and availability of test certificate.

The best solution to this problem is to make the wearing of PPE mandatory for every employee. In organizations where risky jobs are carried out by contract workers, it is generally noticed that the PPE is provided by the contractor. Sometimes the contractor may not provide it at all, or if the contractor does provide it, it may be of substandard quality. This needs to be strictly controlled by management of the organization.

Management of Biomedical Waste

Biomedical waste can be managed through a common biomedical waste treatment facility (CBWTF). It is a set up in which biomedical waste, generated from a number of health-care units, goes through necessary treatment to reduce adverse effects. This treated waste may finally be sent for disposal in a landfill or for recycling purposes. An individual treatment facility in small health-care units requires comparatively high capital investment, separate manpower, and infrastructure development for proper operation and maintenance of treatment systems. The concept of CBWTF not only addresses such problems, but it also prevents proliferation of treatment equipment in a city. In turn, it reduces the monitoring pressure on regulatory agencies. By running the treatment equipment at CBWTF to its full capacity, the cost of treatment of per kilogram gets significantly reduced. Its considerable advantages have made CBWTF popular and proven concept in many developed countries. The various treatment methods could include:

- **Incineration**: A controlled combustion process in which waste is completely oxidized and harmful microorganisms are destroyed or denatured under high temperature. The guidelines for "Design & Construction of Bio-medical Waste Incinerators" prepared by the Central Pollution Control Board (CPCB, 1998) shall be followed for selecting and installing a good biomedical waste incinerator.
- **Autoclaving**: Autoclaving is a thermal process in which steam is brought into direct contact with waste in a controlled manner and for sufficient duration to disinfect the wastes. For easy and safer operation, the system should be of horizontal type and should exclusively design for the treatment of biomedical waste. For optimum results, a prevacuum-based system is preferred against the gravity-type system. It should have a tamper-proof control panel with efficient display and recording devices for critical parameters such as time, temperature, pressure, date, and batch number.
- **Microwaving**: In microwaving, microbial inactivation occurs as a result of the thermal effect of electromagnetic radiation spectrum lying between the frequencies 300 and 300,000 MHz. Microwave heating is an intermolecular heating process. The heating occurs inside the waste material in the presence of steam.
- **Hydroclaving**: It is similar to that of autoclaving except that the waste is subjected to indirect heating by applying steam in the outer jacket. The waste is continuously tumbled in the chamber during the process.
- **Shredding**: It is a process by which waste are deshaped or cut into smaller pieces so as to make it unrecognizable. It helps in prevention of reuse of biomedical waste and also acts as an identifier that the waste material is disinfected and safe to dispose off.

Collection and Transport

The biomedical waste should be collected and transported in a manner so as to avoid any possible hazard to human health and environment. During collection and transportation, the biomedical waste shall come in contact with the public, rag pickers, animals/birds, etc. Therefore, all care shall be taken to ensure that the segregated biomedical waste, handed over by the health-care units, reach CBWTF without any damage, spillage, or unauthorized access by public or animals and is finally properly disposed depending on the category of waste (Table 4.2).

Table 4.2. Disposal of treated biomedical waste.

Waste Category	Disposal Method
Plastic wastes after disinfection and shredding	Recycling or municipal landfill
Disinfected sharps (except syringes)	Municipal landfill
Incineration ash	Secured landfill
Other treated solid wastes	Municipal landfill
Oil and grease	Incineration
Treated waste water	Sewer/drain or recycling

Infection Control Check List

Infection control checklist should include:

1. **Immunization for health-care workers**: Health-care workers should have appropriate immunizations for Hepatitis B, measles, mumps, rubella, varicella, and influenza.
2. **Before patient treatment**: Obtain a thorough medical history of the patient. Place disposable surface barriers to prevent contamination of surfaces or disinfect surfaces after treatment.
3. **During patient treatment**: Treat all patients as potentially infectious. Use PPE, such as gloves, mask, eyewear, and protective clothing. Disinfect hands using alcohol hand sanitizer between patients or whenever changing gloves. Change gloves between each patient or if torn, cut, or punctured. Handle sharp items carefully. Do not bend or break disposable needles. If needles are recapped, use mechanical recapping device or needle protectors. If needles have not recapped, place them in a separate field or dispose of immediately after use.
4. **After patient treatment**: Wear heavy-duty gloves during clean-up. Handle sharp instruments with caution. Place contaminated disposable needles, scalpels/sharp items intact into puncture-resistant containers before disposal. Decontaminate all surfaces that may have become contaminated during treatment. Pre-clean with an appropriate cleaner. Disinfect surface with a suitable liquid disinfectant. Sterilize or discard all instruments or materials that were used intra orally. Ensure instruments stay sterile until next use.

Biosafety Levels

Based on their potency in causing an outbreak and the associated risk, all the biological agents are classified into four biosafety level risk groups by the CDC and the National Institutes of Health (NIH). These groups describe the microbiological techniques, lab practices, safety equipment, and lab facilities necessary to protect workers and the environment against these agents. In the most recent version of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), Closewood and Wilson (2009) describe BSL-1 to BSL-4, agents, factors, and practices. The BMBL is a guide for the use of biological hazards in the laboratory, and it should be consulted regarding use of viruses, fungi, bacteria, and tissues that can harbor hazardous biological agents. Another category includes the Hybrid biosafety levels, which denote combined physical containment and safe work practice levels. For example, BSL-2/3 as used in the HIV labs denotes BSL-2 facilities and BSL-3 work practices.

Biosafety Level-1

BSL-1 risk group contains biological agents that pose a low risk to personnel and the environment. These agents are highly unlikely to cause disease in healthy laboratory workers, animals, or plants. These agents require BSL-1 containment. Examples of BSL-1 organisms are: *Agrobacterium radiobacter, Aspergillus niger, Bacillus thuringiensis, Escherichia coli strain K12, Lactobacillus acidophilus, Micrococcus leuteus, Neurospora crassa, Pseudomonas fluorescens*, and *Serratia marcescens*.

Table 4.3. List of Biosafety Level-2 agents.

BSL-2 Agent	Examples
Bacterial/Rickettsial Agents	*Campylobacter fetus, coli, jejuni, Chlamydia psittaci, trachomatis, Clostridium botulinum, tetani, Corynebacterium diphtheriae, Legionella* spp., *Neisseria gonorrhoeae, Neisseria meningitides, Pseudomonas pseudomallei, Salmonella* spp., *Shigella boydii, dysenteriae, flexneri, sonnei, Treponema pallidum, Vibrio cholera, Vibrio parahaemolyticus, Vibrio vulnificus, Yersinia pestis*
Viral Agents	Adenovirus, Creutzfeldt-Jacob agent, Cytomegalovirus, Eastern equine encephalitis, Epstein-Barr virus, Hepatitis A, B, C, D, E, Herpes simplex viruses, HTLV types I and II, Monkeypox virus, Simian immunodeficiency virus, Spongiform encephalopathies, Vaccinia virus, HIV, Vesicular stomatitis virus (lab adapted strains)
Fungal Agents	*Blastomyces dermatitidis, Cryptococcus neoformans, Microsporum* spp., *Exophiala dermatitidis (Wangiella), Fonsecaea pedrosoi, Sporothrix schenckii, Trichophyton* spp.
Parasitic Agents	*Entamoeba histolytica, Cryptosporidium* spp., *Giardia* spp., *Naegleria fowleri, Plasmodium* spp., *Strongyloides* spp., *Taenia solium, Toxoplasma* spp., *Trypanosoma* spp.

As BSL-1 present minimal potential hazard to laboratory personnel and the environment, BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but it may be used as determined by appropriate risk assessment.

Biosafety Level-2

The BSL-2 risk group contains biological agents that pose moderate risk to personnel and the environment. If exposure occurs in a laboratory situation, the risk of spread is limited, and it rarely will cause infection that will lead to serious disease. Examples of BSL-2 organisms are *Mycobacterium*, *Streptococcus pneumoniae*, *Salmonella choleraesuis*, etc. (Table 4.3). BSL-2 represents hazards primarily related to accidental percutaneous, mucous membrane, nonintact skin exposures, or ingestions of infectious materials. Use PPE as appropriate, including splash shields, face protection, disposable gowns, and gloves. BSL-2 differs from BSL-1:

1. Laboratory personnel of BSL-2 have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.
2. Access to the laboratory is restricted when work is being conducted.
3. Even though BSL-2 organisms are not known to be transmissible by the aerosol route, all procedures in which infectious aerosols or splashes may be created are conducted in biosafety cabinets (BSCs), safety centrifuge, or other physical containment equipment.

Table 4.4. List of Biosafety Level-3 agents.

BSL-3 Agent	Examples
Bacterial/Rickettsial agents	*Bacillus anthracis, Francisella tularensis, Mycobacterium tuberculosis* (MDR), *Mycobacterium bovis, Rickettsia rickettsii, Yersinia pestis* (resistant strains)
Viral agents	Rift Valley fever (Zinga), VSV exotic strains (Piry), Yellow fever (wild type)
Fungal agents	*Coccidioides immitis, Histoplasma capsulatum*

Biosafety Level-3

The BSL-3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents (Table 4.4) that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures. This level requires a development of a separate safety lab, including roles and responsibilities, hazard mitigation, decontamination, and emergency response. Procedures with the live agent are to be performed in a Type II A/B BSC. Use PPE as appropriate, including splash shields, face protection, disposable gowns, and gloves. Entry into and out of a BSL-3 facility must be controlled, allowing the entry of only properly trained individuals.

Biosafety Level-4

The BSL-4 risk group contains biological agents producing very serious diseases that are often untreatable. These agents are usually easily transmitted from one individual to another, from animal to human or vice-versa, either directly or indirectly or by casual contact. These agents pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal for which there are no vaccines or treatments. BSL-4 laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents and the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. The BSL-4 facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell that facilitates fumigation and is an animal- and insect-proof. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to assure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. Within work areas of the facility, all activities are confined to Class III BSCs or Class II BSCs used with one-piece positive-pressure personnel suits ventilated by a life support system.

The BSL-4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment. Personnel enter and leave the facility only through the clothing change and shower rooms, and shower each time they leave the facility. Personal clothing is removed in the outer clothing change room and kept there. A specially designed suit area may be provided in the laboratory facility to provide PPE. The exhaust air from the suit area is filtered by two sets of HEPA filters installed in series. Supplies and materials needed in the facility are brought in by way of

double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. Viruses assigned to BSL-4 include Crimean-Congo hemorrhagic fever, Ebola, Junin, Lassa fever, Machupo, Marburg, and tick-borne encephalitis virus complex (including Absettarov, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian Spring-Summer encephalitis).

Accreditation of Hospitals and Laboratories

In response to the global threat of emerging infectious diseases, the threat of bioterrorism and recent high-profile laboratory-acquired infections and accidents raised the level of concern regarding high-containment laboratories among the public and policy makers. Accreditation using relevant national and international standards is an effective way of ensuring competence in a comprehensive and uniform manner in laboratories working with biohazards. The American Biological Safety Association (ABSA) believes that independent accreditation of high-containment laboratories in the United States will be more effective and less costly than government regulation, inspection, or permitting of laboratories. Such an oversight process would assure lab workers and the community that a bio-containment facility has in place the necessary practices, procedures, personnel, and equipment to protect people, animals, plants, and the environment and minimize the potential of lab-acquired infections and lab accidents. ABSA proposes to lead a process involving all appropriate stakeholders, including representatives from federal agencies and allied professional organizations to develop an accreditation program that organizations would seek to measure and recognize their competence in management of biological hazards.

The National Accreditation Board for Hospitals & Healthcare Providers (NABH) Standards is today the highest benchmark standard for hospital quality in India. Though developed by the Quality Council of India on the lines of International Accreditation Standards such as the Joint Commission International (JCI), the Australian Council on Healthcare Standards (ACHS) and the Canadian Hospital Accreditation Standards, the NABH is, however, seen as a more practical set of standards, topical and relevant to India's unique health-care system requirements. Within just two years of its launch, the Indian Accreditation Standards, the NABH was accepted by the International Society for Quality Assurance in Healthcare (ISQUa), as an International Accreditation. Key components assessed by an effective accreditation program would include:

1. The biosafety expertise and training of personnel managing and conducting the research
2. The adequacy and function of the biosafety management structure supporting the research activities
3. The adequacy and function of bio-containment measures, including facilities, equipment, practices, and record-keeping systems, in place at the facility that is evaluated, either as an organization or through its membership, has had extensive experience in evaluating all three of these components.

The concept of biosafety has paralleled the development of the science of microbiology and its extension into new and related areas (cell culture, recombinant DNA, animal studies, and biotechnology). The knowledge and skill gained by microbiologists necessary to isolate, manipulate, and propagate pathogenic microorganisms require parallel development of containment principles, facility design, and practices and procedures to prevent occupational infections in the biomedical environment or release of organisms into the environment.

References

Barclay L. 2010. World Health Organization issues guidelines on hand hygiene in healthcare. Accessed February 12, 2013, at http://www.medscape.com/viewarticle/702406.

Centers for Disease Control and Prevention (CDC). 1999. *Preventing Needlestick Injuries in Health Care Settings*. Accessed February 27, 2013, at http://www.cdc.gov/niosh/docs/2000-108/pdfs/2000-108.pdf.

Central Pollution Control Board (CPCB). 1998. Biomedical waste management. Accessed February 24, 2013, at cpcb.nic.in/Bio_medical.php.

Chosewood LC, Wilson DE, eds. 2009. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. Washington, DC: US Government Printing Office.

Collins J. 2012. Synthetic biology: Bits and pieces come to life. *Nature* 483:S8–S10 doi:10.1038/483S8a.

Curtis V, Cairncross S. 2003. Effect of washing hands with soap on diarrhoea risk in the community: A systematic review. *Lancet Infect Dis* 3:275–281.

International Civil Aviation Organization (ICAO). 2002. *Technical instructions for the safe transport of dangerous goods by air, 2003–2004 Edition*. Montreal: ICAO.

Iserson KV. 1995. What happens to corpses? Death doesn't have to be a mysterious subject. *Emerg Med Serv* 24:35–43

Jagger J, Hunt EH, Brand-Elnaggar J, Pearson RD. 1988. Rates of needlestick injury caused by various devices in a university hospital. *N Engl J Med* 319:284–288.

Kennedy DA. 1988. Needlestick injuries: mechanisms and control. *J Hosp Infect* 12:315–322.

Krasinski K, LaCouture R, Holzman RS. 197. Effect of changing needle disposal systems on needle puncture injuries. *Infect Control* 8:59–62.

McCormick RD, Maki DG. 1981. Epidemiology of needle-stick injuries in hospital personnel. *Am J Med* 70, 928–932.

McCormick RD, Meisch MG, Ircink FG, Maki DG. 1991. Epidemiology of hospital sharps injuries: a 14-year prospective study in the pre-AIDS and AIDS eras. *Am J Med* 91(Suppl 3B), 301S–307S.

Occupational Safety and Health Administration (OSHA). 1999. OSHA Directives 2-2.44 D Enforcement procedures for the occupational exposure to bloodborne pathogens. Accessed February 12, 2013, at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=DIRECTIVES&p_id=2570.

Rabie T, Curtis V. 2006. Hand washing and risk of respiratory infections: A quantitative systematic review. *Tropical Medicine and International Health* 2:253–267.

Ribeiro S. 2012. Biofuels, bioenergy and biochar: False solutions lead to land-grabbing. Accessed February 12, 2013, at http://uk.oneworld.net/article/view/166491/1/246.

Sengupta S, Sengupta LK, Bisen PS. 2001. Bioengineered crops: The commercial and ethical considerations. *Current Genomics* 2:181–197.

US Department of Health and Human Services. 1999. Public Health Service Centers for Disease Control and Prevention. National Institute for Occupational Safety and Health (NIOSH), DHHS (NIOSH) Publication No. 2000.108. Accessed March 25, 2013 at http://www.cdc.gov/niosh/docs/2000-108/pdfs/2000-108.pdf.

US Department of Health and Human Services/National Institutes of Health (DHHS/NIH). 2002. *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. Bethesda, Md: DHHS/NIH.

US Department of Health and Human Services. 2009. Public Health Service Centers for Disease Control and Prevention. National Institutes of Health, HHS Publication No. (CDC) 21-1112. Accessed March 25, 2013, at http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf.

Yassi A, McGill M. 1991. Determinants of blood and body fluid exposure in a large teaching hospital: hazards of the intermittent intravenous procedure. *Am J Infect Control* 19:129–135.

Chapter 5
Tuberculosis

History

Fransiscus de la Boe gave tuberculosis (TB) its name in the 17th century. Previously, the disease was known by various names such as "white plague," "white swelling," "phthisis," "consumption," "scrofula," and "mesenteric disease." TB of the spine was called "Pott's spine" after Sir Percival Pott. Historically TB has been considered one of the oldest known diseases and its distribution also varies in various eras.

Ancient Times

TB has affected humans since prehistoric times because some of the mummified bodies in ancient Egypt show evidence of TB of bone and spine. The disease was called "Laoping" in ancient Chinese medical literature and mentioned in the *Rigveda* as "Yakshima." The Greek physician and "father of medicine," Hippocrates (460–375 B.C.) warned his colleagues against visiting patients in late stages of phthisis because their inevitable deaths would damage the reputation of the attending physicians. He had identified phthisis as the most widespread disease of his times and noted that it was almost always fatal. Galen (130–205 A.D.), a Roman physician, described the disease as "incurable" and "contagious." According to the famous ancient Indian surgeon Sushruta, TB was difficult to cure (Global Tuberculosis Institute, n.d.).

The Dark Ages: 400–1400 A.D.

During the Dark Ages, the practice of medicine was restricted to superstition and dogma, including the use of a sea voyage (recommended by Celsus), alkaline mineral water, mercury and antimony compounds, cream of tartar, and opium. Rhazes (or Abu Becr 865–925) and Avicenna (or Ibn Sena 980–1037), both Arab physicians, advised fresh milk and

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

dry air. In England during the 11th and 12th centuries, TB was called "King's evil" and touching the monarch's feet were considered a remedy for the disease. Queen Anne was the last English monarch to participate in this ritual (Global Tuberculosis Institute, n.d.).

1400–1800

During this period, the physicians in Europe started studying various aspects of the disease. Fracastorius (1483–1553) of Verona, Italy mooted the "theory of contagion" to describe the infectious nature of the disease (Fracastoro, Girolamo, n.d.). Exact pathological descriptions of the disease began to appear in the 1600s. In 1679, Fransiscus Sylvius of Leyden first used the term *tubercle* in his *Opera Medica*. He described tubercles as consistent lesions in lungs and other viscera of consumptive patients, which could progress to abscesses and cavities (Sylvius Fransiscus, n.d.). In 1702, Manget described the pathological features of military TB (State of New Jersey, n.d.). In 1720, the English physician Benjamin Marten first proposed that the disease could be caused by "wonderfully minute living creatures" in his book *A New Theory of Consumption*. He wrote that frequent and proximity to a consumptive patient led to inhalation of "breathe emitted from the lungs" of the patient, which infected a healthy person. Marten's writings thus displayed remarkable epidemiological insight (Global Tuberculosis Institute, n.d.).

1800s and early 1900s

During the 1800s, many discoveries were made to help in diagnosis of TB. In 1865, French military doctor Jean-Antoine Villemin demonstrated that TB could be transmitted from humans to cattle and from cattle to rabbits. On the basis of this demonstration, he postulated that a specific microorganism was responsible for causing TB (Jean-Antoine Villemin, n.d.). On March 24, 1882, Robert Koch of Wollstein, Germany, described at the Berlin Physiological Society regarding the discovery and culturing the microorganism that caused TB. This news was cabled across the Atlantic, and the unknown village doctor became famous overnight. Koch's great discovery was made in a small cubicle, using a small microscope presented to him on his 28th birthday by his wife, Emmy. Each year, on March 24, this event is commemorated as "World Anti-tuberculosis Day." In 1890, Koch prepared an old tuberculin reagent and described the "Tuberculin test" and was awarded the Nobel Prize for discovering the tubercle bacillus in 1905 (Zwolska, 2010). In the 1890s, Rudolph Carl Virchow described the process of caseation (Nichols, n.d.). In 1895, Wilhelm Konrad von Röntgen invented the X-ray, which was put to clinical use in 1904 and helped in diagnosing some TB-induced lesions. In 1898, the National Association for Prevention of Tuberculosis (NAPT) was formed in England. In 1902, Sir Robert Philip organized an international body, later to be called the International Union against Tuberculosis and Lung Diseases (IUATLD). In 1907, Clemens von Pirquet described the Tuberculin scarification test (Global Tuberculosis Institute, n.d.; Shashidhara and Chaudhuri, 1990).

Treatments in the Past

Hermann Brehmer was a botany student from Silesia (a Central European region, trifurcated after the Second World War). As advised by a physician to overcome TB, Brehmer traveled to the Himalayas pursuing his botanical studies while trying to get cured of the

disease. He returned home cured and began studying medicine. In 1854, he presented his doctoral dissertation titled "Tuberculosis Is a Curable Disease" in Madanapalle TB sanatorium. (Hermann Brehmer, n.d.) It was the world's first sanatorium, and the early cases of TB were started treating in open-air sanatoria, in Western countries. The other sanatorium "Little Rest Cottage" was established in 1884, in Trudeau, New York, which became a model for other sanatoriums in the United States. By 1938, there were 26,000 sanatorium beds in England and Wales. Sanatoriums performed a dual function: isolating the patients (source of infection) and enforcing the rest and balanced diet needed during the healing process. Because no specific cure was available, all that doctors could do was to improve social and sanitary conditions and ensure adequate rest and nutrition. Italian surgeon Forlanini observed that collapsing the infected lung by artificial pneumothorax tended to have a favorable impact on the outcome of the disease. Later, several surgical measures were adopted for preventing the spread of TB, including artificial pneumo-peritoneum, pneumolysis, crushing the phrenic nerve, thoracoplasty, and resection of affected lobe of the lung. These surgical measures were recommended for patients in advanced stages of TB (Global Tuberculosis Institute, n.d.).

Advent of Antitubercular Drugs

In 1940, Selman A. Waksman and his colleagues were working on the inhibitory effect of actinomyces on bacterial growth and isolated an antituberculous compound actinomycin; but it was too toxic for use in animals or humans. Then in 1943, Waksman discovered streptomycin antibiotic from the fungus *Streptomyces griseus*. Animal experiments showed that streptomycin inhibited growth of the tubercle bacillus with relatively low toxicity. Streptomycin was first given to a girl on November 20, 1944; the clinical results were impressive and the girl was declared cured in 1947. This was considered the first successful drug to combat TB, and in 1952, Waksman was awarded the Nobel Prize for discovering streptomycin (Antibiotics: Types and treatments, n.d.). After the Second World War other antitubercular drugs were introduced, including para-aminosalicylic acid (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955), ethambutol (1962), and rifampicin (1963). By 1965, a dozen effective drugs became available, and later it was demonstrated that the emergence of resistant mutants to streptomycin monotherapy could be overcome by using combinations of two or three drugs (Global Tuberculosis Institute, n.d.).

Magnitude

As far as magnitude is concerned, developed countries show a low prevalence of TB as compared to developing countries (Figure 5.1).

Global Situation

Though access to antitubercular treatment improved, it remained low globally. Persons with active TB who receive no treatment can infect an average of 10 to 15 people annually. Numerically, one-third of new TB cases occur in Southeast Asia, but the estimated incidence per capita is highest in sub-Saharan Africa. Both the highest number of estimated deaths as a result of the disease and the highest mortality per capita are in Africa,

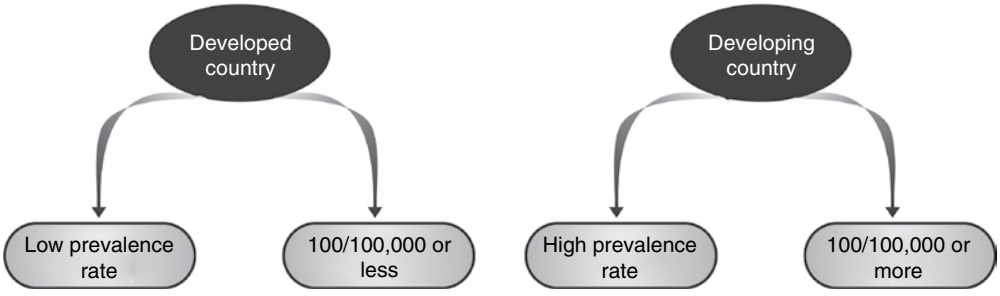

Figure 5.1. Prevalence rate of tuberculosis in developed and developing countries.

Table 5.1. Global tuberculosis control.

	Incidence[a]			Prevalence[b]		Mortality (excluding HIV)	
WHO Region	No. in thousands	Global total (%)	Rate per 100,000 population	No. in thousands	Rate per 100,000 population	No. in thousands	Rate per 100,000 population
Africa	2,800	30	340	3,900	450	430	50
The Americas	270	2.9	29	350	37	20	2.1
Eastern Mediterranean	660	7.1	110	1,000	180	99	18
Europe	420	4.5	47	560	63	62	7
Southeast Asia	3,300	35	180	4,900	280	480	27
Western Pacific	1,900	21	110	2,900	160	240	13
Global total	9,400	100	140	14,000	164	1,300	19

[a] Incidence is the number of new cases arising during a defined period.
[b] Prevalence is the number of cases (new and previously occurring) that exists at a given point in time.
Adapted from World Health Organization (WHO). 2011. Global tuberculosis control. Accessed February 12, 2013, at http://whqlibdoc.who.int/publications/2011/9789241564380_eng.pdf.

driven by an unabated HIV epidemic. TB is a leading cause of death among people who are HIV positive. In Africa, HIV is the single most important factor determining the increased incidence of TB over the past 10 years. HIV weakens the immune system, and a person who is coinfected with HIV and TB is many times more likely to develop clinically overt TB as compared to a person who is HIV negative and infected with the tubercle bacillus. It is paradoxical that despite all the advances in modern scientific medicine, the World Health Organization (WHO), in 1994, declared TB a "global emergency," probably as a result of the advent of HIV epidemic and the emergence of multidrug resistant tuberculosis (MDR-TB). The United Nations Millennium Development Goals include targets to halve the 1990 TB prevalence and death rates by 2015 (WHO, 2007). Table 5.1 details the data of global TB control as declared by WHO in 2011.

In the developed countries, the downward trend in the prevalence of TB was initially as a result of an improved standard of living and socioeconomic changes and later

Table 5.2. Estimated MDR-TB cases and rates in SEAR member countries, 2010.

Country	Source of Estimates	MDR (%) among New TB Cases (%CI)	MDR (%) among Previously Treated TB Cases (%CI)	Number of MDR-TB among Incident Total TB Cases (%CI)
Bangladesh	model	2.2 (0.0–5.6)	14.7 (0.0–39.6)	9,800 (1,000–19,000)
Bhutan	model	2.2 (0.0–5.6)	14.7 (0.0–39.6)	33 (4–61)
DPR Korea	model	2.2 (0.0–5.6)	14.7 (0.0–39.6)	3,900 (658–7,200)
India	DRS[a] (2005)	2.3 (1.8–2.8)	17.2 (14.9–19.5)	99,000 (79,000–120,000)
Indonesia	DRS[a] (2004)	2.0 (0.5–6.9)	14.7 (0.0–39.6)	9,300 (0–21,000)
Maldives	model	2.2 (0.0–5.6)	14.7 (0.0–39.6)	3 (0–6)
Myanmar	DRS (2007)	4.2 (3.2–5.6)	10.0 (7.1–14.0)	9,300 (6,400–12,000)
Nepal	DRS (2007)	2.9 (1.9–4.3)	11.7 (7.6–17.6)	1,700 (990–2,300)
Sri Lanka	DRS (2006)	0.2 (0.0–1.0)	0.0 (0.0–10.2)	63 (0–130)
Thailand	DRS (2006)	1.7 (1.1–2.6)	34.5 (28.2–41.5)	2,900 (2,100–3,800)
Timor-Leste	model	2.2 (0.0–5.6)	14.7 (0.0–39.6)	130 (6–260)

[a] Estimates based on subnational drug resistance data.
CI, confidence interval; DRS, drug resistance surveillance data; MDR-TB- multidrug resistant TB.
Adapted from World Health Organization (WHO). 2007. Global tuberculosis control: Surveillance, planning. Accessed February 12, 2013, at http://www.who.int/tb/publications/global_report/en/index.html.

resulting from improved access to effective antitubercular drugs. The re-emergence of TB in the developed countries in the 1990s is attributed to the HIV epidemic, environmental and social changes, and migration from endemic countries. Thus, the likelihood of elimination of the disease in the developed countries has receded. In the less-developed countries, TB continues to remain a major public health problem and a major cause of mortality because of administrative, epidemiological, and operational reasons.

Asia

In 2008, the estimated deaths by MDR-TB were 150,000 annually. More people have been treated for MDR-TB, and there was estimated prevalence of 650,000 cases of it in 2010. The number of patients enrolled on treatment for MDR-TB was 45,553 in 2010, which was equivalent to 16 percent of the estimated 290,000 cases of MDR-TB among TB patients in 2010 (WHO, 2011). During the past decades well-functioned national TB control programs achieved high cure rates, resulting in maintaining the slow but steady decline in TB incidence rates in the Southeast Asia region. Among the notified cases of pulmonary TB in 2010, it is estimated that 88,000 cases (range: 68,000–110,000) are MDR-TB. In 2010, the total number of MDR-TB cases enrolled for treatment is 3,937 (Table 5.2). However, given the large numbers of TB cases, nearly one-third of the world's MDR-TB cases are in the Southeast Asia region, with India estimated to have the highest number globally (WHO, 2011).

MDR-TB potentially replacing the drug-susceptible TB constitutes a threat to global public health security. In areas of high HIV prevalence, the potential for increased transmission of MDR-TB is high.

Table 5.3. Features of *Mycobacterium tuberculosis* complex.

Feature	*M. tuberculosis*	*Mycobacterium bovis*	*Mycobacterium africanum*
Oxygen preference	Classical strain, obligate aerobe; South Indian strain, aerobe; Asian human, aerobe	Classical strain = microaerophilic on primary isolation; aerobic on serial cultures; BCG, aerobic	African I strain, microaerophilic; African II strain, microaerophilic
Nitrate reduction	Classical strain, +; South Indian strain, +; Asian human, +	Classical strain, –; BCG, –	African I strain, +; African II strain, –
Niacin production	Classical strain, +; South Indian strain, +	Classical strain, –; BCG, –	African I strain, ±; African II strain, –
Effect of 50 microgram per mL of pyrazinamide	Classical strain, sensitive; South Indian, sensitive	Classical strain, resistant; BCG, resistant	African I strain, sensitive; African II strain, sensitive
Thiopen-2-carboxylic acid hydrazide (TCH): 5 microgram per mL	Classical strain, resistant; Asian human strain, sensitive	Classical strain, sensitive; BCG, sensitive	Sensitive
Virulence	Classical strain, humans (+) and Cattle (+); Asian human, humans (+) and Cattle (+)	Classical, cattle (+); BCG, avirulent	Humans, less; cattle, doubtful

BCG, bacille Calmette-Guerin; –, negative; +, positive.

Agent Factors

The causative agent of TB is *Mycobacterium tuberculosis*, and occasionally it is associated with some other strains of *Mycobacteria* as *Mycobacterium bovis* and *Mycobacterium africanum* (Table 5.3). Human, bovine, murine, and avian strains of *Mycobacteria* are also known as "tubercle bacilli" because of the TB lesions they cause. Organisms belong to the genus *Mycobacteria* are either opportunistic pathogens or saprophytes. They grow as branching filaments and appear like mycelia of fungi, thereby, giving it the name *Mycobacteria* (or fungus-like bacteria; Latin: *myces* = fungi). In 1868, Armauer Hansen discovered the first member of the genus *Mycobacteria*, the lepra bacillus (Irgens, 2002). Koch isolated the mammalian tubercle bacillus in 1882.

Mycobacterium Tuberculosis *Complex*

M. tuberculosis is an obligatory aerobic, intracellular pathogen, which has a predilection for the lung tissue rich in oxygen supply (Alamelu, 2004). *M. tuberculosis* complex comprises four species, which are antigenically related and appear to be variants of a single species by DNA hybridization. All of them can cause TB in mammals. Members of this complex includes *M. tuberculosis* (human tubercle bacillus), *M. bovis* (bovine tubercle bacillus), *Mycobacterium microti* (vole tubercle bacillus), and *M. africanum* (an intermediate form between human and bovine tubercle bacilli). Human TB is caused by *M. tuberculosis* and occasionally by *M. bovis* and *M. africanum*. Table 5.4 differentiates *M. tuberculosis* and *M. bovis* strains.

Table 5.4. Difference between *Mycobacterium tuberculosis* and *Mycobacterium bovis.*

Parameters		*M. tuberculosis*	*M. bovis*
Morphology		Long, slender, and usually slightly curved, measures 1–4 x 0.2–0.8 microns (average of 3 x 0.3 microns)	Short, stout and straight
Ziehl-Neelsen staining		May show beading or granular forms	Uniform staining
Growth on LJ medium		Luxuriant (eugonic) growth	Sparse (dysgonic) growth
Effect of glycerol on growth		Improves growth	Inhibits or has no effect on growth
LJ medium: features of colonies		Dry, rough (raised and wrinkled surface), tough or tenacious (colonies do not detach easily), and buff (cream) colored; difficult to emulsify	Moist, smooth (surface), brittle (colonies are friable or break easily when touched), and white colored.
Spread to humans		Via respiratory route	Consumption of raw milk
Biochemicals	Niacin production	+	–
	Nitrate reduction	+	–
Animals	Guinea pig	Highly pathogenic	Highly pathogenic
	Rabbit	Non-pathogenic	Highly pathogenic
	Hamster	Highly pathogenic	–

LJ, Lowenstein Jensen; –, negative; +, positive.

Morphology and Cell Wall

M. tuberculosis is an intracellular, nonmotile, noncapsulated, nonsporing (except *Mycobacterium marinum* and *M. bovis*, which show sporulation, Ghosh et al., 2009) slender, slow-growing organism, occurring singly, in pairs or in small clumps (*see* Table 5.4).

The mycobacterial cell possesses a four-layered cell wall, made up of lipids, proteins, and polysaccharides. About 60 percent of the weight of the cell wall is from its lipid content alone, which are (especially mycolic acid) responsible for the acid-fastness of *Mycobacteria* and the cellular tissue reactions in the host. These four layers (from outer to the inner side), shown in Figure 5.2, are:

1. Mycoside layer: This is the outermost layer made of peptido-glycolipids or phenolic glycolipids. Sugar moieties on the mycosides contain agglutinogenic antigens.
2. Mycolic acid layer: This is the chief constituent of the cell wall, which is a dense band of long-chain alpha-alkyl and beta-hydroxyl fatty acids attached by ester bonds to terminal arabinose units of arabinogalactan.
3. Arabinogalactan layer: This layer located at the inner side to the mycolic acid layer.
4. Peptidoglycan (murein) layer: This is the innermost layer which maintains the shape and rigidity of the cell wall.

Cell Wall Antigens

Mycobacterial cell wall possesses antigens, including arabinomanan, lipoarabinomanan, and arabinogalactan. Except for antigen 60 (a lipo-polysaccharide-protein complex),

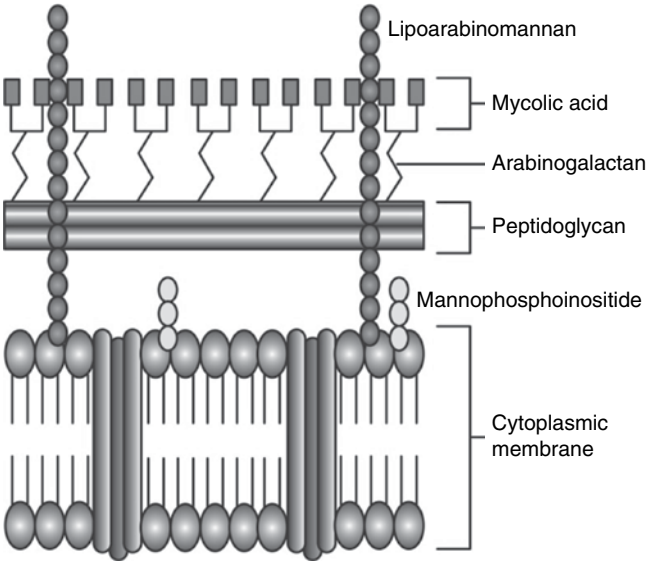

Figure 5.2. Cell wall of *Mycobacteria*.

all cytoplasmic antigens are proteins that are used for the typing of *Mycobacteria*. The biosynthetic pathways of cell wall components are potential targets for new drugs for TB (Bhamidi, 2009).

Staining

Mycobacterial species are difficult to stain, but once stained, they resist decolorization with mineral acids. Hence, they are called *acid-fast bacilli*. They exhibit both acid and alcohol fastness (Ryan and Ray, 2004) depending on the type of staining procedure.

Ziehl-Neelson Method

On staining by the Ziehl-Neelson method, the carbol fuchsin dye binds to the mycolic acid of the mycobacterial cell wall turning it a pink to red color and leaving the blue stained background (tissues, cells, other organisms). Nocardia, actinomyces, and certain fungal and bacterial spores are also weakly acid-fast. Acid-fastness is the property of the intact mycobacterial cell (McMurray, 1996) and is lost when the cell wall got damaged (after exposure to antitubercular drugs, ultraviolet light, heat, and sunlight).

Gram Stain

Tubercle bacilli are also gram positive (alcohol-fast) but are difficult to stain with a Gram stain because the dye cannot easily penetrate the cell wall.

Fluorescent Stain

After the smears are stained with fluorescent dyes (Auramine O, Rhodamine) and examined under ultraviolet light using a fluorescent microscope, the tubercle bacilli are seen as luminous yellow rods against a dark background.

Growth Characteristics

M. tuberculosis is an obligate aerobe. *M. bovis* is microaerophilic on primary isolation, but it also becomes aerobic on subculturing.

Solid Media

On solid media, *Mycobacteria* grow slowly, requiring 18 hours for multiplication. Colonies may take 6 to 8 weeks (minimum of 2 weeks) to appear. Lowenstein-Jensen (LJ), Petragnani, Dorset egg media (egg-based), Middlebrook 7H10, and Middlebrook 7H11 (agar-based media) are the generally used solid media, with LJ being the most common to culture tubercle bacilli.

Lowenstein-Jensen Medium

This is commonly used for growing *Mycobacteria*. It does not contain agar as a solidifying agent but is solidified by inspissation (heating). It consists of beaten eggs (solidifying agent when subjected to inspissation or heating), asparagines, mineral salts, malachite green (a dye that inhibits growth of other organisms and imparts characteristic color to the medium), glycerol (for improving growth of *M. tuberculosis* and inhibiting growth of *M. bovis*), or sodium pyruvate to improve growth of both these organisms.

Liquid Media

Liquid media such as Dubos medium and Middlebrook 7H9 are used for the antibiotic sensitivity testing and preparation of mycobacterial antigens and vaccines. In liquid medium, *Mycobacteria* grow as surface pellicles owing to hydrophobic properties of their cell walls. The addition of Tween 80® (polyoxyethylene sorbitan mono-oleate) to Dubos liquid medium provides uniform growth of bacteria. Tween 80 is a detergent that reduces the surface tension and allows diffuse growth of *Mycobacteria*. Virulent strains grow as serpentine cords in liquid media (Yagupsky et al., 1990), whereas avirulent strains exhibit a more dispersed growth.

Resistance

Sunlight and Ultraviolet Rays

Direct sunlight (5 minutes) and ultraviolet light are lethal to *Mycobacteria* spp. Organisms growing on culture media are killed within 2 hours on exposure to direct sunlight, whereas, in moist sputum samples, they remain viable for up to 20 hours even on exposure to direct sunlight. If protected from the sunlight, they can survive in dried sputum and pus for many months. Tubercle bacilli can remain viable for 8 to 10 days in droplet nuclei when protected from direct sunlight, which is why transmission by droplet nuclei respiratory aerosols generally occurs indoors and in the dark (Figure 5.3).

Heat and Cold

Mycobacteria can survive in freezing conditions but are readily destroyed by heating at 140° F (60° C) for 15 to 20 minutes. At room temperature, cultures remain viable up to 6 to 8 months.

Chemicals

Mycobacteria can survive in 5% phenol, 15% sulfuric acid, 5% oxalic acid, and 4% sodium hydroxide. They are destroyed by tincture of iodine in 5 minutes and 80% ethanol in 2 to

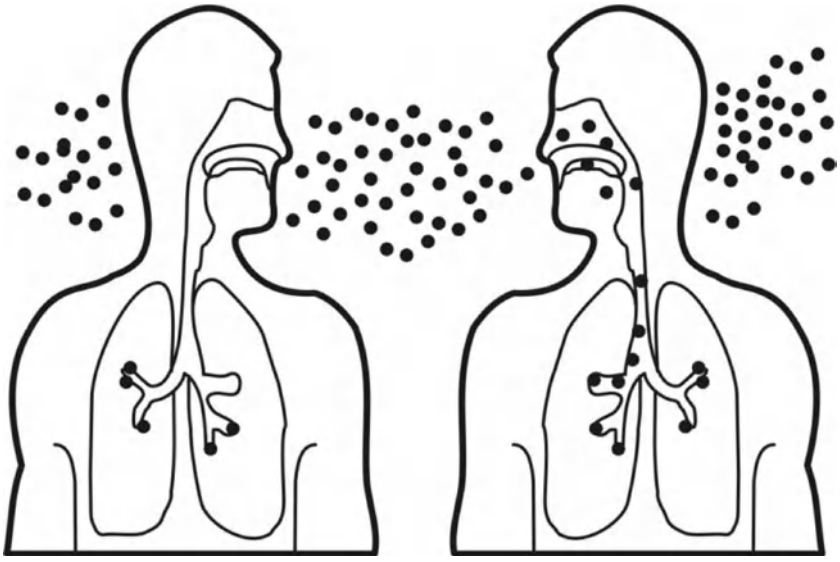

Figure 5.3. Transmission of tuberculosis infection from person to person (aerosol liberated from individual, who is TB^{+ve}, and inhaled by the normal individual, who then acquires infection).

10 minutes. Therefore, 80% ethanol is recommended as a disinfectant for rubber gloves and as a skin antiseptic.

Virulence Factors

Though readily phagocytose, tubercle bacilli are resistant to intracellular killing by macrophages. It grows intracellularly and can remain dormant for years in a host with a good immune status. Hence, many infected persons do not develop clinical manifestations of the disease. The virulence factors associated with *M. tuberculosis* include 65 kDa heat shock protein (highly immunogenic), muramyl dipeptide, mycolic acid, polyanionic trehalose sulfates, Wax D, sulfatides, prevention of fusion of phago-lysosome, ability to grow and multiply intracellularly, and granuloma formation.

Mycobacteriophages

Bacteriophages are viruses that infect and parasitize bacteria. Some tubercle bacilli are infected by temperate phages (bacteriophages that parasitize a bacterium without lysing it). In most of the *Mycobacteria* spp., the phage genome is not integrated with the mycobacterial genome and remains separate by a phenomenon called pseudo-lysogeny (Figure 5.4).

Biochemical Tests

Niacin Production

Most *Mycobacteria* possess enzymes that convert niacin (a metabolic by-product) into niacin ribonucleotide. *M. tuberculosis* and few other *Mycobacteria* lack this enzyme, which accumulates in the culture medium. This accumulation of niacin can be detected

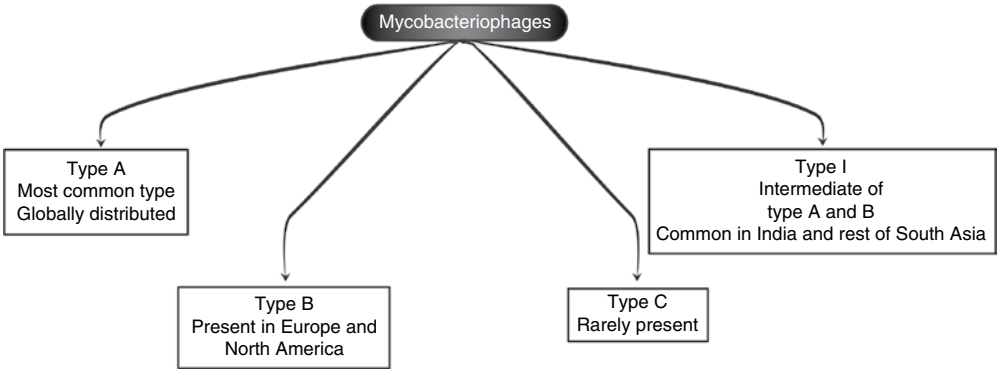

Figure 5.4. Types of mycobacteriophages.

in suspension of mycobacterial culture by adding 10% cyanogen bromide and 4% aniline (in 96% ethanol). Formation of a canary yellow color indicates positive reaction. However, *M. bovis* and bacille Calmette-Guerin (BCG) result a negative test.

Nitrate Reduction Test

A mycobacterial suspension is incubated at 98.6° F (37° C) for 2 hours with a buffer solution containing nitrate followed by the addition of sulfanilamide and n-naphthyl-ethylene diamine dihydrochloride solutions. Development of a pink or red color indicates positive reaction. *M. tuberculosis*, *Mycobacterium fortuitum*, and *Mycobacterium kansasii* produce the enzyme nitroreductase that reduces nitrate to nitrite (positive test), whereas *M. bovis* does not (negative test).

Aryl Sulfatase Test

Certain atypical *Mycobacteria* produce aryl sulfatase enzymes when grown in a medium containing 0.001 M tripostassium phenolphthalein disulfate. In the presence of aryl sulfatase, phenolphthalein is released, which turns pink in the presence of alkali. A positive reaction (aryl sulfatase production) is indicated by development of pink color when 2 N NaOH is added drop-by-drop to a mycobacterial culture in liquid medium.

Neutral Red Test

Virulent strains of *M. tuberculosis*, *M. bovis*, *M. avium*, and *Mycobacterium ulcerans* are capable of binding neutral red in alkaline buffer solution, whereas avirulent strains do not.

Catalase Peroxidase Test

To 5 mL of test culture, a mixture of equal volume of 30% hydrogen peroxide and 0.2% catechol (in distilled water) is added and left for few minutes. "Browning" and "effervescence" indicates peroxidase and catalase activity, respectively. *M. tuberculosis* and *M. bovis* are peroxidase positive and weakly catalase positive. Peroxidase-catalase activity is lost when tubercle bacilli develop resistance to isoniazid. Most atypical *Mycobacteria* are peroxidase negative and strongly catalase positive.

Amidase Test

Mycobacteria that produce pyrazinamidase and nicotainamidase, such as *M. tuberculosis*, can split amides; 0.00165 M solution of an amide (acetamide, benzamide, carbamide, nicotinamide, or pyrazinamide) is incubated at 98.6° F (37° C) in tubes containing mycobacterial suspension. To this, 0.1 mL manganese sulfate, 1.0 mL phenol, and 0.5 mL hypochlorite solution is added. The tubes are placed in boiling water bath for 20 minutes. Development of a blue color indicates a positive test.

Pyrazinamide Sensitivity

M. tuberculosis and *M. africanum* are susceptible to pyrazinamide (50 μg/mL), but other *Mycobacteria*, including *M. bovis* and BCG are resistant.

Thiophene-2-Carboxylic Acid Hydrazide Sensitivity

Classical strains of *M. tuberculosis* are not inhibited by 10 μg/mL concentration of thiophene-2-carboxylic acid hydrazide (TCH). However, *M. bovis* and South Indian strains of *M. tuberculosis* are susceptible.

Tween 80 Hydrolysis

M. kansasii and *Mycobacterium gordonae* possess an enzyme lipase that splits Tween 80 into oleic acid and polyoxy ethylated sorbitol. Change in color of a solution of Tween 80 from yellow to pink indicates its hydrolysis (positive test). *M. tuberculosis* shows variable results. *M. bovis*, *M. africanum*, *M. avium* complex and *Mycobacterium* scrofulaceum do not hydrolyze Tween 80 (negative test).

Bio typing

For many years, human and bovine tubercle bacilli were included under the species *M. tuberculosis*. Other species associated with TB in Africans was named *M. africanum*. A variant of human type of *M. tuberculosis*, differing in its virulence in guinea pigs was first reported in 1948 (Dhaygude and Shah, 1948). This variant was later reporting to be a distinct phage type that caused TB among Asian immigrants in London and was, therefore, named "Asian variant" of *M. tuberculosis* (Grange et al., 1978). Two variants of bovine type (i.e., European and Afro-Asian) have been reported (Marks, 1976). Tsukamura (1996) grouped *M. tuberculosis*, *M. bovis*, *M. bovis* (BCG), *M. microti*, and *M. africanum* under the category "*M. tuberculosis* complex." Grange had described these variants as *M. tuberculosis* with a type designation (Grange, 1979). However, this differentiation of species has been questioned because their DNA is highly related (Collins et al., 1982).

Persisters

In Britain, DNA fingerprinting techniques demonstrated the endogenous reactivation of *M. bovis* infection in elderly patients—even after eliminating the source of infection (viz. contaminated raw milk) through universal pasteurization— which indicates latency in the tubercle bacillus (Grange, 1992). One hypothesis is that, within the lesions, anoxic conditions may induce metabolic changes, enabling tubercle bacilli to enter a dormant state (Wayne, 1976).

Dormancy is also seen during chemotherapy of clinically overt TB. Early bactericidal action (EBA) of drugs rapidly kills the fast replicating extracellular *Mycobacteria* in the

cavity walls. This EBA usually occurs during the first 2 weeks after starting chemotherapy. However, treatment must be continued for at least 6 months because sterilizing action of the drugs kills dormant or semidormant *Mycobacteria* (mostly intracellular forms) at a much slower rate. "Persisters" that survive the intensive phase of chemotherapy are not killed by isoniazid, the drug used in the continuation phase primarily to kill rifampicin-resistant *Mycobacteria* that might start replicating (Mitchison, 1985). Although the persisters in healthy infected individuals (during the latent stage between primary infection and occurrence of postprimary TB) are apparently inactivated by isoniazid monotherapy, but it is not known whether these persisters are identical to the *Mycobacteria* that survive the intensive phase of chemotherapy. Individuals who are HIV positive and are also tuberculin positive (with dual infection) have only about 50 to 60 percent risk of developing overt TB, and it is probably a result of reinfection. This suggests that all infected individuals (i.e., those who are tuberculin-test positive) actually do not harbor viable tubercle bacilli.

Host Factors and High-Risk Groups

Age

Though the disease can occur at any age in either sex, the most vulnerable groups are children, old age (for males), and reproductive age group (for females). Children younger than the age of 6 years are more vulnerable, and the disease usually develops within 1 year of infection. The younger a person is when infected, the shorter the incubation period and the more disseminated the disease is. The prevalence of TB is lower during the ages of 5 to 12 years. The prevalence rises once again in adolescence, when adult-type postprimary TB manifests.

Gender

Female patients with TB face more social stigma and discrimination as compared to their male counterparts. For fear of social ostracism, many families in male-dominated societies do not seek treatment for their womenfolk. In India alone, families abandon more than 100,000 women afflicted with TB each year.

Ethnic Factors

In many situations, higher prevalence in certain ethnic groups seems to be the result of associated socioeconomic factors. However, in isolated nonimmune populations, such as Eskimos and American Indians, initial exposure to TB had caused rapid spread of the disease with death occurring within a few months, a condition known as *galloping consumption*. Similarly, low incidence is reported among Caucasians in the United States as compared to African Americans, probably as a result of associated socioeconomic factors.

Occupation

Some TB cases have been found to be associated with occupation because workers involved in silica industry may develop silico-TB.

Presence of Concomitant Conditions

Conditions that are associated with high risk of developing TB are diabetes mellitus, hypothyroidism, mental stress, alcohol dependence, malignancies, end-stage renal disease, HIV infection, diseases that reduce immunity (Kala azar, diphtheria, pertussis, measles), other congenital and acquired immune deficiencies, and treatment with immune-suppressing agents (e.g., anticancer drugs, corticosteroids). Persons with hyperthyroidism seem to be less vulnerable to TB, probably because their increased metabolic rate enhances phagocytosis of tubercle bacilli.

Immunity

Persons of Jewish descent seem to have a higher level of innate immunity probably as a result of their history of persecution and living in overcrowded ghettos. Individuals are vulnerable during physiological reduction in immunity that occurs in infancy, old age, and pregnancy. Immune suppression may also be a result of malignancy, malnutrition, infections (mentioned previously), cancer chemotherapy, and corticosteroid therapy.

Malnutrition

The previous thought was that a high protein diet would expedite recovery in patients with TB, but trials conducted in India have shown that high protein diets did not contribute significantly to curing it. The observed increase in prevalence of the disease in civilian populations in Europe during the Second World War has been attributed to the chronic shortage of food.

High-Risk Groups

High-risk groups include individuals who are HIV sero-positive, the homeless and the destitute, users of illicit drugs, and inmates of asylums and prisons.

Environmental Factors

Environmental factors also contribute to the likelihood of acquiring TB. Factors, such as overcrowding, congregation in prison settings, poor housing, and inadequate ventilation, predispose individuals to the development of TB (WHO, 2010). In humans, the development of TB is a two-stage process; in the first stage a susceptible person is exposed to an infectious case and gets infected immediately and in the second stage, after an interval of years or decades, the person develops the disease, depending on a variety of factors. Among persons exposed to someone with an infectious case of TB, the risk of becoming infected is determined primarily by the combined action of three factors (Lienhardt et al., 2002):

1. The infectivity of the source case (which is itself, a function of microbial virulence and the density of bacilli in the sputum),
2. The intensity of the susceptible person's exposure to the case
3. The susceptibility of the exposed person to infection.

Sources of Infection

Human Strain

The primary source of infection is an individual with a positive pulmonary TB smear test (Beyers et al., 1997).

Bovine Strain

Milk of infected cattle is the source of human infection with bovine strain TB. Consumption of raw (not boiled or pasteurized) milk from cattle infected by *M. bovis* causes infection of the tonsils that subsequently presents as cervical lymphadenitis. The intestinal tract may also be infected. Thus, bovine tuberculosis is an anthropozoonotic disease (a disease spread from other vertebrate animals to humans).

Reservoir of Infection

Human Strain

Patients with positive pulmonary TB sputum test are the primary reservoir of human strain of tubercle bacilli.

Bovine Strain

Infected cows develop lesions in their udders and *M. bovis* is excreted in their milk. Thus, infected cattle are the main reservoir.

Modes of Transmission

TB is primarily transmitted by inhalation route through droplet infection (pulmonary TB) in which both the portal of entry and exit is the respiratory tract. Any forceful respiratory effort such as coughing, talking aloud, sneezing, spitting, and singing by an individual with sputum-positive pulmonary TB releases droplet nuclei (infectious particles of less than 5 μm in diameter) that contain tubercle bacilli. Owing to their small size, they can directly lodge in the terminal alveoli of the lungs of close contacts by avoiding the host defenses. A single cough by a patient with a positive sputum test (called "open case") can release up to 3,000 droplet nuclei that remain suspended in the air for prolonged periods. Once the droplet nuclei are released, the air remains infectious, even if the patient has moved away from the site of release. Droplet nuclei may settle on dust particles. These infected dust particles may become air-borne during sweeping and dusting and may be inhaled by another host. Transmission by droplet nuclei generally occurs indoors and in the dark. This is because tubercle bacilli can remain viable when protected from direct sunlight for 8 to 10 days in droplet nuclei. Exposure to direct sunlight can kill tubercle bacilli within 5 minutes.

Bovine tuberculosis is results from consumption of raw milk from cattle infected by *M. bovis*. The lactiferous ducts of an infected cow are the portals of exit, and the digestive tract of the human host serves as the portal of entry. Rarely, tubercle bacilli may enter through cuts or abrasions in the skin but not through intact skin. In such cases, primary lesions are present on the exposed parts of the body and likely to be

missed during clinical examination. TB is not transmitted by fomites (inanimate substances, such as utensils and clothing).

Incubation Period

The time period between entry of tubercle bacilli into the body of a human host and the development of tuberculin positivity is about 4 to 6 weeks, whereas progression from stage of infection to stage of clinical disease (pulmonary or extra-pulmonary) may take several months or years.

Period of Communicability

A patient with cavitary TB is infectious to others. The period of communicability starts when a cavitary lesion (a cavity of 2-cm diameter containing 10^8 [100 million] tubercle bacilli) opens into a respiratory passage and the patient starts discharging tubercle bacilli during forceful respiratory efforts such as coughing, talking aloud, sneezing, spitting, and singing. The period of communicability continues until sputum conversion occurs after effective chemotherapy.

Pathology and Immunology

Primary Infection

Primary infection usually occurs in children who have not had any previous exposure to tubercle bacilli, which can be determined by size of the infected droplets. The host defenses against microbial invasion by the respiratory route include filtering systems in the upper respiratory tract, mucociliary escalator (cascade) that clears the microbes, and trapping and killing of microbes by macrophages in the alveoli. Droplets larger than 10 microns in diameter are easily caught by the mucociliary cascade and cleared from the respiratory tract by physiological mechanisms, but because of their smaller size, droplet nuclei usually lodge in subpleural bronchioles or terminal alveoli of the midzone of the lungs by avoiding the mucociliary defenses (cascade) of the bronchi.

Primary infection begins with multiplication of tubercle bacilli in the lungs, and the resulting primary lesion is called Ghon's focus. It is a small area of granulomatous inflammation, detectable only by chest X-ray if it calcifies or grows substantially (Kumar et al., 2007). Ghon's focus and enlarged hilar lymph nodes together constitute the *primary complex*. The initial focus of infection is the subpleural area of the lower part of the upper lobe or the upper part of the middle or lower lobes of the lung. This area is vulnerable because it receives maximal airflow that facilitates deposition of infected droplets. After about 4 to 6 weeks of primary infection, the immune response (delayed type hypersensitivity [Type IV] and cellular immunity) develop based on the quantum of infecting dose and the strength of the immune status. In the case of a healthy immune status, Ghon's focus usually heals by calcification and the only evidence of primary infection is tuberculin conversion, whereas, in condition of poor immune status, the pulmonary component of Ghon's focus may:

- Erode into the pleural cavity causing pleural effusion.
- Open into bronchi causing bronchopneumonia.

- Cause massive enlargement of hilar lymph nodes. This causes partial or complete obstruction of a bronchus and results in emphysema or atelectasis of the affected lung.
- Cause erosion of a caseous lymph node into a bronchus, producing endobronchial TB.
- Spread via lymphatics and blood stream to other lymph nodes, the kidneys, epiphyses of long bones, the vertebrae, and apical posterior areas of the lungs.
- Spread via lymphatics and blood stream to juxtaependymal meninges adjacent to the subarachnoid space forming Arnold Rich's focus.

In cases of milk-borne TB, the portal of entry for *M. bovis* is the mouth; hence, the primary complex may form at the tonsils, cervical lymph nodes, ileocecal region, and mesenteric lymph nodes.

Immunopathology of Primary Infection

Within 3 weeks of primary infection, the bacilli population reaches 10^3 to 10^4 (the number required to trigger an immune response) and then the multiplication suddenly stops triggering a primary reaction, which initially mobilizes neutrophils (the first cell to encounter). If the neutrophils fail to eliminate the bacilli, lymphocytes and macrophages are drawn to the site. Various immunological chemicals activate the resting macrophages to engulf, ingest, and digest the *Mycobacteria*. This process is further enhanced by vitamin D_3, which is tuberculostatic (Kartikeyan et al., 2007). The activated macrophages release chemicals from the cell wall of the digested *Mycobacteria*, resulting in further conversion of monocytes (dormant phase) into macrophages, which ingest free tubercle bacilli. *Mycobacteria* multiply within the macrophages in a logarithmic manner. About after 2 to 3 weeks, the initial infection cell-mediated immune response halts the unhindered multiplication of tubercle bacilli. Activated macrophages start presenting tuberculin antigen along with the class II MHC molecule, which further activates CD4 positive helper T-cells for the development of cell-mediated immunity (CMI). On activation, T-helper cells secrete interferon-γ (IFN-γ), interleukin-2, tumor necrosis factor-α, and other chemicals that exert different biological effects (Figure 5.5). CD4 cells also induce other macrophages to kill the intracellular bacteria, forming epithelioid granulomas. On the other hand if the tubercular antigens are presented together with class-II MHC molecules, CD8-positive suppressor T-cells are activated, which are primarily cytotoxic cells, and they mediate delayed type hypersensitivity (DTH) and also have the ability to recognize infected macrophages and destroy them by direct cytotoxic action.

Macrophages differentiate into epithelioid cells and Langerhans giant cells, forming a granuloma (called the "tubercle"). The center of the granuloma has low partial oxygen pressure (pO_2) and low pH. *M. tuberculosis* is not able to multiply in the acidic extracellular environment within the granuloma and immunologic control can be achieved, by "walling off" the infection. In this stage, the person remains infected, but clinically asymptomatic. About 90 percent of otherwise healthy (and HIV seronegative) persons infected with the tubercle bacillus do not develop clinical TB, which can only be detected by a positive tuberculin reaction or chest X-ray, which is used to visualize if the granuloma gets fibrosed (in adult tuberculosis) or calcified (in childhood tuberculosis).

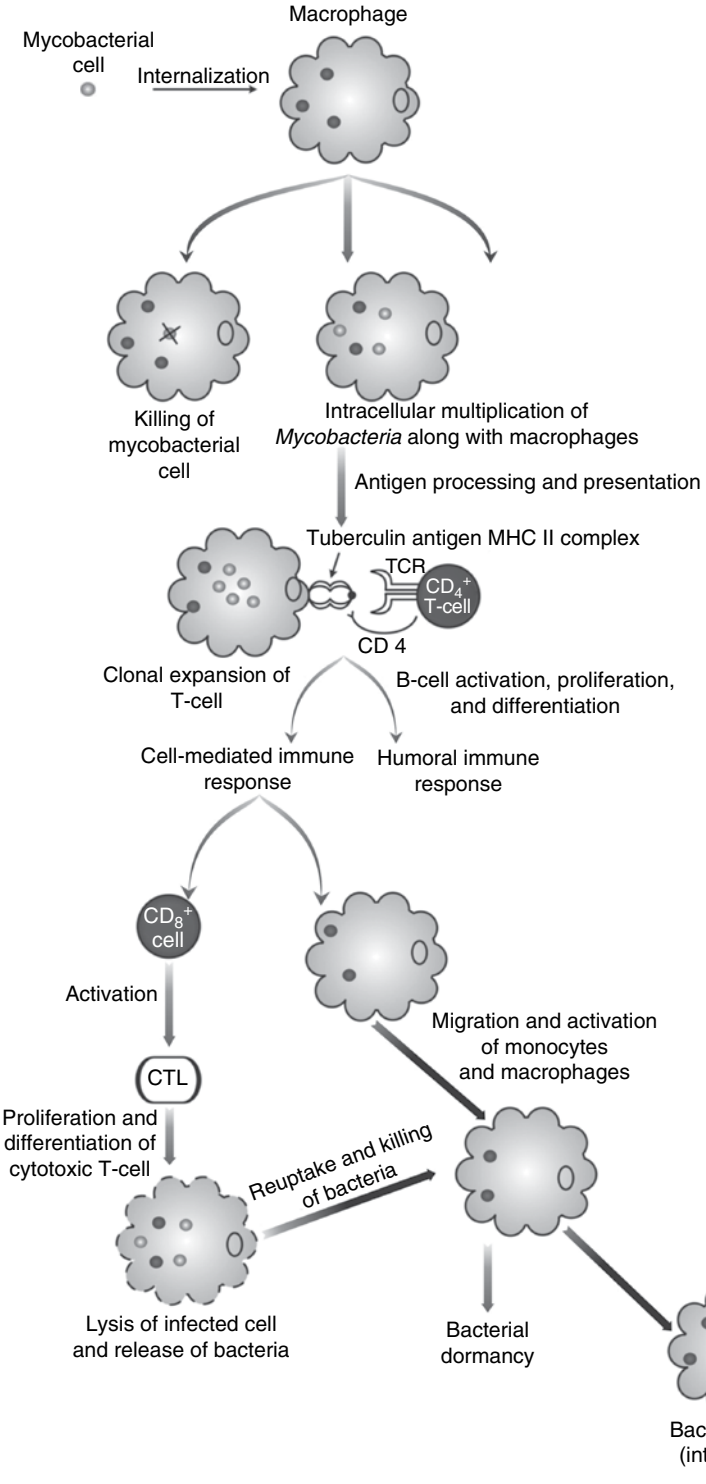

Figure 5.5. Progression of *Mycobacteria tuberculosis* after inhalation, entry into macrophages, and activation of immune system.

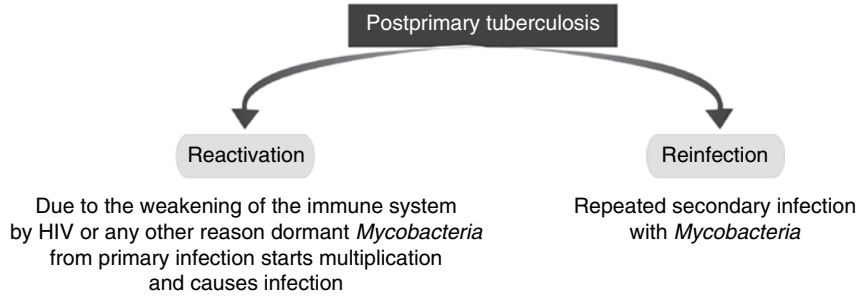

Figure 5.6. Postprimary tuberculosis.

In patients with immune suppression, tubercle bacilli leave the primary focus and invade the body of the host via the lymphatic and bloodstream. Dissemination may occur when caseous material reaches the bloodstream from a primary focus or a caseating metastatic focus in the wall of a pulmonary vein (Weigert's focus). Dissemination results in seeding of tubercle bacilli in all tissues and organs of the body via the lymphatic and bloodstream.

Postprimary Tuberculosis

Postprimary TB, also called secondary or adult-type TB, is usually seen in adults probably as a result of a secondary reaction that occurs either by reactivation or reinfection (Figure 5.6).

Immunopathology of Postprimary Tuberculosis

In the later stages of primary infection, lymphocytes become responsive to tuberculin antigens, and there seems to be a balance between CMI and DTH. If the immunologic control by CMI and DTH is not balanced, then the tissue-damaging action of DTH may predominate. In individuals positive for tuberculin, secondary reaction induces a massive granulomatous reaction, causing extensive destruction of lung parenchyma. Caseous necrosis leads to tissue destruction and formation of cheese-like material in large areas, which may form a tuberculoma (localized nodular parenchymal lesion, which neither cavitates nor gets calcified). If a caseous granuloma erodes into a bronchus and discharges its contents, the spread of caseous material along the bronchial tree causes tuberculous bronchopneumonia. If coughing discharges the contents of a caseous granuloma, it leaves behind a cavity in the lung parenchyma. Thus, tuberculin positivity (DTH) is more harmful, and not helpful, to the host.

Outcome and Complications of Postprimary Tuberculosis

Though postprimary TB usually affects the lung, it can affect any part of the body. Pulmonary TB may manifest as cavities, upper lobes infiltrates, progressive pneumonitis, endobronchial TB (Figure 5.7), and fibrosis. In many instances, tubercle bacilli remain dormant in metastatic foci and do not produce any symptoms. Dormant bacilli may be activated in the presence of immune suppression and results in extrapulmonary TB. The

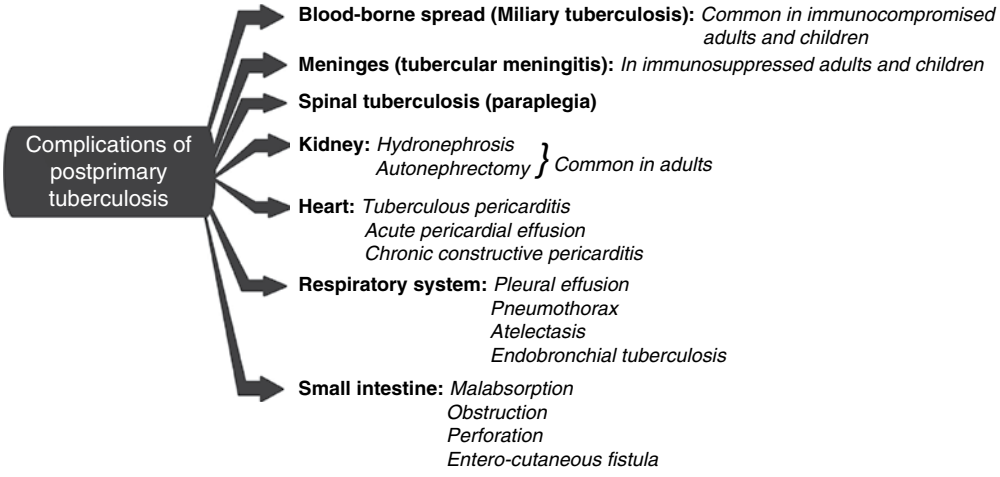

Figure 5.7. Complications of postprimary tuberculosis.

hallmarks of postprimary TB are caseous necrosis, extensive destruction of lung tissue with cavitations, involvement of upper lobe, and positive sputum smear usually with the absence of intrathoracic lymphadenopathy.

The likelihood of complications would depend on stage of the disease when the treatment was started, degree of adherence to treatment, and the presence or absence of immune suppression.

Immune Response in Tuberculosis

As previously mentioned, macrophages stimulate helper T-cells, which further mature via two distinct pathway forming two subsets, Th-1 and Th-2, and each secretes different cytokines, (Mosmann and Moore, 1991) (Figure5.8). The cytokines produced by one cell type tend to inhibit the pathway of maturation of the other cell type. For example, IL-10 produced by Th-2 cells inhibits Th-1 pathway. Thus, the immune response to a given antigen gets locked in to either of the pathways. Th-1 responses induce protective immunity against diseases characterized by chronic intracellular parasitism. Reactions mediated by Th-2 are associated with gross tissue necrosis and production of immunoglobulin E (IgE) antibodies, which cause atopic or allergic reactions.

In TB, Th-1 response produces protective immunity with granuloma formation but without tissue necrosis. This occurs in a majority of infected persons. In cell-mediated immunity ("protective"), tumor necrosis factor (TNF), which is present in the cell membrane of the activated macrophages, helps in the formation, integrity and function of the granuloma. Th-1 cells predominantly respond to common mycobacterial antigens and produce (or stimulate production of) type 1 cytokines such as IFN-γ and interleukin-2 (IL-2), leading to the elimination of mycobacterial challenge.

Th-2 (or mixed Th-1 and Th-2) response causes extensive tissue necrosis as in progressive postprimary TB (Hernandez-Pando and Rook, 1994). Certain components of the cell wall of the tubercle bacillus trigger the release of TNF from macrophages. Type 2 cytokines, such as interleukin-4, -5, and 10 (IL-4, IL-5, and IL-10) cause toxicity in the presence of TNF. This toxicity results in the destruction of sensitized cells leading

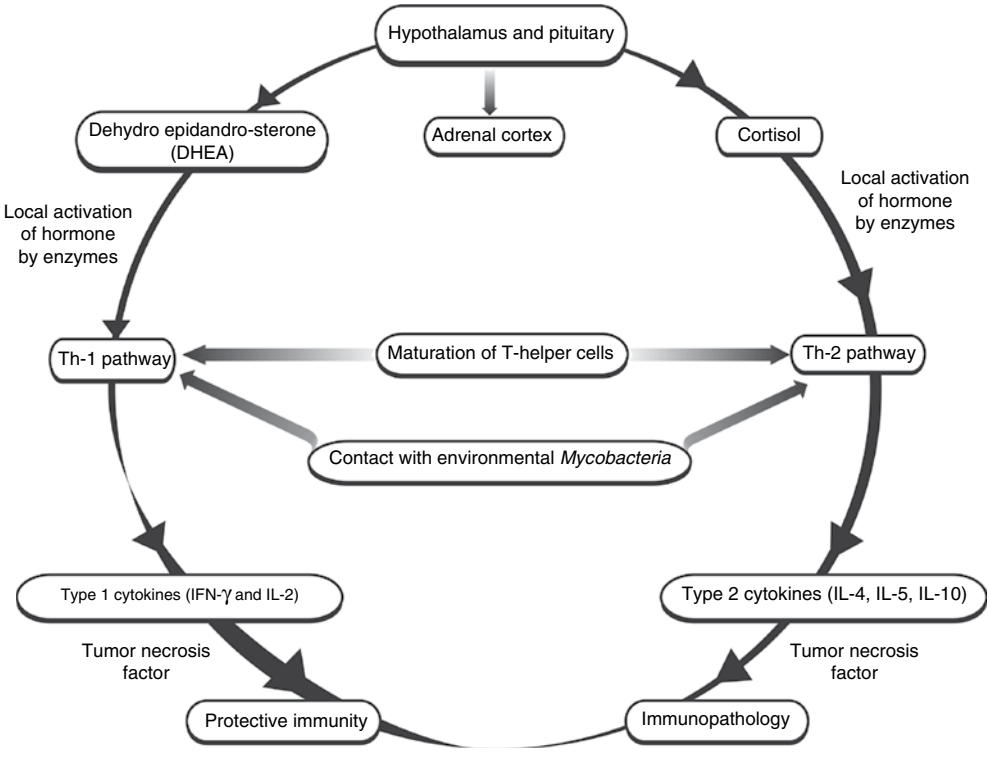

Figure 5.8. Immune response in tuberculosis. IL, interleukin; INF, interferon.

to extensive tissue necrosis and cavitation in the lungs in postprimary TB and systemic symptoms such as weight loss, fever, and night sweats.

Clinical Manifestations

Pulmonary Tuberculosis

The most common symptom of pulmonary tuberculosis is persistence of cough with the production of sputum up to 3 weeks or longer, and it is usually accompanied with expectoration. About 25 percent of people may not have any symptoms (i.e., asymptomatic) (Lawn and Zumla, 2011). Other symptoms include weight loss, loss of appetite, evening rise of temperature, night sweats, chest pain, hemoptysis, shortness of breath, and tiredness. TB may become a chronic illness and cause extensive scarring in the upper lobes of the lungs. The upper lungs are believed to be more frequently affected because of their poor lymph supply rather than a greater air flow (Mandell et al., 2010).

Tuberculous Lymphadenitis

Tuberculous lymphadenitis is a local manifestation of the systemic disease (Kent, 1967). It may occur during primary tuberculous infection or as a result of reactivation

of dormant foci or direct extension from a contiguous focus. Following the primary infection, inhaled droplet nuclei are small enough to pass mucociliary defenses of bronchi and thereby lodge in terminal alveoli of lungs and multiply in the lung, which is called Ghon's focus. The lymphatics drain the bacilli to the hilar lymph nodes. The infection may then spread to regional lymph nodes and might continue to spread via the lymphatic system to other nodes or may pass through the nodes to reach the bloodstream, from where it can spread to virtually all organs of the body. Hilar, mediastinal, and paratracheal lymph nodes are the first sites of infection spread from the lung parenchyma.

Primary lesions, located in the tonsil or mouth, are caused by ingestion of raw milk contaminated with *M. bovis*. These lesions drain into lymph nodes in the upper part of the neck. Primary lesions are not found in some developing countries such as India (because of the universal practice of boiling milk before consumption) and many developed countries (because of universal pasteurization).

In the early stages of the disease, one large lymph node and several small nodes, which are not fixed to the nonerythematous overlying skin, are palpated. These lymph nodes are firm in consistency and non-tender. In advanced disease, matting and caseation of the lymph nodes leads to the formation of a "cold abscess" that may burst through the skin, forming a sinus (if not surgically drained). A cold abscess is characterized by the absence of cardinal signs of inflammation such as local warmth, tenderness, and pain.

Abdominal Tuberculosis

In countries where *M. bovis* infection is rare, the abdominal viscera or peritoneum is probably infected as a result of a hematogenous spread from a primary focus in the lung, which is rare in children. Primary focus may be in the intestine or mesenteric lymph nodes. Caseous lymph nodes may discharge into the peritoneal cavity, causing ascites. In females, pelvic spread of abdominal TB may often involve the fallopian tubes and ovaries, subsequently causing infertility.

Tuberculous Meningitis

Through the hematogenous spread, the bacterium may infects the central nervous system (CNS) resulting in formation of a "tuberculoma," which may cause focal neurological signs or may burst into the meninges, causing a meningeal reaction. Other CNS manifestations include brain abscess and transverse myelitis-like syndrome. Tuberculous meningitis may occur in adults and in 5 to 10 percent of children who are infected when younger than 2 years of age. In children, this condition usually occurs about 3 to 6 months after initial infection. Onset is insidious with nonspecific symptoms such as anorexia, fever, and weight loss. Table 5.5 summarizes the various clinical stages of tuberculous meningitis.

Patients in stage 1 or 2 have a good prognosis if diagnosed and treated early as a medical emergency. Tuberculous meningitis may extend and involve arachnoid membrane that covers the spinal cord causing compression of spinal cord (leading to paraparesis or paraplegia) or spinal nerves a condition called as *tuberculous arachnoiditis*.

Table 5.5. Summarizes clinical stages of *tuberculous meningitis.*

Stages	Signs and Symptoms
Stage I	Headache, occasional projectile vomiting, and irritability (without any neurological symptoms)
Stage II	Lethargy, nuchal rigidity, altered deep tendon reflexes, cranial nerve palsies
Stage III	Major neurological deficits seen, seizures, abnormal movements (choreoathetosis), coma, paresis, or paralysis of one or more extremities
Stage IV or terminal stage	Decerebrate or decorticate posture, opisthotonus, and death.

Tuberculosis of Bones and Joints

The risk of hematogenous spread of TB bacterium from a primary focus in the lung to bones and joints is higher in younger children. Though all bones and joints are vulnerable, those that are weight bearing (i.e., vertebrae, hip, knee, and bones of the ankle and foot) are more likely to be affected.

Tuberculosis of Spine

The spine is the most common site for bone TB. The vertebrae are affected by hematogenous spread of tubercle bacilli, usually from a focus in the lung. The presentation may be acute or insidious. This condition may go unrecognized for months to years. Spinal TB is not seen in infants. The disease usually occurs in children 2 to 3 years old, when they begin to walk and move about, and the vertebrae become weight-bearing bones. After this age, children of all ages and adults are vulnerable. This may involve cervical, thoracic, and lumber vertebra depending on the severity and site of infection.

Tuberculosis of Hip

After vertebrae, the hip is the second most common site for bone TB. The disease is usually seen after 5 years of age. Commonly, the disease begins in the joint capsule, but it may also begin in the neck of femur. If the treatment is delayed, the hip joint may get destroyed, shortening the affected lower limb. Young children usually refuse to walk. Older children and adults may complain of pain, which is usually referred to the knee.

Tuberculosis of Knee

Swelling of the knee may often be the only manifestation in the early stages. Patients may complain of pain and may limp because of pain and associated muscle spasm. The clinical findings include swelling (resulting from fluid accumulation in the joint), warmth on the affected knee, thickened synovium palpable above the patella, and atrophy of the muscles of the thigh.

Tuberculosis of Ankle and Foot

Multiple lesions may be present, and the same bones may be bilaterally affected. Pain and limp are the early manifestations. The calf muscles may undergo disuse atrophy. Swelling over the affected bone or joint indicates the formation of an abscess. Surgical

drainage is indicated to prevent a discharging sinus if the skin over the swelling becomes erythematous or fluctuant. TB of ankle and foot usually responds well to chemotherapy.

Tuberculosis of Upper Limbs

TB seldom affects the bones and joints of upper limbs. Patients rarely complain of pain because the weight bearing is less. Because of the lack of pain, patients may not seek medical help until the bone or joint is considerably damaged. The small bones of the wrist and fingers may be bilaterally affected.

Other Bones and Joints

TB can affect any bone or joint, although the vertebrae and bones and joints of the lower limb are commonly affected. The disease initially manifests as a painless swelling, which gradually develops into an erythematous, soft, and fluctuant abscess. The abscess may discharge, leaving a sinus. Occasionally, these abscesses may be painful and tender, resembling pyogenic abscesses. Systemic symptoms such as fever may be present when multiple bones or joints are involved. Radiographs (antero-posterior and lateral) of the affected area will show loss of bone shadows at the site of swelling. In addition to antitubercular treatment, immobilization of the area may be required, depending on the bone or joint affected.

Tuberculosis of the Eye

M. tuberculosis can affect the eye by one of the three mechanisms, phlyctenular conjunctivitis, primary infection of the conjunctiva, or other tubercular conditions of the eye.

Phlyctenular Conjunctivitis

Phlyctenular conjunctivitis is a painful and sometimes recurring condition usually common between the ages of 5 and 15 years, although it may occur at any age. This is a result of hypersensitivity reaction to tuberculin antigen produced at a primary focus of infection elsewhere in the body and is common in the first year after infection. The condition begins with pain, foreign body irritation, excessive lacrimation, and photophobia in one or both eyes. On examination, single or multiple small gray or yellow spots are seen around the limbus (junction of the cornea and sclera), which may be surrounded by small hyperemic blood vessels. Each spot remains for about 1 week and gradually disappears, which can be further replaced by new spots. Severe attacks are characterized by corneal ulceration, severe pain, and severe photophobia (the patient may close his or her eyes and prefer to sit in a dark area). If complicated by secondary infection, purulent discharge may be seen. In such cases, the cornea may get permanently scarred, leaving white opacities at the site of corneal ulcers.

Primary Infection of the Conjunctiva

Primary infection of the conjunctiva occurs in children who have not had a primary infection with tubercle bacilli. Tubercle bacilli carried by infected dust or droplet nuclei, lodge in the upper or lower eyelid, multiply and form tuberculous lesions that resemble primary foci elsewhere in the body. These tuberculous lesions caseate and can be seen as multiple, small, yellow areas on everting the eyelid. The draining lymphatics involve the

small lymph node near the tragus of the ear, which enlarges, softens, and later ruptures. From the site of conjunctival infection, the tubercle bacilli may be carried to the other parts of the body by the bloodstream. The early stage of primary conjunctival infection is not painful, although there may be excessive lacrimation and mild swelling of the affected eyelid.

Other Tubercular Conditions of the Eye

Other complications are probably the result of the hematogenous spread of tubercle bacilli from a primary focus elsewhere in the body.

- **Acute tuberculous panophthalmitis**: This is highly destructive abscess of the whole eye. The patient progressively loses vision and eventually requires surgical removal of the eye.
- **Uveitis**: "Mutton fat" lesions are seen on the posterior aspect of the cornea and iris.
- **Retinitis**: An inflammation of retina characterized by grayish white ground glass blotches, which is followed by the swelling of retinal veins as a result of local hemorrhages.

All of these eye infections respond well to antitubercular treatment. Corticosteroids may be indicated in the early stages if loss of the eye or sight is imminent.

Skin Tuberculosis

In skin TB, the tubercle bacilli usually spread in the bloodstream from a primary focus to other body parts, which might involve skin and subcutaneous tissues.

Primary Infection of the Skin

M. tuberculosis rarely causes tuberculous infection of the skin. The lesions are small and painless, which might be ignored by the patient or missed by the clinician during examination. Tubercle bacilli may enter the skin on the exposed parts of the body through cuts or abrasions. The face and lower limbs below the knees are more commonly affected as compared to the upper extremities. The patient usually seeks medical care when the draining superficial lymph nodes enlarge and soften. A recent primary focus appears like a thickening in the skin surrounded by tiny, yellowish, macular spots. Sometimes, the primary focus may have healed, leaving a central area of smooth scar with a well-defined irregular edge, and well-defined tiny pits in the skin replace the tiny yellow macular spots. A similar appearance may be seen at the site of BCG vaccination because the vaccine also produces a primary skin infection. If BCG is accidentally given to an immunocompromised patient, it can cause disseminated or life-threatening infection, which is why the WHO stopped recommending BCG for infants with HIV, even if there is a high risk of exposure to TB (WHO, 2007).

Subcutaneous Abscess

A subcutaneous abscess appears as a soft fluctuant swelling below the skin. More than one abscess may be present in different parts of the body. The abscess ruptures to form an ulcer with irregular edge and a clean base, which may allow the ulcer to heal slowly and spontaneously if the nutritional status of the patient is good.

Single Painless Skin Lesion

This is initially small and may gradually enlarge to 2.5 to 5 cm in size. Usually present on the hands or face, the lesion may become covered with rough scaly skin. It may remain unchanged for months before it gradually heals, leaving a scar.

Male Genital Tract

M. tuberculosis causes primary tuberculous infection in male circumcision. The wound heals initially and later breakdown to form the primary focus. Lymphatic drainage involves either one or both inguinal lymph nodes, which enlarge to form abscesses. Tuberculous infection of an infant circumcision leads to the development of miliary or meningeal TB. Similar risks would be associated with female circumcision, which is prevalent among certain African communities.

In young children, testis and epididymis are often involved because of hematogenous spread of bacilli from a primary focus elsewhere in the body. The testis alone or both testes and epididymis may enlarge. Initially, the swelling is painless and hard in consistency. If not treated early, the swelling may gradually become attached to the overlying skin, soften, and discharge as a sinus. This condition is to be differentiated from acute bacterial infections that are characterized by fever, pain, and testicular tenderness. Involvement of the genital tract usually occurs in the reproductive age group for an example, in a study of 40 men with epididymal TB, the median age was 32 years (Viswaroop et al., 2005).

Female Genital Tract

In females, *M. tuberculosis* spreads hematogenously from a primary focus in the lung to the uterus and fallopian tubes after puberty, with an increase of the blood supplies to the pelvic organs. The fallopian tubes are the initial source of infection because both tubes are involved in nearly 100 percent of cases (Nogales-Ortiz et al., 1979). Symptoms include amenorrhea, lower abdominal pain and distension, loss of weight, and loss of appetite. Occasionally, the infection may remain silent and may cause infertility in later years.

Kidneys and Urinary Bladder

TB of the urinary system develops about 10 to 15 years after the primary infection and is, therefore, not seen in younger children. The kidneys are affected by hematogenous spread of tubercle bacilli. The infection starts between the renal pyramid and cortex and slowly progresses to the stage of caseation. With the involvement of renal pelvis, the caseous material is carried in the urine to the bladder, causing tuberculous cystitis. Initially the patients are asymptomatic. Urine may contain pus cells and on culturing may not show the growth of common urinary pathogens.

Diagnosis of Tuberculosis

History and Suggestive Symptoms

Usually the patients remain asymptomatic following tuberculous infection, and it is thereby diagnosed during health check-up or pre-employment medical examination. To

decide the category, previous antitubercular treatment is essential because placement in the wrong category may lead to treatment failure. Misdiagnosis also has a negative impact on TB control program in the community.

Apart from these some other features should also be considered while investigating tuberculosis patients as:

- History of measles or other viral diseases in the recent past
- BCG vaccination history by checking BCG scar on left deltoid region
- Family history of TB or among adults in the neighborhood
- Associated factors as girl child, number of siblings, poverty, etc.

Sputum Microscopy

Rationale

TB affects the lungs in more than 80 percent of patients. Three sputum samples are collected and examined for the presence of acid-fast bacilli (AFB).

Rationale for Primary Symptom

According to the Revised National Tuberculosis Control Program (RNTCP), cough for the duration of 3 weeks or longer is taken as a primary symptom for pulmonary TB. Almost 89.1 percent of patients with positive sputum smears attending health facilities can be diagnosed by screening patients with cough for duration of 2 weeks or longer (Ngadaya et al., 2009). Subjecting other patients (with cough of less than 3 weeks duration or chest pain) to sputum microscopy may increase the laboratory workload by three times, without any additional case yield. Moreover, other clinical manifestations such as fever and chest pain are also seen in other diseases and are thus not typical for diagnosis of TB.

Rationale for Three Sputum Examinations

It is estimated that almost 85.8 percent of TB cases were detected with the first sputum specimen. With the second sputum specimen, the average incremental yield was 11.9 percent, whereas the incremental yield of the third specimen, when the first two specimens were negative, was 3.1 percent (Parsons et al., 2011). Additional case yield from subsequent specimens is minimal; therefore, three sputum specimens are to be examined preferentially.

The examination of a single sputum specimen may yield false-positive results but the likelihood of two smear examinations yielding false-positive results is negligible if quality of sputum microscopy is supervised. Thus, for obtaining high specificity of sputum microscopy as a case finding tool, the criterion of at least two (preferably three) sputum examinations have been fixed, whereas in cases where only two sputum specimens are examined, and one specimen is smear positive for AFB, a third sputum specimen must be collected and examined.

Indications for Sputum Microscopy

- All patients with cough of 3 weeks duration (or longer)
- All patients suspected of having extrapulmonary TB with chest symptoms, irrespective of duration of these symptoms

Table 5.6. Early diagnostic features of tuberculosis.

Category of Patients	Suggestive Signs and Symptoms
Adults	a. Cough for 3 weeks or longer, with or without expectoration, b. Blood-tinged sputum or hemoptysis, especially early morning, c. Low-grade, remittent fever with or without chills and night sweats d. Excessive weight loss, loss of appetite e. Associated factors as advanced age, pregnancy, poverty, overcrowding, poor housing conditions, mental stress, etc.
Children	Children with fever or cough for 3 weeks or longer and a. With or without weight loss b. With history of failure to gain weight c. With history of contact with suspected or diagnosed case of active tuberculosis within the last 2 years.

Table 5.7. Schedule for sputum examination.

Category/Regimen	Schedule after Antitubercular Treatment
Smear positive, CAT-I	After 2, 3, and 6 months
Smear positive, CAT-I, if smear positive at the end of 2 months of intensive phase	After 2, 3, 5, and 7 months
Smear positive, CAT-II	After 3, 5, and 8 months
Smear positive, CAT-II, if smear positive at the end of 3 months of intensive phase	After 3, 4, 6, and 9 months
Smear negative, CAT-I and smear negative, CAT-III	After 2 and 6 months
Non-DOTS regimens	After 2, 6, and 12 months

- All contacts of TB patients with chest symptoms, irrespective of duration of these symptoms
- All patients on antitubercular treatment (as per schedule given in Table 5.6).

Sputum microscopy is more informative than radiology in monitoring the progress of chemotherapy. Table 5.7 provides the schedule for sputum microscopy during the entire course of antitubercular treatment.

If a patient's sputum is found to be positive after 2 to 3 months (end of intensive phase) of antitubercular treatment, positivity may because of dead AFB. Therefore, the intensive phase is to be extended by an additional month. Patients should not be labeled as a "treatment failure" unless the sputum is positive at the fifth month after starting antitubercular treatment.

Importance of Sputum Status

Sputum status at the end intensive phase is a predictor of probability of cure. If the history of previous treatment was taken properly at the time of diagnosis, most patients who continue treatment would get cured. However, the relative risk of treatment failure is higher among those who are sputum positive at the end of the intensive phase, as compared to that among their sputum-negative counterparts. Sputum positivity at the end of

Table 5.8. The method of sputum collection.

Type of Specimen	Procedure
First spot specimen	This specimen is collected under supervision of a staff member on the day a patient reports for sputum examination. The patient is instructed to inhale deeply two to three times with his or her mouth open and then cough out deeply from the chest, open the sputum container and spit out the sputum into the container. The sputum container is immediately closed with a cap.
Early morning sputum	Now the patient is given a sputum container duly labeled with his or her laboratory serial number. The patient is asked to collect an early morning specimen before his or her second visit to the laboratory.
Second spot specimen	When the patient returns with the early morning specimen, a second spot specimen is collected under the supervision of a staff member.

intensive phase is used as a tool for quality control that reflects on quality of laboratory techniques, quality of observation of drug administration, and proportion of defaulters.

Procedure

The reasons for sputum examination ought to be clearly explained to and understood by the patient. The treating physician must record the necessary information (name of the center, department, patient identification details, source of specimen, and reason for sputum examination) on the designated laboratory form. The laboratory personnel must record the patient's full name and address on the TB laboratory register so that the patient can be traced if he or she does not return to the laboratory for the report of the sputum examination. Table 5.8 shows the method of sputum collection.

Concentration of Clinical Specimens

Now the specimens are concentrated to homogenize, kill undesirable organisms, and to achieve proper yield of tubercle bacilli in a small volume without inactivating them. Concentration methods are used for preparation of smear for microscopic examination, inoculation in culture media, and animal inoculation tests. Petroff's (alkali method) method (1915) is a simple and most commonly used for this purpose (Figure 5.9).

Smear Preparation and Staining

Specimen's purulent part is preferred for smear preparation. The smears are then immediately stained and examined under the microscope. The staining procedure commonly used for mycobacterial specimens is called Ziehl Neelsen (ZN) staining, after the people that suggested its use. *Mycobacterium* contains large amounts of lipid substances within their cell walls called *mycolic acids*, which resist staining by ordinary methods like a Gram stain (Morello et al., 2006). ZN staining is based on the principle that the use of a powerful phenol containing stain and heat can make the dye penetrate to the mycobacterial cell wall. Once stained, the mycobacterial cell wall withstands the action of powerful decolorizing agents for a considerable period of time and still retains the stain when everything else in the slide preparation has been decolorized. This method comprises of a primary stain, a decolorizer, and a counterstain. The primary stain is typically a concentrated carbol fuchsin solution, which is made by dissolving the dye

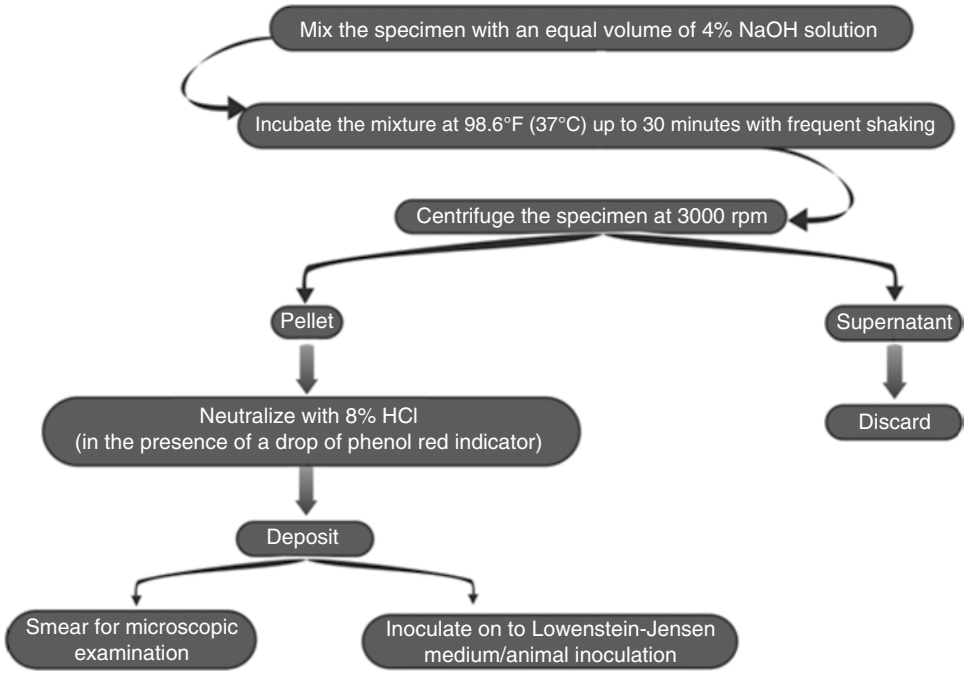

Figure 5.9. Petroff's concentration method.

Table 5.9. Grading of sputum smear.

Observation per OIF	OIFs to be examined	Result	Grading
>10 AFB per field	20	Positive	3+
1–10 AFB per field	50	Positive	2+
10–99 AFB per 100 fields	100	Positive	1+
1–9 AFB per 100 fields	200	Scanty Positive	Record exact figure
No AFB in 100 fields	100	Negative	—

AFB, acid-fast bacilli; OIFs, oil immersion fields.

basic fuchsin in phenol because basic fuchsin dissolves better in phenol than in water. Heating the slide softens the waxy material of cell wall and phenolized dye readily enters the cell. Once stained by this method, these bacteria do not readily decolorize by weak mineral acids, such bacteria (acid-fast bacteria). The non–acid-fast structures in the smear are then visualized by counterstaining with methylene blue solution. The AFB (*Mycobacterium*) appear pink in Color while the non–AFB appear blue in color.

Microscopic Examination and Grading

Only a small proportion of the total number of oil immersion fields (OIFs = 1000x magnification) is to be examined by microscopy (about 100–200 out of 10,000 OIFs). The slides are graded as shown in Table 5.9.

A higher proportion of patients with 2+ and 3+ smears are likely to remain sputum-smear positive at the end of the intensive phase. Such patients may require one additional month of intensive phase. Grading may vary from sample to sample in the same patient because AFBs are not evenly distributed but found in clumps. Specimens that are consistently positive for AFB contain at least 10^5 to 10^6 AFB/mL of sputum.

Limitations of Sputum Microscopy

Microscopic examination of sputum smear provides only a presumptive diagnosis of TB. Only culture provides definitive diagnosis. Sputum microscopy involves a chain of technical steps. Poor performance of any of these steps adversely affects the quality and reliability and may produce erroneous results.

- **Erroneous results**: These are probably the result of erroneous labeling of slides, faulty reporting (sputum results of one patient erroneously assigned to another), poor quality of the microscope, and high workload of examiner.
- **False-positive results**: While preparing smear:
  1. Contamination of smear by other microorganisms from soil and water such as atypical *Mycobacteria*, *Nocardia*, *Actinomyces*, and spores of *Bacillus subtilis*.
  2. Contamination with food particles, pollen from pine trees, fibers from cotton wool, filter paper, blotting paper (used for drying slides after staining).
  3. Retention of dye in scratches on slide.
  4. Previously used slide may contain *Mycobacteria* leading to false-positive result.
- During microscopic examination: Oil dropper or objective of the lens can be contaminated by touching the smear. Because the presence of food particles may yield false-positive results, the patient should be instructed to rinse his or her mouth before sputum collection or sputum should be collected before consumption of food. Use a new wooden stick for preparing the smear on the slide; use a new slide every time and stain the slide separately on a rack; use freshly prepared and filtered stain, and use distilled water during the staining procedure to avoid contamination by environmental *Mycobacteria*.
- **False-negative results**: While collecting the specimens, nasopharyngeal secretions or saliva may be collected instead of bronchial sputum because of inadequate patient guidance on how to produce a sample. Low concentration of tubercle bacilli (less 10,000/mL of sputum) may lead to false-negative results.

  While preparing smear, if the smear on the slide is too thin, bacilli spread out ("diluted"), whereas if the smear is too thick, AFB are poorly stained. If the smear is not prepared from blobs of sputum that contain dead caseous material discharged from cavities, the likelihood of false-negative results is high. Inadequate heat fixation in the smear may get washed out during staining process.

  During staining, inadequate period of staining or intensive counterstaining with methylene blue may cause false-negative results. Overheating with carbol fuchsin may destroy or distort bacilli, leading to loss of acid-fastness.

  While handling slides, exposure to sunlight, ultraviolet light, heat or prolonged storage after heat fixation in a hot climate, retaining oil on smear for prolonged periods before microscopic examination cause loss of acid-fastness.

  During microscopic examination, failure to examine the specified number of OIFs.

Alternative Specimens for Microscopy

Depending on the site of lesions, body fluids may be centrifuged and the sediment stained and examined microscopically for the presence of AFB. However, for a positive result, body fluid should contain about 10,000 AFB per mL. Alternative specimens include gastric aspirate (in children younger than 6 years old), urine (in renal TB), cerebrospinal fluid (in tuberculous meningitis), and other body fluids including bronchoalveolar lavage (BAL), pleural or peritoneal fluid, or aspirated synovial fluid (in bone and joint TB).

Fluorescent Microscopy

Smear stained with fluorescent dyes (Auramine O, Rhodamine) are examined under ultraviolet light using a fluorescent microscope. AFB appear as luminous yellow rods against a dark background, but this method is not used for routine diagnosis.

Tuberculin Test

Tuberculin is the name given to extracts of *M. tuberculosis*, *M. bovis*, or *M. avium* that is used in skin testing in animals and humans to identify a TB infection (Walport et al., 2008). In 1890, Koch first prepared old tuberculin reagent, described the tuberculin test, and began a series of experiments using this reagent in 1891. Rich and Lewis (1932) explained the tuberculin reaction as an allergic response to tubercular infection. von Pirquet (1907) pioneered the skin testing system for diagnosing allergy. He also introduced the words *allergy* and *anergy* (Greek: *allos*=other; *ergos*=energy; *anergos*=lack of energy). He also postulated the existence of suppressor and effector arms in the immune system. Koch had also produced new tuberculin by grinding whole tubercle bacilli to release its cytoplasmic proteins. Different types of tuberculin are used for this purpose, but purified protein derivative (PPD) is the most important, which is a poorly defined complex mixture of antigens.

Types of Antigens

All mycobacterial skin test reagents contain three types of antigens (Stanford and Grange, 1974)

1. those specific for the tubercle bacillus
2. those shared with other slow-growing *Mycobacteria*
3. those common to all *Mycobacteria*

The latter two groups of antigens are responsible for cross-reactivity in skin tests.

When diluted PPD reagents are stored in glass vials, the active principle gets adsorbed on the glass surface of the vials resulting in loss of potency. Tween 80, a detergent, is added to tuberculin reagent. The surface tension is thus reduced preventing the adsorption and enhances the tuberculin reaction.

Reaction to Tuberculin

Tuberculin reaction is a type IV cell-mediated (delayed-type) hypersensitivity (or DTH) to a reagent containing tuberculin antigen. Presence of a tuberculin reaction denotes previous exposure to tubercle bacilli (infection) or exposure to atypical nontuberculous

Mycobacteria. There is a series of reaction followed by the intradermal inoculation of tuberculin antigen ranging from an inflammatory reaction and migration of leukocytes to clinically observable swelling and induration. An inflammatory reaction resembles the intradermal reaction to any mildly irritant foreign substance, such as blood-derived leukocytes cluster around capillaries, sweat glands, and hair follicles. The proportion of different types of leukocytes in this inflammatory focus resembles that in blood. This infiltration probably does not result in the clinically observable swelling and induration. The leukocytes migrate to the interstitial dermis, just below the epidermis. This is followed by swelling and induration as a zone of erythema, with central soft, edematous swelling is observable after 24 hours. In patients with active TB and health-care personnel exposed to patients infected with TB, the erythematous swelling is clearly seen 6 to 8 hours after injecting tuberculin. This 6- to 8-hour reaction is the result of accelerated cell-mediated hypersensitivity, which results a firm induration or a nonindurating edematous swelling, reaches a maximum intensity by 48 to 72 hours.

Indications for Tuberculin Test

Tuberculin test is a screening test for children in developing countries. The test should be performed at 3 to 4 years of age and repeated at 7 to 8 years of age. As per guidelines issued by the Indian Academy of Pediatrics, immediate tuberculin testing is indicated for child contacts of persons with active or suspected TB, children who have migrated from (or traveled to) TB-endemic countries, and children with clinical or radiographic signs suggestive of TB before initiating immune-suppressive therapy. Annual tuberculin testing is indicated for children who are HIV sero-positive.

Method of Tuberculin Testing

Mantoux test using one TU PPD-RT-23 with Tween 80 is the most accepted method for tuberculin testing because it is sensitive and is relatively free from technical errors. It involves administration of a specific quantity of reagent (dose can be regulated) and provides an accurate measurement of tuberculin status; 0.1 ml of one TU PPD RT23 with Tween 80 is injected intradermally on the anterior aspect of the forearm using a tuberculin syringe and 26-gauge needle. After the intradermal injection, a wheal should be raised and should measure 6 to 10 mm in diameter. The reaction is measured after 48 to 72 hours by recording the transverse diameter of induration (not erythema or redness), using a transparent plastic scale.

Use of higher strengths of PPD may give positive reactions in case of nonspecific infections. A tuberculin test should not be repeated at the same site; the previous dose acts as a micro-vaccine and an enhanced reaction may be obtained. A positive Mantoux test in children younger than 6 years of age indicates recent or active tuberculous infection, but it is of limited clinical value for anyone older than 6 years of age.

Recent Advances

Campos-Neto et al. (2001) showed a recombinant antigen (DPPD), a better antigen than PPD, encoded by a gene unique to organisms belonging to *M. tuberculosis* complex. The 6 kDa early secretory antigen TB (ESAT-6) and culture filtrate protein (CFP-10) have been used as alternatives for PPD in in vivo skin tests (Arend et al., 2000). The TB MPB 64-patch test uses MPB 64, a specific antigen for *M. tuberculosis* complex. After

application of the patch, the test becomes positive in 3 to 4 days and lasts for about a week. Its sensitivity and specificity is 98.1 percent and 100 percent, respectively, and has been evaluated only in the Philippines (Nakamura, et al., 1998). IFN-γ assay has been evaluated in patients with TB as a possible candidate to replace the Mantoux skin test, which requires laboratory facilities for stimulating viable lymphocytes and quantifying the released IFN-γ (Ferrara et al., 2006).

Chest X-Ray

To detect TB, the first screening test is sputum microscopy. In most of the cases of sputum-positive pulmonary TB, chest X-rat is not necessary. It is only recommended in certain cases:

- Strong clinical evidence suggestive of TB in a patient with three negative sputum smears
- When only one sample out of three sputum samples are positive for AFB
- Patient has a history of frequent or severe hemoptysis (to rule out aspergillosis, bronchiectasis, or carcinoma)
- Suspected complications in a breathless patient (to exclude pleural effusion, pneumothorax, or pericardial effusion)

Radiographic Findings in Primary Tuberculosis

Primary lesion (Ghon's focus) is seen as a small peripheral area of consolidation. Though calcified lesions (in the parenchyma of lung or lymph node) point to childhood TB, they may also be seen in chest X-rays of adults. Massive lymph node enlargement may cause obstructive atelectasis. Mediastinal lymphadenopathy is seen in TB and also in malignant lymphoma. If a caseating lymph node erodes into a bronchus, the damaged bronchus gets dilated and distorted, causing bronchiectasis. Rarely, tuberculoma (localized nodular parenchymal lesion, which neither cavitates nor gets calcified) may be seen.

Radiographic Findings in Postprimary Tuberculosis

The lesions in the form of patchy or multifocal consolidations are usually located in the apico-posterior segment of the upper lobe or superior segment of the lower lobe. Consolidation may contain nodular elements called *acinar nodules*.

1. **Early infiltration**: Early infiltration is usually in the upper lobes and appears semi-mottled, along with internal streaky shadows. If unilateral, the lesions should be differentiated from that of pneumonia and bronchial carcinoma. If bilateral, it is necessary to exclude pneumonia, pneumoconiosis, connective tissue disorders, allergic bronchopulmonary aspergillosis, central bronchiectasis, and sarcoidosis.
2. **Cavitation**: Cavities may be single or multiple, of varying sizes, occasionally contain air-fluid levels. When a tubercular cavity is secondarily infected by *Aspergillus*, characteristic fungal balls are seen within the cavity. Other communicable diseases that cause cavitations are bacterial (particularly *Staphylococcus* and *Klebsiella*) pneumonias, some fungal infections, melioidosis, nocardiosis, lung abscess, and oriental lung fluke (*Paragonimus westermani*) infestation. Cavitations may be also seen in some noncommunicable diseases such as pneumoconiosis and connective

tissue diseases. A cavitating squamous cell carcinoma of the lung may resemble cavity seen in postprimary TB.

3. **Fibrosis**: These are usually linear or nodular opacities in the upper zone. Features of diminished lung volume are present. Pulmonary fibrosis may also be caused by pneumoconiosis, extrinsic allergic alveolitis, and sarcoidosis. In these conditions, the lesions are bilateral, reticular or reticulonodular, or nodular, with or without hilar lymphadenopathy.
4. **Other lesions**: Pleural effusion, miliary TB, and tuberculoma.

Other Chest X-Ray Findings

1. *Minimal lesions* mimic the radiological appearances caused by scars or viral, mycoplasma, or fungal infections, or infarcts. Hence, findings should be correlated with history, clinical findings, and laboratory reports.
2. *Acute exudation (pneumonitis)* is seen as visible consolidation and air bronchogram. The appearance is similar to that caused by all pneumonias.
3. *Productive lesions* are the diffused nodular shadows intermingled with translucent lung tissue, similar to rosettes of a flower, which mimics that of sarcoidosis, amyloidosis, collagen diseases, fungal diseases, scleroderma, and neoplasms.
4. *Coin lesions (differential diagnosis)* includes tuberculoma, adenoma, hamartoma, neurotibroma, secondaries of neoplasms, syphilic gumma, and fungal lesions.
5. *Other lesions* include fibro-caseous lesions (that resemble coin lesions) and fibroid type lesions that mimic pulmonary fibrosis.

HIV-TB Coinfection

It should be noted that no chest X-ray finding is "typical" of pulmonary TB, especially with concomitant HIV infection. The chest X-ray abnormalities in patients with concomitant HIV infection are indicative of the degree of immune suppression. If the immune suppression is mild, the appearance is often "classical" (presence of cavitations and upper lobe infiltrations). Thus in patients with early HIV infection, the chest X-ray findings are indistinguishable from that in patients who are sero-negative. In severe immune suppression, the appearance is often atypical. Lesions are bilateral, diffuse, and reticular or reticulonodular and less often cavitating and smear positive; miliary pattern, lower lobe infiltration, and mediastinal lymphadenopathy are relatively more frequent than in patients who are sero-negative.

Limitations of Chest X-Ray

Chest X-rays are neither sensitive nor specific for the diagnosis of pulmonary TB. Thus, a negative chest X-ray does not rule out the disease. Abnormal chest X-rays do not necessarily confirm the diagnosis of TB. Interpretation of lung lesions or shadows is prone to intraobserver and interobserver errors. An "active" lesion may not have been visible. Artefacts such as skin folds, thymus, young breast shadow, and braid of hair may give rise to shadows that resemble parenchymal lesions caused by pulmonary TB. Inactive lesions may be seen as intrapulmonary or lymph node calcification, non-cavitating subpleural consolidation, and unilateral (hilar, para tracheal, mediastinal) lymph node enlargement. For the aforementioned reasons, patient's history, physical examination findings, and sputum microscopy reports are essential for interpreting chest X-rays.

Other Imaging Techniques

Extrapulmonary and disseminated forms of TB are also diagnosed by sophisticated imaging techniques such as computerized tomography (CT) scan and magnetic resonance imaging (MRI) (Semlali et al., 2008). MRI is more sensitive than CT scanning in determining the extent of meningeal and parenchymal involvement (Janse van Rensburg et al., 2008). Although these imaging techniques are not routinely indicated when chest X-ray findings are diagnostic, they can still help in diagnosing hilar lymphadenopathy, early cavitation, early bronchiectasis, pericardial effusion, and endobronchial TB. They are also useful for detecting basal exudates, infarcts, and hydrocephalus in infants and diagnosing TB of the central nervous system.

Culture Methods

Mycobacteria can be cultured from clinical specimens such as sputum, lymph node aspirate, cerebrospinal fluid, pleural, peritoneal, and pericardial fluids, and gastric washings. Culturing is highly sensitive and provides definitive diagnosis of TB. As few as 10 to 100 AFB per mL (of digested and concentrated specimen) can be detected by culture methods. This allows the identification of species or strain and also enables drug-sensitivity testing. To get quicker results automated systems, such as BACTEC and Septichek, are used in sophisticated laboratories.

Procedure

Centrifuged deposits are inoculated in two bottles containing LJ medium, the specific media for culturing TB. Residual gastric acid in deposit of gastric washings using alkali should be neutralized. Centrifuged deposit of urine and CSF can be directly inoculated on LJ medium. The deposit should incubate at 98.6° F (37° C) in the dark and in the light. The culture should be examined after 4 days (for rapid growing atypical *Mycobacteria*, fungi, and contaminant bacteria). The culture should be examined each week for up to 8 weeks. Colonies of tubercle bacilli are seen after 6 to 8 weeks of incubation. Incubation is to be prolonged if the clinical sample is from patients who have been treated with antitubercular drugs. Smears (secondary smears) should be prepared from an isolated colony stain by ZN technique and examined using a microscope. The diagnosis should be confirmed by biochemical tests. Slow nonpigmented growth and positive niacin production test are the features of *M. tuberculosis*.

Limitations of Culture Methods

Because *M. tuberculosis* is a slow-growing organism with a doubling time of about 18 hours, it takes about 6 to 8 weeks for colonies to appear on conventional culture media such as LJ and Middlebrook 7H10. Thus, culture reports may not be useful for clinical diagnosis in individual patients. Most developing countries do not have the necessary infrastructure and facilities to sustain a mycobacterial culture facility. These facilities may be available only in a few reference centers and not in health-care facilities. The features of *Mycobacteria* include aggregation, cording, clumping, surface tension caused by waxy cell wall, buoyancy, slow growth, and thick cell wall. Among these the surface tension, buoyancy, and cording compromise the efficacy of concentration methods, which is further complicated by slow growth. As a result, *Mycobacteria* are scarce in processed sediments. The organisms must be viable to compete with contaminating

bacteria, which may be removed by decontaminating procedures. However, decontamination compromises the viability of *Mycobacteria* (Kent and Kubica, 1985). Thus, the processing technique can affect the sensitivity of culture methods.

AFB may not grow in culture medium because of bacteriostatic or bactericidal effect of antitubercular drugs, exposure to heat or sunlight, prolonged storage of sputum specimen before culture, and excessive decontamination procedure before processing sputum sample for culture.

Because of these limitations, culture is not recommended as a routine method of case detection in national control programs. Culture methods are only useful in selected cases as for the diagnosis of extrapulmonary TB, retrospective confirmation of diagnosis, and drug-sensitivity testing. It is also used for differential diagnosis in a few cases after careful evaluation by sputum microscopy and radiology.

Microscopic Observation of Broth: Drug-Susceptibility Assay

In this test, digested and decontaminated sputum samples are concentrated by centrifugation and inoculated into Middlebrook 7H9 broth medium, with or without antitubercular drugs. Growth of *M. tuberculosis* is characteristically observed as strings and tangles of mycobacterial cells under inverted light microscope. This assay has the potential for use on a mass scale in developing countries because of its low cost (about US$ 2 or INR 90 per test), rapidity (less than 2 weeks), and 92 percent sensitivity (Caviedes et al., 2003). If a microscopic observation of drug sensitivity (MODS) culture is negative at 3 weeks, it can be regarded as truly negative. Because cultures need to be stored only for 3 weeks, the required amount of storage space and potential biological hazard in the laboratory is reduced (Moore et al., 2004).

BACTEC

This radiometric method has been developed by Becton Dickinson Instrument Systems, Sparks, MD. Actively metabolizing tubercle bacilli consume radioactive palmitic acid carbon (^{14}C) incorporated in the substrate and produce radioactive $^{14}CO_2$, which is measured in terms of "growth index." A growth index of 10 or more is considered positive. Results are obtained in 9 to 16 hours. Serpentine cording (indicator of virulence) is demonstrated in smears produced from BACTEC systems. However, its high cost and the need for safe disposal of radioactive waste preclude its use in peripheral laboratories. Using BACTEC 12 B bottles (Becton Dickinson) in conjunction with Accuprobe for TB (Gen-Probe Inc., San Diego, CA) may yield results in 1 to 3 weeks but is not cost effective. Classical strains of *M. tuberculosis* are not inhibited by TCH in a concentration of 10 μg/mL however, *M. bovis* and South Indian strains of *M. tuberculosis* are susceptible. Thus, incorporation of TCH in BACTEC culture medium would not inhibit growth of classical strains of *M. tuberculosis*. Inclusion of beta-nitro alpha acetylamine beta hydroxy propiophenone (NAP) helps in differentiating *M. tuberculosis* from other *Mycobacteria*. Growth of *M. tuberculosis* is inhibited by NAP. BACTEC has been used for drug susceptibility testing and is currently used as a comparative standard.

SEPTI-CHEK Acid-Fast Bacilli

This is a nonradiometric method that uses a biphasic broth-based system. It consists of an enriched selective broth and a slide with nonselective Middlebrook agar on one side and two sections on the other side: one with NAP and egg-containing agar and the other

with chocolate agar to detect contamination. This method can simultaneously detect *M. tuberculosis*, atypical *Mycobacteria*, other respiratory pathogens and contaminants (Tiwari et al., 2007).

MB/BacT System

Organon Technika developed this nonradiometric continuous monitoring system with computerized database management. It is based on colorimetric detection of carbon dioxide and is also useful for drug-sensitivity tests. The average time for detecting *M. tuberculosis* by BACTEC and MB/BacT systems was 11.6 and 13.7 days, respectively (Rohner et al., 1997).

ESP Culture System II

ESP Culture system II (Ditco) is a nonradiometric fully automated continuous monitoring technique that detects pressure changes (resulting from gas production or consumption by growing organisms) in the headspace above broth culture in a sealed bottle. As with other liquid culture systems, ESP II is to be used in combination with a solid medium and not as a stand-alone system. A special algorithm is used for slow-growing *Mycobacteria*. The average time for detecting all *Mycobacteria*, *M. tuberculosis*, and *M. avium* complex was 13.1, 15.5, and 10.3 days, respectively (Woods et al., 1997).

Mycobacteriophage-Based Assays

Mycobacteriophages can infect and replicate only in viable *Mycobacteria* and not in dead *Mycobacteria*. Phage-amplified biological (Pha B) assay uses mycobacteriophage D_{29} to detect viable *M. tuberculosis* (lytic cycle = 13 hours), which is demonstrated as plaques on a lawn of *Mycobacterium smegmatis* (lytic cycle = 90 minutes). This assay is also used for drug-susceptibility testing (Wilson and McNemy, 1997). Phage Tek MB assay (Organon Teknika, Durham, NC) is an inexpensive, phenotypic bacteriophage-based assay that rapidly (within 24 hours) detects viable *M. tuberculosis* complex organisms in decontaminated sputum samples with a sensitivity of 31.1 percent and specificity of 86.1 percent. The number of plaques generated from a given sputum sample is related to the number of phage-infected (viable) *M. tuberculosis* complex organisms. Its low sensitivity makes it a poor screening test for TB (Bellen et al., 2003).

Reporter mycobacteriophage specifically infects only viable *M. tuberculosis* (Jacobs et al., 1993). This reporter phage that carries the gene for the firefly enzyme luciferase has been used as a chemiluminescent probe for detecting viable *M. tuberculosis* directly in clinical specimens. Fireflies glow in the dark when this enzyme oxidizes luciferin (in the presence of adenosine triphosphate) to generate light (De Wet et al., 1987). Viable *Mycobacteria* that are infected with this reporter mycobacteriophage emit light when luciferin (substrate of luciferase) is added. This emitted light could be measured by a luminometer. Clinical samples containing 500 to 5000 viable *M. tuberculosis* generate a clear positive signal (Jacobs et al., 1993). This test is economic, easy, and available commercially. It provides results within 48 hours. Because the test can differentiate between live and dead *Mycobacteria*, it can be used for sensitivity testing of antitubercular drugs. FAST Plaque TB assay is a rapid manual test that uses mycobacteriophage for directly detecting *M. tuberculosis* in sputum specimens. This assay has a sensitivity of 70.3 to 75.2 percent and specificity of 98.7 to 99.0 percent (Albert et al., 2002; Muzaffar et al., 2002).

Drug-Sensitivity Tests

Drug-resistant mutants continuously emerge in any bacterial population, with varying pace. Antibiotic sensitivity test has gained importance with the emergence of MDR *Mycobacteria*. Standardized quantity of centrifuged deposit of clinical specimen is inoculated in bottles containing LJ medium incorporated with different concentrations of the antitubercular drugs. Drug sensitivity could be checked by resistance ratio method, absolute concentration method, proportion method, radiometric method, or chemiluminescence.

Resistance Ratio Method

In this method the test strain and a known drug-sensitive strain of *M. tuberculosis*, such as H37Rv, are inoculated in bottles containing LJ medium with doubling concentrations of antitubercular drugs. After 3 weeks of incubation at 98.6° F (37° C), the bottles are examined for growth. The bottle containing the lowest concentration of the drug that shows not more than 20 colonies is taken as the bottle containing minimum inhibitory concentration (MIC) of the drug. The results are expressed as "resistance ratio." Resistance ratio of 1 to 2 indicates that the strain is sensitive to the drug, whereas a resistance ratio of 8 and above points to unequivocal drug resistance. Intermediate values denote doubtful resistance.

Absolute Concentration Method

The MIC of the antitubercular drug is determined only for the test strain. As this method lacks a control, (known sensitive strain) it is inferior to the resistance ratio method.

Proportion Method

In this method, the number of colonies growing from a standard inoculum on a drug-incorporated culture medium is compared with that growing from a similar-sized inoculum on a drug-free culture medium. When more than one percent of *Mycobacteria* grow in the presence of an antitubercular drug, it is regarded as a resistant strain for that drug.

Radiometric Method

BACTEC radiometric method is generally used in which a standardized inoculum is inoculated into drug-incorporated and drug-free liquid media for each drug to be tested. Both these types of media contain ^{14}C-labeled substrate. The rate and quantity of production of $^{14}CO_2$ in the presence and absence of the drug is measured in terms of growth index and then compared.

Chemiluminescence

Isolates of *M. tuberculosis* are grown in the presence and absence of antitubercular drugs then luciferase reporter mycobacteriophage containing genes for the firefly enzyme luciferase are add. Once *Mycobacteria* are infected with mycobacteriophage luciferin (substrate for luciferase) is added, only drug-resistant *Mycobacteria* those that remain viable in the presence of the drug would breakdown luciferin and emit light, whereas the drug-sensitive strains will not emit light. In this method, the amount of emitted light is directly proportional to the number of viable (drug-resistant) *Mycobacteria*.

Newer Diagnostic Techniques

Gamma Interferon Assay

The 6 kDa early secretory antigen TB (ESAT-6) is recognized by T-cells of patients with TB, but not by T-cells of BCG-vaccinated or healthy unvaccinated individuals. Peripheral blood mononuclear cells from infected rats also produced IFN-γ in response to RD-1 antigens, such as the 6-kDa early secretory antigen target (ESAT-6) and the 10-kDa Culture Filtrate Protein (CFP-10) (Foo et al., 2011). The levels of IFN-γ increase in the peripheral blood in treated, as compared to untreated, patients and was associated with improved immunity against TB. Hence, detecting whole blood IFN-γ may be useful for monitoring *M. tuberculosis* infection (Kellar et al., 2011). This assay has been evaluated in immigrants, health-care personnel, and patients with TB. This assay requires laboratory facilities for stimulating viable T-cells and quantifies IFN-γ by enzyme-linked immunosorbent assay (ELISA).

Immunodiagnosis

The proper and accurate diagnosis of TB requires a sensitive immunodiagnostic test, especially when sputum smear microscopy is not helpful (Bothamley, 1995). Conventional antibody-based assays often fail to differentiate between infected and exposed subjects and their results must be cautiously interpreted (Katoch, 2004). Moreover, the response of antibodies and DTH persists for a long time after the subclinical or clinical disease subsides. Serological tests for HIV-TB coinfection are disappointing (Bothamley, 1995). New reagents, both purified antigens and monoclonal antibodies, provide the means to obtain sensitivity and specificity to rival the tuberculin skin test other commonly used diagnostic blood tests (Bothamley et al., 1992).

Detection of an Antigen

Free mycobacterial antigens, in a concentration of 3 to 20 μg/mL, can be detected in body fluids. The most commonly used antigens are PPD, glycolipids, sulpholipids, lipopolysaccharides, antigen 5 (38kDa), antigen A60, 45/47 KDa antigen, antigen KP90, 30 KDa antigen, P32 antigen, lipoarabinomannan, cord factor (trehalose-6, 6'dimycolate), and phenolglycolipid-lipid antigen (PLG Tb 1). The methods for detecting antigens of *M. tuberculosis* include sandwich ELISA, inhibition ELISA, latex agglutination, and reverse passive hemagglutination tests (Pereira Arais-Bouda et al., 2000). ELISA is useful for early diagnosis of all forms of TB. Capture ELISA is a quantitative test that detects lipoarabinomannan in urine samples (Ramachandran and Paramasivan, 2003). The dipstick method detects lipoarabinomannan in both pulmonary and extrapulmonary specimens semi-quantitatively. In a field test, the dipstick method had sensitivity and specificity of 93 percent and 95 percent, respectively (Del Prete et al., 1998).

Detection of an Antibody

These can be detected either by using monoclonal or polyclonal antibodies. Cross-reaction by environmental *Mycobacteria* may produce false-positive results. The currently available methods for purifying mycobacterial antigens are not reproducible and, therefore, results of antibody detection assays are variable in different settings. It is also unlikely that immune systems of all patients will recognize a single mycobacterial antigen (Ramachandran and Paramasivan, 2003). TB STAT PAK, an immunochromatographic test, can differentiate between active and dormant TB by testing whole blood, plasma, or serum (Bothamley, 1995). Superoxide dismutase, a secretory protein of *M. tuberculosis*,

has also been evaluated for serodiagnosis. The ELISA test for detecting superoxide dismutase has a positive predictive value (PPV) of 93 to 94 percent in low prevalence countries, but PPV drops to 77 to 88 percent in high endemic countries (Ahmad et al., 1998). In a comparative study on seven serodiagnostic tests, a combination of two tests (ICT and Pathozyme-Myco) yielded the best results, with a sensitivity of 66 percent and specificity of 86 percent (Pottumarthy et al., 2000).

"Cocktail" of Antigens

Though a vast array of potential diagnostic antigens is available, no single antigen-based kit gives 100 percent sensitivity. Hence future research needs to focus on identifying the best possible combination ("cocktail") of antigens of *M. tuberculosis* complex. In the recently patented Liposomal Agglutination Card test or TB screen test (Bundelkhand University, Jhansi, Uttar Pradesh and National Research Development Corporation, Government of India), a cocktail of purified cell wall-associated antigens (H37Rv) of *M. tuberculosis* has been incorporated on to the surface of liposome particles. This cocktail of antigens reacts with specific antibodies present in clinical samples to give a blue agglutination. The TB screen test has the potential for use on a mass scale because of its low cost (about US$0.12 or INR 5 per test) rapidity (4 minutes), high sensitivity (94 percent) and high specificity (98.3 percent) (Tiwari et al., 2005, 2007). ASSURE TB rapid test (Genelabs Diagnostics Pte Ltd., Singapore) is an indirect solid-phase immunochromatographic assay for detecting antibodies in clinical samples (plasma, serum, or whole blood). The test employs an antibody-binding protein conjugated to colloidal gold particle and a cocktail of novel antigens (Mtb 11 [CF-10], Mtb 8, Mtb 48, Mtb 81 and 38 kDa protein), immobilized on the membrane, in lateral flow devices. Mtb 81 has been found to be a promising antigen in the serodiagnosis of HIV-TB coinfection (Houghton et al., 2002).

Limitations of Serological Tests

The highest sensitivity is seen in sputum-positive cases. Poor sensitivity is seen in children, sputum-negative cases of pulmonary TB, extrapulmonary TB, and HIV-TB coinfection. None of the currently available serological techniques shows necessary sensitivity and specificity for routine diagnosis of TB in children.

Chromatography

Thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and high-performance liquid chromatography (HPLC) are used for chromatographic separation of the ester to detect the unique pattern of mycolic acid of *Mycobacteria*. Tuberculostearic acid is a component of *M. tuberculosis* that can be easily detected even in infinitesimal (femtomole) quantities by GLC. The presence of tuberculostearic acid in CSF is considered to be a diagnostic marker for tuberculous meningitis (Brooks et al., 1987) and is perhaps also useful for diagnosing pulmonary tuberculosis (Savic et al., 1992). HPLC is a rapid and highly specific method for identifying mycobacterial species. Sherlock *Mycobacteria* identification system (SMIS) uses computer software to identify mycobacterial species on the basis of mycolic acid pattern generated by HPLC (Kellogg et al., 2001).

Molecular Methods

Molecular diagnostic methods are indicated when available culture methods have low sensitivity or the organisms cannot be grown in vitro require complex culture media and need prolonged incubation time (Rattan, 2000). Nucleic acid-based amplification and

hybridization assays detect biomolecules in clinical specimens and thereby shorten the time required to detect and identify organisms. In any molecular diagnostic technique, a specific sequence of nucleic acid in the clinical sample is hybridized with a complementary sequence (probe), which is followed by the detection of the hybrid. The sequence of nucleic acid can be amplified by using DNA polymerase. For diagnosing TB, the most frequently used target sequence is the IS 986 or IS 6110 repetitive element that is present in multiple copies (up to 20), in most strains of *M. tuberculosis* complex, (Rattan, 2000). In general, amplification systems can detect as few as 1 to 10 organisms in clinical specimens and clearly distinguish *M. tuberculosis* in specimens containing nucleic acids from human cells, other mycobacterial species, and common contaminating organisms. These amplification techniques provide results in less than 1 day and have been used on a variety of clinical specimens.

These assays are usually designed for use with specimens that have been processed for mycobacterial culture because of the need for culturing organisms for drug-susceptibility tests or identifying mycobacterial species (Kent and Kubica, 1985). In most assays, the *Mycobacteria* in the collected specimens are first lysed by various methods ranging from simple boiling to sonication, treatment with sodium dodecyl sulphate (SDS) plus lysozyme and heat. Each clinical sample is to be decontaminated by the gentler n-acetyl l-cysteine method rather than the harsh sodium hydroxide method. Two media (one egg-based and another agar-based) are to be used to maximize the likelihood of isolating *Mycobacteria*. Up to one-fifth of processed clinical specimens may contain inhibitors of Taq polymerase. Hence, most amplification assays include internal controls to assess the efficiency of amplification and to detect the presence of inhibitors (Rattan, 2000).

DNA-Targeting Probes

Well-defined DNA-targeting probes are commercially available for identifying clinically relevant *Mycobacteria*. When used along with other methods for detecting early growth (e.g., BACTEC, MGIT), DNA probes can confirm the isolates within 1 to 2 days.

Gene Amplification Methods

- Restriction fragment length polymorphism (RFLP): This technique is feasible in clinical laboratories. RFLP analysis of *hsp* 65 kDa gene, *kat G* and *rRNA* genes is used for epidemiological purposes. The clinical specimen, when treated with restriction endonuclease enzyme, yields nucleic acid fragments of different lengths. The patterns of these fragments are species specific. DNA probes are used to identify the species of *Mycobacteria* that are grown on solid or liquid culture media. RFLP patterns of 36-bp direct repeat (DR) sequence and polymorphic GC-rich repetitive sequence (PGRS) being stable, these sequences can be used for identifying the isolates (Van Soolingen et al., 1994).
- Polymerase chain reaction (PCR): It is a rapid technique that is currently available in reference laboratories for detecting *M. tuberculosis* directly in clinical specimens (Mullis and Faloona, 1987). This could be based on conventional DNA amplification, nested PCR, or real time (Katoch, 2004).
  (a) Advantages: The PCR amplification process can be completed in 2 to 4 hours after obtaining processed clinical sample. The detection assay requires an additional 2 to24 hours. Storage of sputum samples on filter paper for 5 days at room temperature reportedly had no apparent effect on performance of nested PCR (Tansuphasiri et al., 2001). This implies that the assay can also be carried out on samples from peripheral laboratories.

(b) Disadvantages: PCR tests are currently expensive (about US$ 15 per test). Though available at research institutions, they are not feasible in most clinical laboratories in developing countries. Another feature is these tests are unable to differentiate between viable and dead AFB, which may lead to discordance between sputum smear and PCR results. Under field conditions, the specificity of PCR may be low. PCR lacks sensitivity in smear-negative but culture-positive cases (Rattan, 2000). False-positive PCR results, usually as a result of laboratory-introduced contamination, can be reduced by improved laboratory design, strict laboratory discipline about collecting and processing clinical samples and handling of reagents, using certain blocking agents, and using in situ PCR methods (Katoch, 2004). Effectiveness of the PCR technique in diagnosing TB is related to DNA concentration in the clinical sample, size of target DNA sequence, repetitiveness of amplified sequence, choice of primer, and expertise of personnel conducting the assay (Rattan, 2000).

- Strand displacement amplification (SDA): It is a semi-quantitative, isothermal (does not require a thermal cycler) amplification technique. This technique takes advantage of Hinc II to nick the unmodified single strand of hemiphosphorothiote from off its recognition site and the ability of exonuclease-deficient Klenow fragment of *Escherichia coli* DNA polymerase to start at the site of the single-stranded nick in the double-stranded DNA, extend one strand from the 3-foot end, and displace the downstream strand of DNA (Walker et al., 1992). The process proceeds exponentially to produce 10^7 to 10^8 fold amplification in about 2 hours. When coupled with a chemiluminescence-based hybridization detection system, this entire assay can be completed within 4 hours after obtaining a processed clinical specimen (Spargo et al., 1993). The amplification is usually carried out in a single tube (multiplexing) and products can be differentiated by the detection system, without significant loss of sensitivity. Species-specific SDA assays have already been developed for *M. tuberculosis*, *M. avium*, and *M. kansasii*.
- Transcription-mediated amplification (TMA): TMA is based on isothermal amplification of ribosomal RNA (rRNA). It is commercially available Amplified *Mycobacterium tuberculosis* direct (AMTD) (Gen-Probe Inc., San Diego, CA) test. Earlier radiolabeled rRNA probes were used in this technique, but now this is a well-developed chemiluminescent technique. Probes that target rRNA are 10- to 100-fold more sensitive than those that target DNA. The lowest detection limit is 100 organisms. When combined with a homogeneous detection method, TMA can detect *M. tuberculosis* in clinical specimens (Jonas et al., 1993). In the AMTD test, sediments are prepared from the clinical sample by the standard sodium hydroxide method (Kent and Kubica, 1985) and the *Mycobacteria* are lysed by sonication. rRNA is amplified by copying rRNA target sequences on to a transcription complex using reverse transcriptase. RNA polymerase is then used to make numerous copies of the target sequence from the transcription complex. This process is then repeated autocatalytically. The amplified sequences are detected by an acridinium ester-labeled DNA probe specific for *M. tuberculosis*. The entire AMTD test is done in a single test tube thereby minimizes the likelihood of laboratory-introduced contamination. The test can be completed in 3 to 4 hours after obtaining the processed clinical specimen but like PCR, the AMTD test lacks sensitivity in smear-negative but culture-positive cases (Rattan, 2000).
- Q-Beta (QB) replicase-based gene amplification: QB replicase enzyme is used for production of RNA in the amplification reaction at a fixed temperature. When suitable combination of capture and detector probes is used, *M. tuberculosis* can be detected

with a sensitivity of up to one colony-forming unit (Shah et al., 1995). An advantage is that the inhibitors of PCR do not affect this assay.

- Ligase chain reaction (LCR): It is a variant of PCR, which is potentially useful for screening persons at high risk for developing TB and detecting extrapulmonary TB (Gamboa et al., 1998). In this technique, a pair of oligonucleotides is made to bind to one of the DNA target strands so that they are adjacent to each other. A second pair of oligonucleotides is designed to hybridize to the same regions on the complementary DNA. DNA polymerase helps in filling the gap between the primers with suitable nucleotides while ligase binds primers. The LCX *M. tuberculosis* assay (Abbot), mainly used for respiratory specimens, has high sensitivity and specificity for smear-positive and -negative specimens (Fadda et al., 1998). Currently, the use of this technique is limited by its high cost and the need for skilled personnel.
- Peptide nucleic acid (PNA) assay: PNA is a DNA replica in which sugar phosphate backbone of DNA is replaced by a polyamide backbone. PNA probes hybridize DNA or RNA with excellent affinity. The sensitivity of PNA probes targeting *M. tuberculosis* and atypical *Mycobacteria* is reported to be 98 percent and 57 percent, respectively. The latter probes did not target all species of atypical *Mycobacteria* (Hongmanec et al., 2001).
- Other modifications of PCR: These include nucleic acid sequence-based amplification (NASBA) that uses 16S rRNA and line probe assay (LiPA), which employs IS 6110 or 65 kDa protein as target sequence (Ramachandran and Paramasivan, 2003).

Directly Observed Treatment, Short Course (DOTS)

Directly observed treatment, short course (DOTS) was first introduced in 1991 and recommended by WHO as an effective interventional strategy for the control of TB. Since then, incidence rates have decreased in high-burden countries. High cure rates have been reported in selected areas, but these areas may be isolated islands of excellence. Studies in many countries have shown that

- At least one-third of the patients receiving self-administered drugs do not adhere to treatment.
- It is not possible, to predict whom these patients will be and also to ensure compliance by patient education alone.
- It is essential to verify adherence at least in the intensive phase to ensure sputum conversion.
- A patient who misses one dose can be traced immediately and counseled,
- It ensures treatment with the right drugs, in the right doses and at the right intervals.
- No other method (other than direct observation) has been able to achieve 85 percent cure rate in patients who are new and smear positive.

The WHO and the IUATLD recommend DOTS as the most effective and affordable strategy to control TB. The DOTS strategy involves:

- Diagnosis of cases by sputum microscopy from among patients with symptoms suggestive of TB
- Free-of-cost intermittent therapy with a standardized drug regimen
- Direct observation of drug consumption by a trained health worker to ensure adherence
- Reliable and regular drug supply

- Adequate health infrastructure and trained health personnel
- Government commitment
- Monitoring and Evaluation of the program.

Several concepts that form the basis for DOTS strategy are based on research studies in India conducted at Tuberculosis Research Centre (Chennai) and National Tuberculosis Institute (Bangalore). These studies demonstrated that patients with TB could be treated on domiciliary basis; supervised treatment was essential; intermittent treatment using biweekly or triweekly doses was effective, and sputum microscopy was an effective case-finding tool. India has been at the forefront of TB control and research since the 1940s (Mahadev and Kumar, 2003). In the 1960s, the concept of DOTS was introduced in Chennai as "Supervised Chemotherapy of Tuberculosis." This was reworded as DOTS by Centers for Disease Control and Prevention (CDC) and later endorsed by WHO, based on extensive World Bank–funded field studies. The cover page of the DOTS pamphlet issued by WHO carried a photograph showing a patient swallowing pills in the presence of a nurse. As of 2010, India has more reported cases of TB than any other country (Bhargava et al., 2011). This is in part as a result of severe mismanagement of diagnosis and treatment of TB within the private health-care sector of India that serves about 50 percent of the population. Therefore, private sectors are invited to engage in the public RNTCP, which has proved effective in reducing TB among the patients receiving health care through the government (Bhargava et al., 2011).

Basis of Intermittent Therapy

When the tubercle bacilli are exposed to an antimycobacterial drug, they do not multiply for varying duration, called the "lag period," and this forms the basis of intermittent therapy. Multiple clinical trials have established that a thrice a week (alternate day) regimen is as effective as daily regimens. The doubling time for *M. tuberculosis* is about 18 hours as compared to 10 to 20 minutes for most bacteria. Hence it is not necessary to maintain a MIC of drugs in the tissues. These trials have not found any differences in severity of side effects, although one clinical trial reported a lower frequency of arthralgia among patients on intermittent therapy. In experimental animals, intermittent therapy is more effective than daily regimens possibly because the pulsed intermittent dosage permits the *Mycobacteria* to re-enter the phase of active metabolism. Once *Mycobacteria* enter this phase, mycobactericidal drugs such as isoniazid and rifampicin are more effective. Under DOTS, only thrice a week (alternate day) regimens should be used. Though biweekly regimens have been shown to be effective, they have some disadvantages, such as:

- If a patient misses one dose, it results in a once weekly dose, which is more than the lag period for most antitubercular drugs. Hence, missing a single dose may increase the risk of developing drug resistance.
- Biweekly regimens are also associated with higher risk of immunologically mediated side effects.

Prerequisites for Directly Observed Treatment, Short Course

An adequate public health infrastructure is a prerequisite for starting DOTS. Critics point out that the DOTS strategy will only be as efficient as the public health services of the country where it is being implemented. In countries where the basic health services are

inadequate, the long-term sustainability of DOTS is endangered. A strong political will and commitment are essential for ensuring adequate funding and collaboration between several stakeholders. Good record keeping is essential for audit and review of the program.

Diagnosis

The diagnosis of the disease is primarily based on sputum microscopy. Three samples of sputa (two spot samples collected under the supervision of a health worker and one morning sample) are collected from patients with symptoms suggestive of TB, who attend health facilities. Thus, there is no active case finding in the community. Sputum microscopy is a low-cost appropriate technology, which can be made available even in remote health facilities. Patients with positive sputum smears with pulmonary TB are responsible for almost 95 percent of transmission of tubercular infection in the community. If not detected early and treated adequately, they are at a higher risk of dying and may transmit drug-resistant tubercle bacilli to others. Sputum microscopy is also useful for monitoring response to treatment and finally confirming that the patient is cured.

Treatment

The drugs are supplied free of cost to public sector organizations and nongovernmental organizations (NGOs) selected to take part in the DOTS program. Intermittent therapy, using standardized regimens, has been shown to be as effective as daily treatment. Depending on the local situation, the administration of drugs is supervised by a person (health worker or a community volunteer), who is accessible and acceptable to the patient. This person, called "treatment supporter" or "DOTS provider," also provides encouragement and support for the person taking antitubercular treatment.

Basis of Directly Observed Treatment, Short Course Regimens

- Demonstrated efficacy of regimens in clinical and field trials in terms of sputum conversion and non-occurrence of relapse and different modes of action.
- Dose of each drug in a regimen depends on the MIC of the drug, which inhibits the growth of tubercle bacilli in vitro, and minimum bactericidal concentration (MBC) at which the bacilli are killed. MBC is usually higher than MIC.
- Minimal side effects, cost-effectiveness, prevention of drug resistance, and operational feasibility.

Different Modes of Action Drugs

DOTS treatment involves three types of drug therapy: the first-line drug, the second-line drug, and the third-line drug.

The First-Line Drug

All first-line antituberculous drug names have a standard three-letter and a single-letter abbreviation; for example, ethambutol is EMB or E; isoniazid is INH or H; pyrazinamide is PZA or Z and rifampicin is RMP or R. Drug regimens are abbreviated in a standardized manner. The drugs are listed using their single letter abbreviations. A prefix denotes the number of months up to the treatment should be given, a subscript denotes intermittent dosing (as $_3$ means three times a week) and no subscript means daily dosing. Most

regimens have an initial high-intensity phase which is to be followed by a continuation phase (consolidation or eradication phase). The high-intensity phase is written first, followed by the continuation phase and both of these two phases are divided by a slash.

For example, 2HREZ/4HR_3: It shows isoniazid, rifampicin, ethambutol, pyrazinamide should be given daily for 2 months, followed by 4 months of isoniazid and rifampicin, which should be given three times a week. Isoniazid has early bactericidal activity and kills rapidly multiplying extracellular tubercle bacilli. This drug alone accounts for 85 percent reduction in bacillary population. Isoniazid also prevents resistance to other antitubercular drugs, whereas other drugs are not so effective in preventing isoniazid resistance. Consequently, isoniazid resistance is more common. Although rifampicin acts on rapidly multiplying (extracellular AFB) and intermittently multiplying (AFB inside macrophages) bacteria, its bactericidal action starts after that of isoniazid, but the prevalence of rifampicin (RMP) resistance is 1.4 percent in the United Kingdom (Anderson et al., 2010). Pyrazinamide acts on AFB inside macrophages that are inhibited by the acidic intracellular environment and ethambutol is the companion drug to prevent drug resistance.

Second-Line Drug

The second line drugs are less effective than the first-line drugs (e.g., p-aminosalicylic acid) or may have toxic side effects (e.g., cycloserine). These include aminoglycosides (amikacin, kanamycin), polypeptides (capreomycin, viomycin, and enviomycin), fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin), and thioamides (ethionamide and prothionamide).

The Third-Line Drug

These include other drugs, which are useful, but not listed in the WHO list of the second-line drugs such as rifabutin, linezolid, macrolides, thioridazine, etc. These drugs are considered third-line drugs because they are not effective (e.g., clarithromycin a macrolide) or their efficacy has not been proven (e.g., linezolid). Rifabutin is effective but is not included on the WHO list because it is expensive for use in most developing countries.

Packaging

The current treatment regimens involve the use of four or five antitubercular drugs to be taken for at least 6 months. The drugs are supplied in patient-wise boxes containing the full course of antitubercular treatment and packaging in "blister packs." Tablet pyridoxine is given along with antitubercular drugs. This innovative approach ensures that each patient is administered a full course of treatment. Fixed-dose combinations contain two or more drugs within the same tablet. Currently, fixed-dose combinations are more expensive than the total cost of single drugs. The situation is likely to change with an increase in production of fixed-dose combinations. WHO recommends the use of fixed-dose combinations for the following reasons:

- The probability of patients forgetting to take a particular medication is reduced because they have fewer pills to swallow
- Prescribing becomes easier
- Managing drug supplies becomes easier because fewer drugs need to be procured
- Reduces the possibility of drug resistance by reducing the likelihood of using wrong drug combinations.

Monitoring Drug Administration

Each patient who is placed on DOTS must have a TB treatment card containing essential information about patient's identification, past treatment history, sputum reports, regimen prescribed, duration of treatment, and record or drugs to be taken by the patient during intensive and continuation phases. Direct observation of treatment is an integral component of DOTS strategy. A health worker (or DOTS provider) observes while the patient swallows the prescribed drugs for all doses during the intensive phase and for the first dose of each week during the continuation phase. Direct observation is necessary because

- At least one-third of patients receiving self-administered drugs do not adhere to treatment.
- It is impossible to predict which patient will regularly take treatment.
- It is essential to verify adherence at least in the intensive phase to ensure sputum conversion.
- A patient who misses one dose can be traced immediately and counseled.
- It ensures treatment with the right drugs, in the right doses, and at the right intervals.

During the intensive phase, every dose is fully supervised, whereas during the continuation phase, only the first dose of each week is supervised. Adherence is sustained by continued motivation and education of the patient. It may not be operationally feasible to supervise properly the administration of each dose during the continuation phase. However, for patients with history of irregularity, alcoholism, or other problem, each dose during the continuation phase should be fully supervised.

Any person who is accessible and acceptable to the patient and accountable to the health system (such as health-care personnel, NGOs, community volunteers lay midwives, and religious leaders) can act as "observers." By identifying such observers in communities, the feasibility of DOTS can be ascertained. Health workers (or DOTS providers) also need to be periodically observed while they administer drugs. This is done by first referring the TB treatment card to determine the drugs the patient should be taking. Then in private, the patient is asked to describe how he or she is receiving the drugs. If the patient's response is inconclusive, leading questions should be asked.

Monitoring Drug Collection by Patients

The chart on the front side of the TB treatment card is used to record the days (1–31) on which, the drugs are to be taken by the patient during the intensive phase. The chart on the reverse of the card is used to record when a patient should collect his or her drugs during the continuation phase. The reasons for missing any dose and the actions taken to return the patient to treatment are to be recorded under Remarks on the reverse of the card. The stock of drugs available in the patient-wise boxes is also compared with the dosages recorded on the card for drug monitoring.

Patient Education

Patient education is an integral component of treatment of TB. If possible, it should also be discussed with the patient's family members. It is essential to ensure that all health-care personnel (doctors, health workers, and DOTS providers) communicate with the patient on a continuous basis, especially during the intensive phase of treatment. Frequency of patient education is at least once a week and once a month during the

intensive and continuation phases, respectively, in groups or individual basis, depending on the situation. Topics for patient education should include:

- Explanation of the mode of transmission of TB, the need for regular treatment as per DOTS schedule, and the need for examination of contacts (in case sputum positive).
- Explain the different types of oral drugs and injections that the patient will be taking and the duration of treatment.
- Discuss the color of the drugs so that the patient can identify whether he or she is being given the correct drugs.
- Provide information about the number of tablets of each oral drug to be taken from the blister pack and the frequency of administration.
- Inform the patient about the possible side effects of drugs and also the hazards of taking only selected drugs.
- Inform the patient about the frequency of sputum examination and the meaning of "positive" and "negative" sputum smear results.

Counseling

Counseling is a confidential dialogue between the client and the health-care provider, which is aimed at enabling the client to cope with stress and to take realistic personal decisions. It is a challenging task that requires patience, dedication and commitment. Any person (professionally trained counselor, doctor, nurse, paramedical worker, or volunteer), who has the necessary aptitude, values, attitude, knowledge, and skills, can undertake counseling. Counseling is indicated in all conditions in which the client is likely to be under psychological stress. The purpose is to provide the means to overcome the stress-inducing condition and to take personal decisions that are not affected by moods, emotions, or sentiments. In the past, traditional systems were in place for counseling apparently healthy persons, patients, and their families for stress-related problems. Usually, respectable elderly persons who had the necessary aptitude and know-how undertook this challenging task within their own communities. In recent times, the need for counseling has increased as a result of the breakdown of traditional support systems and values, and stressful situations caused by "modern" lifestyle. Counselors have to develop a wide range of skills to provide services to individuals from various social backgrounds.

Directly Observed Treatment, Short Course for HIV-Tuberculosis Coinfection

The principles of control of TB are the same even for patients with coinfection. The standard treatment regimens used in DOTS are equally effective in patients who are HIV positive. DOTS strategy has improved survival of patients with coinfection. In areas with high prevalence of coinfection, the rise in number of patients with TB may increase the workload of public health facilities, with the following possible outcomes:

- Excess laboratory workload causing erroneous diagnosis of pulmonary tuberculosis
- Inadequate supervision of anti-tubercular chemotherapy
- low cure rates and high rate of recurrence
- High morbidity and mortality during treatment
- Poor adherence of patients due to adverse drug reactions
- Increased transmission of MDR-TB among patients infected with HIV.

Case Definitions

There are uniform case definitions to be used by all categories of DOTS personnel.

Case Definitions for Disease

Pulmonary Tuberculosis: Smear Positive
TB in a patient with at least two initial smear examinations by direct microscopy positive for AFB, or TB in a patient with one initial smear examinations by direct microscopy positive for AFB and radiographic abnormalities consistent with active pulmonary TB, as determined by the treating physician, or TB in a patient with one initial smear examination by direct microscopy that is positive for AFB and culture also positive for AFB.

Pulmonary Tuberculosis: Smear Negative
TB in a patient with symptoms suggestive of the disease and at least three initial smear examinations by direct microscopy negative for AFB and radiographic abnormalities consistent with active pulmonary TB as determined by the treating physician, followed by a decision to treat the patient with a full course of antitubercular treatment (ATT) or TB in a patient with symptoms suggestive of the disease and positive culture but negative sputum smear report.

Extrapulmonary Tuberculosis
This is TB that affects organs other than the lungs (e.g., pleura, abdomen, genitourinary tract, lymph nodes, meninges and brain, skin, or skeletal system). Diagnosis should be based on one culture-positive specimen from the extrapulmonary site or histological evidence or strong clinical evidence consistent with extrapulmonary TB, followed by a physician's decision to treat the patient with a full course of ATT. Pleurisy is classified as extrapulmonary TB. A patient diagnosed with both pulmonary and extrapulmonary TB should be classified as pulmonary TB.

Case Definitions for Types of Cases

- **New case**: A patient who has never taken ATT or has taken ATT for less than 1 month.
- **Relapse**: A patient declared cured of TB by a physician, but who reports back to a health facility and is found to be *Mycobacteria* positive.
- **Transferred in**: A patient got admitted into a tuberculosis unit (TU) or district after getting treatment in another TU or district.
- **Transferred out**: A patient who has transferred to another TU or district and his or her treatment results are not known.
- **Treatment after default**: A patient who received ATT for 1 month or more from any health facility and who returns for treatment after having defaulted (i.e., not taken ATT consecutively for 2 months or more).
- **Failure**: A patient who was smear positive initially, is found to be smear positive 5 months after starting ATT or a patient who was smear negative initially becomes smear positive during the course of ATT.
- **Chronic**: A patient who remains smear positive even after completing ATT.
- **Others**: A patient with TB who does not fit into the aforementioned categories; the physician must specify the reason for putting a patient into this category.

Table 5.10. Categories and drug regimens for DOTS (Adults).

Category	Patients	Regimen
CAT- I	New smear positive Seriously ill smear negative Seriously ill patients with extrapulmonary TB	$2(HRZE)_3 \rightarrow 4(HR)_3$*
CAT- II	Retreatment Relapse Failure, Treatment after default "Others"	$2(HRZES)_3 + 1(HRZE)_3 \rightarrow 5(HRE)_3$*
CAT- III	Sputum smear negative cases Extrapulmonary TB patients *not* seriously ill	$2(HRZ)_3 \rightarrow 4(HR)_3$*

E, ethambutol; H, isoniazid; R, rifampicin; S, injectable streptomycin; Z, pyrazinamide.
*Prefix number refers to the duration (number of months) of treatment, and subscript refers to the number of doses per week.

Case Definitions for Treatment Outcomes

- **Cured**: A patient who is smear positive, who has recently completed ATT and still having negative sputum smears on at least two observations, one of which was after completing ATT.
- **Treatment completed**: A patient who was initially smear positive, who has completed ATT and has had negative sputum smears at the end of the intensive phase, but none at the end of ATT, or a patient who was smear negative, who has received a full course of ATT and has not become smear positive during or at the end of ATT, or a patient with extrapulmonary TB who has received a full course of ATT and has not become smear positive during or at the end of ATT.
- **Died**: A patient with TB who died during ATT, irrespective of the cause of death.
- **Defaulted**: A patient with TB, who at any time after got registered, has not taken ATT consecutively for 2 months or more.

Directly Observed Treatment, Short Course for Adults

Category I (CAT-I)

Indications for including patients in this category are: (a) patient with a new positive sputum smear, (b) patients with history of previous treatment of less than 1 month, (c) patients who are seriously ill and are sputum smear negative, and (d) seriously ill patients with extrapulmonary TB. Cut-off point used is history of previous ATT of less than 1 month because such patients have been observed to respond to treatment in the same way as patients who have never received treatment.

If more than 20 percent of CAT-I patients are smear negative, it indicates that too many patients are being placed in CAT-I and requires review and correction of diagnostic practices. Intensive phase consists of 2 months (24 doses) of isoniazid, rifampicin, pyrazinamide, and ethambutol, all given under direct observation, three times a week (on alternate days) (Table 5.10). The continuation phase starts immediately after the intensive phase and comprises 4 months (18 weeks, 54 doses; Table 5.11) of isoniazid and rifampicin given three times a week (on alternate days). The first dose every week is directly

Table 5.11. Summary of Duration of DOTS Regimens (Adults).

Category	Intensive Phase	Continuation Phase	Total Duration
CAT- I	24 doses (8 weeks)*	54 doses (18 weeks)	78 doses (26 weeks)
CAT- II	36 doses (12 weeks)*	66 doses (22 weeks)	102 doses (34 weeks)
CAT- III	24 doses (8 weeks)	54 doses (18 weeks)	78 doses (26 weeks)

*Patients on CAT-I and CAT-II regimens, who are smear positive at the end of intensive phase, must receive an additional 1 month (4 weeks, 12 doses) of intensive phase.

observed. Clinical trials have shown that pyrazinamide provides no additional benefit beyond the initial 2 to 3 months of treatment and is, therefore, not included in the continuation phase. Ethambutol (the companion drug to prevent drug resistance) is also not given because during the continuation phase, the number of AFB is drastically reduced and the chances of drug resistance to isoniazid or rifampicin are negligible. If the sputum is positive after 2 months of starting treatment, the intensive phase drugs are continued for another 1 month (12 doses) before starting the 4-month (18 weeks) continuation phase (*see* Table 5.10). If the sputum is positive after 5 or more months of treatment, the patient is categorized as "failure" and is placed on Category II afresh (i.e., to start from the beginning).

Category II (CAT-II)

This includes the patients, who are generally sputum smear positive: (a) retreatment, (b) relapse, (c) failure, (d) treatment after default, and (e) others.. Because previously treated patients are at an increased risk of harboring drug-resistant strains of *M. tuberculosis*, they are given a more intensive regimen. If a patient receiving Category-III regimen has a positive sputum smear at the end of the second month of ATT, he or she should be recorded as a failure and re-registered and placed on CAT-II afresh (i.e., to start from the beginning). Streptomycin has limited penetration of membranes. Consequently, CAT-II regimen is not recommended for seriously ill extrapulmonary cases. However, streptomycin can be given intrathecally in serious cases of tuberculous meningitis.

Intensive phase consists of 2 months (24 doses) of isoniazid, rifampicin, pyrazinamide, ethambutol, and injection streptomycin, all given under direct observation, three times a week (on alternate days; *see* Table 5.10). This is followed by 1 month (12 doses) of isoniazid, rifampicin, pyrazinamide, and ethambutol, all given under direct observation, three times a week (on alternate days). If the sputum smear is positive for AFB after 3 months of intensive phase, the four oral intensive phase drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) are continued for another 1 month (12 doses), before starting the 5-month continuation phase. The extended intensive phase is to be completed within 6 weeks. The continuation phase starts immediately after the intensive phase and comprises 5 months (22 weeks, 66 doses) of isoniazid, rifampicin, and ethambutol given three times a week (on alternate days). The first dose every week is directly observed. Three drugs are given in the continuation phase of CAT-II because retreatment cases are likely to harbor drug-resistant strains of AFB, at least to isoniazid. Therefore, ethambutol (the companion drug to prevent drug resistance) is added to CAT-II regimen to prevent resistance to isoniazid and rifampicin. Most patients with TB are effectively cured by the

CAT-II regimen. Patients who relapse generally have better outcomes than failure or treatment after default cases, but even these cases respond to ATT provided it is taken regularly.

Rationale for Using Streptomycin in Category-II Regimen

Only a small proportion of patients on CAT-II (retreatment) regimen are treatment failure cases, with risk of drug resistance to one or more drugs. Hence an additional drug is added. Studies in India reveal that two-thirds of patients on CAT-I regimen, who were sputum positive at the end of 5 months of ATT, were subsequently treated successfully afresh with the CAT-II regimen (Banu Rekha et al., 2007).

Category III (CAT-III)

This includes sputum smear negative cases and patients with extrapulmonary TB, who are not seriously ill. The intensive phase consists of 2 months (24 doses) of isoniazid, rifampicin, and pyrazinamide, all given under direct observation, three times a week (on alternate days). Only three drugs are used in the intensive phase of CAT-III because sputum negative cases harbor few AFB in their lesions, and there are negligible chances of emergence of drug-resistant strains. The continuation phase starts immediately after the intensive phase and comprises 4 months (18 weeks, 54 doses) of isoniazid and rifampicin given three times a week (on alternate days; *see* Table 5.10). The first dose every week is directly observed. If the sputum is positive after 2 months of ATT, the patient is categorized as failure and is placed on CAT-II afresh (i.e., to start from the beginning). The most common cause of treatment failure is failure to supervise (observe) drug administration. Children generally receive CAT-III regimen because they are rarely sputum positive. Ethambutol should not be given to children who are too young to have their visual acuity assessed or to report reduced vision.

Interruption of Treatment

Most of the patients stop treatments after the initial 2 to 3 months because the symptoms are relieved. Poor compliance to treatment increases the risk of multidrug resistance (Ormerod, 2005). If the treatment is stopped, after 2 months, the estimated risk of relapse is 70 percent, whereas after 4 months, it is 40 percent. If a patient does not present as per schedule during the course of treatment, home visits are essential to bring the patient back under treatment. Health personnel or community health workers should carry out home visits no later than the day of missed dose of treatment (for intensive phase) and within 1 week of the missed dose of treatment (for continuation phase). Adequate counseling of the patient is essential. If a patient, in the intensive phase of ATT, misses a scheduled dose, he or she should be traced and given the medication on the very next day. The medication for the following day is then given as scheduled. If a patient completely misses any dose of medication, these missed doses must be made up at the end of the scheduled period.

Prolonging Treatment

Intensive phase is prolonged by 1 month if sputum smear is positive for AFB at the end of the intensive phase; continuation phase is prolonged up to 7 months with isoniazid and rifampicin for patients with miliary TB, tuberculous meningitis, and spinal TB.

Precautions during Treatment for All Directly Observed Treatment, Short Course Regimens

1. At the end of each month of ATT, evaluate each patient for side effects by history-taking and clinical examination.
2. Provide pyridoxine supplementation especially for pregnant women, diabetics, and chronic alcoholics.
3. Discourage alcohol consumption during the period of ATT.
4. Advise the use of nonhormonal contraceptives.
5. Monitor for signs and symptoms suggestive of liver damage and carry out liver function tests every 2 to 3 months for those at high risk.
6. Avoid antacids that reduce drug absorption.
7. Streptomycin and ethambutol is contraindicated in pregnancy and renal disease.
8. Monitor side effects of streptomycin especially in the elderly (i.e., tinnitus, vertigo, hearing tests for higher frequencies that are affected first Romberg's test). Avoid loop diuretics (these potentiate side effects of streptomycin).
9. In case of suspected pre-existing ophthalmic disease, assess visual acuity before starting ethambutol; if side effects occur, stop ethambutol because these side effects are reversible. Avoid in children younger than 6 years old because they are too young to have their visual acuity assessed or report diminished vision.

Patients with Liver Disease

In acute hepatitis, with serious tuberculosis, start ATT with 3 months of streptomycin and ethambutol (3SE) *or* 3 months of streptomycin, ethambutol, and ofloxacin. When patient recovers from hepatitis after 3 months, start 6 months of isoniazid and rifampicin (6HR). In chronic liver disease, 2HRZE/6HR is to be given unless liver damage is severe. In the presence of ascites or portal hypertension, treat with 2HES/10HE.

Management of Jaundice during Treatment

Stop all drugs if the rise in serum transaminase levels is more than fourfold. Restart ATT with same regimen after serum transaminase levels return to normal. If TB is serious or life threatening:

1. Start ATT with 3 months of streptomycin and ethambutol (3SE) *or* 3 months of streptomycin, ethambutol, and ofloxacin. The least hepatotoxic regimen is 3SE.
2. Monitor liver functions every 1 to 2 weeks
3. Once liver functions return to normal, restart ATT with isoniazid, rifampicin, and pyrazinamide (in that order). Initially, give only half the recommended dose of each drug.
4. Increase to full dose gradually if there is no rebound increase in level of serum transaminases.

Renal Patients

Drugs eliminated by nonrenal routes (i.e., isoniazid, rifampicin, pyrazinamide, and thioamides) may be administered in normal doses. 2HRZ/6HR is a safe regimen. If streptomycin or ethambutol is to be given, decrease the dose in relation to results of renal function tests.

Directly Observed Treatment, Short Course for Children

Features of Childhood Tuberculosis

- Children younger than the age of 6 years are vulnerable, and the disease usually develops within 1 year of infection.
- The younger the child gets the infection, the shorter the incubation period is and the disease is more disseminated.
- Prevalence of TB is lower during the ages of 5 to 12 years. The prevalence rises once again in adolescence, when adult-type postprimary TB manifests.
- The ratio of pulmonary to extrapulmonary cases is 3:1.
- As compared to adults, children are more vulnerable to disseminated extrapulmonary forms of the disease.
- Lesions are closed caseations with low bacillary load.
- As compared to adults, children tolerate higher doses of antitubercular drugs per kilogram of body weight.

Spectrum of Manifestations

In children, TB has a wide spectrum of manifestations. Failure to thrive, failure to gain weight or loss of weight (over a period of more than 1 month), low-grade fever for more than 1 week, night sweats, and anorexia are among the nonspecific manifestations. Endobronchial TB with focal lymphadenopathy, progressive pulmonary disease, signs of fluid (dullness) in the chest indicating pleural involvement, reactivated pulmonary disease, and presence of nonproductive cough or wheeze are seen if the respiratory system is involved. Abdominal TB is characterized by the presence of lump in the abdomen or chronic diarrhea, which is not responding to medication, including deworming agents. Headache, irritability, occasional projectile vomiting in a child who wishes to be left alone, gradual onset of drowsiness in a child over 2 to 3 weeks, and gradual onset of weakness over one side of the face or one limb indicate involvement of the central nervous system. Other manifestations include:

- Lump in vertebral region with or without stiffness in walking
- Swelling in any bone or joint, not associated with trauma
- Discharging sinus or wound near any joint
- Painless, enlarged lymph node, firm or soft, which may or may not be matted
- Lymph node abscess or soft, painless, fluctuant swellings under the skin
- Painless ulcer on the skin with undermined edges and usually clean base
- Painless, chronic discharge from the ear.

Classification of Childhood Tuberculosis

As, per clinico-radio-immunological criteria, childhood TB is classified as:

- Asymptomatic Mantoux positive (ASMP): tuberculin positivity may be associated with erythema nodosum and phlyctenular conjunctivitis
- Symptomatic Mantoux positive
- Primary pulmonary complex
- Progressive primary disease

- Miliary TB and tuberculous meningitis
- Extrapulmonary TB, such as lymphadenitis and bone and joint TB

This classification is not used under DOTS program.

Progressive Primary Tuberculosis

Progressive primary TB is observed in 5 to 10 percent of patients with primary TB. It is most commonly seen in children younger than 1 year of age, teenagers, and in black people (Kilincer, 2010). This condition may follow an attack of measles or any other viral disease that diminishes immunity. Progressive primary TB may result in pneumonia or atelectasis. The systemic manifestations include fever, cough, malaise, and weight loss.

Endobronchial Tuberculosis

This is the most common variety of pulmonary TB in children (Kashyap et al., 2003). Lymph nodes become enlarged, which may cause bronchial obstruction (persistent cough may be the only indication); compression of oesophagus (causing difficulty in swallowing), and paralysis or vocal cord (indicated by hoarse voice or difficulty in breathing).

Pleural Effusion

It is usually seen in older children. The manifestations include acute onset of fever, chest pain that increases in intensity on deep inspiration, difficulty in breathing or respiratory distress, tachypnea, dullness on percussion, decreased breath sounds over the affected area, and features of mediastinal shift (occasional).

Miliary Tuberculosis

This condition usually has a subacute presentation with low-grade fever, malaise, fatigue, and weight loss, which are sometimes followed by abrupt onset of fever and associated symptoms. Cough and respiratory distress may be present.

Other Sites of Tuberculosis in Children

Any chronic, painless discharge from the ear (chronic otitis media) in a child should arouse suspicion of TB. In an infant or a young child, the mastoid process or ear may be infected by hematogenous spread from a primary focus in the lung. If the focus of infection is inside the ear, the draining lymph node is located between the mastoid process and the angle of the mandible. Involvement of the facial nerve may result in ipsilateral (same side) facial paralysis. The gastrointestinal tract (ascites, subacute obstruction, stricture), skin (lupus vulgaris, tuberculids), and kidneys are unusual sites for TB in children.

Congenital Tuberculosis

This is an extremely rare condition manifested in the second or third week after birth probably as a result of the passage of tubercle bacilli from the maternal circulation to the fetal circulation through the placenta. The umbilical veins carry the bacilli to the liver, causing primary infection of the liver. The manifestations in the second or third week after birth include lethargy or irritability, poor feeding and poor weight gain, cough or respiratory distress, and hepatosplenomegaly. Usually AFB can be demonstrated by microscopic examination of gastric lavage. For diagnosing congenital TB, the neonate should have proven tubercular lesions and at least one of the following:

- Apparently healthy at birth
- Proven tubercular lesions in placenta or maternal genital tract
- Presence of primary complex in the liver
- Exclusion of possible postnatal transmission.

Neonatal Tuberculosis

Neonates may be infected by *Mycobacteria* during or immediately after birth by the inhalation route from an adult who has sputum-positive pulmonary TB. After a silent period of 3 to 4 weeks, the disease rapidly progresses and resembles acute pneumonia. Neonatal TB is characterized by cyanotic attacks, cough, and bilateral fine crepitations. Radiologically, the disease resembles bilateral acute pneumonia. Because a tuberculin test is negative, diagnosis is made only by microscopic examination of gastric lavage, where numerous AFB are seen. Neonates usually respond if full course of ATT is started promptly.

Reactivation of Tuberculosis

Reactivation is usually seen in children older than 7 years of age and adolescents. Like in adult-type of TB, this condition has a subacute presentation with fever, cough, weight loss, and rarely, hemoptysis. The patient may discharge tubercle bacilli in their sputum and thus, is infectious to others.

Indications for Investigations

Any child with the following signs and symptoms needs to be evaluated for TB: fever or cough for 3 weeks or longer and

- With or without significant weight loss
- With history of failure to gain weight
- With history of contact with suspected or diagnosed case of active TB within the last 2 years.

In addition, the following conditions in children also need investigations to rule out TB:

- Fever of unknown origin or unexplained lymphadenopathy
- Pneumonia, pleural effusion cavitary lesion, or a mass in the lung that does not improve with antimicrobial therapy
- Fever, night sweats, anorexia, nonproductive cough, failure to thrive, or failure to gain weight.

Suspect Case

A suspect case is any child with fever or cough for 3 weeks or longer, and

- With or without weight loss
- With history of failure to gain weight
- With history of contact with suspected or diagnosed case of active TB within the last 2 years.

Table 5.12. DOTS Regimens for Children.

Category	Type of Patient	Intensive Phase	Continuation Phase
CAT-I	New sputum-positive pulmonary tuberculosis Seriously ill sputum negative pulmonary tuberculosis Seriously ill extrapulmonary tuberculosis	$2(HRZE)_3$*	$4(HR)_3$*
	Meningeal tuberculosis	$2(HRZS)_3$*	$6(HR)_3/7(HR)_3$*
	Spinal tuberculosis with neurological complications	$2(HRZE)_3$*	$6(HR)_3/7(HR)_3$*
CAT-II	Sputum smear positive: Relapse Treatment failure Treatment after default	$2(HRZES)_3 + 1(HRZE)_3$*	$5(HRE)_3$*
CAT-III	Sputum negative pulmonary tuberculosis Not seriously ill extrapulmonary tuberculosis	$2(HRZ)_3$*	$4(HR)_3$*

E, ethambutol; H, isoniazid; R, rifampicin; S, injectable streptomycin; Z, pyrazinamide.
*Prefix numbers refer to the duration (number of months) of treatment, and subscripts refer to the number of doses per week.

Diagnosis in Children

Observing a child for a few minutes (behavior, skin, hairs, thoracic movements during respiration, wheeze, and cough) may yield a lot of clinical information. If in doubt, the examining doctor should refer the suspect case to a pediatrician. Case definitions are to be used for all diagnosed cases. Diagnosis in children is based on a combination of:

- History of contact with suspected or diagnosed case of active TB within the last 2 years
- Clinical presentation
- Sputum examination (if possible)
- Chest radiography (postero-anterior view)
- Mantoux test (using one TU PPD RT23 with Tween 80 in endemic countries). The test is considered positive if induration is 100 mm or more after 48 to 72 hours.

Treatment

All pediatric patients with TB should also be registered for DOTS therapy. Intermittent regimens (Table 5.12) are given under direct observation. "Seriously ill" sputum negative pulmonary TB includes all forms of pulmonary TB other than primary complex, whereas "not seriously ill" extrapulmonary TB includes lymph node TB and unilateral pleural effusion. Seriously ill extrapulmonary TB includes:

- Disseminated/military TB
- Bilateral or extensive pleurisy

Table 5.13. Dosages for children.

Name of Antitubercular Treatment Drug	Thrice-a-Week Dose
Isoniazid (H)	10–15 mg/kg body weight
Rifampicin (R)	10 mg/kg body weight
Pyrazinamide (Z)	35 mg/kg body weight
Ethambutol (E) *	30 mg/kg body weight
Injectable streptomycin (S)	15 mg/kg body weight

*Not to be given to children younger than 6 years of age

- TB of meninges, pericardium, peritoneum, intestines, genitourinary tract, and skeletal system
- TB of spine with or without neurological involvement.

Children with tuberculous meningitis are placed on a CAT-I regimen, but the four drugs in the intensive phase are isoniazid (H), rifampicin (R), pyrazinamide (Z), and streptomycin (S), instead of HRZE (Tables 5.12 and 5.13). The duration of continuation phase for tuberculous meningitis and TB of spine with neurological involvement should be 6 to 7 months (instead of 4 months). Before starting a CAT-II regimen, the child should be examined by a pediatrician or TB expert, where available. Steroids are used initially in hospitalized patients with meningeal or pericardial TB, and doses are tapered gradually over 6 to 8 weeks.

Guidelines for Treating Children

- Children (under 7 years of age) rarely suffer from smear-positive pulmonary TB. Consequently the lesions harbor few AFB, and there are negligible chances of emergence of drug-resistant mutant tubercle bacilli. Therefore, most of the child cases (under 7 years of age) are placed on a CAT-III regimen.
- For all cases, the dose of drugs is to be calculated in mg per kilogram body weight.
- To enable administration of the correct pediatric dose medications may be made available in combi-packs in patient-wise boxes, linked to the child's weight.

Special Situations

Tuberculosis Diagnosed in Pregnancy

Isoniazid, rifampicin, and ethambutol are safe for use in pregnancy. According to American Thoracic Society (2003), pyrazinamide is reserved for pregnant women in the United States with MDR-TB, but allowed elsewhere in the world.

Baby Born to Mother with Tuberculosis

- Mother to continue breastfeeding
- Mother to wear a mask, especially while breastfeeding
- If the baby's chest X-ray is normal at birth, give isoniazid for 3 months and perform Mantoux test at 3 months of age. If the baby is Mantoux positive, start on 7HR.

- If the baby is Mantoux negative, give BCG vaccine. If the baby's chest X-ray is abnormal at birth, start on 2EHR/7HR.

Tuberculosis and HIV

HIV destroys the immune system and makes the infected person vulnerable to multiple infections. With the high prevalence of TB, the problem of HIV-TB coinfection is overwhelming. An estimated 40 percent of adults in India are already infected with *M. tuberculosis* (Kartikeyan et al., 2007). HIV is the most common opportunistic infection and the most frequent cause of death among persons infected with HIV in Africa.

- HIV testing for patients with TB: In the developed countries, all newly diagnosed patients with TB are tested for HIV sero-status. However, in India, this strategy is neither feasible nor cost-effective because of the large number of new cases of TB that are detected each year. Pulmonary TB is diagnosed in individuals who are HIV positive by sputum microscopy.
- Effect of HIV infection on pre-existing TB: HIV infection causes immune suppression, which predisposes to opportunistic infection. The impact of HIV infection on pre-existing TB is as follows:
    1. Expedites the spread of TB by reactivating latent tubercular infection
    2. Accelerates progression of recently acquired tubercular infection
    3. Increases risk of recurrence of TB by endogenous reactivation or exogenous reinfection
    4. Increases risk of acquiring nosocomial TB, especially that resulting from drug-resistant strains
    5. Affects TB control programs by lowering cure rates, overdiagnosing sputum negative pulmonary TB, underdiagnosing sputum-positive pulmonary TB, and increasing the case load
    6. May deter patients with TB from accessing health care because of social stigma associated with HIV infection
    7. Increases morbidity (resulting from HIV-related opportunistic infections) and mortality.
- Among those infected by tubercle bacilli, the estimated life time risk of progression from latent to clinically active TB is 50 to 60 percent in individuals who are HIV positive as compared to counterparts who are HIV negative (5–10 percent).
- HIV infection along with active TB leads to the depletion of CD_4 lymphocytes, increased multiplication of HIV, and elevated plasma levels of HIV-RNA. This results in predominance of CD_8 cells and DTH, which helps the reactivation of primary tubercular infection and dissemination of the disease. In pulmonary TB, HIV also impairs the innate resistance of alveolar macrophages. Thus, HIV and *M. tuberculosis* form a deadly alliance.
- Effect of TB on HIV/AIDS epidemic: TB occurs earlier in the course of progression of HIV infection. When CD_4 counting is 400 cells/μL, the TB intensifies the HIV epidemic by:
    1. Accelerating progression of HIV infection by causing six- to sevenfold rising in viral load. This increases susceptibility to other opportunistic infections.

2. Shortening the survival period of patients who are HIV positive by increasing mortality rates. TB accounts for one-third of deaths worldwide among individuals who are infected with HIV. The disease is a leading cause of death among individuals who are HIV positive.
3. Affecting TB control program by increasing caseload of active patients with TB, increasing the incidence of extrapulmonary (especially lymphadenitis) and disseminated forms of TB and causing difficulties in diagnosis of TB in patients who are HIV positive (who excrete fewer AFB per mL of sputum).

Pulmonary Tuberculosis

Though disseminated and extrapulmonary TB is relatively more common in patients with coinfection, pulmonary TB is still the most common manifestation. Features of pulmonary TB in individuals who are HIV positive are summarized in Table 5.14.

- Clinical features in patients who are HIV positive: Cough is less frequent, probably because there is less cavitation, inflammation, and endobronchial irritation as a consequence of diminished cell-mediated immunity. Hemoptysis, which is the result of caseous necrosis of endobronchial blood vessels, is also less common in these patients.
- Sputum microscopy: Sputum examination is the key to diagnosis of TBs even in areas with high prevalence of HIV sero-positivity. Patients who are infected with HIV and have positive sputum tend to excrete fewer AFB per mL of sputum as compared to their counterparts who are HIV negative. Therefore, if the recommended minimum number of OIFs is not examined, AFB may be missed, leading to false-negative sputum report.
- Chest X-ray: Chest X-ray (postero-anterior view) is indicated for patients with symptoms suggestive of TB, who are smear negative, and do not respond to a course of broad-spectrum antibiotics for 10 to 14 days. No radiographic pattern is diagnostic of TB. Sometimes a chest X-ray may be normal. The classical hallmarks of the disease are cavitation, apical infiltration, pulmonary fibrosis, shrinkage, and calcification. Chest X-ray of patients who are infected with HIV with intact immune function will show these "classical" features. However, as immune suppression sets in, chest X-ray findings become more atypical, with lower lobe infiltration and mediastinal lymphadenopathy. Infections (other than tuberculosis) and noncommunicable diseases may

Table 5.14. Pulmonary tuberculosis in patients who are HIV positive.

Feature	Early HIV Infection	Late HIV Infection
Clinical manifestations (children)	Similar to children who are HIV negative	Disseminated forms of tuberculosis
Clinical manifestations of pulmonary tuberculosis (adults)	Often resembles postprimary pulmonary tuberculosis	Often resembles primary pulmonary tuberculosis
Sputum smear (adults)	Frequently positive	Frequently negative
Chest X-ray (adults)	Cavities are more common	Usually diffuse infiltrates, with no cavities

produce both "classical" and "atypical" chest X-ray findings. If sputum smear is negative for AFB, other conditions need to be considered in the differential diagnosis.

Extrapulmonary Tuberculosis

The most frequent forms of extrapulmonary TB in patients who are HIV sero-positive are pleural and pericardial effusions, widespread tubercular lymphadenitis, and military or disseminated TB. The definitive diagnosis of the extrapulmonary disease is often complicated by inadequate diagnostic facilities.

- **Tuberculous lymphadenitis**: The clinical manifestation is similar in both patients who are HIV positive and negative, but in individuals who are HIV positive, the onset of lymphadenitis may be acute, resembling acute bacterial infection. Diagnostic techniques include needle aspiration and histopathological examination of lymph node biopsies.
- **Meningeal TB**: CSF may be normal in patients who are HIV positive.
- **Miliary or disseminated TB**: may be difficult to diagnose.
- **Tuberculous pericarditis**: This condition is not rare and may be presumptively diagnosed on the basis on characteristic "balloon-shaped" cardiac shadow on chest X-ray.

Childhood Tuberculosis

HIV has been cultured from breast milk of infected mothers. There are several reports of HIV infection in babies apparently caused by breastfeeding. On the other hand, there are also reports of babies who were breastfed by their mothers who are infected with HIV, but who did not become infected. Clearance of HIV infection in a perinatally infected infant has also been reported (Bryson et al., 1995).

Diagnosis of HIV Infection in Children

Because maternal antibodies to HIV, cross the placenta, almost all babies born to mothers, who are HIV positive, have HIV antibodies in their blood. Most of the uninfected babies lose their maternal antibodies when they are 15 months old, but they may continue to test positive for HIV antibodies even after the age of 15 months. Some babies born to mothers infected with HIV may have a negative test even if they are infected. For this reason, laboratory tests that detect HIV antibodies are unreliable in babies less than 15 months old. If sophisticated tests (e.g., p24 antigen) are not available, HIV infection may be diagnosed on the basis of HIV antibody test in the mother and presence of clinical manifestations in the baby. Overt manifestations of HIV infections are usually seen in babies who are HIV sero-positive by the time they reach the age of 2 years. In some babies, the clinical manifestations are seen in the first few weeks after birth whereas in others, these may be delayed for many years. The reasons are still unclear. A wide spectrum of manifestations is seen in children and may be confused with other diseases. The usual manifestations include cough, failure to thrive, chronic diarrhea, and pruritic rash.

Effect of Antitubercular Treatment

The DOTS regimens are the same, regardless of the patient's HIV status. This is because the sputum conversion and cure rates are similar if effective drugs are administered

regularly. Patients who complete ATT show the same clinical, radiographic, and bacteriological response to short-course chemotherapy, irrespective of their HIV status.

Prognosis in Children with HIV-Tuberculosis Coinfection

A child with HIV-TB coinfection usually responds well to ATT. Periodic clinical assessment and weight record help in deciding the response to ATT. When overt AIDS develops the condition usually worsens, and the child dies by the age of 2 to 3 years of age. When the disease develops for the first time in the second or third year of life, the child may continue to grow despite recurrent illnesses. Some well-nourished children infected with HIV may remain apparently healthy and may become sick years later. An undernourished child infected with HIV is susceptible to fatal infections.

Mortality Rates in Patients with Coinfection

Mortality in patients with HIV-TB coinfection, 1 year after starting ATT, is higher than that for their counterparts who are HIV negative. The excess mortality is partly attributed to TB itself and partly to other HIV-related opportunistic infections such as diarrhea, pneumonia, Kaposi's sarcoma, and cryptococcal meningitis. The mortality is less if patients with HIV-TB concomitant infection are treated with short-course chemotherapy as compared to that with old standard ATT regimens. This is because of a broad-spectrum bactericidal activity of rifampicin, in addition to mycobactericidal activity, it may control HIV-related bacterial infections during the course of short-course chemotherapy. Mortality rates among patients with HIV-TB concomitant infection treated with DOTS (under supervised administration) and similar regimens under self-administration were 15 percent and 43 percent, respectively. Relapse rates among patients, who are HIV sero-positive, treated with DOTS and similar regimens under self-administration were 3 percent and 5 percent, respectively.

Guidelines for Treating HIV-Tuberculosis Coinfection

- The duration of treatment will be as per the regimen and category. If required, the duration of treatment may be extended within the current DOTS guidelines.
- Full supervision is essential in the continuation phase because lower rates of adherence and higher mortality have been observed in patients who are HIV sero-positive.

Side Effects of Antitubercular Drugs

Adverse drug reactions as a result of ATT are more common in patients who are HIV sero-positive as compared to that in their counterparts who are sero-negative. Risk of adverse reactions increases with a degree of immune suppression. Most reactions occur within the first 2 months of starting ATT.

Antiretroviral Therapy in Coinfection

In patients with coinfection (especially sputum-positive pulmonary TB), the priority is to treat TB to stop transmission of the disease. With careful evaluation and management, patients with coinfection can be administered antiretroviral therapy (ART) and anti-tubercular drugs at the same time. In a patient with risk factors (CD_4 count less than 200 per mm^3 and disseminated TB), it may be necessary to start ART and antitubercular drugs simultaneously. In patients infected with HIV with smear-positive TB, who are not at risk

of rapid progression of HIV or death, ART may be deferred until completion of intensive phase of TB treatment. This decreases the risk of immune reconstitution syndrome and prevents risk of interaction between ATT and ART drugs. WHO recommends that patients with TB-HIV coinfection complete their ATT prior to starting ART, unless there is a high risk of progression of HIV or death during the period of ATT (Agarwal et al., 2012).

Difficulties in Treating Coinfection

Around 350,000 people are estimated to have died of TB and HIV coinfection in 2010, in addition to the 1.1 million, people who died from TB alone (WHO, 2011). People having HIV infection and TB when they die are internationally reported as having died of HIV infection (WHO, 2010). This is probably because one-third of patients with HIV-TB coinfection do not complete treatment. HIV infection is an independent risk factor for drug-resistant TB (Suchindran et al., 2009). Higher incidence of drug toxicity is reported among patients who are HIV sero-positive. In New York, previously treated patients, those with HIV infection, and injection drug users were all at increased risk of having drug-resistant TB (Frieden et al., 1993). In 90 percent of cases, these side effects occur during the first 2 months of treatment. Occurrence of opportunistic fungal infections in patients with HIV-TB coinfection can complicate treatment because rifampicin and isoniazid reduce the serum levels of ketoconazole and fluconazole.

If atypical *Mycobacteria* cause opportunistic pulmonary infections, treatment regimens need to be specifically devised because these organisms have varied drug susceptibility patterns. Over all highest resistance (32.9 percent by both the types) was reported against pyrazinamide, followed by isoniazid resistance 26 percent and 59.9 percent, 22 percent and 16.9 percent for streptomycin, 14.2 percent and 15.9 percent for ethambutol and 6.2 percent and 9.68 percent for rifampicin, for both typical and atypical mycobacterial isolates, respectively (Khanum et al., 2011). The most suitable drug to control both typical and atypical *Mycobacteria* is rifampicin (Khanum et al., 2011) (Table 5.15). Infection with *M. avium* complex (MAC) is treated with conventional antitubercular regimens with the addition of other drugs such as ciprofloxacin, rifabutin, cycloserine, and imipenem.

Table 5.15. First-line drug resistance profile of typical and atypical *Mycobacteria* based on resistance ratio method.

	Typical *Mycobacteria*			Atypical *Mycobacteria*		
Drugs	R (%)	S (%)	MDR (%)	R (%)	S (%)	MDR (%)
Rifampicin	6.2	93.8	6 (5.68)	9.68	90.32	2 (3.2)
Isoniazid	26	74		59.9	40.1	
Streptomycin	22	78		16.9	83.1	
Ethambutol	14.2	85.8		15.9	84.1	
Pyrazinamide	32.9	67.1		32.9	67.1	

MDR, multidrug resistant; R, resistance; S, sensitive.
Adapted from Khanum T, Rasool AJ, Ajaz M, Khan AS. 2011. Isolation—Drug resistance profile and molecular characterization of indigenous typical and atypical Mycobacteria. *Pak J Pharm Sci* 24:527–532.

Drug-Resistant Tuberculosis

Definitions

- **Primary resistance**: This occurs when someone who harbors drug-resistant forms of *M. tuberculosis* infects another individual. Sometimes, a patient may withhold information on previous treatment with antitubercular drugs. Such cases may be wrongly labeled as that of primary drug resistance.
- **Secondary or acquired resistance**: This is as a result of the emergence of drug-resistant strains as the dominant population. Secondary drug resistance is attributable to:
  1. Use of correct combinations for inadequate duration. This can occur because of interruptions in drug supply.
  2. Another factor is poor adherence of patients to the prescribed drug regimen because of ignorance, poverty, or relief of symptoms after partial treatment. DOTS program requires biweekly visits to a designated health facility. During each visit, the patient loses the day's wages and has to bear the additional cost of to and from travel.
  3. Ignorance of patients because some health personnel do not care to provide information about the disease and its treatment.
  4. Use of wrong combinations: Non-adherence of doctors to current recommendations on treatment of TB is either because of ignorance or recalcitrance.
- WHO and the IUATLD suggest the replacement of the *primary resistance* with the term *drug resistance among new cases* and *secondary resistance* with the term *drug resistance among previously treated cases*.
- **MDR-TB**: This is a disease caused by *M. tuberculosis* and is resistant to at least isoniazid and rifampicin. The global pandemic of MDR-TB is characterized by
  1. High rates of transmission to patients' household contacts, health-care providers and other individuals
  2. Institutional outbreaks-
  3. Ineffectiveness of standard antitubercular drugs.
- **Extensively Drug-Resistant Tuberculosis (XDR-TB)**: This is a disease caused by *M. tuberculosis* and is resistant to isoniazid, rifampicin, fluoroquinolone, and at least one of the three injectable drugs—amikacin, capreomycin, and kanamycin. XDR-TB is the latest emerging disease recognized as a global health threat (Calain and Fidler, 2007). It has so far been identified in at least 27 countries covering all regions of the world, except Oceania.

Magnitude of Drug Resistance

Global Magnitude

Among infectious diseases, TB is the leading cause of death. The control of Ts has been declared as a global emergency by WHO, mainly because of the increasing incidence of MDR-TB in several parts of the world. The HIV/AIDS epidemic has also contributed to the resurgence of TB. MDR-TB has been reported in Eastern Europe, Iran, China (Henan province), and India (state of Tamil Nadu). In the WHO/IUALTD Global Drug resistance surveillance project, the median value for resistance to any drug among new cases of TB was 11 percent, whereas among the previously treated cases, it was 33.4

percent. The median prevalence of MDR-TB in new cases was 1 percent, whereas that for previously treated cases was 9.3 percent.

India

Most surveys on drug resistance in India are deficient in varying technical aspects such as methodology, recording of previous treatment history, sample selection, uniformity in bacteriological procedures, etc. There is no clear evidence of increase in prevalence of primary drug resistance in India, in the post-rifampicin era. The prevalence of MDR-TB is also low in most parts of India. However, there is a relatively higher prevalence of acquired drug resistance in many regions of the country. The estimated annual incidence of TB and MDR-TB in India is 187 cases and 24.9 cases, respectively, per one lakh population. According to the Tuberculosis Research Centre, Chennai (Tamil Nadu), the prevalence of MDR-TB is 25 percent for previously treated cases and 3.4 percent for newly detected cases.

Etiology of Drug-Resistant Tuberculosis

The occurrence of drug-resistant TB is primarily a man-made amplification of a natural phenomenon (i.e., inherent bacterial resistance). Patients failing to complete their recommended regimens of antitubercular drugs accelerate the evolution of drug-resistant strains. From the public health point of view, poorly supervised or incomplete treatment of TB is more detrimental than no treatment at all. Emergence of MDR-TB is an indication of ineffective TB control probably as a result of:

- Inadequately functioning antituberculosis activities
- Use of standard drug regimens for variable periods of time
- Use of varying drug combinations that may have doubtful bioavailability.

The existence of MDR and XDR-TB reflects weaknesses in TB management, which should minimize the emergence of drug resistance. Early diagnosis and prompt initiation of supervised treatment are hallmarks of good TB management. Inadequate drug regimens favor the emergence of drug-resistant strains, which then proliferate. Further treatment errors lead to emergence of strains that are resistant to other drugs until MDR-TB is created. XDR-TB is created by the inappropriate use of second-line drugs in a patient for whom first-line drugs are failing. Patients then spread the infection to close contacts, who acquire primary XDR-TB. Wild strains of *M. tuberculosis*, which have never been exposed to antitubercular drugs, are almost always susceptible. Resistance to both isoniazid and rifampicin is the product of probability of potential resistance of the individual drugs. Hence, multidrug regimens are used in the treatment of TB. Fortunately, the mutations that cause resistance to the different classes of antitubercular drugs are not genetically linked. Some bacilli are inherently resistant to some drugs. If a single drug is used to treat a patient, only those bacilli that are sensitive to that drug are killed, allowing resistant bacilli to multiply. Multiple drugs are used in the intensive phase of TB treatment so that the number of viable *M. tuberculosis* is greatly reduced. The best way to prevent MDR-TB is to ensure that patients with drug-sensitive disease are given the correct drug regimens for the prescribed duration until they are declared cured.

Laboratory Tests for Drug-Resistant Tuberculosis

Conventional methods of testing for drug susceptibility of *M. tuberculosis* require 8 to 10 weeks. Newer methods that provide results in less than 3 weeks have been developed, but because of the high cost and the need for technical expertise, these new methods are not available for routine use in poor countries. Potentially, the PCR could provide specific diagnosis of TB in a few hours, but this technique is expensive and is not yet available for routine diagnosis. Advances in molecular epidemiology can be used to track the sources of infection and transmission of individual strains of organisms, including drug-resistant strains. Individual isolates or strains of *M. tuberculosis* have distinct banding patterns in DNA fingerprinting analysis. When isolates obtained from different patients' exhibit the same DNA fingerprint pattern, it is highly suggestive of a common source of the isolates.

Treatment of Drug-Resistant Tuberculosis

Short-course chemotherapy with first-line antituberculous drugs is not effective in treating cases with MDR-TB. The "DOTS-Plus" is advocated for treatment of MDR-TB. It is based on DOTS, but with the following modifications:

- Individualized treatment
- Provision of on-site laboratory facilities for culture and drug-sensitivity testing. Treatment of MDR-TB comprises a minimum of five drugs, of which, at least two (preferably three) drugs should not have cross-resistance with previously administered drugs. Reserve second-line drugs (amikacin, capreomycin, ciprofloxacin, cycloserine, ethionamide, kanamycin, levofloxacin, ofloxacin, or prothionamide) are prescribed. Second-line drugs are expensive (therefore, not available in many developing countries), cause serious side effects, and are often unsuccessful. Treatment is to be taken for up to 2 years to prevent relapse. For these reasons, ensuring the cure of smear-positive patients is a much higher public health priority, as compared to treatment of MDR-TB.

The intensive phase of treatment for MDR-TB includes five drugs (to which the patient's isolate is susceptible) for a period of 6 months. The continuation phase incorporates three drugs (to which the patient's isolate is susceptible). The current WHO recommendations for MDR-TB specify daily treatment for a minimum duration of 18 months (Orenstein et al., 2009).

Prevention and control

Case Finding

Basically two types of case finding are there active and passive. In active case finding, health workers visit people's homes, inquire about persons with symptoms suggestive of TB, and obtain sputum samples from such persons. Active case finding is time consuming, expensive, and yields fewer positive cases. In the passive case finding, sputum samples are obtained from attending a health facility with symptoms suggestive of TBs. Passive case finding is used in DOTS because it is more economical and yields more

positive results. Sputum smear microscopy is cheap, easy to perform, and can yield results rapidly. Because sputum-positive cases contribute to spread of TB, detecting such cases is of epidemiological importance. Tuberculin test is not useful as a case finding tool in older children and adults.

Case Holding

Case holding implies ensuring adherence to treatment for the prescribed duration. Non-adherence is seen in all conditions requiring the long-term use of medication (e.g., TB, leprosy, hypertension, diabetes, epilepsy, prophylaxis for rheumatic fever, malaria prophylaxis, and oral contraception). There are two types of defaulters:

1. "Tablet dodgers" who may attend the health facility regularly, but may skip few doses, and
2. "Clinic dodgers" who fail to report to the health facility on the scheduled date.

Causes for Non-Adherence

Patient-Related Causes

- Relief from symptoms (patient "feels better").
- Preference for injections.
- Repetition of the same medication for prolonged periods leading to "boredom," inconvenient timings, or distance of the clinic.
- Miscellaneous causes like marriage or death in the family, festivals, sowing. or harvesting season.

Provider-Related Causes

- Failure to explain (in a simple language that is understood by the patient and his or her family members) the need for prolonged treatment and possible side effects.
- Too much information is given at one sitting, confusing the patient.
- Perceived "rude" behavior of health-care personnel.
- Prolonged waiting time at the clinic.

Drug-Related Causes

- Frustration due to shortage of drugs.
- Occurrence of side effects (that have not been told beforehand) may make the patient suspect that the wrong medication has been given.

Detection of Non-Adherence

Non-adherence may be detected either by tablet count (gives a count of unused tablets) or by detecting the presence of antitubercular drugs in urine as:

- Isoniazid can be detected by the addition of 10% potassium cyanide and chloramine T to urine, which gives it a pink or red color.

- Rifampicin can be detected by the addition of analytical grade of chloroform to urine, which gives it a yellow or orange color.

Remedial Measures

- Counseling the patient and follow up.
- Determining the cause of non-adherence and taking corrective measures, if possible.
- Using innovative measures for combating non-adherence.

Information, Education, and Communication

Information, education, and communication (IEC)is a preplanned, concerted effort, with specific objectives, and focuses toward specific program goals to reach specific target audiences. The objective of each of the components of IEC is clear and specific. Knowledge, attitude, and practice (KAP) studies, act as a barometer of the level of knowledge, the prevailing attitudes, and the type of behavior, which needs to be undertaken before and after IEC programs to know the impact of these programs.

- Information component attempts to generate awareness in the target audience and improve their knowledge by dissemination of information, either in individual, group, or mass settings.
- Education component deals with the development of favorable attitudes.
- Communication component aims at bringing about desired behavior changes, through motivation and persuasion.

IEC is an essential ingredient of a control program for any disease. Use of methods (e.g., folk songs, storytelling, role play) and media (group or mass media) are determined by the local situation. Physical, psychological, linguistic, and sociocultural barriers in communication may hamper IEC efforts for the general population. Certain subgroups in the population (e.g., school and college students, office employees, industrial workers) are more accessible at a given time and place for IEC activities. The messages ought to be repeated periodically for reinforcement.

Topics for IEC
These include:

- Information about TB, its mode of transmission, and organs affected.
- Emphasis on curability of the disease if detected and treated in the early stages.
- Possible outcomes (spread to contacts, complications, death) if incompletely treated.
- Available methods for preventing the disease (BCG vaccination).
- Essential behavioral changes (covering the mouth while coughing, sneezing, etc., discouraging indiscriminate spitting).

Screening of Contacts

Patients with TB ought to be informed that if any of their family members have symptoms suggestive of TB (especially children younger than 6 years of age) they should be brought to the nearest health facility for examination. When an infant or preschool child is diagnosed with TB, the source of infection is usually an adult family member, baby sitter,

domestic servant, or a neighbor. When a school-going child is diagnosed to have TB, the source of infection may be any infected adult (at home, school, or in public transport on the way to school) who coughs without covering his or her mouth or spits indiscriminately. The infection usually spreads inside buildings and within closed spaces, such as buses.

Immunotherapy

Immunological agents such as IL-2 and TNF may stimulate host defense mechanisms (especially Th-1) to recognize mycobacterial antigens and eliminate semi-dormant and dormant *Mycobacteria*. Actively metabolizing tubercle bacilli are killed by the first few doses of isoniazid and rifampicin. Few surviving tubercle bacilli are those that are not susceptible to these drugs (because of the prolonged duration of the treatment) or those that have low or no metabolic activity inside or outside the macrophages. Such dormant and semi-dormant organisms may be eliminated by immunotherapy. Immunotherapy shortens the duration of chemotherapy and improves the chances of a cure in drug-resistant cases; improves patient adherence (because of shortened duration of regimens, thus reducing the likelihood of emergence of drug-resistant mutants; brings about rapid relief from symptoms, and rapidly renders patients non-infectious (Stanford and Stanford, 1996). Immunotherapy, as an adjunct to chemotherapy, may be useful for newly diagnosed drug-sensitive cases, patients with HIV-TB coinfection, and in drug-resistant cases.

Disinfection

Exposure to direct sunlight kills tubercle bacilli in 5 minutes, but these organisms may survive for many years in the dark. For this reason, there is a higher risk of disease transmission in ill-ventilated, dark rooms. Exposure to air and sunlight is a simple method for disinfecting bedding, blankets, clothes, etc. However, this method is not recommended for disinfecting sputum, which is the infective material for spread of the disease. Sputum may be collected in paper cups, paper napkins, or old newspapers and incinerated as soon as possible. One percent solution of sodium hypochlorite (NaOCl) rapidly kills tubercle bacilli. Because this chemical corrodes metals, glass containers are to be used. It also bleaches colored clothes and other materials. Tubercle bacilli may not be destroyed for several hours by 5% phenol. However, heat (140° F [60° C] for 20 minutes or 158° F [70° C] for 5 minutes) effectively kills these organisms.

Social and Cultural Factors

Socioeconomic Factors

- Social stigma: In many communities, patients with TB are stigmatized, which leads to bias and marginalization. Because of actual or perceived discrimination, patients with symptoms suggestive of TB may not seek health care. It is also difficult to deal with non-adherence to treatment in such circumstances.
- **Poverty and associated conditions**: Poverty forces people to live in overcrowded conditions that increase the risk of disease transmission. Poverty is also accompanied by undernutrition, lack of access to health care, and poor living conditions such as poor ventilation, lack of safe drinking water, and inadequate sanitation. These factors compromise the body's ability to fight infections. Therefore, poor people are more

vulnerable to TB as compared to their relatively affluent counterparts. Thus, socioeconomic development ought to be an integral part of TB control programs. As a result of losses in earnings, many families are forced to sell their land or livestock or take their children out of school. These school dropouts help out with domestic chores or work outside their homes as child laborers.

- **Population migration**: Migrants are pushed to poverty if they are unable to find work. Actual or perceived discrimination and social maladjustment prevent these migrants from seeking health care. Illegal international migrants do not avail of health-care services for fear of detection and deportation. Because migration of patients (for economic or other reasons) cannot be prevented in a democratic society, it is difficult to ensure their regular visits to DOTS facilities. If all diagnosed patients with TB are provided with an identity card, it may enable them to receive treatment from any part of the country.

Ethical Aspects and Human Rights

Patients with TB are stigmatized in many communities. Stigma leads to bias, marginalization, and even denial of basic human rights such as women being abandoned by their families or children being forced to discontinue schooling. Using services of volunteers from the same community would deter patients who fear social stigma.

Cultural Factors

In culturally heterogeneous societies, health-care personnel need to overcome linguistic and cultural barriers because doctors may unknowingly offend or fail to understand patients' perceptions (Grange and Festenstein, 1993).

Involving Private Medical Practitioners

Training and cooperation of private medical practitioners is essential for success of the DOTS program. In most developing countries, patients tend to seek treatment from private medical practitioners where services are perceived to be good, but costly. Ayurveda, Unani, Siddha, Homeopathy (AYUSH) medical practitioners need to be acquainted with DOTS program and persuaded to refer patients with TB to the nearest DOTS centre. After adequate orientation, their services may be pooled for case holding.

General Measures

The decline in prevalence of TB in the developed countries occurred before the advent of antitubercular drugs. This decline is attributed to improved socioeconomic conditions and better nutrition. Tobacco smoking and high alcohol consumption reduce body defenses. Discouraging the use of these substances may help in reducing vulnerability to TB. Health-care personnel themselves may set an example and contribute to reducing consumption of these substances.

Organizations Associated with Tuberculosis

- National Association for the Prevention of Tuberculosis (NAPT): It was formed in England in 1898 and advocated for free sanatorium beds for patients under health

insurance schemes, scholarships for TB workers, and held the first conference on TB in 1901. NAPT campaigned for compulsory notification of TB, which became law in England and Wales in 1913.

- International Union Against Tuberculosis and Lung Diseases: In 1902, Sir Robert Philip organized this international body which adopted the double-barrelled Cross of Lorraine as its symbol in 1903. The IUATLD is involved in producing educational and training materials and organizes a biennial (two-yearly) international conference. It publishes a peer-reviewed medical journal.
- Tuberculosis Association of India (TAI): It was established on 22nd February 1939, due to the efforts of Lady Linlithgow, wife of the then Viceroy of India. TAI was involved in managing a TB center at New Delhi and sanatoria at Kasauli (Himachal Pradesh) and Mehrauli (near Delhi). The activities of TAI include education of the public, training of personnel, promoting research, and publishing a peer-reviewed medical journal *Indian Journal of Tuberculosis*. TAI published the first Indian text-book on TB. The director general of health services, government of India, is the ex-officio chairman of the TAI. The planning commission considers the recommendations of the Standing Committee of the TAI in 5-year plans. The organization maintains working relations with IUATLD, WHO, and UNICEF.

Role of Voluntary Organizations

The voluntary organizations play a role in:

- Case detection, supervised drug administration, defaulter action, and ensuring high cure rate,
- Health education campaigns,
- Cooperation with governmental and municipal organizations to prevent duplication of efforts.
- Covering gaps in the program.

Low Priority to Childhood Tuberculosis

Unfortunately, children are not the priority targets for national TB control programs, which is probably because of resource constraints and also because it is postulated that childhood TB is rarely infectious to others, more difficult to diagnose, and there is a misplaced faith in efficacy of BCG against childhood TB. Childhood TB ought to be prioritized in a DOTS program because children represent a reservoir of future adult cases because children older than 7 years of age may suffer from reactivated (adult-type) TB that is infectious to others. Any immune suppressive condition, such as malnutrition, measles, vitamin deficiency, and HIV infection, can predispose children to the risk of developing active TB, and young children with TB are more vulnerable to develop severe complications of the disease.

Inadequate Health Education

Majority of patients with active diseases do not seek treatment because of the lack of knowledge about the disease. Others are deterred by the social stigma attached to TB. Some still believe that the disease is incurable and that seeking treatment would be futile and would

expose the patient to social ostracism. Illiteracy is also a causative factor for non-adherence to the prescribed drug regimen. Once relieved of symptoms, patients stop medication in the belief that they are cured. This could be overcome by continued patient education.

Poverty

Even before the advent of antitubercular drugs, there was a decrease in the incidence of the disease in England and Wales. This was as a result of the improvement in socioeconomic conditions following the Industrial Revolution. Currently, in both developed and developing countries, TB is a disease that afflicts the impoverished and the underprivileged. This issue can be solved only by sociopolitical interventions.

References

Agarwal U, Kumar A, Behera D, French MA, Price P. 2012. Tuberculosis associated immune reconstitution inflammatory syndrome in patients infected with HIV: meningitis a potentially life threatening manifestation. *AIDS Research and Therapy* 9:17 doi:10.1186/1742-6405-9-17.

Ahmad A, Afghan S, Raykundalia C, Catty D. 1998. Diagnosis of tuberculosis by using enzyme-linked immunosorbent assay (ELISA) to detect anti-mycobacterial superoxide dismutase in patients. *J Islamic Acad Sci* 11:1–8.

Alamelu R. 2004. Immunology of tuberculosis. *Indian J Med Res* 120:213–232.

Albert H, Heydenrych A, Brookes R, Mole RJ, Harley B, et al. 2002. Performance of a rapid phage-based test, FAST Plaque TB, to diagnose pulmonary tuberculosis from sputum specimens in South Africa. *Int J Tuberc Lung Dis* 6:529–537.

American Thoracic Society. 2003. Treatment of Tuberculosis. *Am J Respir Crit Care Med* 167: 602–662.

Anderson L, Moore J, Kruijshaar M, Pedrazzoli D, Bradshaw L, et al. 2010. *Tuberculosis in the UK: Annual Report on Tuberculosis Surveillance in the UK 2010*. London: Tuberculosis Section, Health Protection Agency Centre for Infections.

Antiobiotics: Types and treatments. EMedExpert.com. Accessed March 25, 2013, at www.emedexpert.com/classes/antibiotics.shtml.

Arend SM, Andersen P, van Meijgaarden KE, Skjot RL, Subronto YW, et al. 2000. Detection of active tuberculosis infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10. *J Infect Dis* 181:1850–1854.

Banu Rekha VV, Balasubramanian R, Swaminathan S, Ramachandran R, Rahman F, et al. 2007. Sputum conversion at the end of intensive phase of Category-1 regimen in the treatment of pulmonary tuberculosis patients with diabetes mellitus or HIV infection: An analysis of risk factors. *Indian J Med Res* 126:452–458.

Bellen AL, Ang C, Montoya J, Mendoza MT. 2003. Accuracy of a bacteriophage-based assay in the rapid diagnosis of pulmonary tuberculosis. *Phil J Microbiol Infect Dis* 32:1–10.

Beyers N, Gie RP, Schaaf HS, Van Zyl S, Talent JM, et al. 1997. A prospective evaluation of children under the age of 5 years living in the same household as adults with recently diagnosed pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1:31–37.

Bhamidi S. 2009. *Mycobacterial Cell Wall Arabinogalactan Bacterial Polysaccharides. Current Innovations and Future Trends*. Norfolk, UK: Caister Academic Press.

Bhargava A, Pinto L, Pai M. 2011. Mismanagement of tuberculosis in India: Causes, consequences, and the way forward. *Hypothesis* 9:e7.

Bothamley G, Batra H, Ramesh V, Chandramui A, Ivanyi J. 1992. Serodiagnostic value of the 19 kilodalton antigen of *Mycobacterium tuberculosis* in Indian patients. *Eur J Clin Microbiol Infect Dis* 11:912–915.

Bothamley GH. 1995. Serological diagnosis of tuberculosis. *Eur Respir J* 8 (Suppl. 20):676s–688s.
Brooks JB, Daneshvar MI, Fast DM, Good RC. 1987. Selective procedures for detecting femtomole quantities of tuberculostearic acid in serum and cerebrospinal fluid by frequency-pulsed electron-capture gas-liquid chromatograph. *J Clin Microbiol*;25:1201–1216.
Bryson YJ, Pang S, Wei LS, Dickover R, Diagne A, et al. 1995. Clearance of HIV infection in a perinatally infected infant. *N Engl J Med* 332:833–838.
Calain P, Fidler DP. 2007. XDR Tuberculosis, the New International Health Regulations, and Human Rights. *Global Health Governance I*(1): January.
Campos-Neto A, Rodrigues-Júnior V, Pedral-Sampaio DB, Netto EM, Ovendale PJ, et al. 2001. Evaluation of DPPD, a single recombinant *Mycobacterium tuberculosis* protein as an alternative antigen for the Mantoux test. *Tuberculosis (Edinb.)* 81:353–358.
Caviedes L, Lee TS, Gilman RH, Sheen P, Spellman E, et al. 2003. Rapid, efficient detection and drug susceptibility testing of *Mycobacterium tuberculosis* in sputum by microscopic observation of broth cultures. *J Clin Microbiol* 38:1203–1208.
Collins CH, Yates MD, Grange JM. 1982. Subdivision of *Mycobacterium tuberculosis* into five variants for epidemiological purposes: methods and nomenclature. *J Hyg* 89:235–242.
De Wet JR, Wood KV, DeLuka M, Helsinki DR, Subrahmanyam S. 1987. Firefly luciferase gene: Structure and expression in mammalian cells. *Mol Cell Biol* 7:725–737.
Del Prete R, Picca V, Mosca A, D'Alagni M, Miragliotta G. 1998. Detection of anti-lipoarabinomannan antibodies for the diagnosis of active tuberculosis. *Int J Tuberc Lung Dis* 2:160–163.
Dhayagude RG, Shah BR. 1948. Variation in the virulence of *M. tuberculosis* and its correlation with the clinical type of tubercular disease. *Ind J Med Res* 36:79–89.
Fadda G, Ardito F, Sanguinetti M, Posteraro B, Ortona L, et al. 1998. Evaluation of the Abbott LCx *Mycobacterium tuberculosis* assay in comparison with culture methods in selected Italian patients. *New Microbiol* 21:97–103.
Ferrara G, Losi M, D'Amico R, Roversi P, Piro R, et al. 2006. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. *Lancet* 367:1328–1334.
Foo DG, Tay HC, Siew JY, Singhal A, Camacho L, et al. 2011. T cell monitoring of chemotherapy in experimental rat tuberculosis. *Antimicrob Agents Chemother* 55:3677–3683.
Fracastoro, Girolamo. n.d. Universalium. Accessed March 25, 2013, at http://universalium.academic.ru/239379/Fracastoro,_Girolamo.
Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen G, et al. 1993. The emergence of drug resistant tuberculosis in New York City. *N Engl J Med* 328:521–526.
Gamboa F, Dominguez J, Padilla E, Manterola JM, Gazapo E, et al. 1998. Rapid diagnosis of extrapulmonary tuberculosis by ligase chain reaction amplification. *J Clin Microbiol* 36:1324–1329.
Ghosh J, Larsson P, Singh B, Fredrik Pettersson BM, Islam NM, et al. 2009. Sporulation in mycobacteria. *Proc Natl Acad Sci USA* 106:10781–10786.
Global Tuberculosis Institute. n.d. A history of tuberculosis treatment. Accessed March 25, 2013, at http://www.umdnj.edu/ntbc/tbhistory.htm.
Grange J. 1992. The mystery of the mycobacterial "persistor." *Tubercle and Lung Disease* 73:249–251.
Grange JM. 1979. Tuberculosis: The changing tubercle. *Br J Hosp Med* 22(6):540–548.
Grange JM, Aber VR, Allen BW, Mitchison DA, Goren MB. 1978. The correlation of bacteriophage types of *Mycobacterium tuberculosis* with guinea-pig virulence and *in vitro* indicators of virulence. *J Gen Microbiol* 108:1–7.
Grange JM, Festenstein F. 1993. The human dimension of tuberculosis control. *Tuber Lung Dis* 74:219–222.
Hermann Brehmer. n.d. Wikipedia. Accessed March 25, 2013, at en.wikipedia.org/wiki/Hermann_Brehmer.
Hernandez-Pando R, Rook GAW. 1994. The role of TNFα in T cell-mediated inflammation depends on the Th1/Th2 cytokine balance. *Immunology* 82:591–595.

Hongmanec P, Stender H, Rasmussen OF. 2001. Evaluation of a fluorescence in situ hybridization assay for differentiation between tuberculous and non-tuberculous mycobacterium species in smears of Lowenstein–Jensen and mycobacteria growth indicator tube culture using peptide nucleic acid probes. *J Clin Microbiol* 39:1032–1035.

Houghton RL, Lodes MJ, Dillon DC, Reynolds LD, Day CH, et al. 2002. Use of multiepitope polyproteins in serodiagnosis of active tuberculosis. *Clin Diagn Lab Immunol* 9:883–891.

Irgens L. 2002. The discovery of the leprosy bacillus. *Tidsskr nor Laegeforen* 122:708–709.

Jacobs WR Jr, Barletta RG, Udani R, Chan J, Kalkut G, et al. 1993. Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. *Science* 260:819–822.

Janse van Rensburg P, Andronikou S, van Toorn R, Pienaar M. 2008. Magnetic resonance imaging of miliary tuberculosis of the central nervous system in children with tuberculous meningitis. *Pediatr Radiol* 38:1306–1313.

Jean-Antoine Villemin. n.d. Wikipedia. Accessed March 25, 2013, at en.wikipedia.org/wiki/Jean_Antoine_Villemin.

Jonas V, Alden MA, Curry JI, Kamisango K, Knott CA, et al. 1993. Detection and identification of Mycobacterium tuberculosis directly from sputum sediments by amplification of rRNA. *J Clinc Microbiol* 31:2410–2416.

Kartikeyan S, Bharmal RN, Tiwari RP, Bisen PS. 2007. Co-infection with HIV and Tuberculosis. In: Kartikeyan S, Bharmal RN, Tiwara RP, Bisen PS, *HIV and AIDS: Basic Elements and Priorities*, pp. 183–196. Dordrecht: Springer.

Kashyap S, Mohapatra PR, Saini V. 2003. Endobronchial tuberculosis. *Indian J Chest Dis Allied Sci* 45:247–256.

Katoch VM. 2004. Infections due to non-tuberculous mycobacterial. *Indian J Med Res* 120:209–304.

Kellar KL, Gehrke J, Weis SE, Mahmutovic-Mayhew A, Davila B, et al. 2011. Multiple cytokines are released when blood from patients with tuberculosis is stimulated with Mycobacterium tuberculosis antigens. *PLoS One* 6:e26545.

Kellogg JA, Bankert DA, Withers GS, Sweimler W, Kiehn TE, et al. 2001. Application of the Sherlock *Mycobacteria* identification system using high-performance liquid chromatography in a clinical laboratory. *J Clin Microbiol* 39:964–970.

Kent DC. 1967. Tuberculous lymphadenitis: not a localized disease process. *Am J Med Sci* 254:866–874.

Kent PT, Kubica GP. 1985. *Public Health Mycobacteriology: A Guide for the Level III Laboratory*. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention.

Khanum T, Rasool AJ, Ajaz M, Khan AS. 2011. Isolation—Drug resistance profile and molecular characterization of indigenous typical and atypical Mycobacteria. *Pak J Pharm Sci* 24:527–532.

Kilincer A. 2010. Progressive primary tuberculosis. *Imaging Science Today* 335.

Koch R. Die 1882. Atiologic der Tuberkulose. *Berliner Klinische Wochenschrift* 15:221–230.

Kumar V, Abbas AK, Fausto N, Mitchell RN. 2007. *Robbins Basic Pathology*, 8th ed., pp. 516–522. Amsterdam: Elsevier.

Lawn SD, Zumla AI. 2011. Tuberculosis. *Lancet* 378:57–72.

Lienhardt C, Bennett S, Del Prete G, Bah-Sow O, Newport M, et al. 2002. Investigation of environmental and host-related risk factors for tuberculosis in Africa I. Methodological aspects of a combined design. *Am J Epidemiol* 155:1066–1073.

Mahadev B, Kumar P. 2003. History of tuberculosis control in India. *J Indian Med Assoc* 101:142–143.

Mandell GL, Bennett JE, Dolin R. 2010. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 7th ed. Philadelphia: Elsevier.

Marks J. 1976. A system for the examination of tubercle bacilli and other mycobacteria. *Tubercle* 57:207–225.

McMurray DN. 1996. Mycobacteria and Nocardia. In: Baron S ed., *Medical Microbiology*, 4th ed., chapter 33. Galveston: University of Texas Medical Branch at Galveston.
Mitchison DA. 1985. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle* 66:219–225.
Moore DA, Mendoza D, Gilman RH, Evans CA, Hollm Delgado MG, et al.; Tuberculosis Working Group in Peru. 2004. Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug-resistant tuberculosis suitable for use in resource-poor settings. *J Clin Microbiol* 42:4432–4437.
Morello JA, Granato PA, Wilson ME, Morton V. 2006. *Laboratory Manual and Workbook in Microbiology: Applications to Patient Care*, 10th ed. Boston: McGraw-Hill.
Mosmann TR, Moore KW. 1991. The role of 11-10 in cross regulation of Thl and Th2 responses. *Immunol Today* 12:A49–A53.
Mullis KB, Faloona FA. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155:335–350.
Muzaffar R, Batool S, Aziz F, Naqvi A, Rizvi, A. 2002. Evaluation of the FAST Plaque TB assay for direct detection of *Mycobacterium tuberculosis* in sputum specimens. *Int J Tuberc Lung Dis* 6:635–640.
Nakamura RM, Velmonte MA, Kawajiri K, Ang CF, Frias RA, et al. 1998. MPB 64 mycobacterial antigen: a new skin test reagent through patch method for rapid diagnosis of active tuberculosis. *Int J Tuberc Lung Dis* 2:541–546.
Ngadaya ES, Mfinanga GS, Wandwalo ER, Morkve O. 2009. Detection of pulmonary tuberculosis among patients with cough attending outpatient departments in Dar Es Salaam, Tanzania: Does duration of cough matter? *BMC Health Services Research* 9:112.
Nichols EH. n.d. Tuberculosis of bones and joints. Accessed March 25, 2013 at http://jbjs.org/data/Journals/JBJS/981/353.pdf.
Nogales-Ortiz F, Tarancion I, Nogales FF Jr. 1979. The pathology of female genital tuberculosis: A 31-year study of 1436 cases. *Obstet Gynecol* 53:422.
Orenstein EW, Basu S, Shah NS, Andrews JR, Friedland GH, et al. 2009. Treatment outcomes among patients with multidrug-resistant tuberculosis: Systematic review and meta-analysis. *Lancet Infect Dis* 9:153–161.
Ormerod LP. 2005. Multidrug-resistant tuberculosis (MDR-TB): Epidemiology, prevention and treatment. *Br Med Bull* 73:17–24.
Parsons LM, Somoskövi A, Gutierrez C, Lee E, Paramasivan CN, et al. 2011. Laboratory diagnosis of tuberculosis in resource poor countries: Challenges and opportunities. *Clin Microbiol Rev* 24:314–350.
Pereira Arias-Bouda LM, Nguyen LN, Ho LM, Kuijper S, Jansen HM, et al. 2000. Development of antigen detection assay for diagnosis of tuberculosis using sputum samples. *J Clin Microbiol* 38:2278–2283.
Petroff SA. 1915. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces *J Exp Med* 21:38–42.
Pottumarthy S, Wells VC, Morris AJ. 2000. A comparison of seven tests for serological diagnosis of tuberculosis. *J Clin Microbiol* 38:2227–2231.
Ramachandran R, Paramasivan CN. 2003. What is new in the diagnosis of tuberculosis? Part I: Techniques for diagnosis of tuberculosis. *Indian J Tuberc* 50:133–141.
Rattan A. 2000. PCR for diagnosis of tuberculosis: Where are we now? *Indian J Tuberc* 47:79–82.
Rich AR, Lewis MR. 1932. Bull. *Johns Hopkins Hosp* 50:115.
Rohner P, Ninet B, Metral C, Emler S, Auckenthaler R. 1997. Evaluation of the MB/Bac T system and comparison with BACTEC 460 systems and solid media for isolation of mycobacteria from clinical specimens. *J Clin Micribiol* 35:3127–3131.
Ryan KJ, Ray CG. 2004. *Sherris Medical Microbiology*, 4th ed. New York: McGraw Hill.
Savic B, Sjobring U, Alugupalli S, Larsson L, Miörner H. 1992. Evaluation of polymerase chain reaction, tuberculostearic acid analysis and direct microscopy for the detection of *Mycobacterium tuberculosis* in sputum. *J Infect Dis* 166:1177–1180.

Semlali S, El Kharras A, Mahi M, Hsaini Y, Benameur M, et al. 2008. Imaging features of CNS tuberculosis. *J Radiol* 89:209–220.

Shah JS, Liu J, Buxton D, Stone B, Nietupski R, et al. 1995. Detection of *Mycobacterium tuberculosis* directly from spiked human sputum by Q-beta replicase-amplified assay. *J Clin Microbiol* 33:322–328.

Shashidhara AN, Chaudhuri K. 1990. The tuberculin skin test: Emerging 100 years since its first use. Bangalore, India: National Tuberculosis Institute. Accessed March 25, 2013, at http://openmed.nic.in/419/01/NBTSSH90.PDF.

Spargo CA, Haaland PD, Jurgensen SR, Shank DD, Walker GT. 1993. Chemiluminescent detection of strand displacement amplified DNA from species comprising the *Mycobacterium tuberculosis* complex. *Mol Cell Probes* 7:395–404.

Stanford JL, Grange JM. 1974. The meaning and structure of species as applied to mycobacteria. *Tubercle* 55:143–152.

Stanford JL, Stanford CA. Immunotherapy with *Mycobacterium vaccae* and the treatment of tuberculosis. *Soc Appl Bacteriol Symp Ser* 1996;25:81S–86S.

State of New Jersey. n.d. History of tuberculosis. Accessed March 25, 2013, at http://www.state.nj.us/health/tb/documents/tbstats/history_of_tuberculosis.pdf.

Suchindran S, Brouwer ES, Van Rie A. 2009. Is HIV Infection a risk factor for multi-drug resistant tuberculosis? A systematic review. *PLoS ONE* 4:e5561.

Sylvius Fransiscus. n.d. Wikipedia. Accessed March 25, 2013, at en.wikipedia.org/wiki/History_of_tuberculosis.

Tansuphasiri U, Chinrat B, Rienthong S. 2001. Evaluation of culture and PCR-based assay for detection of *Mycobacterium tuberculosis* from sputum collected and stored on filter paper. *South East Asian J Trop Med Public Health* 32:844–855.

Tiwari RP, Hattikudur NS, Bharmal RN, Kartikeyan S, Deshmukh NN, et al. 2007. Modern approaches to a rapid diagnosis of tuberculosis: Promises and challenges. *Tuberculosis (Edinb)* 87:193–201.

Tiwari RP, Tiwari D, Garg SK, Chandra R, Bisen PS. 2005. Glycolipids of *Mycobacterium tuberculosis* strain H37Rv are potential serological markers for diagnosis of active tuberculosis. *Clin Diagn Lab Immunol* 12:465–473.

Tsukamura M. 1966. Adansonian classification of *Mycobacteria*. *J Gen Microbiol* 45:253–273.

Van Soolingen D, de Haas PE, Hermans PW, van Embden JD. 1994. DNA fingerprinting of *Mycobacterium tuberculosis*. *Methods Enzymol* 235:196–205.

Viswaroop BS, Kekre N, Gopalakrishnan G. 2005. Isolated tuberculous epididymitis: A review of forty cases. *J Postgrad Med* 51:109–111.

von Pirquet CP. 1907. Tuberkulindiagnose durch cutane Impfung. *Berliner klinische Wochenschrift* 44:644–645.

Walker GT, Little MC, Nadeau JG, Shank DD. 1992. Isothermal in vitro amplification of DNA by a restriction enzyme/DNA polymerase system. *Proc Natl Acad Sci USA* 89:392–396.

Walport M, Kenneth M, Janeway C, Travers PJ. 2008. *Janeway's Immunobiology*, 7th ed. New York: Garland Science.

Wayne LG. 1976. Dynamics of submerged growth of Mycobacterium tuberculosis under aerobic and microaerophilic conditions. *Am Rev Respir Dis* 114:807–811.

Wilson SM, McNemy R. 1997. Evaluation of a new rapid bacteriophage-based method for drug susceptibility testing of *M. tuberculosis*. *Nat Med* 3:465–468.

Woods GL, Fish G, Plaunt M, Murphy T. 1997. Clinical evaluation of Difco ESP culture system II for growth and detection of mycobacteria. *J Clin Microbiol* 35:121–124.

World Health Organization (WHO). 2011. Global tuberculosis control. Accessed February 12, 2013, at http://whqlibdoc.who.int/publications/2011/9789241564380_eng.pdf.

World Health Organization (WHO). 2007. Global tuberculosis control: Surveillance, planning. Accessed February 12, 2013, at http://www.who.int/tb/publications/global_report/en/index.html.

World Health Organization (WHO). 2010. *International Classifications of Diseases*. Accessed February 12, 2013, at http://apps.who.int/classifications/icd10/browse/2010/en.

World Health Organization (WHO). 2007. Revised BCG vaccination guidelines for infants at risk for HIV infection. *Wkly Epidemiol Rec* 2007;82:193–196.

World Health Organization (WHO). 1994. TB: A global emergency. Accessed February 12, 2013, at http://whqlibdoc.who.int/hq/1994/WHO_TB_94.177.pdf.

Yagupsky PV, Kaminski DA, Palmer MK, Nolte FS. 1990. Cord formation in BACTEC 7H12 medium for rapid, presumptive identification of *Mycobacterium tuberculosis* complex. *J Clin Microbiol* 28:1451–1453.

Zwolska Z. 2010. Koch i jego dokonania-Rys historyczny. *Post. Mikrobiol* 49(3):139–150. Accessed March 25, 2013, at www.pm.microbiology.pl/web/pelne/vol4932010.pdf.

Chapter 6
Plague

History of Plague

In 1894, two bacteriologists, Alexandre Yersin of France and Kitasato Shibasaburo of Japan independently isolated the bacterium *Yersinia pestis* in Hong Kong during the third pandemic. Although both reported their findings, the statements given by Kitasato were confusing and so Yersin was considered as the primary discoverer of the bacterium. He named it *Pasteurella pestis* in honor of the Pasteur Institute in France where he worked, but in 1967, this name was replaced by *Yersinia pestis* in honor of Yersin (Bibel and Chen, 1976). Yersin reported that plague affected rats not only during plague epidemics, but also often preceding such epidemics in humans, and many locals regarded plague as a disease of rats. Villagers in China and India asserted that, when large numbers of rats were found dead, plague outbreaks soon followed.

In 1898, the French scientist Paul-Louis Simond suggested that the rat-flea vector transmitted the disease. He reported that persons who became ill did not acquire the disease by close contact. In Yunnan, China, inhabitants would flee from their homes as soon as they saw dead rats, whereas the residents of the island of Formosa (Taiwan) thought that the handling of dead rats heightened the risks of developing plague. These observations led Simond to suspect that the flea might be an intermediary factor in the transmission of plague because people acquired plague only if they were in contact with recently dead rats that had died less than 24 hours before. Through another experiment, he demonstrated the plague death of a healthy rat after infected fleas had jumped to it from a rat that had recently died of the plague.

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Magnitude

Global

Although so many cases of plague in ancient times may be attributable to *Y. pestis*, the cycles of plague epidemics can be divided into three pandemic eras.

- **The First Pandemic** (between 541 and 750 A.D.): On record, the first known attack was the Justinian plague (541–544) that began in Pelusium, Egypt, after arriving from Ethiopia. This was the first firmly recorded evidence of bubonic plague. The disease appeared in China (Nicholas, 2010), then spread to Africa from where the vast city of Constantinople imported massive amounts of grain, mostly from Egypt, to feed its citizens. The grain ships were the source of contagion for the city, with massive public granaries nurturing the rat and flea population. At its peak, the plague killed 10,000 people in Constantinople every day and ultimately destroyed perhaps 40 percent of the city's inhabitants. It went on to destroy up to a quarter of the human population of the eastern Mediterranean. This pandemic eventually affected the known world as North Africa, Europe, central and southern Asia (eastern Asia was largely unaffected), and Arabia. The death rates were between 15 and 40 percent for locales and from 541 to 700, there was an estimated population loss of 50 to 60 percent (Duplaix, 1988; Russell, 1968). This depopulation is not exactly as a result of the plague because other epidemics such as smallpox probably also occurred during this period.
- **The Second Pandemic or the Black Death** (between 1347 and1351): The plague that spread from 1330 to 1346 started from the central Asia and then migrated into Sicily in 1347. This was the start of the second pandemic, referred to as the Black Death, a massive and deadly pandemic originating in China. It reduced the world's population from 450 million to between 350 and 375 million. China lost around half of its population, from around 123 million to around 65 million; Europe around one-third of its population, from about 75 million to about 50 million; and Africa approximately one-eighth of its population, from around 80 million to 70 million (mortality rates correlated with population density so Africa, being less dense overall had the lowest death rate). This makes the Black Death the largest death toll from any known nonviral epidemic. On the other hand, northeastern Germany, Bohemia, Poland, and Hungary suffered less, and there are no estimates available for Russia or the Balkans. Russia was not terribly affected probably because of its extremely cold climate and large size and often less close contact with the contagion.

  This second pandemic was more accelerated and directed significant changes in economic, social, political, religious, and medical systems and convictions.
- **The Third Pandemic**: This began in China in 1855, spreading plague to the whole continent, and ultimately killed more than 12 million people in India and China alone. Casualty patterns showed that waves of this pandemic might have come from two different sources. The first primarily was bubonic and carried around the world through ocean-going trade, transporting infected persons, rats, and cargoes harboring fleas. The second, more virulent strain was primarily pneumonic, with a strong person-to-person transmission. This strain largely confined to Manchuria and Mongolia. Researchers during this pandemic identified plague vectors, and the plague bacterium lead to the discovery of modern treatment methods.

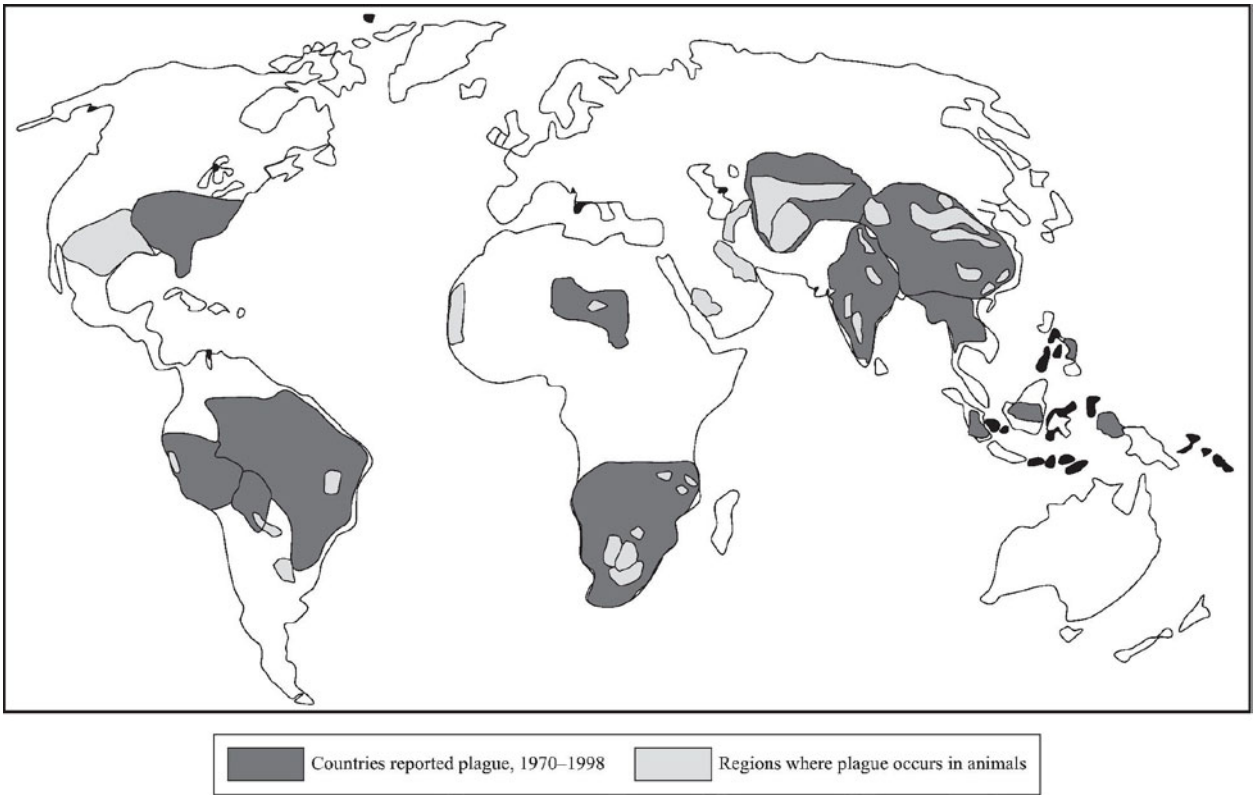

Figure 6.1. World distribution of plague, 1998.

Apart from these pandemics, some sporadic cases of plague have also been reported throughout the world, for example, according to the *Latin American Herald Tribune* (2010) plague cases were reported in Peru in 1994 and 2010. Other cases were reported in Oregon in the United States in 2010 and in 2012 (Nogueras, 2010; Adetunji, 2012). Figure 6.1 shows the worldwide distribution of the plague bacterium.

India

In India, there was a pneumonic plague epidemic in Surat in 1994, which resulted in 52 deaths, and in a large internal migration of about 300,000 residents, who fled fearing quarantine (Plague (disease), n.d.). The cause of this epidemic was heavy monsoon rain and clogged sewers, which led to massive flooding and resulted in unhygienic conditions and a number of uncleared animal carcasses (*New York Times*, 1994). There was widespread fear that the flood of refugees might spread the epidemic to other parts of India and finally the world, but that scenario was averted, probably as a result of effective public health response mounted by the Indian health authorities. The Indian health authorities were unable to culture *Y. pestis* and that might be as a result of poor laboratory facilities, so the reason behind the epidemic remains unresolved (Hazarika, 1995). Yet, there are several lines of evidence strongly suggesting that it was a plague epidemic because blood tests for *Yersinia* were positive; people showed antibodies against *Yersinia*; and the clinical symptoms displayed by the affected were all consistent with the plague. In February 2002, a small outbreak of pneumonic plague took place in the Shimla District of Himachal Pradesh state, in northern India (Gupta and Sharma, 2007).

According to the National Institute for Communicable Diseases in New Delhi, India, the plague endemic areas are Shimla (Himachal Pradesh), Uttarkashi (Uttaranchal), Surat (Gujrat), Beed (Maharashtra), Kolar and Attibele (Karnataka), Chittor (Andhrapradesh), Nilgiris, Krishnagiri, and Dharmapuri (Tamil Nadu).

Agent Factors

Y. pestis is a gram-negative rod-shaped, nonmotile, facultative anaerobic bacterium that can infect humans and other animals (Rayn and Ray, 2004). Virulent forms of the bacteria can survive dormant in soil, animal carcasses, grains, flea feces, buried bodies, and dried sputum (Ayyadurai et al., 2008; Casman and Fischhoff, 2008).

Classification

Yersinia was previously classified in the family *Pasteurellaceae*, but based on DNA-DNA hybridization similarities to *Escherichia coli* the *Yersinia* group had been reclassified as members of the *Enterobacteriaceae* family (Farmer, 1995). *Enterobacteriaceae* family members could be differentiated on the basis of biochemical and antigenic characteristics. Recently, nucleic acid techniques are applied to assist the definition of genera and species within this family, and as more techniques are applied, newly defined genetic relationships sometimes lead to changes in classification. Though there are 11 named species in the genus *Yersinia*, only 3 are considered fundamental human pathogens: *Y. pestis*, the etiologic agent of plague, and the enteropathogenic strains, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. *Y. pseudotuberculosis* is the closest genetic relative to *Y. pestis*,

but can easily be distinguished from the plague bacteria, based on its clinical manifestations and by laboratory test results. Both *Y. pestis* and *Y. pseudotuberculosis* infect humans rarely in contrast to *Y. enterocolitica*, which is more commonly found in clinical specimens.

Morphology and Cultural Characterists

Y. pestis exhibits a striking bipolar staining when stained with Wayson's, Wright, Giemsa, or methylene blue stain and shows classical safety pin–like appearance, but this is not always seen with all the isolates of *Y. pestis*. Growth is rapid in media containing blood or tissue fluids and shows fastest growth at 86° F(30° C). It is catalase positive and oxidase negative, which is non motile at 98.6° F (37° C) and room temperature. This is the only *Yersinia* species that is non motile at room temperature. It grows well on most of the nonselective standard laboratory media (e.g., sheep blood, chocolate, and tryptic soy agars) and produces pinpoint, gray white, nonhemolytic colonies after 24 hours. After 48 hours, these colonies resemble typical enteric bacterial colonies, and incubation up to 72 hours produces colonies that are gray-white to slightly yellow, opaque, raised, irregular fried-egg appearance or alternatively may have a hammered copper shiny surface. On MacConkey's agar medium, it produces nonlactose fermenting colonies.

Antigenic Structure

Being a gram-negative bacterium, *Y. pestis* possesses a lipopolysaccharide cell wall that on release shows endotoxic activity. The envelope contains a protein fraction (fraction I), produced at 98.6° F (37° C), conferred antiphagocytic activity and then involved in complement activation. Virulent strains of *Y. pestis* carry V-W antigens, which are encoded by plasmid genes.

Host Factors

Age and Sex

The disease typically occurs in persons younger than 20 years. It can occur in both male and female, but it is more common in males rather than females the frequency is approximately 50 percent.

Risk Groups

Persons exposed to rodent fleas, wild rodents, or other susceptible animals in enzootic areas. Highest rates are seen in American Indians, especially Navajos; other risk groups, include hunters, veterinarians, and pet owners handling infected cats and campers or hikers.

Risk Factors

The following risk factors may be associated with plague epidemic:

- Living in a rural area and especially in areas where plague is common.
- Contact with sick animals, small rodents, or other possible hosts.

- Participating in wilderness activities, such as camping, hiking, sleeping on the ground, and hunting.
- Exposure to flea bites.
- Exposure to naturally occurring plague in the community.
- Occupation as a veterinarian.
- Outdoor activity during the summer months.
- Travel: Although contracting plague while visiting another country is rare, doctors may suspect that a flea might have bitten a patient with plague-like symptoms who has recently traveled abroad to areas where plague is present. Fewer than 10 percent of flea-bitten people remember a flea having bitten them.
- Animal contact: Close contact with infected animals and travel through rural areas.

Environmental Factors

In Surat, India, a combination of heavy monsoon rain and clogged sewers led to massive flooding resulting in unhygienic conditions and a number of uncleared animal carcasses. These factors probably contribute in the emergence of plague epidemic.

Reservoir

More than 200 different rodent species can serve as a reservoir for plague bacterium. These include domestic cats and dogs, squirrels, chipmunks, marmots, deer mice, rabbits, hares, rock squirrels, camels, and sheep. Fleas transfer the bacteria to susceptible animals such as ground squirrels, prairie dogs, and chipmunks. When a large numbers of host animals are dying off, hungry fleas search out for new food sources. This is an epizootic stage and ensures the spread of the organism to new territory. In contrast, humans are infected from wild animals in sylvatic stage (Figure 6.2). Rats have been the principal reservoir hosts of the plague. In the United States, ground and rock squirrels are the most common reservoir. In recent years, the domestic cat has emerged as a prominent reservoir of fleas that transmit the plague to veterinarians.

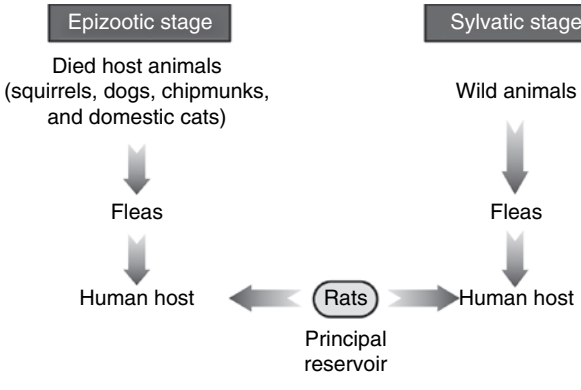

Figure 6.2. Reservoir for *Yersinia pestis*.

Mode of Transmission

Biological Transmission

Plague is mainly a disease in the fleas (*Xenopsylla cheopis*) that infested rats, making the rats the first victims of the plague. Human infection occurs, when a flea that has already been infected during biting a rodent (which itself has already been infected by the bite of a flea carrying the disease) bites a person. Biological transmission requires growth of *Y. pestis* in the digestive tract of flea to produce a proventricular infection. The bacteria multiply inside the flea, sticking together to form a plug that blocks flea's stomach and starve it. The flea then bites a host and continues to feed, even though it cannot quell its hunger, and consequently it vomits blood tainted with the bacteria back into the bite wound. The plague bacterium then infects a new victim, and the flea eventually dies from starvation. *Y. pestis* infection of the flea is only confined to the digestive tract of the infected flea. Storage, digestion, and absorption of the blood meal all occur in the simple midgut made of a single layer of columnar epithelial cells and associated basement membrane. The proventriculus, a valve located at the base of the esophagus (Figure 6.3), which guards the entrance to the midgut, plays a central role in this transmission mechanism.

Apart from rat flea, there are 80 other species of flea associated with 200 species of wild rodents, which found to be infected with *Y. pestis* in nature and can transmit the disease to human beings.

Mechanical Transmission

Although biological transmission is the only reliable means for the transmission of plague bacterium (Burroughs, 1947; Pollitzer, 1954), mechanical transmission may also play a crucial role in the ecology of plague. For mechanical transmission, vector infection is not necessary; it just requires high septicemia levels in which *Y. pestis* survive on the blood-stained mouthparts of fleas between consecutive feedings. In this, the flea feeds on unin-

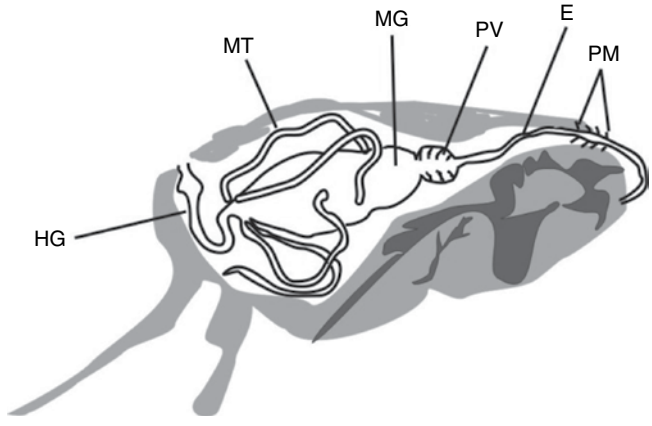

Figure 6.3. Digestive tract anatomy of fleas. E, esophagus; HG, hindgut; MG, midgut; MT, the malpighian tubules; PM, the muscles that pump blood into the midgut; PV, proventriculus.

fected mice 1 day after feeding on a highly septicemic mouse and transmits the disease. Because the time interval is too short for the development of proventricular infection, transmission presumably occurred by mechanical transfer of bacteria through contaminated mouth parts. The phenomenon of mechanical or mass transmission provides a potential mechanism for fleas that do not develop proventricular blockage readily, such as *Malaraeus telchinum* and the human flea *Pulex irritans*, to transmit *Y. pestis* during epidemics.

Others

Pneumonic plague can be also transmitted by inhaling infected droplets expelled by coughing, by a person or animal, especially domestic cats. Person-to-person transmission is uncommon; the organism enters through a break in the skin by direct contact with tissue or body fluids of an animal infected with the plague, for instance, especially in the process of skinning a rabbit or other animal.

Clinical Manifestations

Common clinical symptoms of a plague outbreak include fever, chills, body aches, sore throat, headache, weakness, general feeling of illness, nausea, vomiting (sometimes containing blood), constipation, diarrhea, black or tarry stools, stomach pains, cough (may contain blood), shortness of breath, stiff neck, heart irregularities, low blood pressure, confusion, and seizures, which appear later in the infection period. Based on the site of infection, *Y. pestis* causes three types of infection: bubonic plague, septicemic plague, and pneumonic plague.

Bubonic Plague

The term *bubonic plague* originated from the Greek word *bubo*, meaning "swollen gland." It is particularly an infection of the lymphatic system, resulting from the bite of an infected flea. The fleas found on infected rodents seek out other prey when their rodent hosts die. The bacteria form aggregates in the gut of infected fleas and this results in the flea regurgitating ingested blood, which becomes infected, into the bite site of another rodent or human host. Plague bacillus enters the skin from the site of the bite and migrates through the lymphatic system to the nearest lymph node. The lymph node then becomes inflamed because of the replication of *Y. pestis* in huge numbers. The organism can resist phagocytosis and even reproduce inside phagocytes and kill them. As the disease progresses, the lymph nodes can hemorrhage and become swollen and necrotic, exhibiting the most famous symptom of bubonic plague: painful, swollen lymph glands, called *buboes*. Buboes are commonly found in the groin region (Figure 6.4) but may also occur in the armpits or neck and are usually most often at the site of the initial infection (bite or scratch).

Septicemic Plague

In some cases, bubonic plague can progress and leads to the development of lethal septicemic plague. Septicemic plague is a blood infection of *Y. pestis*, which can cause disseminated intravascular coagulation and is almost always fatal (the mortality rate in the

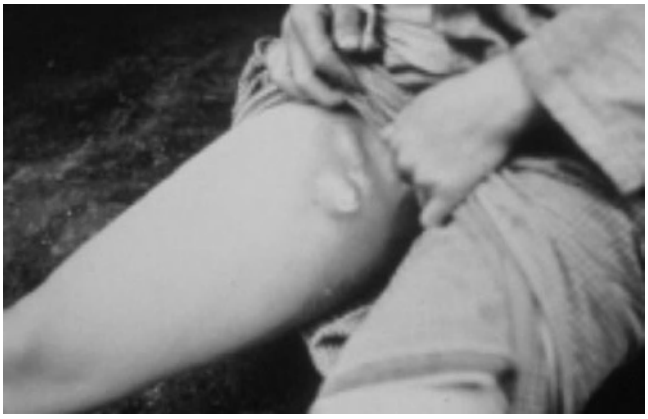

Figure 6.4. Bubo, a swollen lymph node in the groin area of a plague victim.

medieval times was 99–100 percent). It is the rarest of the three plagues that struck Europe in 1348. This disease is contracted through the bite of an infected rodent or bug, but it can also be contracted through cuts in the skin or by cough from another infected human. In the septicemic plague, bacteria multiply in the blood, causing bacteremia and severe sepsis and ultimately releasing bacterial endotoxins, which cause disseminated intravascular coagulation (DIC); tiny clots are formed throughout the body and ischemic necrosis (tissue death as a result of lack of circulation or perfusion to that tissue) from the clots is also possible. DIC results in depletion of the body's clotting resources, so that it cannot further control bleeding. Consequently, there is bleeding into the skin and other organs, which can cause red or black patchy rash and hemoptysis (coughing up or vomiting of blood). There are bumps on the skin that look like insect bites, which are usually red but are sometimes white in the center. People that contract this disease must receive treatment in at most 24 hours or death is inevitable. In some cases, people may even die on the same day they contract it.

Pneumonic Plague

Pneumonic plague is a lung infection that is more virulent, severe, and rarer than bubonic plague. The pneumonic form occurs as a result of a secondary spread from advanced infection of an initial bubonic form. Primary pneumonic plague results from inhalation of infective droplets containing bacilli and can be transmitted from human to human without involvement of fleas or animals. Untreated pneumonic plague has an extremely high fatality rate. This infection of the lung can be caused by two ways: primary, through the inhalation of aerosolized plague bacteria, or secondary, when septicemic plague spreads into lung tissue from the bloodstream. Pneumonic plague can spread from person to person and can result from the dissection or handling of contaminated animal tissue. This is formerly known Black Death (Benedictow, 2004), which could kill 90–95 percent of a population if the victims coughed and passed on the bacteria. In this infection, bleeding occurs into the tissues and turns the tissue black (Figure 6.5), hence the name "Black death," which originated from the deeply darkened skin, bleeding, and tissue death associated with septicemic and pneumonic plague.

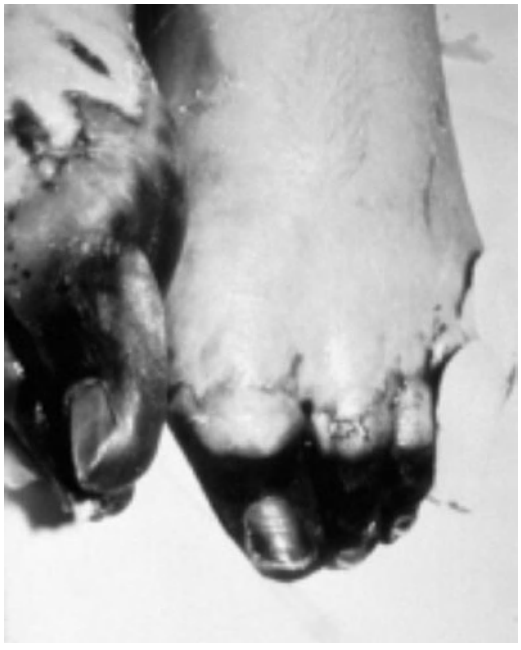

Figure 6.5. Toes showing gangrene following infection with *Yersinia pestis*. *Source*: www.emedicinehealth.com/image_gallery/article.htm.

This form of the disease is highly communicable because the bacteria can be transmitted in droplets emitted when coughing or sneezing and through physical contact with victims of the plague or flea-bearing rodents that carry the plague. The pneumonia may cause respiratory failure and shock, and patients will die without early treatment within 36 hours. Without diagnosis and treatment, the infection can be fatal in 1 to 6 days, and mortality in untreated cases is approximately 100 percent (Hoffman, 1980; Ryan and Ray, 2004).

Table 6.1 shows various signs and symptoms of all the three forms of plague. Apart from these three classical forms of plague, some other clinical manifestations found associated with the *Y. pestis* infection, such as:

- **Pharyngeal plague**: It is an uncommon form of plague that resembles tonsillitis and is found in cases of close contact of patients with other forms of plague (Centers for Disease Control and Prevention [CDC], 2011). Plague pharyngitis results from the contamination of the oropharynx with material infected with *Y. pestis*. Sources of infection include respiratory droplets expelled during coughing by a patient (or animal) with a respiratory plague infection (Meyer, 1961; LaForce et al., 1971) or ingestion of undercooked or raw tissues of an infected animal (Christie et al., 1980). Asymptomatic colonization of the pharynx has been reported in contacts of pneumonic plague patients (Marshall et al., 1967). Symptomatic plague pharyngitis is clinically similar to streptococcal or viral pharyngitis although the cervical lymphadenopathy of plague is often more severe and painful.

Table 6.1. Signs and symptoms of three classical types of plague.

Type of Infection	Incubation Period	Signs and Symptoms
Bubonic plague	days	Chills, malaise, high fever (102° F, 38.9° C), muscular cramps, seizures, smooth, painful lymph gland, skin color changes to a pink and bleeding out of the cochlea will begin after 12 hours of infection. Other symptoms include heavy breathing, continuous blood vomiting, aching limbs, coughing, extreme pain, extreme fatigue, gastrointestinal problems, lenticulae (black dots scattered throughout the body), delirium, and coma
Septicemic plague	2–7 days	Abdominal pain, bleeding because of blood clotting problems, diarrhea, fever, low blood pressure, nausea, organ failure, and vomiting
Pneumonic plague	1–3 days	Initial pneumonic plague symptoms include fever, weakness, headache, nausea, rapidly developing pneumonia with shortness of breath (Dennis and Mead, 2009), chest pain, cough, bloody or watery sputum; typical symptom is coughing with hemoptysis

- **Meningeal plague**: It occurs when bacteria cross the blood-brain barrier, leading to infectious meningitis. Plague meningitis is characterized by fever, headache, stiff neck (nuchal rigidity/meningismus), and delirium, confusion, obtundation, or coma (Butler et al., 1976). Spinal fluid shows pleocytosis, predominantly polymorphonuclear leukocytes, and often gram-negative plague bacilli. Meningeal plague may be a primary manifestation, but it usually occurs a week or more after the onset of bubonic or septicemic plague. It is usually associated with delayed, inappropriate, or bacteriostatic antibiotic therapy and is more common in patients with axillary (as opposed to inguinal) buboes (Butler et al., 1976; Becker et al., 1987). Bacteriostatic antibiotic, such as the tetracyclines, may not eradicate *Y. pestis* before meningeal invasion, and once the meninges become infected, the organisms there may be protected by the blood-brain barrier. In these cases, the clinical course is subacute, and permanent neurological sequelae are rare (Becker et al., 1987).
- **Cellulocutaneous plague**: It results in infection of the skin and soft tissue, often around the bite site of a flea.

Laboratory Diagnosis

Y. pestis is a highly infectious pathogen (in Hazard Risk Group 3), so all specimens should be handled with care. If possible, procedures should be carried out in a safety cabinet and the creation of aerosols minimized. In all the suspected cases, isolation should be performed in a central public health laboratory.

Specimens for Laboratory Diagnosis

Specimen include bubo aspirates, sputum, and blood (if possible, four samples taken at 30-minute intervals) for culture, cerebrospinal fluid in patients with plague meningitis, and scrapings from skin lesions if present. In cases in which septicemic plague is

suspected, blood is also collected into ethylenediaminetetraacetic acid (EDTA) to examine blood smears for bipolar-stained organisms.

Methods for Laboratory Diagnosis

Staining Techniques

Y. pestis shows bipolar staining when stained with Giemsa, methylene blue, or Wayson's stain. Staining techniques such as the Gram, Giemsa, Wright, or Wayson stain can provide supportive but not presumptive or confirmatory evidence of a plague infection. In 70 percent of patients, the gram-negative, bipolar-stained coccobacilli are visualized if present.

Wayson's Bipolar Staining

Wayson's is a rapid method that clearly shows the bipolar morphology of *Y. pestis*.

Preparation of Reagents To make 220 mL of Wayson's stain, the required quantity of basic fuchsin and methylene blue are dissolved in 20 mL of alcohol (methanol or ethanol 95%) solution. It is then transferred into a brown bottle of 250-mL capacity and 200 mL of phenol (5%w/v) solution is added and mixed well. The stain is filtered, for staining purposes.

Procedure

1. Prepare the smear and fix it.
2. Cover it with Wayson's stain for 10 to 20 seconds.
3. Wash off the excess stain with clean water. For this purpose use filtered water or clean boiled rain water.
4. Wipe the back of the slide clean, and place it in a draining rack for the smear to air dry.
5. Examine the smear microscopically, first with the 40x objective to see the distribution of material and then with the oil immersion objective to look for bipolar-stained bacteria.

The *Y. pestis* can be seen as blue-colored bacterium with pink ends and a safety pin appearance (Figure 6.6).

Giemsa Staining

Giemsa stain is a Romanowsky stain that can be used to stain plague bacterium in case Wayson's stain is not available.

Preparation of Reagents To make 500 mL of the reagent, 3.8 g of Giemsa powder should be weighed and transferred to a dry brown bottle of 500-mL capacity, which contains a few dry glass beads. Then, 250 mL of methanol and 250 mL of glycerol are added separately to the stain and mixed well. The bottle is placed in a waterbath at 122 to 140° F (50 to 60° C), or if not available, at 98.6° F (37° C) for up to 2 hours to dissolve it properly. Finally, it is filtered and stored.

To make 1 litre of buffered water (pH 7.0), 0.58 g of anhydrous di-sodium hydrogen phosphate (Na_2HPO_4) and 0.35 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) are added to 1 L of distilled water and then the pH is maintained.

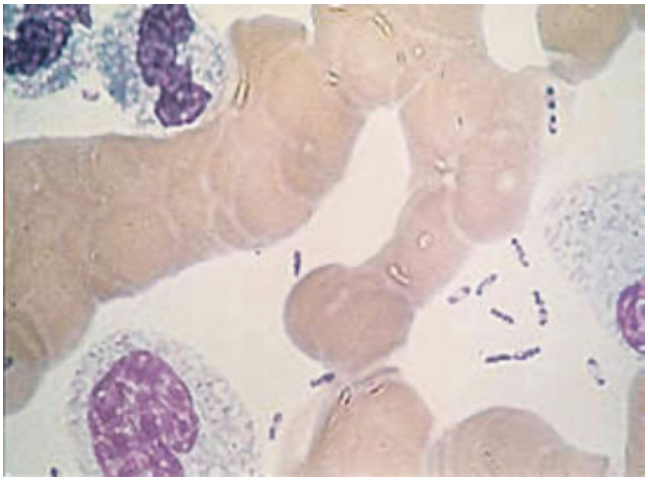

Figure 6.6. Wayson's stain of *Yersinia pestis* with the characteristic safety pin appearance of the bacteria. For color detail, please see color plate section. http://www.cdc.gov/ncidod/dvbid/plague/wayson.htm.

Procedure

1. Fixed the dry smear by covering it with methanol for 2 to 3 minutes. Allow it to air dry.
2. Dilute the Giemsa stain in buffered water as 1 in 20.
3. Place the slide, smear downward in a petri dish or other small container supported on each side by a thin piece of stick.
4. Pour the diluted stain, into the dish and cover with lid.
5. Leave the smear to stain for 25 to 30 minutes.
6. Wash the stain from the dish and rinse the smear with buffered water.
7. Wipe the back of the slide clean, and place it in a draining rack for the smear to air dry.
8. Examine the smear microscopically under oil immersion objective.

The *Y. pestis* cells can be seen as blue-colored coccobacilli with darkly stained ends (bipolar). Figure 6.7 shows the flow diagram for the stain identification of plague bacterium.

Isolation of Yersinia pestis in Culture

For the definitive identification of the plague, *Y. pestis* should be cultured. It is an aerobe and facultative anaerobe, which grows at the temperature range of 57.2 to 98.6° F (14–37° C) with an optimum of 80.6° F(27° C). Cultures should be, therefore, incubated at room temperature. The plague bacillus grows readily on most of the culture media. The colonies are opaque and smooth with irregular edges that have a hammered-metal appearance when magnified (Poland and Barnes, 1979). Three types of culture media are used for culturing the plague bacilli:

1. **Blood agar**: On blood agar *Y. pestis* grows well, producing small shiny, nonhemolytic colonies after 24 to 48 hours incubation at room temperature.

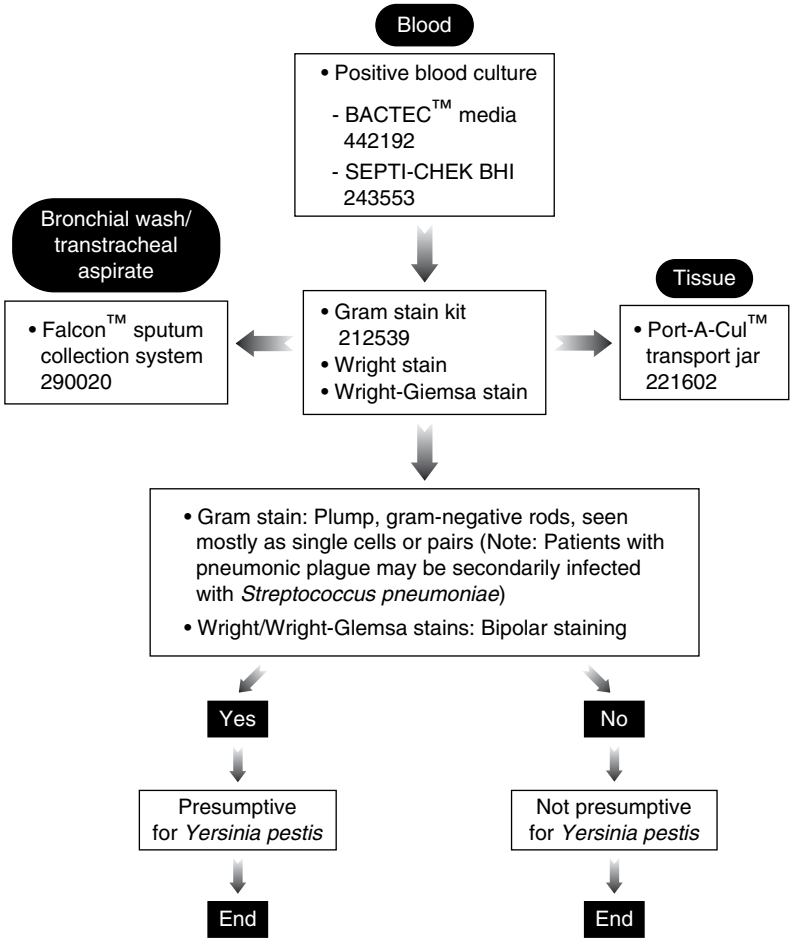

Figure 6.7. Stain identification of *Yersinia pestis* (for Level A Labs).

2. **MacConkey's agar**: After 24 to 48 hours of incubation on MacConkey's agar, it forms small translucent pink colonies. *Y. pestis* does not ferment lactose but appears lactose fermenting because it takes up the red dye of the indicator in the medium.
3. **Cefsulodin-Irgasan-Novobiocin (CIN) agar**: This is the selective medium that is used in specialist microbiology laboratories to isolate *Y. pestis*.

Apart from these, Congo red agar is also used for the isolation of *Y. pestis*.

Biochemical Tests

On the basis of biochemical tests, presumptive identification of *Y. pestis* can be done (Figure 6.8), however, not all of the automated microbiological test systems are programmed to identify *Y. pestis* (Doll et al., 1994). In addition, rapid biochemical identification systems may not be reliable for identification of *Y. pestis* probably because of its lower growth rate. Lysis by a specific bacteriophage is used by the CDC to

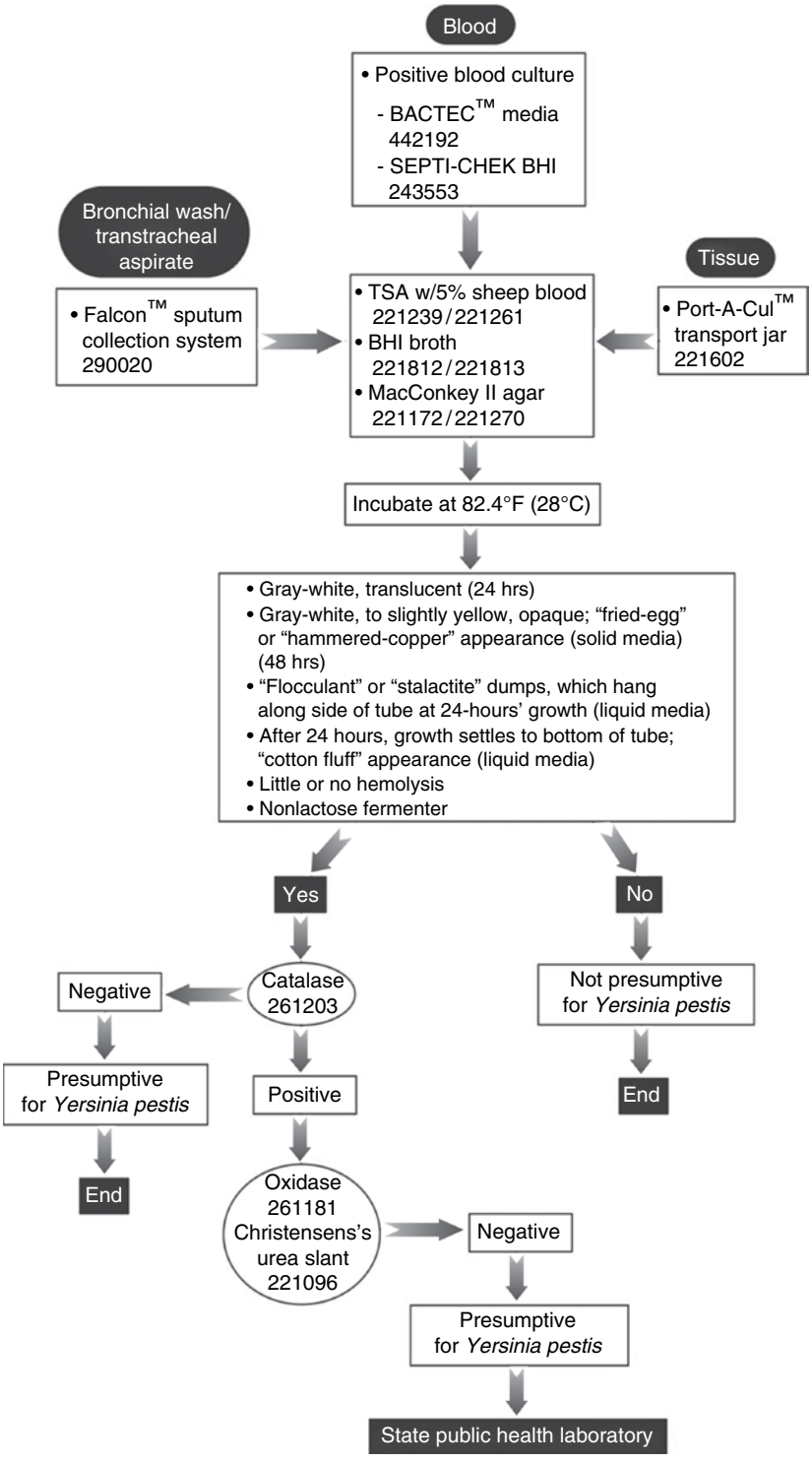

Figure 6.8. Culture identification of *Yersinia pestis* (for Level A Labs).

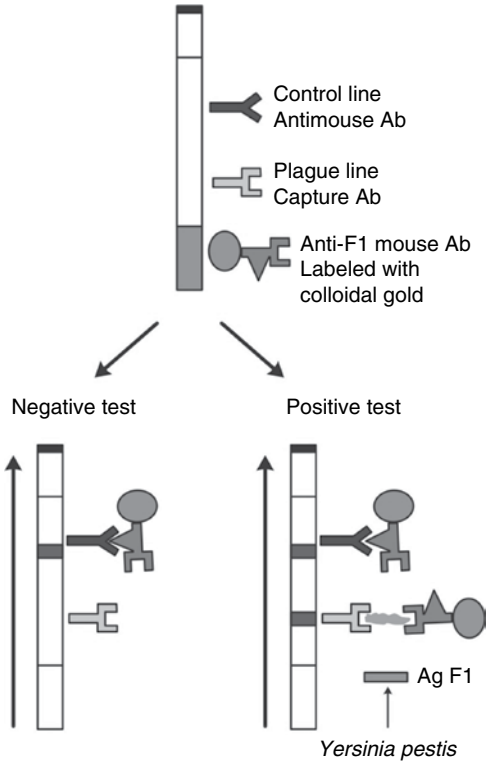

Figure 6.9. F1 detection principle of the Pasteur Institute, rapid diagnostic test.

conclusively identify *Y. pestis* (Baltazard, 1956). *Y. pestis* is catalase positive and oxidase negative. This oxidase reaction is helpful in differentiating *Y. pestis* from *Burkholderia pseudomallei*, which is oxidase positive.

Serological Identification

Although serological identification is not a rapid diagnostic technique, it is used retrospectively to confirm plague diagnosis. For confirmation, paired serum samples, either from acute or convalescent phases or from convalescent and postconvalescent phases, are used because a single serum sample can only provide presumptive evidence of plague. The samples are analyzed at the CDC for the presence of anti-F1 antibodies by a passive hemagglutination test (Chen and Meyer, 1966). A fourfold rise or fall in the titer of paired serum samples is considered confirmatory for plague. A single serum sample with a titer greater than 10 in a person not previously infected or vaccinated against plague is presumptive evidence of recent infection.

Dipstick Test

A dipstick test is a rapid, easy-to-perform immunochromatographic strip test (dipstick), which has been developed in the Pasteur institute to detect specific F1 antigen of *Y. pestis* in bubo aspirates and in sputum from patients with bubonic and pneumonic plague (Figure 6.9). This test has a low detection limit (1–5 ng/mL) and takes only 15 minutes to

perform, which can also prove to be useful to test samples obtained from diseased rats. It shows 100 percent sensitivity and specificity. Like other dipstick assays, it is a semi-quantitative test involving manual reading and a subjective threshold, and so it must be performed by specific, trained health staff.

Other alternative methods for diagnosing plague disease include enzyme linked immunosorbent assays (ELISAs; Williams et al., 1984), polymerase chain reaction (PCR) analysis (Norkina et al., 1994), and DNA hybridization studies (McDonough et al., 1988). ELISAs have been used to measure levels of either F1 antigen (direct) or antibodies to F1 (indirect) in serum. Both antigen and antibody need to be assayed because patients who are positive for one are negative for the other. *Y. pestis* fluorescent antibody stain is also proved to be useful if available.

Differential Diagnosis

Bubonic Plague

Bubonic plague may be confused with streptococcal or staphylococcal lymphadenitis, infectious mononucleosis, cat-scratch fever, lymphatic filariasis, tick typhus, tularemia, and other causes of acute lymphadenopathy. Involvement of intra-abdominal lymph nodes may mimic appendicitis, acute cholecystitis, enterocolitis, or other intra-abdominal surgical emergencies (Hull et al., 1986). Inguinal buboes have even been mistaken for an inguinal hernia.

Septicemic Plague

Septicemic plague resembles a nonspecific sepsis syndrome or a gram-negative sepsis. Fortunately, some empiric antibiotic treatments for gram-negative sepsis (e.g., aminoglycosides or fluoroquinolones) are effective against *Y. pestis*, but use of advanced generation cephalosporins is problematic. As in other sepsis syndromes, gastrointestinal complaints of abdominal pain, nausea, vomiting, and diarrhea may be prominent and misleading. An improperly decolorized Gram's stain examination of a blood smear or lymph node aspirate may result in the interpretation of *Y. pestis* bipolarity as a gram-positive diplococcus.

Pneumonic Plague

Pneumonic plague may be confused with other causes of acute, severe community-acquired pneumonia, such as pneumococcal, streptococcal, *Haemophilus influenzae*, anthrax, tularemia, *Legionella pneumophila*, leptospiral, hanta virus pulmonary syndrome, and influenza virus pneumonia. Regional lymphadenitis may indicate plague or tularemia pneumonia arising secondary to a cutaneous infective exposure.

Clinical Management

There are series of steps that should be followed after a clinical suspicion of an outbreak of plague (Figure 6.10).

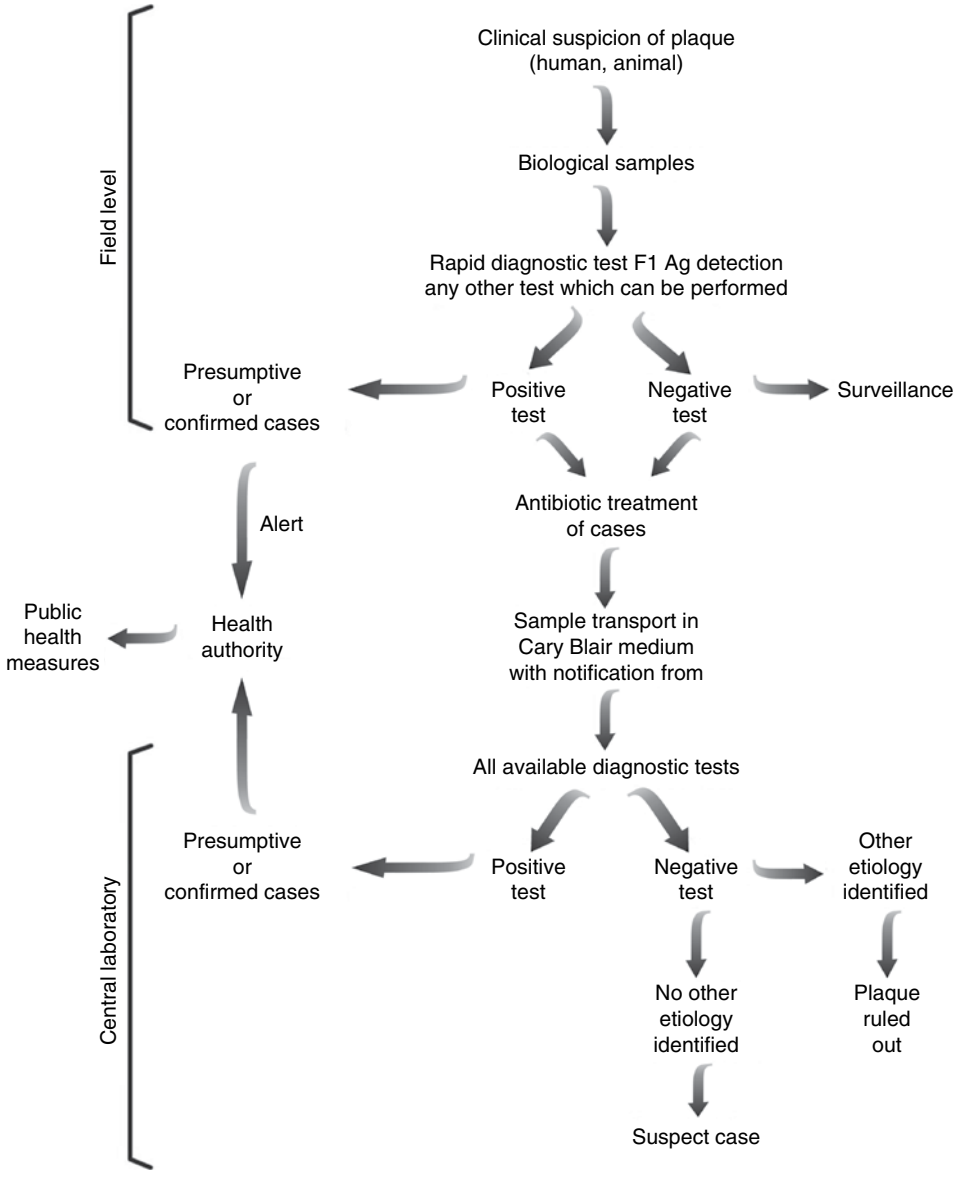

Figure 6.10. Diagnosis procedural indications following clinical suspicion of plague.

Treatment should be initiated as soon as the diagnosis is suspected. Many antibiotics are active against *Y. pestis* (streptomycin, gentamicin, doxycycline, ciprofloxacin [Russell et al., 1996]), chloramphenicol, sulfadiazine, trimethoprim-sulfamethoxazole) (Harris, 1994). Gentamicin or streptomycin can be used as first-line therapy with ciprofloxacin as an alternative (Table 6.2). Chloramphenicol should be used for the treatment of meningitis. Persons who come in contact with patients with pneumonic plague should receive

Table 6.2. Recommendations for treatment and post-exposure prophylaxis of plague.

Patient Category	Type of Therapy	Treatment of Suspected or Confirmed Clinical Cases (10 days)	Postexposure Prophylaxis (7 days)
Adults Pregnant women It is recommended, when possible, to cease breast feeding	First-line treatment	Gentamicin: 5 mg/kg IV in one or two doses daily or Streptomycin: 1 g IM twice daily	
	Second-line treatment, first-line prophylaxis	Ciprofloxacin: 400 mg IV BID followed by 500 mg PO BID or Ofloxacin: 400 mg IV BID followed by 400 mg PO BID or Levofloxacin: 500 mg IV once a day, followed by 500 mg PO once a day	Ciprofloxacin: 500 mg PO BID or Ofloxacin: 400 mg PO BID or Levofloxacin: 500 mg PO once a day
	Third-line treatment, second-line prophylaxis	Doxycycline: 100 mg IV bid followed by 100 mg PO BID	Doxycycline: 100 mg PO BID
Children	First-line treatment	Gentamicin: 2.5 mg/kg IV in three doses daily or streptomycin: 15 mg/kg IM twice daily (max, 2g)	
	Second-line treatment, first-line prophylaxis	Ciprofloxacin: 10–15 mg/kg IV BID followed by 10–15 mg/kg PO BID	Ciprofloxacin: 10–15 mg/kg PO BID
	Third-line treatment, second-line prophylaxis	Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg IV BID followed by 2.2 mg/kg PO BID (max 200 mg/d)	Doxycycline: >8 years and >45 kg: adult dose >8 years and <45 kg or <8 years: 2.2 mg/kg PO BID (max 200 mg/d)

BID, twice daily; IM, intramuscularly; IV, intravenously; PO, by mouth.

antibiotic prophylaxis with doxycycline or ciprofloxacin for 7 days. Other antibiotics (chloramphenicol, sulfadiazine, or trimethoprim-sulfamethoxazole) could also be used.

Prevention of human-to-human transmission from patients with plague pneumonia can be achieved by implementing standard isolation procedures until at least 4 days of antibiotic treatment have been administered. For the other clinical types of the disease, patients should be isolated for the first 48 hours after the initiation of treatment. Healthcare workers should wear high efficiency respirators.

Prevention and Control

Plague is primarily spread by fleas and it is impractical to eliminate fleas and wild rodents from the natural environment in plague-infected areas; however, to prevent human disease, rodents and fleas around areas where people live, work, and play can be controlled. Therefore, preventive measures are directed to home, work, and recreational settings

where the risk of acquiring plague is high. These measures are environmental sanitation, educating the public on ways to prevent plague exposures, and preventive antibiotic therapy.

Environmental Sanitation

Effective environmental sanitation reduces the risk of persons being bitten by infectious fleas of rodents and other animals in places where people live, work, and recreate. It is important to remove food sources used by rodents and make homes, buildings, warehouses, or feed sheds rodent-proof. Chemicals should also be applied that kill fleas and rodents, which can be done by trained professionals. Rats that inhabit ships and docks should be controlled by trained professionals who can inspect and, if necessary, fumigate cargoes. Plague prevention also involves controlling rat populations in both urban and rural areas. This goal has been reached in the cities, towns, and villages of most developed countries.

Public Health Education

Plague imposed a great threat in people living, working, and playing areas where there are widespread wild rodents. Hence it is important to educate the general public and the medical community about how to avoid exposure to disease-bearing animals and their fleas. These precautions include:

- Plague activity in rodent populations should be monitored. Reporting should be done to the local health department or law enforcement officials about sick or dead animals.
- Sources of food and nesting places for rodents around homes, work places, and recreation areas should be eliminated; brush, rock piles, junk cluttered firewood, and potential food supplies, such as pet and wild animal food, should be removed. Homes should be made rodent-proof.
- In a condition of exposure to rodent fleas, insect repellents should be applied to clothing and skin to prevent flea bites. Gloves should be worn when handling potentially infected animals.
- In case people living in areas where rodent plague occurs, pet dogs and cats should be treated for flea control regularly and these animals should not be allowed to roam freely.
- Health authorities may use appropriate chemicals to kill fleas at selected sites during animal plague outbreaks.

Prophylactic (Preventive) Antibiotics

Antibiotics should be administered by the people who have been exposed to the bites of potentially infected rodent fleas especially during a plague outbreak or those people handling an animal known to be infected with the plague bacterium. Experts recommend that antibiotics should also be given if a person has had close exposure to a person or an animal with suspected plague pneumonia. During a plague outbreak, persons can protect themselves for 2 to 3 weeks by taking antibiotics. The preferred antibiotics for prophylaxis against plague are the tetracyclines or sulfonamides.

References

Adetunji J. 2012. Plague patient in critical condition at Oregon hospital. *The Guardian* June 14. Accessed June 15, 2012, at http://www.guardian.co.uk/science/2012/jun/15/plague-patient-critical-condition-oregon.

Ayyadurai S, Houhamdi L, Lepidi H, Nappez C, Raoult D, et al. 2008. Long-term persistence of virulent *Yersinia pestis* in soil. *Microbiology* 154:2865–2871.

Baltazard M, Davis DHS, Devignat R, Girard G, Gohar MA, et al. 1956. Recommended laboratory methods for the diagnosis of plague. *Bull WHO* 14:457–509

Becker TM, Poland JD, Quan TJ, White ME, Mann JM, et al. 1987. Plague meningitis: a retrospective analysis of cases reported in the United States, 1970–1979. *Western Journal of Medicine* 147:554–557.

Benedictow OJ. 2004. *The Black Death, 1346–1353: The Complete History*. Woodbridge, England: Boydell Press.

Bibel DJ, Chen TH. 1976. Diagnosis of plague: An analysis of the Yersin-Kitasato controversy. *Bacteriol Rev* 40:633–651.

Burroughs AL. 1947. Sylvatic plague studies. The vector efficiency of nine species of fleas compared with *Xenopsylla cheopis*. *J Hygiene* 45:371–396.

Butler T, Levin J, Nguyen NL, Duong MC, Adickman M, et al. 1976. *Yersinia pestis* infection in Vietnam II. Quantitative blood cultures and detection of endotoxin in the cerebrospinal fluid of patients with meningitis. *J Infect Dis* 133:493–499.

Casman EA, Fischhoff B. 2008. Risk communication planning for the aftermath of a plague bioattack. *Risk Anal* 28:1327–1342.

Centers for Disease Control and Prevention (CDC). 2011. Plague Information. Accessed February 15, 2013, at http://emergency.cdc.gov/agent/plague/.

Chen TH, Meyer KF. 1966. An evaluation of *Pasteurella pestis* fraction-1-specific antibody for the confirmation of plague infections. *Bull WHO* 34:911–918.

Christie AB, Chen TH, Elberg SS. 1980. Plague in camels and goats: their role in human epidemics. *Journal of Infectious Diseases* 141:724–726.

Dennis DT, Mead PS. 2009. *Yersinia* species, including plague. In: Mandell GL, Bennett JE, Dolin R, eds., *Principles and Practice of Infectious Diseases*, 7th ed., chapter 229 Philadelphia: Elsevier Churchill Livingstone.

Doll JM, Zeitz PS, Ettestad P, Bucholtz AL, Davis T, et al. 1994. Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. *Am J Trop Med Hyg* 51:109–114.

Duplaix N. 1988. Fleas: The lethal leapers. *Natl Geogr* 173:672–694.

Farmer JJ. 1995. *Enterobacteriaceae*: Introduction and identification. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds., *Manual of Clinical Microbiology*, 6th ed., pp. 438–448. Washington DC: ASM Press.

Gupta ML, Sharma A. 2007. Pneumonic plague, northern India, 2002 letter. *Emerg Infect Dis* 13:4.

Harris S. 1994. *Factories of Death*. New York: Routledge.

Hazarika S. 1995. Plague's origins a mystery. *New York Times* March 14. Accessed February 15, 2013, at http://www.nytimes.com/1995/03/14/science/plague-s-origins-a-mystery.html.

Hoffman SL. 1980. Plague in the United States: The "Black Death" is still alive. *Ann Emerg Med* 9:319–322.

Hull HF, Montes JM, Mann JM. 1986. Plague masquerading as gastrointestinal illness. *Western Journal of Medicine* 145:485–487.

LaForce FM, Acharya IL, Stott G, Brachman PS, Kaufman AF, et al. 1971. Clinical and epidemiological observations on an outbreak of plague in Nepal. *Bulletin of the World Health Organization* 45:693–706.

Latin American Herald Tribune. 2010. One dead from plague in northern Peru. Accessed November 24, 2011, at http://www.laht.com/article.asp?CategoryId=14095&ArticleId=361749.

Marshall JD, Quy DV, Gibson FL. 1967. Asymptomatic pharyngeal plague infection in Vietnam. *American Journal of Tropical Medicine and Hygiene* 16:175–177.

McDonough K A, Schwan T G, Thomas R E, Falkow S. 1988. Identification of a *Yersinia pestis*-specific DNA probe with potential for use in plague surveillance. *J Clin Microbiol* 26:2515–2519.

Meyer K. 1961. Pneumonic plague. *Bacteriologic Review* 25:249–261.

Nicholas W. 2010. Europe's plagues came from china, study finds. *New York Times* October 31. Accessed February 15, 2013, at http://www.nytimes.com/2010/11/01/health/01plague.html?_r=1&hp.

Nogueras D. 2010. Rare case of Bubonic plague shows up in Lake Country. *Oregon Public Broadcasting* October 1. Accessed November 24, 2011, at http://www.opb.org/news/article/rare-case-bubonic-plague-shows-lake-county/.

Norkina OV, Kulichenko AN, Gintsburg AL, Tuchkov IV, Popov YA, et al. 1994. Development of a diagnostic test for *Yersinia pestis* by the polymerase chain reaction. *J Appl Bacteriol* 76:240–245.

Poland JD, Barnes AM. 1979. Plague. In: Steele JH, ed., CRC *Handbook Series in Zoonoses. Section A. Bacterial, Rickettsial, and Mycotic Diseases*, I: pp. 515–559. CRC Press Inc., Boca Raton: CRC Press.

Pollitzer R.1954. Plague. Geneva: World Health Organization.

Russell P, Eley SM, Bell DL, Manchee RJ, Titball RW. 1996. Doxycycline or ciprofloxacin prophylaxis and therapy against *Yersinia pestis* infection in mice. *J Antimicrob Chemother* 37:769–774.

Russell JC. 1968. That earlier plague. *Demography* 5:174–184.

Ryan KJ, Ray CG. 2004. *Sherris Medical Microbiology: An Introduction to Infectious Diseases*, 4th ed., pp. 484–488. New York: McGraw-Hill.

Plague (disease). n.d. Wikipedia. Accessed March 25, 2013, at en.wikipedia.org/wiki/Plague_(disease).

New York Times. 1994. Surat: A victim of its open sewers. September 25. Accessed April 26, 2008, at http://query.nytimes.com/gst/fullpage.html?

Williams JE, Gentry MK, Braden CA, Leister F, Yolken RH. 1984. Use of an enzyme-linked immunosorbent assay to measure antigenaemia during acute plague. *Bull WHO* 62:463–466.

Chapter 7

Leptospirosis

Introduction

Leptospirosis was first described by Adolf Weil as a disease entity in 1886 (WHO, 2003), while reporting an "acute infectious disease with enlargement of spleen jaundice and nephritis." Leptospirosis is caused by a spirochete *Leptospira*, which is primarily a zoonotic disease with an accidental human infection. Recent outbreaks of the disease indicated that it has become an emerging infection in human beings. The disease usually appears wherever humans come in contact with the urine of infected animals as walking in a urine-polluted environment. Thereby, whole communities living in tropical regions with a wet environment could be at risk (Chan et al., 1987).

The magnitude of the problem in tropical and subtropical regions can be ascribed to the various environmental and behavioral factors such as the local agricultural practices, poor housing, and unsafe methods of waste disposal, which gives rise to a *Leptospira*-contaminated environment. The disease is underreported in India, and its transmission is linked to occupational, recreational, behavioral, and environmental factors. If control measures are not implemented, the disease may become a public health problem. Other names for leptospirosis include canicola fever, autumnal fever, swine herd's disease, rice-field fever, cane cutter's disease, dairy farm fever, and mud fever (WHO, 2003; Gulati et al., 2002).

Magnitude

Global

Globally the estimated annual incidence of leptospirosis ranges from 0.1 to 1.0 person per 100,000 population in the temperate climates, and in tropical countries, it ranges from 10 to 100 persons per 100,000 population (WHO, 2003). In developed countries, people

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

traveling to tropical countries and those who are occupationally and recreationally exposed may acquire the disease. The largest recorded outbreak in the United States affected 110 persons during June and July 1998. It is estimated that about 200 cases are identified annually in the United States, with about 50 percent of these occurring in the Hawaiian islands (Meites et al., 2004).

India

In India, the first confirmed report of leptospirosis came from Andaman and Nicobar islands in 1929, when Taylor and Goyle (1931) isolated leptospires from patients with Weil's disease and reported the isolation of the causative organism. Subsequently, few reports on leptospirosis originated from India till the 1980s (Sehgal et al., 1994; Sehgal, 1998). In 1988, there were annual outbreaks of acute febrile illness with hemorrhagic manifestations and pulmonary involvement in North and South Andamans. Case fatality ratios in these outbreaks were in the range of 10 to 50 percent. In the absence of a specific diagnosis, this clinical entity was named Andaman haemorrhagica fever (Sehgal et al., 1995; Singh et al., 1999). After that, leptospirosis became an endemic disease in the Andaman and Nicobar Islands, which usually occurs as a postmonsoon outbreak (Sehgal et al., 1995). Sero-prevalence among the settler population ranges between 50 and 60 percent, but it is comparatively low among indigenous tribes (Sehgal et al., 1994). The median age of patients has shown a decreasing trend, and pulmonary hemorrhage is a common clinical manifestation of the disease in the Andamans (Singh et al., 1999).

During the late 1980s, outbreaks of leptospirosis were reported from Tamil Nadu, Mysore (Karnataka), and Nagpur (Maharashtra). In 1997, thousands of people were affected by the disease in Surat (Gujarat). After the super-cyclone in Orissa in October 1999, fever outbreaks with pulmonary hemorrhage occurred in the villages affected by floods. In July and August 2000, cases of leptospirosis were also reported from Gujarat, Maharashtra, Kerala, and Andaman and Nicobar Islands (Sehgal, 2000).

Agent Factors

Leptospira was first observed in 1907 during the postmortem of a renal tissue slice (Stimson, 1907). It was first identified as the causative organism by Inada and Ito (1915), and in 1916, its presence was noted in rats (Inanda et al., 1916). Leptospira are thin, highly motile and antigenically complex spiral bacteria, belonging to the family *Leptospiraceae* of order Spirochaetales. They are 0.1- to 0.2-μ wide and 5- to 15-μ long and differ from other spirochetes by the presence of hooked ends. They are too thin to be visible under the light microscope on a bright field, but they are visible by dark field illumination (WHO, 2003). They do not stain readily with aniline dyes and require silver impregnation techniques for staining purpose. Leptospires show two types of motility: they dart quickly holding the middle part rigid and bending the ends like coat hangers, showing "flexuous motility," and sometimes they also rotate along a longitudinal axis showing "corkscrew motility."

Serological Classification

The serovar (or "serological variant") is the basic unit of classifying leptospires (Figure 7.1), which is defined on the basis of antigenic similarities. Serovars have

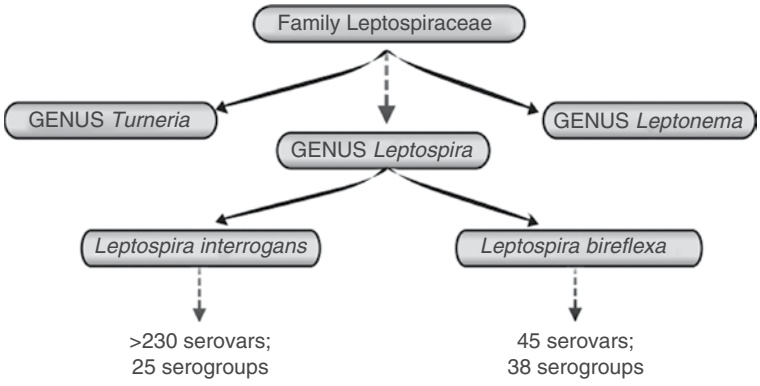

Figure 7.1. Serological classification of Genus *Leptospira*.

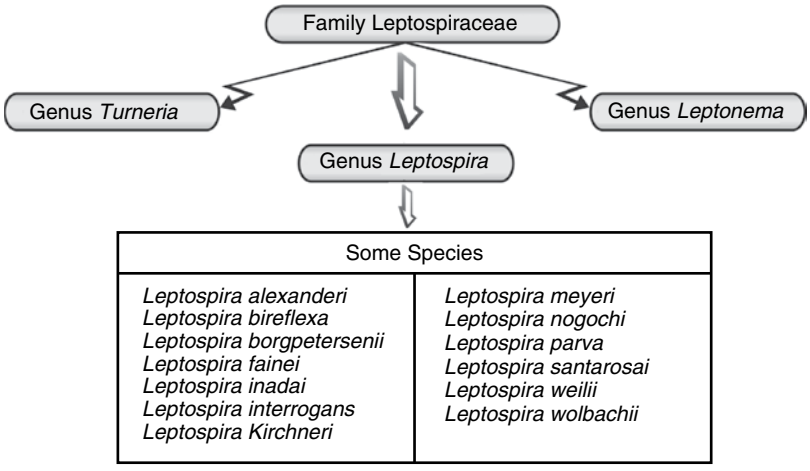

Figure 7.2. Species classification of Genus *Leptospira*.

characteristic antigenic features and also exhibit cross reactivity. For the genus *Leptospira* more than 200 pathogenic serovars, which are further subdivided into 25 sero-groups, have been described (WHO, 2003; Sehgal, 1998). Serovar is epidemiologically important because certain serovar may develop a commensal relationship with a certain vertebrate host species such as serovar *hardjo* in cattle, serovar *canicola* in dogs, serovars *icterohaemorrhagiae* and *copenhageni* in rats (WHO, 2003). In India, the predominant serovars of the organism are *andamana*, *pomana*, *hebdomadis*, *semoranga*, *javanica*, *canicola*, *autumnalis*, *australis*, and *grippotyphosa*. The latter three serovars and *icterohaemorrhagica* are common in Southern India (Gulati et al., 2002).

Species Classification

There is another method of classifying leptospires (Figure 7.2). Because all the *Leptospira* look alike with minor differences, morphology does not help in differentiating between pathogenic and saprophytic *Leptospira*. Thereby, differentiation is based on culture characteristics

and by antigenic and genetic properties. Unlike pathogens, saprophytes grow well at 55.4° F (13° C) and in the presence of 8-Azaguanine in a concentration of 225 mg/mL. Saprophytes also get converted in to spherical forms in one molar solution of sodium chloride. Polymerase chain reaction (PCR) techniques have been developed to detect oligonucleotides specific for pathogenic and saprophytic leptospires occurring in water (WHO, 2003).

Pathogenic leptospires are usually maintained in the renal tubules of vertebrate host species (animal reservoirs), whereas saprophytic leptospires are free living and are generally not associated with disease outbreaks (WHO, 2003).

Leptospires are obligate aerobes with a generation time of 7 to 12 hours at 86° F (30° C). They grow well at 7.2 to 7.4 pH, but are susceptible to acidic pH. Unlike other spirochetes, leptospires can also easily be grown in artificial culture media. They are resistant to antimicrobial action of pyrimidine analogue 5-fluorouracil because they use purines and not pyrimidines for their metabolism.

Host Factors

Natural Maintenance Hosts

Pathogenic leptospires are the commensals in the convoluted renal tubules of certain vertebrate animals that do not harm their host animals. These host animals are the natural maintenance hosts, which are asymptomatic and excrete the causative organism in their urine, without clinically manifesting the disease (Chosewood and Wilson, 2009).

Accidental or Incidental Hosts

When the other animals and humans (that are not natural maintenance hosts) are infected by pathogenic leptospires, they start developing signs and symptoms of the disease. A host that becomes infected accidentally or incidentally with an organism or serovar of it (for which the animal is not a natural maintenance host) is called an *accidental* or *incidental* host. The natural maintenance host for a particular disease can also develop clinical manifestations of the disease, in which case they are infected by another serovar. In animals that are natural maintenance hosts for a particular serovar, the clinical manifestations might be absent or mild. If infected by another serovar, the same animals will develop illness because they probably would be the incidental hosts for that serovar. Leptospirosis is primarily an animal infection, which is transmitted to humans only under certain environmental conditions. There are at least five serotypes of *Leptospira* causing disease in dogs and reported in the United States and Canada as icterohaemorrhagiae, canicola, pomona, grippotyphosa, and bratislava (Klopfleisch et al., 2011).

Human infection is only accidental because it may spread from wild animals to domestic livestock and to humans. Because leptospires can adapt to new animal host species, the distinction between natural, accidental, and incidental hosts cannot be clearly defined (WHO, 2003).

Animal Reservoir

More than 200 serovars of leptospires are naturally carried by rodents and wild and domestic animals, resulting in successful maintenance of leptospires in the environment

(National Institute of Communicable Disease [NICD], 2006). Animals harboring leptospires include:

- Rodents, such as rats, mice, and voles, are the most widely distributed animal reservoir of leptospiral infection (WHO, 2003). The alkaline pH of the rodent's urine favors permanent colonization and lifelong urinary shedding of leptospira; hence *Rattus norvegicus* (Norway rat) and *Mus musculus* (domestic mouse) are considered to be an important reservoir (Sehgal, 1998).
- Insectivores such as shrew and hedgehog (WHO, 2003).
- Domestic animals such as cattle, pigs and dogs. Sheep, goats, cats, horses, and buffaloes rarely harbor leptospires (WHO, 2003).
- Fur-bearing animals such as silver foxes, mink, and nutria that are reared in captivity for fur production (WHO, 2003).
- Reptiles and amphibians (WHO, 2003).

Hence, most of the animals are considered as potential reservoirs of leptospiral infection and thereby all should be handled cautiously. In cows, acute infection with leptospires may cause malaise and drop in milk production, whereas chronic infection may lead to abortion or low fertility (WHO, 2003).

In a cross-sectional study, in Andaman and Nicobar Islands, sero-positivity rate for leptospirosis was found to be highest among cows (40.3 percent), followed by buffaloes (37.0 percent), goats (36.3 percent), pigs (18.0 percent), and dogs (9.1 percent). Though rats (*Rattus rattus*) had a lower sero-positivity (7.1 percent), few of them were carriers of pathogenic leptospires. The sero-positivity rates in animals were found to correlate with that in humans. The affected animals continued to excrete leptospires in their urine for several months (Sharma et al., 2003).

Human Infection

Age and Sex

Children are more vulnerable because they use to play with dogs and are more exposed to water bodies and damp soil and are prone to get infected. Males probably outnumber females because of more risk of exposure to infected animals and contaminated environment. For the same reason, young and middle-aged men (of 20- to 30-age group) have a higher prevalence than children and old men (WHO, 2003; NICD, 2006). In situations in which both men and women are at equal risk, the gender differences are not apparent (NICD, 2006).

Leptospirosis is transmitted by occupational and recreational contact with fresh water, damp soil, and vegetation etc. that is contaminated by urine of infected animals (Table 7.1). Water ingestion during water sports is also one of a mode of transmission; for example, in 1997, nine sportsmen involved in white-water rafting (a water sport), were infected in Costa Rica in Central America (Centers for Disease Control [CDC], 1997). If drinking water gets contaminated during flooding or hurricanes, the whole population may be at risk. New risk groups further may be formed as a result of changes in environment, human behavior, and agricultural practices (WHO, 2003). Rowers are also sometimes known to contract the disease (Langston and Heuter, 2003).

Behavioral Factors

Walking barefoot is a behavioral risk factor to acquire leptospirosis (Faine, 1982). Working in the fields, defecating in open fields, and bathing in ponds are associated with

Table 7.1. Occupational and recreational exposure.

High-Risk Group	Exposure
Cattle farmers, dairy workers, and cow herds	Handling cattle; milking; contact with infectious droplets when animals urinate; contact with amniotic fluid, placenta, and dead aborted fetuses
Pig farmers and swine herds	Handling and tending pigs; contact with urine
Rice farmers, vegetable growers, sugarcane cutters, and gardeners	Direct or indirect exposure to rodent urine; working barefoot in contaminated water or damp soil
Veterinarians, animal handlers and trainers, and pet owners	Direct or indirect exposure to asymptomatic carriers, urinary shedders, or ill animals
Health-care personnel and laboratory workers	Exposure to blood and urine of patients; exposure to zoonotic tissues and organs
Abattoir workers and butchers	Slaughtering asymptomatic carriers and handling infected organs (e.g., kidneys) and carcasses
Sewer workers	Exposure to sewage contaminated with rat urine
Miners	Exposure to contaminated water or soil
Fishermen and prawn farmers	Exposure to contaminated surface waters
Forest workers, soldiers, hunters, hikers, and laborers engaged in de-silting irrigation canals	Contact with contaminated vegetation, damp soil, water, or animals; exposure while wading through contaminated surface water or swamps
Persons involved in water sports and outdoor sports	Contact with contaminated vegetation, damp soil. or water

Adapted from World Health Organization (WHO). 2003. *Human Leptospirosis: Guidance for Diagnosis, Surveillance, and Control*. Geneva: WHO; and Mitra DK. 1999. Leptospirosis. In: Sainani GS, ed., *API Textbook of Medicine*, 6th ed., p. 62. Mumbai: Association of Physicians of India.

risk infection with *australis*, *grippotyphosa*, and *canicola* species of *Leptospira*, respectively. Truck drivers who use contaminated water to wash their vehicles and masons using contaminated water to prepare a cement-sand mixture are also at risk (NICD, 2006). Crossing stagnant water bodies on the way to school or workplace is associated with risk of infection with serovars of *Leptospira* (Murhekar et al., 1998). Migration of the rural population to urban slums has been implicated in the spread of leptospirosis to urban areas.

Immunity

Leptospira infection induces serovar-specific immunity (i.e., the host only persists the immunological memory against a specific serovar of *Leptospira* which infected it recently). More than 200 pathogenic serovars of *Leptospira* have been described (WHO, 2003). In a study on school children in Andaman and Nicobar Islands, an overall sero-positivity rate of 23.6 percent was observed.

High-Risk Groups

During an outbreak that occurred one month after the collection of the first sample, the infection rate among sero-negatives was 33.5 percent, whereas the reinfection rate among sero-positives was 16.7 percent. More than 90 percent of leptospiral infections were subclinical or unnoticed. Morbidity and mortality were higher among individuals who

were sero-negative as compared to individuals who wee sero-positives. The inhabitants of these islands are exposed to infection from childhood and exposure level increases with age as their outdoor activities increase. Repeated exposures result in build-up of partial immunity. During outbreaks, children and adolescents who are newly infected (without partial immunity conferred by prior exposure) have a higher rate of severity and frequency of fatal complications (Vijayachari et al., 2004).

Environmental Factors

Natural events, such as heavy rainfall and flooding are the predisposing factors for epidemics of leptospirosis (NICD, 2006). Recent outbreaks of leptospirosis have shown a strong association with the rainy season or flooding (Mitra, 1999). In 1995, after widespread flooding in Nicaragua in Central America, an epidemic of leptospirosis affected about 2,000 persons and killed at least 13 individuals (CDC, 1995). Outbreaks of fever in association with pulmonary hemorrhage were reported in the flooded villages, following the super-cyclone in the Indian state of Orissa in October 1999 (Sehgal, 2000).

The environment favorable for the survival of leptospires is the key factor in maintaining the infection among carrier animals that further transmit it to humans. Salinity of the soil provides a favorable environment for prolonged survival of leptospires (NICD, 2006). In places with sandy soil (that has low water retaining capacity), chances of survival of leptospires in the soil are low (Sharma et al., 2003). *Leptospira*, when shed in urine by carrier animals, can survive for 1 to 4 weeks in soil and water, and environmental contamination may reach high levels in areas where carrier animals frequently urinate (Sehgal, 1998).

In India, leptospirosis outbreaks have been reported in Andaman and Nicobar Islands and in the coastal districts of Andhra Pradesh, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Orissa, and Tamil Nadu. The changes in the incidence and geographical distribution of leptospirosis are proportional to alterations in the ecosystem. Reclamation of wastelands, afforestation, and changes in agricultural technology are among the man-made factors responsible for converting previously infection-free areas into potentially endemic zones for leptospirosis. Construction of irrigation canals, roads, and railway lines obstruct the natural drainage of rain water, causing prolonged water logging of soil. Water logging and flooding forces rodents to abandon their burrows. Humans who come in contact with stagnant water or soil contaminated with rodent urine get infected with leptospires (NICD, 2006).

Leptospires thrive optimally at temperatures of 82.4 to 86° F (28–30° C) and in areas where the annual rainfall is more than 200 cm and vegetation is luxuriant (Faine, 1982). In endemic areas, the mean annual soil temperature at a depth of 50 cm from the surface is 71.6° F (22° C) or higher. The difference between the mean summer and winter temperatures is less than 41° F (5° C). These factors also favor prolonged survival of leptospires (NICD, 2006).

Location of houses in low-lying areas, presence of ponds near houses, and presence of rat infestation are risk factors for leptospiral infection. Use of well or stream water for domestic use is associated with risk of infection with the *grippotyphosa* and *australis* species of *Leptospira*. Presence of cattle or dogs in the household is associated with risk of infection with *australis* and *grippotyphosa*, respectively (Murhekar et al., 1998). During

an outbreak in Andaman and Nicobar Islands, a new risk factor found to be associated was mud flooring of houses (Vijayachari et al., 2004).

Unplanned urbanization, especially with inadequate facilities for sanitation, provides the environmental conditions that are conducive to the spread of leptospirosis. Open dumping of garbage is usually the norm in poor countries; this garbage attracts animal reservoirs like rodents and stray dogs. Flooding and water logging during the rainy season also increases the risk of human contact with water that may be contaminated with animal urine infected with leptospira (Sehgal, 1998).

Mode of Transmission

The transmission cycle of *Leptospira* infection involves interaction between one or more species of carrier animals, an environment conducive for the survival of leptospires, and humans (Andre et al., 2000). *Leptospira* enters the human body through the cuts and burns in the skin or mucous membrane, through the intact skin (hair follicles) or mucous membranes of the nose, mouth, and eyes (Mitra, 1999), and can probably enter through intact waterlogged skin (WHO, 2003). Ingestion of contaminated food and water is another mode of infection (WHO, 2003).

Infection is transmitted to humans by droplet infection while milking cows or goats, by inhalation of aerosol polluted with droplets of animal urine (WHO, 2003), and by direct contact of humans with urine or blood of an infected animal or with contaminated water, soil, and vegetables (Mitra, 1999; Figure 7.3). Direct human-to-human transmission is rare.

Leptospires are actually present in the blood of patients during the first week of illness and are also excreted in patient's urine. After recovery, they persist in immunologically privileged sites, such as the eyes for prolonged periods (WHO, 2003). In humans, the infection is seldom transmitted sexually, transplacentally, or via breastfeeding (WHO, 2003; Gulati et al., 2002). Congenital or neonatal infection is seen in case of animals, whereas sexual transmission is reported in rats, cattle, dogs, and pigs (WHO/ILS, 2003). It is also possible to modify the carrier state among domestic animals because treatment regimens are available (Sharma et al., 2003), but it is difficult to modify some links in transmission chain of leptospirosis as:

- Animal activity and their contamination of the environment.
- Suitability of environment for survival of leptospires.
- Load of rodent population.
- Carrier state among rodents.

Pathology and Immunology

Following infection, pathogenic leptospires appear in the blood and invade almost all tissues and organs of the host. The host's immune system starts clearing leptospires from blood and other tissues, but the organisms may settle in the convoluted tubules of the kidney thereby may be shed in urine for a few weeks or even longer. Even after they are cleared from kidneys and other organs, they may persist in the eyes for a longer duration.

Human body produces specific anti-*Leptospira* (genus-specific, serovar-specific, and serogroup-specific) antibodies in response to *Leptospira* infection. Sero-conversion usually takes 10 days, but the duration may be variable (WHO, 2003).

Anti-leptospiral immunoglobulin M (IgM) antibodies appear earlier than anti-leptospiral immunoglobulin G (IgG) antibodies and remain detectable at low titers for months or even years. Detection of IgG antibodies is variable. During the initial phase of the disease, genus-specific antibodies are produced, which react with several serovars (i.e., they are cross reacting). In the later course of the disease, these cross-reactive (i.e., genus-specific) antibodies disappear and the host's body starts producing the serovar-specific and serogroup-specific antibodies that persists for years. Weak cross-reactions might occur with other groups of microorganisms, depending on the serological technique employed. Serovar-specific antibodies are considered to be protective, and a patient is immune to reinfection with the same serovar as long as their titer is high. They may not, however, provide protection against infection with other serovars (WHO, 2003).

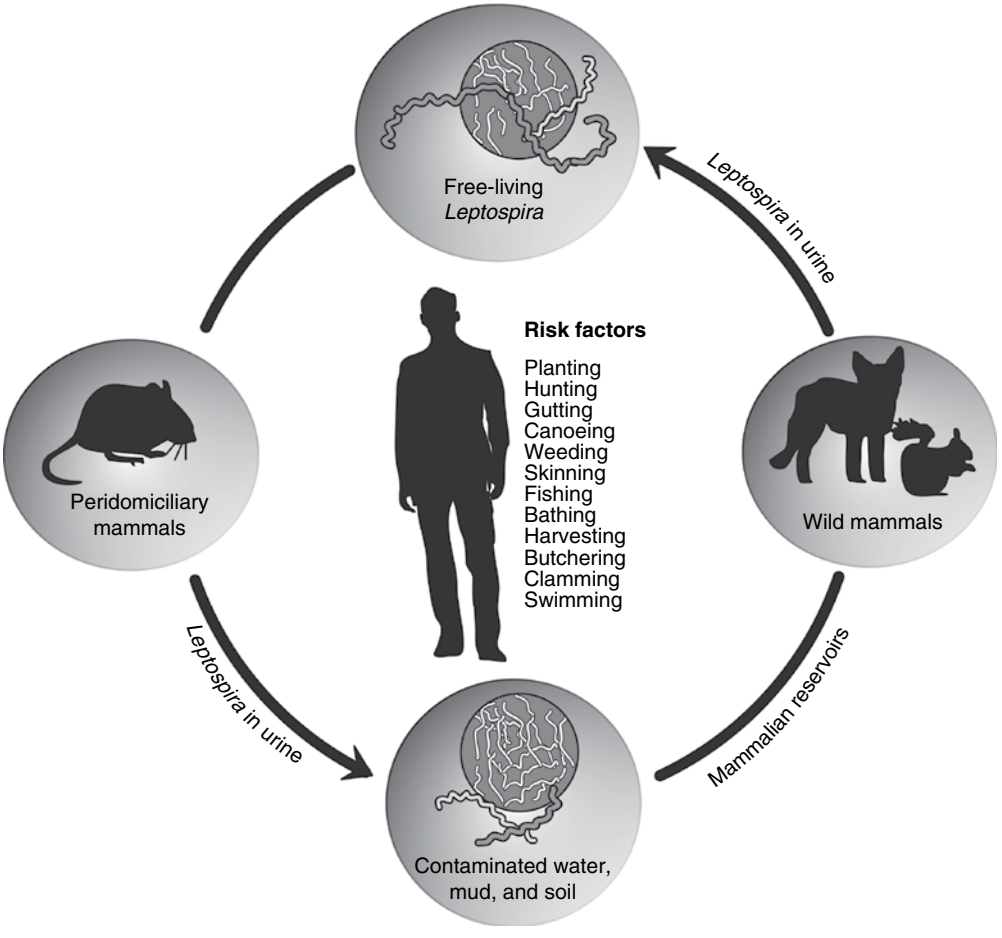

Figure 7.3. Transmission of leptospires.

Clinical Manifestations

Spectrum of Manifestations

The incubation period (time of exposure to first symptoms of leptospirosis is about 2 to 14 days, with an optimum range of 2 to 30 days (WHO, 2003). There is no serovar-specific differentiation in clinical manifestations, although some serovars tend to cause mild disease. The same serovar may cause mild or severe disease in different hosts depending on the host's immune status. The risk factors for severe illness are old age, multiple underlying health problems, and the infective dose, which may influence the course of the disease (WHO, 2003). The clinical presentations of the disease are not a diagnostic feature, and therefore, it must be confirmed by laboratory tests. Clinical presentation ranges from nonspecific febrile illness to severe icteric disease, acute renal failure, and adult respiratory distress syndrome (Figure 7.4).

In animals, the incubation period is from 2 to 20 days. In dogs, the liver, and kidney are most commonly affected by leptospirosis. In addition, a pulmonary form of canine leptospirosis found to be associated recently with severe hemorrhage in the lungs similar to the human pulmonary hemorrhagic syndrome (Klopfleisch et al., 2011; Kohn et al., 2010).

First Phase of Illness

Leptospirosis is classically a biphasic illness. The first phase is called the *leptospiromic phase* and includes high-grade fever, headache, retro-bulbar pain, transient maculopapular skin rashes, photophobia, subconjunctival hemorrhage, severe myalgia, prostration, tender hepatomegaly, and rarely, splenomegaly (Gulati et al., 2002; Faine, 1982; Andre et al., 2000). These nonspecific symptoms last for 4 to 9 days and may be misdiagnosed as influenza, dengue, rickettsiosis, or pyrexia of unknown origin (Gulati et al., 2002). In Chennai, 38 percent patients, labeled as cases of pyrexia of unknown origin, were later

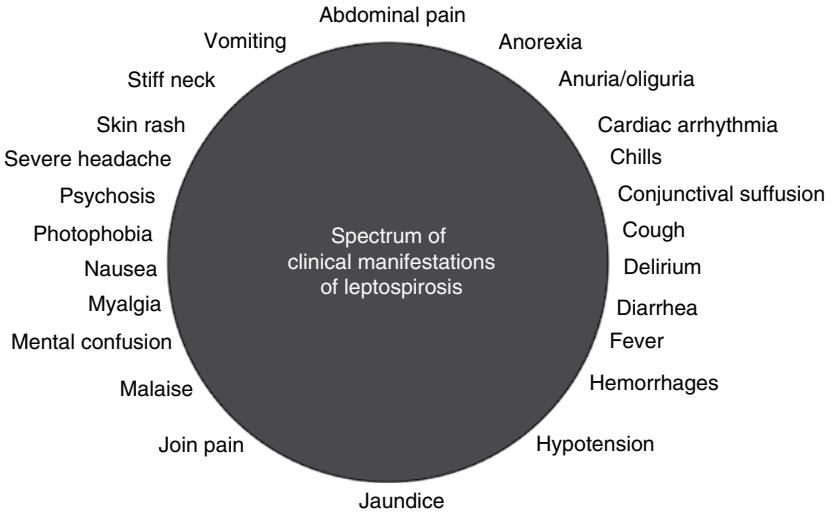

Figure 7.4. Spectrum of clinical manifestations.

diagnosed to be cases of leptospirosis (Vijayachari et al., 2004). Following the first phase, the patient may be asymptomatic for 1 to 3 days (Mitra, 1999).

Second Phase of Illness

The second phase is the immune phase, which coincides with the appearance of circulating IgM antibodies and the disappearance of *Leptospira* from the blood. This phase may last 4 to 30 days. The symptoms of this phase may be easily mistaken for worsening after improvement or as a continuation of the first phase (Mitra, 1999).

Most of the patients recover after this phase. Instead, a small proportion of patients develop various complications as a result of the involvement of multiple organ systems. In such patients, the clinical manifestations depend on the predominant organs involved (Scott and Coleman, 2003). The signs and symptoms are the result of damage the endothelium of the blood vessels, causing microhemorrhages. The mechanism of damage is not clearly understood. Though hepato-renal and central nervous system involvements are the most common complications of leptospirosis, any parenchymatous organ and even striated muscles may also be affected (Gulati et al., 2002). Hemorrhages may occur in intestines, lungs, or skin (Kuriakose et al., 1997). In addition, there are recent reports of a pulmonary form of canine leptospirosis associated with severe hemorrhage in the lungs similar to the human pulmonary hemorrhagic syndrome. (Kohn et al., 2010; Klopfleisch et al., 2011). During this phase, the clinical outcome probably depends on the involvement of body organ system. For example

- **Central nervous system**: Aseptic meningitis (10–15 percent), cranial nerve palsy, convulsions and sometimes Guillain-Barré syndrome may also occur (Faine, 1982; Andre et al., 2000).
- **Liver**: There will be elevated levels of serum alkaline phosphatase (normal values are 5–12 and 1.4–4.0 Bodansky units per dL in children and adults, respectively). Serum glutamic oxaloacetic transaminase (SGOT; or aspartate transaminase [AST]) and serum glutamic-pyruvic transaminase (SGPT; or alanine transaminase [ALT]) are either normal or mildly elevated (normal values are 8–40 and 0–40 Karmen units per dL, respectively). With liver involvement, leptospirosis has to be differentiated from alcohol-induced and viral hepatitis. SGOT levels are markedly elevated in alcohol-induced hepatitis, whereas SGPT levels are increased in viral hepatitis. High level of serum creatine phosphokinase (CPK) is suggestive of leptospirosis (NICD, 2006); normal level of serum creatine phosphokinase is 0 to 3.4 mm/dL and its MB fraction isoenzyme constitutes 0 to 5 percent.
- **Kidney**: Renal involvement is the most common complication of leptospirosis (Muthusethupathi et al., 1995, Dassanayake et al., 2012), which may range from mild asymptomatic transient albuminuria and pyuria to severe nephritis, which requires peritoneal dialysis or hemodialysis (Gulati et al., 2002). Severe renal involvement is characterized by oliguria or anuria, pedal and facial edema, and features of uremia (breathlessness, convulsions, delirium, and altered sensorium). Renal function starts improving after the second week of infection and complete recovery occurs usually by the end of the fourth week.
- **Heart**: Hemorrhages occur as a result of thrombocytopenia, disseminated intravascular coagulation, or coagulation factor deficiency, which is secondary to hepatic involvement. Patients may have petechial hemorrhages, purpura, epistaxis,

or gastrointestinal bleeding. In severe cases, ecchymosis or intracranial hemorrhage may also occur (NICD, 2006). Hypotension is another observable manifestation occurs probably as a result of the dehydration or peripheral vasodilatation. Clinical features are cold clammy, extremities; tachycardia; and thready pulse. Echocardiography reveals normal systolic function of the left ventricle (NICD, 2006). Myocarditis, cardiac dilatation, and failure may be seen in some cases. Atrial fibrillation and conduction defects have also been documented in some patients (Gulati et al., 2002). There may be evidence of rhabdomyolysis and hypotension (Mitra, 1999).

- **Pulmonary involvement**: It was first reported in India from North Andamans (Sehgal et al., 1995). Outbreaks of fever with pulmonary hemorrhage have also observed in persons living in flooded villages of Orissa, following the super-cyclone in October 1999 (Sehgal, 2000). Lung involvement occurs quite early in the course of the disease, during the first phase, which might indicate the possibility of another pathogenesis. Lesions appear in other organ failures are actually caused by endothelial injury to the microvasculature. This endothelial injury typically occurs during the second phase (second or third week) of illness when *Leptospira* starts invading the tissues. The cause of pulmonary involvement could be disseminated intravascular coagulation or adult respiratory distress syndrome. Because pulmonary complications occur early in the course of the disease, patients often report to the hospital at critical stages, when management is difficult (Singh et al., 1999). More than 90 percent of deaths in leptospirosis are the result of pulmonary alveolar hemorrhage, which is the underlying pathology in pulmonary involvement. In mild cases, patients present with cough, chest pain, and blood-tinged sputum. Severe pulmonary involvement is characterized by cough, hemoptysis, and rapidly worsening dyspnea, which may lead to respiratory failure and death. Patients have basal crepitations which rapidly spread to middle and upper lobes of the lungs. Chest X-ray reveals basal and midzone opacities in severe cases, but it may be normal in mild cases (NICD, 2006).
- **Weil's Syndrome**: This rare, severe icteric form of leptospirosis (also called Weil's disease) is named after Adolf Weil, who described this clinical entity (Gulati et al., 2002). Weil's disease has a high mortality rate and is characterized by hepatic dysfunction, icterus, hemorrhage, azotemia, and neurological changes (Gulati et al., 2002). Of an unknown hepatorenal syndrome in Pune, 34 percent of cases were diagnosed to have leptospiral infection (Gulati et al., 2002; Sharma et al., 2003).

Recovery and Late Sequel

- Most patients present only with mild fever and recover completely without any complications (Scott and Coleman, 2003). In some patients, recovery may take months or years, and late sequel may occur.
- Infection during pregnancy may lead to foetale death, abortion, stillbirth, or congenital leptospirosis in infants.
- The late sequel includes neuropsychiatric symptoms such as chronic fatigue, headache, paresis, paralysis, mood swings, and depression.
- Ophthalmic conditions, such as uveitis and iridocyclitis, are also part of late sequel because of the persistence of leptospires in the eyes, where they are sheltered from the patient's immune response (WHO, 2003).

Table 7.2. Differential diagnosis.

Aseptic meningitis
Borrelia infection
Brucellosis
Chemical poisoning
Dengue and dengue hemorrhagic fever
Enteric fevers
Food poisoning
Hantavirus infection and pulmonary syndrome
HIV sero-conversion illness
Infectious mononucleosis
Influenza
Legionnaire's disease
Malaria
Pharyngitis
Pyelonephritis
Pyrexia of unknown origin
Rickettsial infection
Toxoplasmosis
Viral hepatitis
Yellow fever or viral hemorrhagic fevers

Adapted from World Health Organization (WHO). 2003. Human Leptospirosis: Guidance for Diagnosis, Surveillance, and Control. Geneva: WHO.

Fatality

The fatality ranges from less than 5 percent to 30 percent in different parts of the world. Because the disease is not well documented, these estimates are not accurate. In recent times, excellent supportive care and dialysis have reduced the mortality to some extent. Causes of death in leptospirosis are renal failure, cardiopulmonary failure, and widespread hemorrhage (WHO, 2003).

Differential Diagnosis

The symptoms of leptospirosis may mimic those of other acute bacterial and viral diseases such as influenza, meningitis, dengue, or viral haemorrhagica fevers (Table7.2).

There is a vicious cycle in the awareness about leptospirosis, which is misunderstood and underdiagnosed because of the low index of clinical suspicion, confusion with other diseases, nonutilization of laboratory investigations in case of mild disease, and lack of confirmatory laboratory tests in developing countries, even when required. Consequently, few studies are carried out, which results in the disease being overlooked. Like leptospirosis in animals, the infection in humans may often be subclinical, and also among the symptomatic individuals, more than 90 percent have relatively mild (and usually nonicteric) forms of the disease (Speelman, 1998).

Laboratory Diagnosis

Laboratory examinations for leptospirosis should be considered for any patient presenting with abrupt onset of fever, chills, conjunctival suffusion, headache, myalgia, and jaundice. Conjunctival suffusion and muscle tenderness (usually in the calf and lumbar region) are the characteristic features (Singh et al., 1999). Patients may also present with respiratory symptoms (cough, dyspnea) or gastrointestinal symptoms (nausea, vomiting, diarrhea) or musculoskeletal symptoms (arthralgia) and skin rash. The physician should have specifically inquired for history of occupational or recreational exposure to an environment potentially contaminated with animal urine (WHO, 2003). In 1982, Faine devised a scoring system for the presumptive and probable diagnosis of leptospirosis (Table 7.3).

Leptospirosis is diagnosed presumptively if the total scores of Part A or Parts A and B is more than or equal to 26 or the total scores of Parts A, B, and C is more than or equal to 25. Scores that range from 20 to 25 suggest a probable diagnosis of the disease.

Importance of Laboratory Diagnosis

Patients exhibiting nonspecific clinical manifestations are usually subjected to various laboratory investigations, which generally do not include serology for the detection of leptospirosis. Because leptospirosis can also occur sporadically, health-care providers should have a high index of clinical suspicion of the disease so that the investigations are done at an early stage. All suspect cases need to be investigated thoroughly because it is not possible to diagnose the disease with certainty on clinical grounds alone (WHO, 2003).

Leptospirosis can affect any of the organ systems in the human body and thereby leads to a variety of clinical syndromes. In the acute stage, symptoms and signs mimic that of other acute bacterial and viral diseases. Clinical diagnosis is difficult because of its protean manifestations, and if not diagnosed and treated at an early stage, the disease can result in fatal complications. Hence early laboratory confirmation of the disease is necessary (Sehgal et al., 1995).

Suggestive Biochemical Parameters

Leptospirosis is suspected only if, in addition to the clinical parameters, at least three of the following seven biochemical parameters are positive (Gulati et al., 2002; Kuriakose et al., 2008).

1. Urine analysis: albuminuria, microhematuria, pus cells in urine, oliguria, presence of bile pigment casts, and creatinine more than 70 u/L.
2. Erythrocyte sedimentation rate (ESR) more than 50 mm at the end of 1 hour.
3. Leukocytosis with relative neutrophilia (neutrophils more than 80 percent).
4. Thrombocytopenia (platelet count of 100,000 per cubic mm or less).
5. Blood urea more than 40 mg/dL (normal is 7–21 mg/DL).
6. Serum bilirubin more than 2 mg/dL.
7. Serum amylase more than 200 Somogyi units.

Table 7.3. Faine's scoring system for presumptive diagnosis of leptospirosis.

Question/Finding		Answer	Score
Part A: Clinical Findings			
Headache of sudden onset		Yes = 2 No = 0	
Fever		Yes = 2 No = 0	
If "yes," is temperature more than 102.2° F (39° C)?		Yes = 2 No = 0	
Bilateral conjunctival suffusion		Yes = 2 No = 0	
Meningism		Yes = 2 No = 0	
Muscle pain		Yes = 2 No = 0	
Are any two features (conjunctival suffusion, meningism, muscle pain, jaundice, and oliguria) present together?		Yes = 2 No = 0	
Jaundice		Yes = 2 No = 0	
Albuminuria or nitrogen retention		Yes = 2 No = 0	
Subtotal			
Part B: Epidemiological Findings			
Question/Finding		Answer	Score
History of contact with any animal?		Yes = 10 No = 0	
Subtotal			
Part C: Laboratory Findings			
Question/Finding		Answer	Score
Isolation of leptospires in culture		Yes	Diagnosis certain
Positive serology in an endemic area	Single, positive, low titre	Yes = 2 No = 0	
	Single, positive, high titre	Yes = 10 No = 0	
	Paired sera, rising titre	Yes = 25 No = 0	
Positive serology in a nonendemic area	Single, positive, low titre	Yes = 5 No = 0	
	Single, positive, high titre	Yes = 15 No = 0	
	Paired sera, rising titre	Yes = 25 No = 0	
Subtotal			

Adapted from Faine S. 1982. *Guidelines for Leptospirosis Control*. WHO offset Publication 67. Geneva: World Health Organization.

Table 7.4. Specimens for laboratory diagnosis.

Specimen	Time Period
Blood for culture	During the first 7 days of illness
Cerebrospinal fluid (CSF)	From the 4th to 10th day of illness
Urine for culture	After the 10th day, up to 4 weeks
Postmortem specimens for culture	As soon as possible after death
Serum for sero-diagnosis	At any stage of illness

Adapted from World Health Organization (WHO). 2003. *Human Leptospirosis: Guidance for Diagnosis, Surveillance, and Control*. Geneva: WHO; and Gulati S, Menon S, Kabra M, Caudhry R, Kalra V. 2002. Leptospirosis: A case report. *Pediatr Today* 7:428–433.

Specimens for Laboratory Diagnosis

For laboratory diagnosis, the type of clinical sample actually depends on the phase and severity of infection (Table 7.4).

Clinical specimen containers should be clearly labeled with the information specified below:

- Name of the patient and his or her registration number.
- Name of the patient's mother and father.
- Age, sex, and complete residential address of the patient.
- Name and address of the institution sending the sample.
- Dates of onset of illness, hospitalization, and collection of sample.
- Brief clinical history, examination findings, and provisional diagnosis.
- Results of clinical laboratory investigations (Gulati et al., 2002).

Methods for Laboratory Diagnosis

Various microscopical, bacteriological, immunological, and molecular methods can be used for the laboratory diagnosis of leptospirosis (Figure 7.5).

Wet Mounts

Dark-Field Microscopy

Leptospires are too thin and therefore cannot be detected in wet mounts by ordinary microscopes. In dark-field microscopy, oblique light is cast on the microorganism by a special condenser. The central light is interrupted so that light incident on the microorganism gets reflected upwards and enters the objective.

Specimens and Procedure

Leptospires can be demonstrated in blood during the first 7 days of illness (when the patient is in leptospiremic phase) and in urine 5 to 10 days after onset (during the phase of leptospiruria). They may be occasionally demonstrated in cerebrospinal fluid (CSF), aqueous humor, and amniotic fluid.

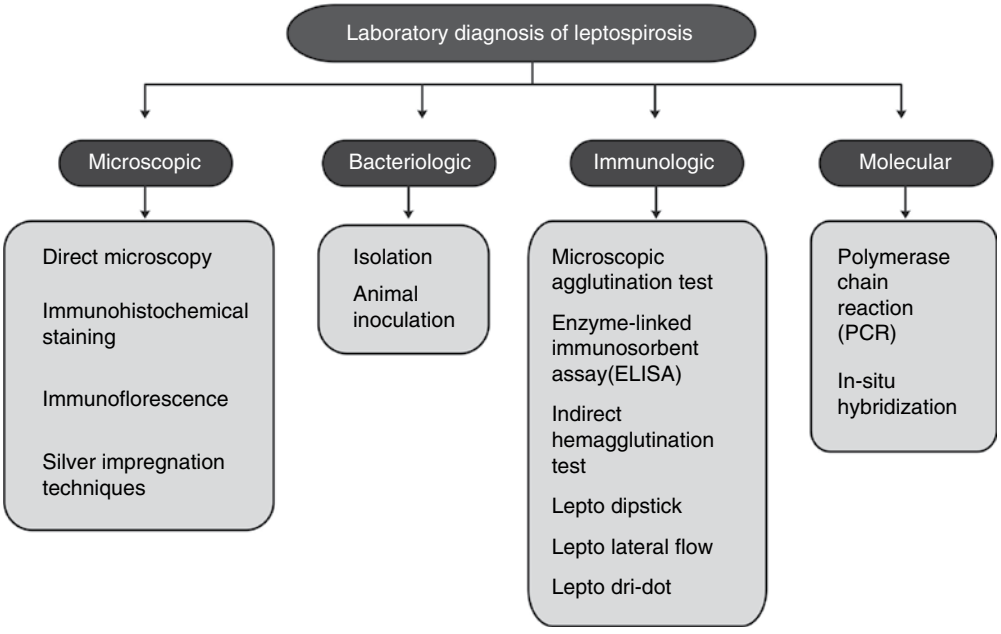

Figure 7.5. Laboratory diagnostic methods for leptospirosis.

Urine Sample: Midstream urine samples should be collected early in the morning on three successive days because the urinary shedding of leptospires may be intermittent. Because leptospires are rapidly killed in acidic urine, the patient should be given one teaspoon of sodium bicarbonate in water on the previous evening to alkalinize the urine. The same dose should also be repeated on the morning of sample collection. If this is not possible, check the pH of the collected urine sample at the bedside and neutralize the collected sample with one drop of sterile sodium bicarbonate from an ampoule before transporting the sample to the laboratory. To increase the likelihood of visualizing leptospires, 5 to 10 mL of the urine sample is centrifuged at 1500 RPM for 20 minutes. Larger debris settles downward. The supernatant is transferred to another centrifuge tube, which is centrifuged at 5000 RPM for 20 minutes by a technique of double centrifugation. The deposit is transferred on to a glass slide and examined under dark ground microscope using a 40x objective.

Blood The blood sample should be 5 mL of venous blood collected in an ethylenediaminetetraacetic acid (EDTA) bulb. The sample is centrifuged at 1000 RPM for 15 minutes to separate the plasma. A drop of plasma is then transferred on to a glass slide and examined under dark ground microscope using a 40x objective. If leptospires are visualized, centrifuge the plasma at 3000 to 4000 RPM for 20 minutes. Remove the supernatant and examine a drop of the sediment under dark ground microscope using a 40x objective.

Other Body Fluids Use 2 mL CSF or bronchoalveolar lavage fluid and collect in a sterile tube.

Interpretation
Leptospires are seen as silvery threads against a dark background. They have a typical flexuous motility (darting quickly, holding the middle part rigid and bending the ends like coat hangers), and rotation along a longitudinal axis, called corkscrew motility.

Advantages Dark field microscopy is an adjunct to clinical diagnosis if history and clinical examination findings are suggestive of leptospirosis. This technique is also used to examine leptospires in culture and to detect agglutination in MAT.

Disadvantages The procedure is technically demanding and requires skilled personnel. It is difficult to detect low numbers of leptospires. There is a high risk of false-positive results because serum protein, fibrin strands, and cellular debris resemble leptospires, and negative results do not exclude the possibility of leptospirosis. Sometimes, the concentration of leptospires in urine may be too low to be detected by this method. For these reasons, dark ground microscopy is not recommended as a single diagnostic tool. Detection of leptospires by this method ought to be confirmed by other diagnostic tools (WHO, 2003).

Staining Techniques

Silver Impregnation Technique (Fontana's method)
Leptospires cannot be stained properly by conventional Gram stain or aniline dyes, so special stains are used to visualize them. Silver impregnation methods are used for contaminated specimens such as urine and fixed tissues.

Preparation of Solutions

- **Fixative**: Acetic acid (1 mL) + Formalin (2 mL) + Distilled water (100 mL)
- **Mordant**: Phenol (1 g) + Tannic acid (5 g) + Distilled water (100 mL)
- **Ammoniated silver nitrate**: Take 0.5% solution of silver nitrate in distilled water. Add 10% ammonia till the precipitate formed just dissolves. Further add more silver nitrate solution drop by drop until the precipitate returns and does not redissolve.

Procedure

1. Prepare smear by placing centrifuged urine deposits on a clean slide and allow it to dry.
2. Treat the film three times each for 30 seconds with the fixative.
3. Wash off the fixative with absolute alcohol followed by the treatment with absolute alcohol again for 3 minutes.
4. Drain off excess alcohol and carefully burn off the remainder until the slide is dry.
5. Pour the mordant on the slide and apply heat with a spirit lamp till steam rises.
6. Allow action of the hot mordant on the slide for 30 seconds.
7. Wash the slide in distilled water and allow to air dry.
8. Pour ammoniated silver nitrate solution on the slide and heat with a spirit lamp till steam rises. Allow action of the hot mordant on the slide for 30 seconds, when the smear film becomes brown in color.
9. Wash the slide in distilled water and air dry it.

10. Mount in Canada balsam and cover with a cover slip because some immersion oils cause immediate fading of the stained film.
11. Examine the stained slide using a 100x objective (oil immersion lens). Leptospires are seen as brownish black spiral organisms against a brownish yellow background.

Disadvantages The number of organisms may be too low to permit visualization. Silver impregnation technique has low sensitivity and specificity because artefacts such as serum protein, fibrin strands, and cellular debris resemble leptospires and may lead to false-positive reports. Therefore, this is not recommended as a routine method for diagnosis.

Isolation of Leptospires in Culture

Leptospires may also be cultivated from clinical specimens (blood, CSF, urine, aqueous humor, or peritoneal or amniotic fluids) and postmortem specimens for definitive diagnosis of leptospirosis.

Advantages

Culture provides definite proof of leptospiral infection. Isolated leptospires can be sero-typed to identify locally pathogenic serovars and to detect new serovars (useful as a tool for epidemiological surveillance). Local isolates can be used as antigens for microscopic agglutination test (MAT). Culture is a useful for postmortem diagnosis of infection in patients who died in the early phase of infection before antibodies could be detected (WHO, 2003).

Disadvantages

Leptospires grow slowly with a maximum doubling time of 6 to 8 hours and require the maintenance of optimal temperature of 82.4 to 86° F (28–30° C), which makes it time consuming and laborious. Clinical specimens need to be collected before starting antibiotic therapy because the use of antibiotics adversely affects growth of leptospires in culture media. As a result of the prolonged incubation period, there is a likelihood of contamination with additional bacteria. By the time diagnosis is made, antibodies are already detectable by serological techniques. Thus, culture is not useful as a routine diagnostic tool for treating patients (WHO, 2003).

Clinical Specimens for Culture

Blood Venous blood (two to three drops) is collected aseptically at the bedside and inoculated in 10 to 12 mL of culture medium in blood culture bottles during the first 7 days of illness before administering antibiotics. Anticoagulants may be detrimental to successful isolation therefore, should not be used. Blood culture is not recommended after 7 days because the leptospires would have disappeared from the bloodstream. Moreover, sero-diagnosis is possible after 10 days of illness because of the presence of detectable antibodies. A blood sample collected for culturing purpose should be stored and transported at ambient temperature because low temperatures are detrimental to pathogenic leptospires. In the laboratory, a few drops of blood (a small inoculum) are inoculated into tubes containing 5 mL of appropriate culture medium. Cultures are to be incubated at 86° F (30° C) and observed for growth for a period of four to six months (WHO, 2003).

Cerebrospinal Fluid On the 4th to 10th day of the illness, 0.25 to 0.5 mL of CSF is collected and inoculated in 10 to 12 ml of culture medium.

Urine *Leptospira* may be voided in urine of suspected individuals for 1 to 4 weeks. The pH of urine is made neutral to increase the survival of *Leptospira* (WHO, 2003). The urine samples are collected and alkalinized similar to that described for microscopy. Before inoculation, the urine sample is either filtered through a 0.22-μ filter or diluted with sterile phosphate buffer to minimize the likelihood of contamination. Three to four dilutions are made from collected urine sample (1:10, 1:20, 1:40, and 1:80) using sterile phosphate buffer (pH 7.2). Finally, 0.5 mL of each dilution is inoculated in 5 mL of medium preferably within 2 hours of voiding because leptospires die quickly in urine.

Postmortem Specimens In fatal cases of human and animal leptospirosis, the organism can be cultured from minced postmortem tissue specimens, such as kidney or liver or aborted animal fetuses. A small piece of tissue is kept inside the barrel of a syringe (without needle), and pressure is exerted on the plunger. The minced tissue from the nozzle is taken on culture medium. Alternatively, the tissue is placed in a mortar along with phosphate-buffered saline at pH 7.2 and minced. The resulting suspension is then inoculated in culture media. Postmortem specimens should be collected aseptically from as many organs as possible (brain, CSF, aqueous humor, lungs, kidney, liver, pancreas, and heart) and inoculated in suitable culture medium as soon as possible. Samples may be stored and transported at 39.2° F (4° C). Fresh-fixed tissue can be examined using fluorescent antibody technique. Blood from the heart is collected for serological tests (WHO, 2003). Postmortem changes can rapidly reduce the number of viable leptospires. These organisms are rapidly killed in tissues stored at 68° F (20° C) or at higher temperatures because of the autolysis of cells and consequent decline in pH. Freezing of tissues will also reduce the viability of leptospires (WHO, 2003).

Culture Media

Leptospires are fastidious organisms having complex growth requirements. Vitamin B_1, B_{12}, and long-chain (>15 carbon atoms) fatty acids are the only known essential nutrients for leptospires. Rabbit serum contains the highest concentration of bound vitamin B_{12}. The organism use fatty acids (not carbohydrates) as a source of energy but cannot synthesize them. Pyruvate is a nonessential nutrient that enhances growth of fastidious leptospires. In contrast to most other bacteria, leptospires do not use external sources of pyrimidine bases for their DNA or RNA synthesis. They are thus resistant to antimicrobial action of 5-fluorouracil (a pyrimidine analogue). For this reason, 5-fluorouracil is used for the selective isolation of *Leptospira* from contaminated clinical samples (WHO, 2003). There are three categories of culture media used for the isolation of leptospires:

1. **Liquid media**: Generally used for isolating leptospires as well as useful for the preparation of antigens for serological testing because agar particles, which are present in semi-solid and solid media, interfere with interpretation of serological tests. Growth of leptospires (to be confirmed by microscopic examination) is visualized by the naked eye as turbidity or by granular precipitate at the bottom of culture tubes.
2. **Semi-solid media**: Consisting agar (0.1–0.5% w/v) and are used in screw-capped bottles for the maintenance of stock cultures and primary isolation of various strains

of *Leptospira*. The growth (to be confirmed by microscopic examination) is visualized by the naked eye as rings or dense growth slightly beneath the surface.

3. **Solid media**: Contains higher agar concentration (0.8–1.3% w/v) and requires proper sealing of cultures to retain moisture and prevent dehydration. Growth occurs beneath the surface and is visible within 7 to 14 days for most of the serovars (WHO, 2003).

Choice of Culture Medium

Five types of culture media are available for growing leptospires.

1. **Traditional media containing about 8 to 10% rabbit serum**: Fletcher, Korthof, Schüffner, Stuart, and Vervoort media are commonly used (Gulati et al., 2002). For MAT test, Korthof and Schüffner media are not useful because they contain phosphate, which might precipitate. Cultures become positive in 1 to 6 weeks (WHO, 2003). If rabbit serum is easily available, it is preferable to use Fletcher's semi-solid medium for isolation and modified Korthof medium for antigen preparation.
2. **Ellinghausen-Mccullough-Johnson-Harvis (EMJH) medium**: This contains Tween 80 or Bovine serum albumin (BSA), as detoxicant (WHO, 2003). This medium can be used in case rabbit serum is not easily available (Wuthiekanun et al., 2007).
3. **Protein-free or low-protein media**: For example Shenberg, Bey, and Johnson media particularly used for vaccine production.
4. **Enriched media**: Enriched media can be prepared by adding 1 to 4% fetal calf serum and rabbit serum. EMJH medium may also be enriched by adding 1% rabbit serum and 1% fetal calf serum, which is used for increasing the growth of fastidious leptospires such as serovar *hardjo*.
5. **Selective media**: Selective media are used to suppress the growth of contaminating bacteria in clinical samples. For this purpose, we can add:
   (a) 5-fluorouracil (50–1,000 μg/mL), or
   (b) A combination of nalidixic acid (50 μg/mL), vancomycin (10 μg/mL), and polymixin B sulphate (5 units/mL), or
   (c) Antimicrobial agents such as Acti-Dione, amphotericin B, neomycin, rifampicin, or sulphathiazole). However, these additives (particularly sulfathiazole) might also suppress the growth of leptospires (WHO, 2003).

Culture Technique

After inoculation, the media is incubated at 82.4 to 86° F (28–30° C) and should be inspected for growth of leptospires or of contaminating organisms after Days 1, 3, and 5 and weekly thereafter. The cultures need to be incubated for at least 30 days before they are discarded as negative. Growth may be visualized as a subsurface ring called as *Dinger's ring*. The culture media must be examined by dark ground microscopy even if growth is not visible to the naked eye. Immediately after growth appears, several replicate cultures are to be made in fresh media using relatively large inocula.

Identification of Isolates

Pathogenic and saprophytic leptospira could not be differentiated on the basis of morphology because they all look alike with minor differences. Therefore, differentiation is based on culture and antigenic and genetic properties. Unlike pathogens, saprophytes grow at 55.4° F (13° C) in the presence of 8-Azaguanine (concentration of 225 mg/mL) and get converted into spherical forms in 1-M solution of sodium chloride. The infecting

serogroup and serovar can be further identified by agglutination with specific antisera. Because these techniques are not available at most of the diagnostic laboratories, the isolate has to be sent to a reference laboratory.

Quality Control Paired samples are taken from each batch of medium and tested for sterility. One sample from each pair is incubated at 86° F (30° C) to detect possible leptospiral contamination, whereas the other sample is incubated at 98.6° F (37° C) to detect possible contamination with other bacteria. Turbidity (in most media) or sediment deposits (in serum-containing media) indicates contamination, which is further followed by subculturing on conventional bacteriological media.

Purification of Contaminated Cultures

Contaminated cultures can be purified by using liquid, solid, or selective media or through animal inoculation procedures.

- **Liquid media**: Contaminated cultures are first filtered through a membrane filter (with average pore diameter of 0.22 μ). The filtrate is centrifuged subsequently and then inoculated in fresh medium to remove toxic microbial products.
- **Solid media**: A loopful of inoculum from the contaminated culture is plated on to the surface of a fresh solid medium. The contaminants remain at the site of inoculation, but the leptospires migrate to other sites from where they can be subcultured.
- **Selective media**: Contaminated cultures are subcultured in media containing antimicrobial agents.
- **Animal inoculation**: About 1 mL of the contaminated culture is inoculated intraperitoneally into a hamster, guinea pig, or mouse. Leptospires are recovered from blood cultures taken after 20 minutes to 1 hour.

Maintenance of Routine Cultures

Strains of leptospires that are routinely used as sources of antigens are maintained in 5 to 10 mL of liquid media in high quality hard-glass screw-capped tubes. Cultures are incubated at 86° F (37° C) for 5 to 7 days and then kept at room temperature. After every 2 to 3 weeks, about 10 percent of the volume is transferred to fresh media to make subcultures. These subcultures are further examined microscopically to confirm the presence of viable leptospires and the absence of contaminating organisms.

Stock Cultures

Stock cultures should be maintained in semi-solid media and stored in the dark at room temperature in screw-capped bottles. These cultures remain viable for at least three months and usually up to 1 year. Semi-solid agar containing rabbit serum, neomycin sulfate (20 μg/mL) and 5-fluorouracil (250 μg/mL), is useful for maintaining stock cultures for many months. Cultures of leptospires may be preserved for years by lyophilization or by storing in liquid nitrogen (using 5% dimethyl sulfoxide or 5–10% glycerol as cryoprotectant). Leptospires also survive for up to 8 years at room temperature in media containing serum and hemoglobin, and in biphasic agar medium containing 0.5% activated charcoal. The pH should be maintained at 7.4 because leptospires do not survive in acidic pH. Bottles containing the media are to be labeled indicating the date of preparation of the media and date of inoculation with leptospires.

Serological Tests for Detecting Antigens

All the three types of antibodies (i.e., genus-specific, serovar-specific, and serogroup-specific) in the host's immune system react with leptospiral antigens when patient's blood is brought in contact with antigens in the serological test kits. Some tests use live leptospires as antigens, whereas others employ extracts of the organism. After diluting patient's serum serially, the highest dilution that shows a detectable reaction is determined as the *titer*. A titer above a cut-off point is called a positive or significant titer. The detectable titer in serum will depend on relative concentrations of antigens and antibodies and the strength of the reaction between them (WHO, 2003). A variety of serological methods is used for the detection of anti leptospiral IgM as well as IgG antibodies. Some are used as screening tests for leptospirosis. To make a reliable diagnosis, it is essential to use multiple techniques (together or in succession).

As IgM is the antibody of primary immune response, it can be detected within 3 to 10 days after infection. IgG antibodies appear late (usually after 15–30 days) but persist for years. A fourfold rise in IgG titers confirms diagnosis of leptospirosis. Antibody levels usually drop over a period of weeks or months but may be detectable for over 2 to 10 years. Genus-specific antibodies usually appear and fall earlier, but the serovar-specific antibodies fall slowly and variable titers may be detectable even years after infection. Testing of paired sera is necessary to detect sero-conversion or rise in titers between the paired samples.

The serological tests that helps in the diagnosis of leptospirosis include:

- Enzyme-linked immunosorbent assay (ELISA) is a rapid, sensitive, and specific test, which can be designed to detect IgM antibodies.
- MAT: Although it is technically complex and cumbersome, it is highly sensitive and specific and is currently accepted as the gold standard. It helps in identifying the infecting serovar and is, therefore, useful for epidemiological surveillance (Ahmad et al., 2005).
- Complement fixation test (CFT): Standardization of reagents is a technically complex procedure. Its limitations include short shelf life of reagents and anticomplement activity of sera.
- Counterimmuno electrophoresis (CIEP): Its is commercially not available.
- Lepto dipstick: Simple to perform, it can be used as a screening test in field situations. It detects leptospira-specific IgM antibodies (Sehgal et al., 2003).
- Lepto Tek lateral flow: This screening test can be performed directly on whole blood.
- Dried latex agglutination test (Lepto Tek Dri Dot): It is commercially available for screening and has good sensitivity but low specificity.
- Indirect fluorescent antibody test (IFAT): Requires fluorescent microscope (expensive) and, therefore, is not used in routine diagnostic laboratories.
- Indirect hemagglutination test (IHA): Though it has low specificity, IHA is easy to perform and can be used as a screening test.
- Microcapsule agglutination test (MCAT): Though simple to perform and easy to read, this is not a confirmatory test.
- Macroscopic slide agglutination test (MSAT): It is a good screening test with high sensitivity but has low specificity.
- Patoc-slide agglutination test (PSAT).

Advantages

The probability of successful sero-diagnosis is high in the early phases of leptospirosis (leptospiremia). Serology is the most appropriate method for diagnosis because other methods are time consuming or have limited reliability and patients seeking medical care would have been ill for sufficiently long period and would have produced detectable antibodies.

Limitations

The antibody titer increases gradually during the course of illness, peaks and decreases after recovery. Weak serological reaction may probably be the result of the administration of a high dose of antibiotics during the early phase of illness, presence of nonspecific antibodies, severe illness, immune suppression, or very early or very late phase of immune response. The presence of IgG antibodies does not clearly indicate recent infection, but IgM antibodies indicate acute infection. Instead, fourfold rise in IgG titers in paired sera confirms the diagnosis of leptospirosis (Levett et al., 2001). An IgG titer of 1:100 can be present because of a past infection (Mitra, 1999). Positive serological tests do not always indicate current infection because some antibodies persist for a long time. Hence, two consecutive samples should be sent to the laboratory, and a fourfold rise in titer is considered indicative of recent or current infection. The results of serological tests should always be correlated with clinical presentation and history of risk factors. The only definitive proof of leptospiral infection is the isolation of pathogenic leptospires (WHO, 2003). Sero-diagnostic tests for leptospirosis have demonstrated cross-reactivity with hepatitis A and E viruses. Hence results of serological tests should be interpreted with caution (NICD, 2006).

Choice of Serological Test

As already reported, a wide variety of serological tests are available for diagnosing leptospirosis. These tests are genus-specific, serogroup-specific, or serovar-specific. Genus-specific tests are more sensitive, less specific, and rapid. The serogroup- or serovar-specific tests are useful for confirming the diagnosis and also for sero-epidemiological purposes (identifying the sources of infection, reservoirs, and the circulating serovars in the community). Ideally, the serum sample is to be screened using a rapid or simple test that detects IgM antibodies. Subsequently, the diagnosis is to be confirmed by the gold standard (MAT).

Enzyme-Linked Immunosorbent Assay Several ELISA methods are available, depending on the type of antigens and reagents used. This test detects only genus-specific IgM antibodies and does not identify infecting serovar or serogroup. Conventional ELISA, such as PanBio IgM ELISA, is performed using antigen-coated microcells and a sonicated preparation of different antigens. The type of antigens used depends on the manufacturer of the test kit. Though in-house ELISA is a cheap alternative to commercially available ELISA kits, it requires standardization. The conventional tests have evolved to more convenient and rapid ELISA-based tests:

- For detecting leptospira-specific IgM: DOT ELISA and Dipstick ELISA.
- For detecting leptospira antigens: Sandwich ELISA.

Advantages Being more sensitive to IgM antibodies as compared to MAT, ELISA can detect IgM antibodies about 6 to 8 days after the onset of first clinical manifestations, but it may be delayed if antibiotic treatment has been started. The titer of IgM antibodies rises and falls rapidly; thus, a positive ELISA test is suggestive of current infection. In this test, only a single genus-specific antigen is used, and there is no need to maintain a panel of cultures in local laboratory because ELISA kits are commercially available (WHO, 2003). Because of its high specificity and sensitivity, ELISA test can be used in epidemiological studies to determine sero-incidence or sero-prevalence of leptospirosis (NICD, 2006).

Disadvantages ELISA is less specific as compared to MAT, hence why the latter is used as a screening test. In co-infection with other pathogens, weak cross-reactions may occur. Because ELISA is based on a genus-specific antigen, it does not indicate the infecting serovar (WHO, 2003). It requires an ELISA reader, micropipettes, ELISA washing system, and trained personnel. Reagents need to be stored under refrigeration and continuous electric supply is required for storing reagents and for performing the test.

DOT ELISA In this test, minute quantities of the antigen are dotted on nitrocellulose discs and the sera is allowed to react with chromogenic substrate that can be precipitated. The test is rapid as takes about 2 hours, economizes on quantity of antigen used, and can be performed in field settings because of its portability. Its sensitivity and specificity matches that of the conventional ELISA techniques.

Dipstick ELISA The dipstick assay is easy to perform quickly, and it does not require electricity or special equipment (Sehgal et al., 2003). An additional advantage is that the dipstick and the staining reagent can be stored for prolonged periods at tropical temperatures (Smits et al., 1999). For this purpose generally, an antigen derived from serovar *hardjo* of *Leptospira interrogans* is used with horse radish peroxidase enzyme. An antispecies antibody conjugated to enzyme is then added. The activity of the enzyme is determined by adding a specific chromogenic substrate. Within a certain range of concentration, intensity of color reaction is proportional to the quantity of antibody present in the serum sample. IgM titer of 1:80 to 1:100 is considered suggestive of leptospiral infection. Diagnosis can further be confirmed by MAT.

Sandwich ELISA A two-tip nitrocellulose dipstick (after loading with conjugate and incubating at room temperature for 45 minutes) is incubated in substrate solution for 3 to 5 minutes until a colored dot appears at the upper tip of the dipstick. The colored dots develop in both upper and lower tips of the dipstick, which indicates a positive result. If only the upper tip of the dipstick shows a colored dot, the test is considered negative.

Microscopic Agglutination Test Martin and Pettit (1918) originally developed MAT as an agglutination-lysis test, and it was subsequently modified. The notion of lysis was later abandoned as a misinterpretation. The test uses multiple antigen pools; serovars from each serogroup are pooled and used as antigens so that each serum sample has reacted with the maximum possible number of different leptospiral serovars (WHO, 2003). Only experienced laboratories that can maintain a large number of live, locally prevalent strains of *Leptospira* use the microscopic MAT test, which employs multiple live antigens (Adler and Faine, 1978).

The macroscopic MAT test uses killed (formalized) antigens, which are less specific, but more sensitive than live antigenic preparations. Moreover, they are safe to use and can be stored for a few weeks (WHO, 2003).

Table 7.5. Panel of cultures for microscopic agglutination test.

Serogroup	Serovar	Strain
Andamana	*andamana*	CH 11
Australis	*australis*	Balico
	bratislava	Jez Bratislava
Autumnalis	*autumnalis*	Akiyami
	rachmati	Rachmati
Ballum	*ballum*	S 102
Bataviae	*bataviae*	V Tienen
Canicola	*canicola*	Hond Utrecht IV
Celledoni	*celledoni*	Celledoni
Cynopteri	*cynopteri*	3522 C
Grippotyphosa	*grippotyphosa*	Moskva V
Hebdomadis	*hebdomadis*	Hebdomadis
Icterohemorrhagiae	*icterohemorrhagiae*	RGA
	cpoenhageni	M 20
Javanica	*javanica*	Veldrat Bat 46
	poi	Poi
Panama	*panama*	CZ 214
Pomona	*pomona*	Pomona
Pyrogenes	*pyrogenes*	Salinem
Shermani	*shermani*	LT 821
Sejroe	*sejroe*	M 84
	hardjo	Hardjo prajitno
Semaranga	*patoc*	Patoc 1
Tarassovi	*tarassovi*	Perepelitsin

Adapted from World Health Organization (WHO). 2003. *Human Leptospirosis: Guidance for Diagnosis, Surveillance, and Control*. Geneva: WHO.

Both microscopic and macroscopic MAT tests become positive about 10 days after the onset of first clinical manifestations, with peak titers at 3 to 4 weeks after onset. Sero-conversion may sometimes be detected by MAT earlier (5 to 7 days after the onset of first clinical manifestations). The antibody response might be delayed if antimicrobials are administered before collecting the serum samples. Heterologous antibodies (that cause cross-reactions) are the first to appear and vanish rapidly. Homologous antibodies appear slightly later and persist for prolonged periods (WHO, 2003).

Panel of Cultures For use as antigens in MAT, a panel of cultures comprising all locally occurring serovars of live leptospires are subcultured in EMJH medium in the local laboratory (Table 7.5). Cultures between the 4th and 10th days of growth at 86° F (30° C) are used. Before use, the strains are diluted 1:2 in physiologically buffered water to obtain a density of 1 to 2 x 10^8 leptospires per mL. Culture maintenance is essential because:

- Agglutinating antibodies often react with only a particular serovar or serogroup.
- Multiple serovars may cause illness in a given locality and their relative prevalence may change with changes in human behavior and agricultural practices.
- New serovars may be introduced in a locality (WHO, 2003).

Patoc 1 is a saprophytic serovar strain, which is also included along with the pathogenic serovars. It behaves like genus-specific antigen and would, therefore, detect antibodies against serovars not yet known to exist in a particular region. If the panel of cultures is incomplete, antibodies to the missing serovar (from the panel) may not be detected, leading to false-negative results. If the locally occurring serovars are unknown, the panel should consist of serovars representing all serogroups (WHO, 2003). In laboratories where it is not possible to maintain a panel of pathogenic leptospires, clinical suspicion of leptospirosis can be confirmed by genus-specific MAT using the Patoc 1 strain. Ideally, two consecutive serum samples should be examined for sero-conversion. Alternatively a fourfold (or greater) rise in titer should be considered significant. If a single serum sample is sent to the laboratory, different cut-off points are applied in different localities. These cut-off points vary from 1:100 to 1: 800 (WHO, 2003). When information about the circulating serovars is lacking, the panel of cultures in Table7.5 may be used.

Method Several serial doubling dilutions of sera are mixed with *Leptospira* antigens. If antileptospiral antibodies are present in the patient's serum, the leptospires tend to stick together to form clumps (agglutination). Movement of the free ends of agglutinated leptospires is visible by dark field microscopy. After completion of agglutination, the microscopic field is devoid of free leptospires. Agglutinating antibodies may belong to both IgM and IgG classes.

Interpretation The end point is the dilution, which shows 50 percent agglutination, leaving 50 percent free cells, when compared with a control culture diluted in 1:2 phosphate-buffered saline (PBS). If the proportion of free leptospires is 50 to 100 percent, the test is considered as negative, and it is considered positive if the proportion of free leptospires is less than 50 percent (WHO, 2003). Antibody titers should be interpreted after considering:

- Date of collecting serum sample, in relation to date of onset of first clinical manifestations.
- Treatment given.
- Rising antibody titer between two or three successive serum samples.
- Causative serogroup.

Advantages Currently, this test is the gold standard for detecting leptospiral antibodies and determining their titer because of its high diagnostic specificity in comparison with currently available serological tests (WHO, 2003; Gulati et al., 2002). Both IgM and IgG antibodies can be detected by this test (WHO, 2003). It helps in identifying the infecting serovar and is useful for epidemiological purposes.

Disadvantages of MAT MAT is a technically demanding and time-consuming procedure requiring skilled personnel. If only a single serum specimen is available, the significant titer has to be calibrated. False-negative results may be obtained in the early phase of the disease. It is necessary to maintain a panel of cultures; if a strain is not represented on the panel, it

may lead to false-negative result. Moreover, it is never possible to ensure that the panel is complete because new, unidentified strains may cause illness. MAT cannot be standardized (unlike ELISA) because live leptospires are used as antigens and several factors (age and density of antigen cultures) affect the agglutination titers. There may be day-to-day variations in test results. To overcome this problem, paired samples should be tested together. MAT may indicate the serogroup to which the infective serovar belongs, but it only rarely identifies it. MAT detects both IgM and IgG antibodies. Because IgG antibodies persist for prolonged periods, the test cannot differentiate between agglutinating antibodies as a result of current, recent or past infection. High titers may persist for many years (Sehgal, 1998). In the first few weeks of illness, a heterologous cross-reaction with other serovars may be positive, but the homologous reaction with the infecting serovar may be negative. This is termed *paradoxical reaction* (WHO, 2003). Because of these disadvantages, a genus-specific screening test such as ELISA is used before performing MAT.

Lepto Dipstick Test Lepto dipsick test detects leptospira-specific IgM antibodies in human sera. The dipstick contains two horizontal bands, lower and upper:

1. **Lower band**: It is an antigen band comprising *Leptospira*, which are broadly reactive.
2. **Upper band**: It is an internal control bound to a nitrocellulose strip. Human *Leptospira*-specific IgM antibodies that bind to the *Leptospira* antigen of the lower band are detected by an anti-human IgM-dye conjugate.

To perform this test: white end of the test strip is dipped in the dipstick fluid for 1 minute to moist it, followed by the incubation for 3 hours at room temperature in a mixture containing detection agent and serum. Development of a visible red colored antigen band indicates a positive test and if the red colored band doesn't appear, the text is negative. The upper band (internal control) should also be stained in all cases, otherwise the assay is considered invalid. The possibility of weak staining of the antigen band can be overcome by repeating the test with the same sample or, if possible, with a fresh sample.

Advantages Lepto dipstick test is easy to perform and read and operates only at a single dilution and does not require any special equipment. The dipsticks and its reagents have a long shelf life, even at room temperature.

Disadvantages This test is not able to detect the infecting serovar and is expensive also especially for large-scale use.

Lepto Dri Dot It is a card agglutination test developed by the Royal Tropical Institute (KIT; Amsterdam, Netherlands) for the rapid diagnosis of leptospirosis (Smits et al., 2000). This assay is based on the binding of *Leptospira*-specific antibodies present in patient's serum to the broadly reactive antigen coated on latex particles, which can be observed by a visible agglutination reaction, and results are ready within 1 minute. The test kit can easily be stored at room temperature and can be performed by relatively low-skilled personnel without any sophisticated equipment. Unlike ELISA, a test with a single sample gives reasonably reliable information by the fifth day of illness. In an evaluation study, the sensitivity and specificity of Lepto Dri Dot was 67.6 percent and 66.0 percent, respectively, during the first week of illness. From the second to fourth week, the values increased to 85.5 percent and 80.0 percent, respectively (Vijayachari et al., 2002).

Table 7.6. Diagnostic indices obtained at different stages of illness.

Technique	Stage of illness	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Lepto Lateral Flow	First week	52.9	93.6	92.5	57.1
	Second–fourth week	86.0	89.4	90.7	84.0
Lepto dipstick	First week	48.6	85.1	82.9	52.6
	Second–fourth week	87.7	85.1	87.7	85.1
IgM ELISA	First week	50.0	78	77.8	51.4
	Second–fourth week	87.7	87.2	89.3	85.4

NPV, negative predictive value; PPV, positive predictive value.

Adapted from Sehgal SC, Vijayachari P, Sugunan AP, Umapathi T. 2003. Field application of Leptolateral flow for rapid diagnosis of leptospirosis. *J Med Microbiol* 52:897–901.

Lepto Lateral Flow The Royal Tropical Institute also developed another test, the Lepto Lateral Flow for the rapid diagnosis of leptospirosis, based on the binding of leptospira-specific IgM antibodies to the broadly reactive heat-extracted antigen prepared from nonpathogenic Patoc 1 strain. These bound antibodies are detected with an antihuman IgM gold conjugate contained within the test devices.

This is a simple test that can be performed at the bedside of the patient, using a drop of blood obtained by finger prick. The test kit and sample do not require any special storage. Therefore, it is suitable for use in peripheral health centers and in field settings. The diagnostic indices for Lepto lateral flow are comparable to that for IgM ELISA and Lepto dipstick tests (Sehgal et al., 2003). Table 7.6 compares the sensitivity and specificity of various diagnostic tests.

Indirect Immunofluorescence Assay This is specific and sensitive test that can detect IgM antibodies in the serum in the first week of illness. It uses fluorescein isothiocyanate (FITC) dye conjugated with anti-IgG and detect IgG antibodies. This is also commercially available assay in which Patoc 1 strain is used for antigen preparation.

Latex Agglutination Assay In this assay, the serum is mixed with an equal volume of stabilized, antigen-coated dyed test and control beads on an agglutination card. The test is read within 2 minutes and the overall sensitivity and specificity is 82.3 percent and 94.6 percent, respectively. This is easy to perform and does not require any special skills or equipment. The reagents have a long shelf life, even at tropical temperatures thus, the latex agglutination assay is suitable for use as a rapid screening test in field settings (Smits et al., 2000).

Macroscopic Slide Agglutination Test It was originally described by Mazzonelli-Mailoux and was subsequently modified by Coghlan and later by Galton et al. This test is a rapid and reliable for screening purposes (Sumathi et al., 1997). This test employs a fixed quantity of concentrated killed antigen mixed with a fixed quantity of patient's serum sample on a slide. Agglutination (clumps) occur, which indicates the presence of

genus-specific *Leptospira* antibodies in the serum sample (i.e., positive test). For this purpose, 10 mL of the antigen is added to 10 mL of patient's serum on a venereal disease research laboratory (VDRL) test slide and mix thoroughly using an applicator stick. The slide is placed on a VDRL rotator and rotated at 120 RPM up to 4 minutes. The test will be considered positive when obvious clumps are seen with the naked eye with the clearing of the suspension. When it is negative, the suspension of serum and antigen remains unchanged.

Advantages MSAT is easy to perform and read and the antigen is stable for up to six months at 39.2° F (4° C). In the early stage of the disease, it is more sensitive than MAT. The visual reading of the result can be improved by staining the antigen with a drop of gentian violet.

Disadvantages There may be the auto-agglutination of antigen if old cultures are used, which gives a false-positive result.

Detecting Leptospiral DNA

The specific segments of leptospiral DNA (in clinical and postmortem samples) can be amplified to detectable levels (WHO, 2003) by PCR. It is a useful diagnostic tool during the diagnostic window in the first week of illness, when the antibodies cannot be detected by the other available methods (Adler and Faine, 1978). Short DNA sequences specific for leptospires (primers) are combined with heat-stable DNA polymerase in the presence of nucleotides and subjected to temperature cycles that amplify a stretch of leptospiral DNA, which is easily detected in gels. PCR is a highly specific technique for diagnosis of leptospirosis, both early phase of infection before antibody titers reach detectable levels and also late in the course of the disease. Several PCR techniques protocols have been described for diagnosis of leptospirosis (Bal et al., 1994; Brown et al., 1995; Mérien et al., 1992, 1995; Zhang and Dai, 1992).

Specimens

Urine samples used frequently because they are the most sensitive and continue to remain positive even after the clinical disease has regressed. Blood, serum, CSF, and postmortem specimens may also be used.

Multiplex PCR

It is used for simultaneous identification of pathogenic as well as saprophytic leptospires. For differentiating between pathogenic and saprophytic leptospires, genus-specific primers of 23S rRNA and pathogenic species-specific primers of 16S rRNA are used in combination with ISSR $(AG)_8$ T repeat primer.

Advantages

PCR enables the rapid diagnosis in early phases of infection before antibody titers reach detectable levels.

Disadvantages

PCR requires special equipment and skilled personnel. False-positive results may be obtained even if a minute quantity of extraneous DNA contaminates the sample (WHO, 2003).

Criteria for Diagnosis

Diagnosis can only be confirmed by the presence of any one of the following criteria:

1. Detection of *Leptospira* in blood, cerebrospinal fluid, or urine.
2. Suggestive clinical symptoms associated with either
   (a) Fourfold increase in titers by MAT or a single MAT titer of 400 of more.
   (b) Detection of specific IgM antibodies by Dot ELISA or dipstick ELISA (Gulati et al., 2002; Andre et al., 2000).

Clinical Management

Leptospires are sensitive to a wide range of antibiotics therapy, but antibiotics should be initiated before the fifth day of illness and should not be delayed until the results of the laboratory tests are available. This is because the serological tests do not become positive till about a week after the onset of illness. Cultures may not be positive for several weeks (WHO, 2003).

At Primary Care Level

In leptospirosis endemic areas, all febrile patients showing any of the two symptoms among myalgia, conjunctival suffusion, history of contact with animals, or agriculture-related occupation, should be clinically suspected for leptospirosis. Primary-level management includes historical observation, clinical examination including assessment of vital signs, provision of basic life support (maintenance of airway, manual resuscitation, intravenous alimentation, and nursing care) and use of case definitions. Investigations include hemoglobin, total and differential leukocyte count, and peripheral smear especially for malarial parasites.

Adults with mild disease and positive immunodiagnostic test results should be given injectable crystalline penicillin, 2 million units intravenously, every 6 hours, for 7 days. Penicillin should be administered only after an intradermal test dose. In children, the dose of is 200,000 to 400,000 units per kg/body weight per day, in four divided doses, for 7 days (NICD, 2006).

Clinically stable adults with negative ELISA and negative rapid immunodiagnostic test results should receive capsule doxycycline 100 mg twice daily for 7 days. In children, younger than 6 years of age, capsule amoxicillin/ampicillin 30 to50 mg/kg body weight per day should be administered in four divided doses six hourly for 7 days (NICD, 2006).

Penicillin decreases the duration and severity of the disease, even in cases where it is started late (Gulati et al., 2002). For individuals allergic to penicillin, any one of the following drugs may be used orally: Erythromycin 500 mg four times a day; ciprofloxacin 250 to 750 mg twice a day; doxycycline 100 mg twice daily; or Tetracycline 2 to 4 grams daily, in four divided doses (WHO, 2003; Gulati et al., 2002; Mitra, 1999; Figure 7.6). These drugs are effective when the treatment is initiated early (Mitra, 1999). Quinolones and third-generation cephalosporins (ceftizoxime and cefotaxime) are also effective (WHO, 2003).

All suspected cases of leptospirosis with or without positive immunodiagnostic tests manifesting signs and symptoms of organ dysfunction (such as hypotension, urine output less than 400 mL/day, hemorrhagic tendencies, irregular pulse, jaundice, altered sensorium,

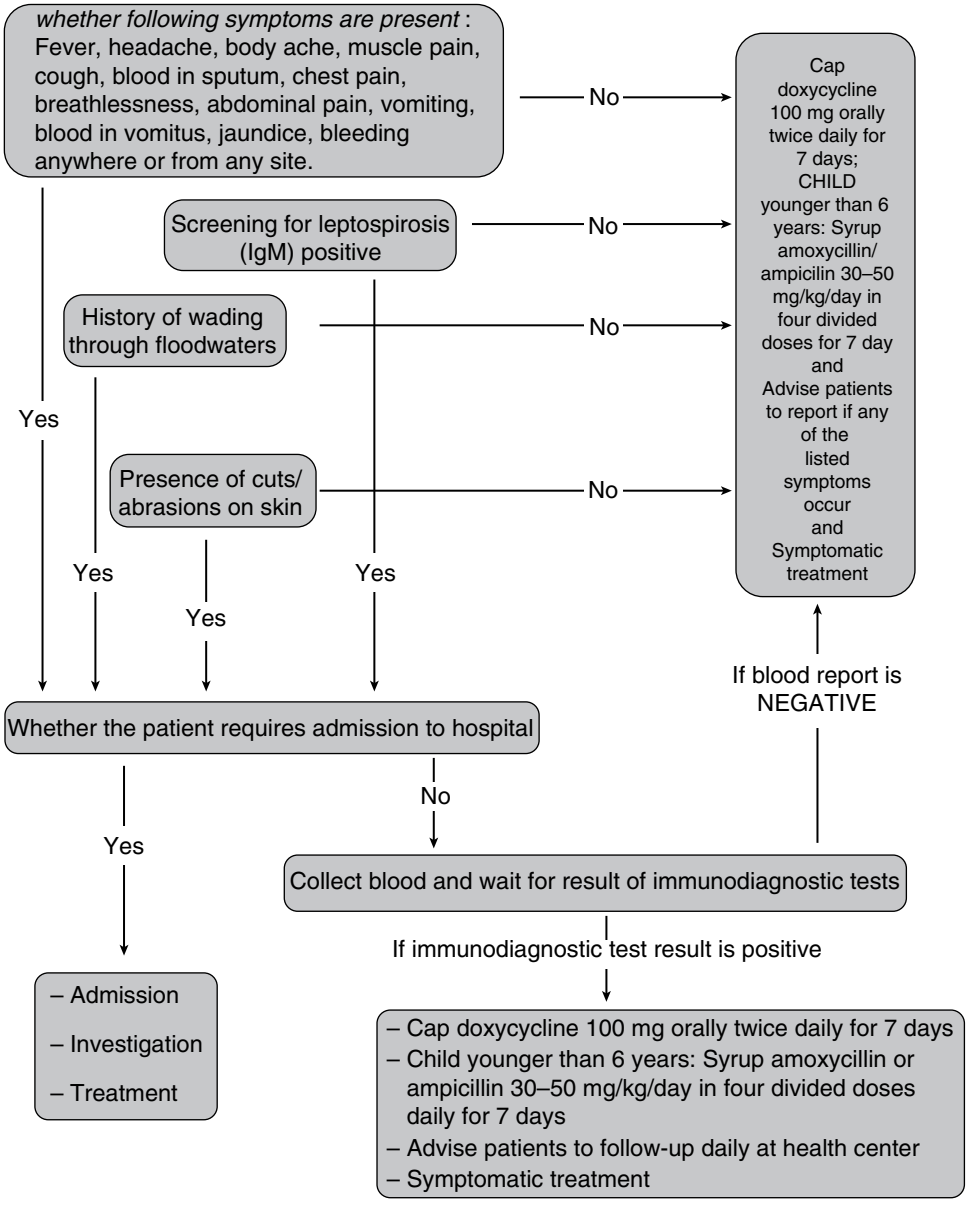

Figure 7.6. Management of leptospirosis cases.

confusion, delirium, clinical features of uremia) should be immediately transferred to a secondary level health-care facility.

Pulmonary involvement (manifested as breathlessness or hemoptysis) progresses rapidly and is a common cause of death in leptospirosis. Therefore, such patients should be immediately transferred to a tertiary level health-care facility. In all cases, the referral is to be accompanied by continued basic life support and a detailed referral slip mentioning

the patient's details such as age, sex, occupation, clinical symptoms, date of onset, serological test results, and treatment given (NICD, 2006).

At Secondary Care Level

It includes immediate hospitalization of severe cases, historical observation, clinical examination, provision of basic life support (maintenance of airway, manual resuscitation, intravenous alimentation, nursing care), and chemotherapy. Correction of fluid and electrolyte imbalance is essential (Sharma et al., 2003). Laboratory investigations include detection of IgM antibodies against leptospires by rapid screening tests as Lepto dipstick, Lepto lateral flow, or Lepto Tek Dri Dot; blood investigations such as total and differential leukocyte count, ESR, platelet count, bleeding time, clotting time, prothrombin time, blood urea nitrogen, serum creatinine, serum electrolytes, serum bilirubin, serum alkaline phosphatase, SGOT, SGPT, serum creatinine phosphokinase (CPK) and its MB isoenzyme, and urine tests for proteinuria, hematuria, and casts. During the monsoon, in leptospirosis-endemic areas, all febrile patients clinically suspected for leptospirosis should immediately receive presumptive treatment for both leptospirosis and malaria. Adults with mild disease and positive immunodiagnostic test results and clinically stable adults with negative ELISA and negative rapid immunodiagnostic test results should be treated as previously mentioned. Platelet or whole blood transfusion is to be given in case of thrombocytopenia or bleeding episodes. Patients are referred to the tertiary level health facilities in cases of:

- Blood observations: thrombocytopenia, bleeding tendencies.
- Nervous system defects: altered sensorium, confusion, delirium.
- Liver associated problems: icterus, elevated levels of direct serum bilirubin (more than 3.0 mg/dL).
- Pulmonary complications: breathlessness, hemoptysis, tachypnea, chest X-rays showing opacities.
- Kidney complications: clinical features of uremia, urine output less than 400 mL/day, blood urea more than 60 mg/dL, serum creatinine more than 2.5 mg/dL.

Referral should be accompanied by continued basic life support and a detailed referral slip mentioning the patient's details such as age, sex, occupation, clinical profile, serological test results, and treatment given (NICD, 2006).

At Tertiary Care Level

During the monsoon, any febrile patient in leptospirosis endemic areas should receive chemotherapeutic treatment for both leptospirosis and malaria. The treatment of organ dysfunction in leptospirosis is similar to that resulting from nonleptospiral causes. Laboratory investigations such as ELISA, MAT, culture, and PCR may be carried out are tertiary-care facilities (NICD, 2006). Management of aseptic meningitis is symptomatic and supportive.

Pulmonary Involvement

Pulmonary involvement is the commonest cause of death in leptospirosis; therefore, patients with pulmonary involvement, such as breathlessness, tachypnea, or hemoptysis

have to be immediately shifted to tertiary-care facility. Management should include continuous administration of oxygen, and mechanical ventilation with positive end-expiratory pressure may be required in case of respiratory failure. (NICD, 2006).

Bleeding Episodes

In patients with thrombocytopenia, (platelet count of 100,000 or less per cubic mL of blood), platelet concentrate or platelet-rich plasma is proved to be useful, whereas fresh-frozen plasma is to be transfused in cases with disseminated intravascular coagulation. Intravenous injection of vitamin K 5 to 10 mg for 3 days corrects increased prothrombin time. The normal values for bleeding time, clotting time, and prothrombin time are 1.7 minutes (Ivy's method), 4 to 8 minutes (Lee and White's method), and control ± 1 second (one stage by Quick's method), respectively, and need to be monitored.

Cardiac Involvement

Hypovolemic shock responds to fluid replacement therapy. If the blood pressure is not restored despite fluid replacement, vasopressor drugs (i.e., dopamine, dobutamine) should be given. Cardiac monitoring is essential to identify and treat cardiac arrhythmias.

Renal Involvement

General measures include dietary restriction of sodium, potassium, and phosphorus; observation for signs of hypovolemia, hypotension, infection, and prompt treatment of these conditions. Dosages of commonly used antimicrobials are to be reduced in severe azotemia. Nonsteroidal anti-inflammatory drugs (NSAIDs), tetracyclines, vancomycin, and aminoglycosides are contraindicated. In the presence of proteinuria with no signs of azotemia, the patient is kept under observation, and chemotherapy for leptospirosis is administered. Severe renal involvement (acute renal failure) requires of hypovolemia by intravenous normal saline. If the urine output is inadequate despite correction of hypovolemia, the quantity of intravenous fluid infused should equal the urine output plus insensible loss (about 500–700 mL) or 400 mL per square meter of body surface plus urine output of the previous day. This varies with the ambient temperature and patient's respiratory rate. Peritoneal dialysis or hemodialysis is indicated if acidosis is refractory to conservatory treatment or clinical features of uremia develop. Other indications are hyperkalemia, fluid overload, encephalopathy, lethargy, convulsions, myoclonus, asterixis, and pericarditis (NICD, 2006).

Hepatic Involvement

General measures include high carbohydrate diet; protein restriction in severe cases; avoidance of hepatotoxic agents (alcohol, paracetamol, rifampicin, isoniazid, pyrazinamide); avoidance of precipitating factors for hepatic encephalopathy (sedatives, hypnotics, tranquilizers, opioids, constipation, surgery). Hypokalemia and alkalosis, usually caused by diuretics and diarrhea, also precipitate encephalopathy. Upper gastrointestinal bleeding is another precipitating factor that requires prompt aspiration of bleeding followed by bowel wash. If hepatic encephalopathy occurs, lactulose is given initially in the dose of 15 to 45 mL two to four times daily and later, the dose is adjusted to produce three

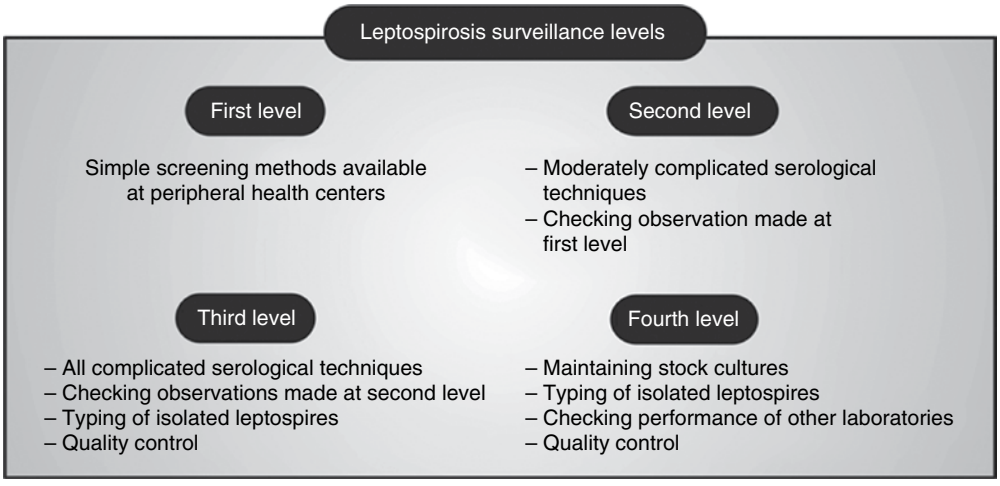

Figure 7.7. Levels of leptospirosis surveillance.

to four stools per day. Injection ampicillin is administered in the dose of 2 grams every 6 hours (adults) and 200 mg per kg/body weight per day in four divided doses (children). A dose of 250 mg metronidazole is given orally three times a day or, 1 gram of neomycin is given orally four times a day (NICD, 2006).

Surveillance

Surveillance is a mandatory prerequisite for monitoring the problem of leptospirosis in a given locality. Regular surveillance is useful for early detection of an outbreak and initiation of timely interventions for prevention and control. Leptospirosis is grossly underreported in India because of lack of awareness, low index of clinical suspicion, wide spectrum of clinical manifestations, and inadequate facilities for laboratory diagnosis (Gulati et al., 2002). There are four levels of leptospirosis surveillance (Figure 7.7).

Personnel at peripheral health centers should be vigilant about any increase or clustering of cases of leptospirosis. In such situations, local epidemiological and serological investigations should be carried out.

Epidemiological Surveillance

This includes clinical surveillance, confirmation of clinical diagnosis, and notification of cases. All cases of leptospirosis should be reported each month to the district health authority, who should further inform superior officials. Any sudden increase in cases, clustering of cases, or deaths resulting from leptospirosis should be reported immediately to the district health authority through telephone, fax, or e-mail. The details of the outbreak, including investigations, and control measures should also be monitored and reported.

Serological Surveillance

Sero-surveillance can be useful in identifying high-risk individuals, high-risk geographical areas, outbreaks, animal reservoirs, new serological variants and their geographical distribution, and new strategies for prevention and control of the disease. Screening tests for serological surveillance of leptospirosis are MSAT and IHA (Gussenhoven et al., 1997).

Sero-Surveillance in Humans

Sources of serum samples include hospital patients with clinical manifestations resembling that of leptospirosis, known risk groups, and random blood samples. Periodic re-examination of the same risk group in a population may provide information on sero-conversion. The participants in such a survey may be asked about history of exposure to possible risk factors during the time interval between consecutive examinations (WHO, 2003).

Sero-Surveillance in Animals

Serological testing of animals is useful in determining primary reservoirs in a locality, though animals might harbor leptospires without having detectable antibodies in serum. Most leptospiral screening tests have not been evaluated in animals. An ELISA test requires conjugates developed from animals species. However, culture of animal tissues or urine may identify the locally prevalent serovars. Serological surveillance in rodents requires live-trapped animals and underestimates infection because many rodents do not show antibody response, although they may be urinary shedders of leptospires. Serodiagnosis in rodents requires MAT, and the sensitivity of diagnosis may be augmented by kidney culture (WHO, 2003).

Surveillance Using Culture Methods

Saprophytes interfere with detection of pathogenic leptospires shed in animal urine. Because leptospires grow slowly, it may take months to obtain results, and because leptospires are not evenly distributed in an environment, negative culture results do not exclude the presence of pathogenic leptospires. Moreover, the environment or surface water may have been free of pathogenic leptospires at the time of sample collection but may have been subsequently contaminated by urine of an infected animal (WHO, 2003).

Investigation of an Outbreak

The principles involved in the investigation of outbreaks of leptospirosis are quite similar to that for other epidemic diseases. In case of a major outbreak or if leptospirosis is a major public health problem, a committee should be formed to monitor outbreak investigation and control. The committee should be comprised of:

- Specialists in infectious disease surveillance and epidemiology, infectious diseases, environmental health and safety, veterinary medicine, agriculture, rodent control.
- Staff of public health laboratory.
- Representatives of dairy farmers and pig farmers.
- Officials responsible for parks and recreations, forestry, and fisheries and wildlife (WHO, 2003).

Investigation to Define the Case

Once the first information is received, the validity of the information should be verified. Initial sources of information are epidemiological surveillance (early warning system), reports from curative facilities, and reports from other sources such as press reports and community sources.

Preliminary Steps for Case Definition

Case definition includes common and uncommon signs and symptoms of mild, moderate, and severe forms of the disease; criteria for deciding suspect, probable, and confirmed cases; and laboratory tests for confirming disease. Case definitions for suspect and confirmed cases of leptospirosis given by government of India's National Surveillance Programme for Communicable Diseases (NSPCD) are as follows.

Suspect Case

Acute febrile illness with headache, myalgia, and prostration associated with any of the following signs (NICD, 2006):

- Conjunctival suffusion
- meningeal irritation
- Anuria, oliguria, or proteinuria
- Jaundice
- Hemorrhages (petechial, gastrointestinal, or pulmonary)
- Cardiac arrhythmia or failure
- Skin rash
- History of exposure to infected animals or an environment contaminated with animal urine
- Presence of other symptoms such as nausea, vomiting, abdominal pain, or diarrhea
- Arthralgia

Confirmed Case

A case can be confirmed when a suspect case is associated with the following laboratory evidence of leptospiral infection:

- Isolation of leptospires from blood or cerebrospinal fluid (during the first week of illness) and from urine (after 10 days of illness)
- Positive serology (preferably MAT), or *Leptospira* agglutination titer of more than 1:200 in one or more serum samples.

Case Finding

Health-care providers should be alerted to report increased or clustering of cases. Case finding can be carried out by institution-based and community surveys. For tracing the source and contacts, line listing of cases, (reporting cases by age, sex, detailed address, and clinical details) is used to report to the health authorities. The purpose of active search is to detect more cases and to trace their contacts. Once the clinical signals are received from the field, samples should be collected and sent for laboratory confirmation of the diagnosis.

Epidemiological Surveillance

It is necessary to obtain a detailed history of exposure to infected animals or exposure to an environment contaminated with animal urine (Sehgal, 1998). The organisms may persist in urine for 1 to 4 weeks (Milind and Sunil, 2009).

Early Application of Control Measures

Assess the status of case management facilities in endemic areas. Once the outbreak of leptospirosis is confirmed, precautionary measures should be taken in other high-risk areas in the neighborhood. After the control of the outbreak, the details (including report of the action taken, both corrective and preventive) should be sent to superiors.

Prevention and Control

Prevention and control of leptospirosis is complicated because of the presence of more than 200 serovars, many sources of infection, presence of animal reservoir, and wide differences in transmission conditions (WHO, 2003; Sehgal, 1998). Moreover, leptospirosis is also not a disease in many endemic areas.

Control of Source of Infection

Environmental Modification

Changes in environmental factors are a prerequisite for long-term prevention of leptospirosis (WHO, 2003; NICD, 2006). Environmental measures for prevention of leptospirosis ought to include:

- Mapping water bodies and human activities in areas prone to water logging.
- Carrying out health-impact assessment before undertaking developmental activities.
- Determining animals that are carriers or urinary shedders of leptospires in a locality.
- Reducing animal reservoir population (especially rodents and stray dogs).
- Preventing environmental contamination by urine of carrier animals.
- Preventing water logging in peridomestic areas and building residences in areas not prone to flooding.
- Planned urbanization with adequate water supply and sanitation.
- Safe methods of garbage disposal so that animal reservoirs, such as rodents and stray dogs, are not attracted to garbage dumps.
- Preventing contact with reservoir animals (fences, screens).

Immunization of Livestock and Pets

Vaccination should be used as a preventive intervention only in the hyperendemic areas for leptospirosis. The vaccine must contain the dominant serovars. Animal vaccines confer a limited duration of immunity and boosters are required every 1 to 2 years.

- Vaccines for cattle: Leptavoid (Schering Plough Animal Health, USA), Spirovac (USDA, USA), and Leptoferm-5 (USDA, USA).
- Vaccine for cattle and pigs: Farrowsure-Plus (Pfizer Animal Health, USA).

- Vaccines for dogs: Novivac-DHPPI-2L (Intervet, Norway), Eurican- DHPPI-2L (Merial, France), Vanguard- DHPPI-2L (Pfizer Animal Health, USA), Duramax-DHPPI-2L (Fortdodge Lab, USA).

Though vaccines prevent clinical illness in animals, they do not prevent urinary shedding of leptospires (NICD, 2006; WHO, 2003).

Rodent Control

Because rodents are major reservoirs of leptospirosis, rodent control measures are necessary in endemic areas. The basic principle is to deny food and shelter to rodents. This involves keeping peridomestic areas clean and safely disposing leftover food, particularly in recreational areas (WHO, 2003).

Interruption of Chain of Transmission

Avoiding contact with animal urine, an environment likely to be contaminated with animal urine, or potential animal reservoirs can prevent transmission (WHO, 2003). Figure 7.8 reflects various targets for controlling the transmission of leptospirosis.

Information, Education, and Communication

Information, education, and communication (IEC) constitutes the main preventive intervention in leptospirosis. It is essential to disseminate information on the behavioral and environmental factors that predispose humans to leptospiral infection. The objective is to bring changes in high-risk behavior that predisposes to leptospirosis. If an outbreak is suspected or confirmed, it is essential to update health-care providers on recognition of illness, case definitions for leptospirosis, and provision of appropriate treatment. The population at risk need information (in the locally used languages) on symptoms of the disease so that if they experience similar symptoms, they will seek medical help. They also need to be told about the modes of transmission of the disease, use of protective gear and other methods of preventing or limiting exposure, and availability of chemoprophylaxis (WHO, 2003; Gulati et al., 2002; Mitra, 1999). Basic information may also be transmitted through mass media (television, radio, press). Warning signs may be put up at vantage points (where people are likely to notice and read warnings), near places of recreation, and near rivers, ponds, and lakes. Farmers need to be educated to drain out urine from cattle sheds into a soakage pit and to prevent contamination of water bodies with cattle urine (NICD, 2006).

Chemoprophylaxis

When there is short-term exposure, 200 mg of doxycycline orally, once a week (Speelman, 1998). The duration of chemoprophylaxis should not exceed 6 weeks. Chemoprophylaxis may be administered to high-risk groups in areas where clustering of cases has been reported during the peak transmission season (NICD, 2006). Chemoprophylaxis is generally not considered as a disease control measure in endemic areas, but even in endemic areas, outbreaks of leptospirosis occur seasonally and are of short duration. This is comparable to the risk of infection faced by a visitor from a nonendemic area who enters an endemic area (Sehgal et al., 2000). The primary objective of chemoprophylaxis is to reduce morbidity and mortality. Doxycycline, in a dose of 200 mg per week orally,

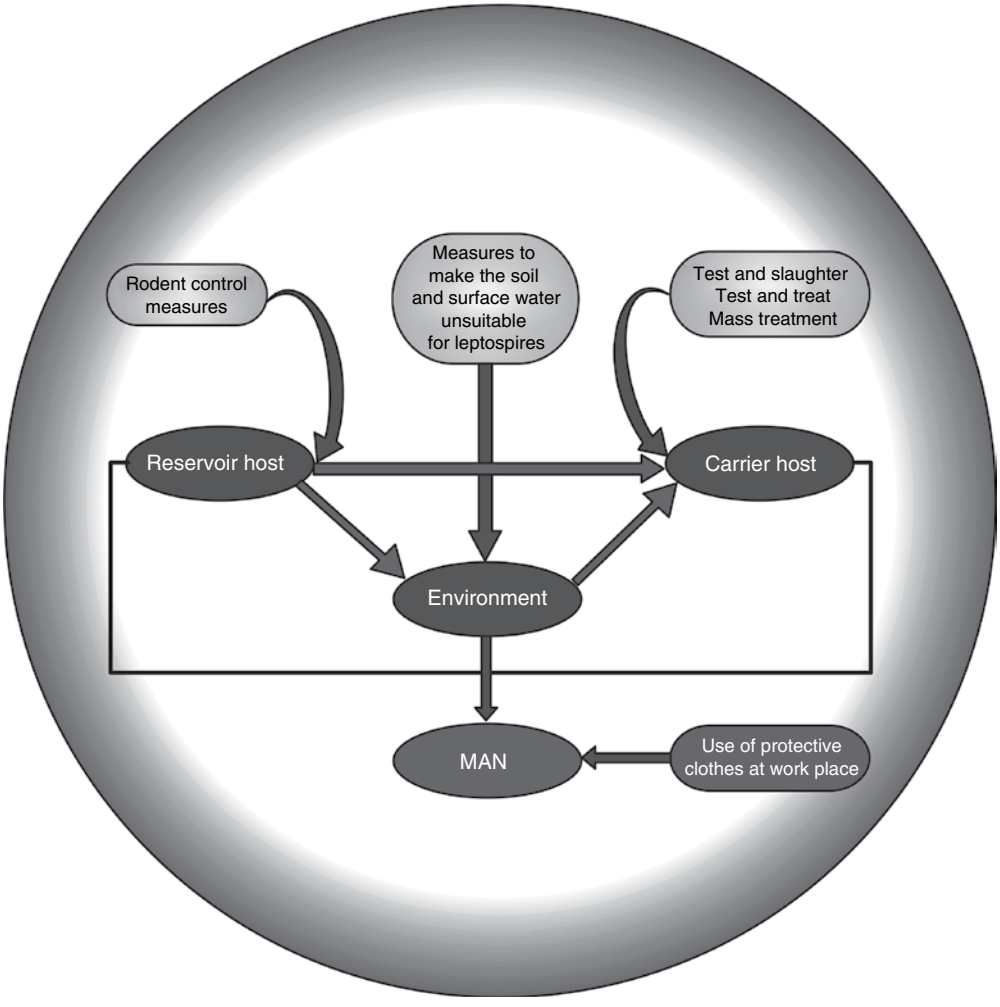

Figure 7.8. Targets for control strategies on the transmission cycle of leptospirosis.

achieves this objective to some extent even in highly endemic areas where the occurrence of leptospirosis is short-term and seasonal (Sehgal et al., 2000).

After ingestion of 200 mg doxycycline, plasma levels of 3 mg/L or higher, are achieved within 2 hours of ingestion and plasma levels of 1 mg/L or higher are maintained for 12 to 16 hours (Kapusnik-Uner et al., 1996). In the subsequent week, when the oral dose of 200 mg is repeated, sufficient plasma level of the drug is achieved to suppress leptospiral multiplication in the bloodstream. If doxycycline is pulsed at weekly intervals, the suppressing effect of the drug would be related to frequency of exposure, mean incubation period, and the infecting dose of *Leptospira*. The incubation period of leptospirosis is inversely related to the infecting dose; the heavier the infecting dose, the shorter the incubation period (Sehgal et al., 2000).

If the exposure to infection is frequent and the infecting dose heavy, the infection occurring when the blood level of doxycycline is low would progress sufficiently to show

Figure 7.9. Vanguard canine distemper combined with a *Leptospira* vaccine. http://www.factsonlepto.com/VanguardPlus5L4CV.htm.

serological evidence of infection, but because of the suppressive effect of the next dose of doxycycline, the infection might not appear as a clinical infection requiring medical attention (Sehgal et al., 2000).

Immunoprophylaxis

Though antileptospiral vaccines are available for human use in some countries such as Italy, Russia, and China, they are currently used only in individuals employed in certain high-risk jobs. Vaccine containing one serological variant does not provide protection against infection by other serological variants of *Leptospira*. For effective immunoprophylaxis, any antileptospiral vaccine should incorporate all locally prevalent serological variants (WHO, 2003). Current vaccines those are available contain inactivated leptospires or their outer membrane fractions (Fraga et al., 2011). Furthermore, these vaccines do not produce a T-cell dependent response, and therefore, requires annual booster shots. Another complication associated with *Leptospira* vaccines is that there is no cross-protection against leptospiral serovars not included in the vaccine preparation, which results in the emergence of new serovar requiring an entirely new vaccine. Because different geographical populations have such varied pathogenic serovars, the continuation of epidemiological studies is of the utmost importance for a successful vaccination program. Canine vaccines generally contain the serovars *canicola, icterohaemorrhagiae, grippotyphosa, and pomona*, and pig leptospiral vaccinations generally contain the serovars *pomona* (Figure 7.9), *grippoyphosa*, *bratislava*, *canicola*, and *icterohaemorrhagiae* (Adler and Moctezuma, 2010).

Personal Protection

This includes protective clothing and waterproof dressing on cutaneous cuts and abrasions to reduce the risk of occupational or recreational exposure (NICD, 2006; WHO,

2003). Persons in agriculture-related occupations or those likely to come in contact with an environment contaminated with animal urine should be advised to use rubber boots and India rubber gloves (NICD, 2006).

References

Adler B, Faine S. 1978. Serological and protective antibody responses of rabbits to lepospiral antigens. *J Med Microbiol* 11:401–409.

Adler B, Moctezuma AP. 2010. *Leptospira* and leptospirosis. *Veterinary Microbiology* 140:287–296.

Ahmad SN, Shah S, Ahmad FMH. 2005. Laboratory diagnosis of leptospirosis. *J Postgrad Med* 51:195–200.

Andre VL, Diamen D, Jaime RT. 2000. Leptospirosis in Latin America. *Infect Dis Clin North Am* 14:23–29.

Bal AE, Gravekamp C, Hartskeerl RA, de Meza-Brewster J, Korver H, et al. 1994. Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. *J Clin Microbiol* 32:1894–1898.

Brown PD, Gravekamp C, Carrington DG, van de Kemp H, Hartskeerl RA, et al. 1995. Evaluation of the polymerase chain reaction for early diagnosis of leptospirosis. *J Med Microbiol* 43:110–114.

Centers of Disease Control and Prevention (CDC).1995. Outbreak of acute febrile illness and pulmonary hemorrhage—Nicaragua, 1995. *MMWR* 44:839–843.

Centers of Disease Control and Prevention (CDC). 1997. Outbreak of leptospirosis among white-water rafters - Costa Rica occupational infections. *MMWR* 46(25):577–578.

Chan OY, Paul DR, Sng EG. 1987. Leptospirosis among abattoir workers-a serological study. *Singapore Medical Journal* 28:293–296.

Chosewood DE, Wilson LC, eds. 2009. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. Atlanta: Centers for Disease Control and Prevention. Accessed March 25, 2013, at http://www.cdc.gov/biosafety/publications/bmbl5/.

Dassanayake DLB, Wimalaratna H, Nandadewa D, Nugaliyadda A, Ratnatunga C, et al. 2012. Predictors of the development of myocarditis or acute renal failure in patients with leptospirosis: An observational study. *BMC Infect Dis* 12:4.

Faine S. 1982. *Guidelines for Leptospirosis Control*. WHO offset Publication 67. Geneva: World Health Organization.

Fraga T R, Barbosa AS, Isaac L. 2011. Leptospirosis: Aspects of innate immunity, immunopathogenesis, and immune evasion from the complement system. *Scandinavian Journal of Immunology* 73:408–419.

Gulati S, Menon S, Kabra M, Caudhry R, Kalra V. 2002. Leptospirosis: A case report. *Pediatr Today* 7:428–433.

Gussenhoven GC, vander Hoorn MA, Goris MG, Terpstra WJ, Hartskeerl RA, et al. 1997. LEPTO dipstick, a dipstick assay for detection of *Leptospira* specific immunoglobulin M antibodies in human sera. *J. Clin. Microbiol* 35:92–97.

Inada R, Ito Y. 1915. A report of the discovery of the causal organism (a new species of Spirocheta) of Weil's disease. *Tokyo Ijishinshi* 1908:351–360.

Inanda R, Ido Y, Hoke R, Kaneko R, Ito H. 1916. The Etiology, Mode of Infection and Specific Therapy of Weil's Disease. *J Exper Med* 23:377.

Kapusnik-Uner JE, Sandre MA, Chambers HF. 1996. Chemotherapy of microbial diseases. In: Goodman LS, Limbird LE, Milinoff PB, Ruddon RW, Gilman AE, eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th ed., pp. 831–836. New York: Mc Graw-Hill.

Klopfleisch R, Kohn B, Plog S, Weingart C, Nöckler K, et al. 2011. An emerging pulmonary haemorrhagic syndrome in dogs: Similar to the human leptospiral pulmonary haemorrhagic syndrome? *Vet Med Int* 33:928541. doi:10.4061/2010/928541.

Kohn B, Steinicke K, Arndt G, Gruber AD, Guerra B, et al. 2010. Pulmonary abnormalities in dogs with leptospirosis. *J Vet Intern Med* 24:791–807.
Kuriakose M, Eapen CK, Paul R. 1997. Leptospirosis in kolenchery, Kerala, India: Epidemiology, prevalent local serogroups and serovars and a new serovar. *Eur. J. Epidemiol* 13:691–697.
Kuriakose M, Paul R, Joseph MR, Sugathan S, Sudha TN. 2008. Leptospirosis in a midland rural area of Kerala State. *Indian J Med Res* 128:307–312.
Langston CE, Heuter KJ. 2003. Leptospirosis. A re-emerging zoonotic disease. *Vet Clin North Am Small Anim Pract* 33:791–807.
Levett PN, Branch SL, Whittington CU, Edwards CN, Paxton H. 2001. Two Methods for Rapid Serological Diagnosis of Acute Leptospirosis. *Clin Diagn Lab Immunol* 8:349–351.
Martin L, Pettit A. 1918. Sero-diagnostic de la spirochaetose icterohaemorrhagique. *Bulletin et Mémoires de la Société Médicale des Hôpitaux de Paris* 42:672–675.
Meites E, Jay MT, Deresinski S, Shieh W-J, Zaki SR, et al. 2004. Reemerging leptospirosis, California. *Emerg Infect Dis*. Accessed February 27, 2013, at http://wwwnc.cdc.gov/eid/article/10/3/03-0431.htm.
Merien F, Amouriauz P, Pérolat P, Baranton G, Saint Girons I. 1992. Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. *J Clin Microbiol* 30:2219–2224.
Merien F, Baranton G, Pérolat P. 1995. Comparison of polymerase chain reaction with microagglutination test and culture for diagnosis of leptospirosis. *J Infect Dis* 172:281–285.
Milind T, Sunil K. 2009. Leptospirosis in children: A review for family physicians. *Indian Journal of Medical Sciences* 63(8):368–378.
Mitra DK. 1999. Leptospirosis. In: Sainani GS, ed., *API Textbook of Medicine*, 6th ed., p. 62. Mumbai: Association of Physicians of India.
Murhekar MV, Sugunan AP, Vijayachari P, Sharma S, Sehgal SC. 1998. Risk factors in the transmission of leptospiral infection. *Indian J Med Res* 107:218–223.
Muthusethupathi MA, Shivakumar S, Suguna R, Jayakumar M, Vijaykumar R, et al. 1995. Leptospirosis in Madras-a clinical and serological study. *J Assoc Physicians India* 43:456–458.
Scott G, Coleman TJ. 2003. Leptospirosis. In: Cook GC, Zumla A, eds. *Manson's Tropical Diseases. Jakarta*, 21st ed., pp. 1165–1171. London: Saunders.
Sehgal SC. 1998. Emergence of leptospirosis as a public health problem. In: *Round Table Conference Series-Leptospirosis*, pp. 7–16. New Delhi: Ranbaxy Science Foundation.
Sehgal SC, Murhekar MV, Sugunan AP. 1994. A serosurvey for leptospirosis in North Andaman. *Indian J Med Microbiol* 12:289–291.
Sehgal SC, Murhekar MV, Sugunan AP. 1995. Outbreak of leptospirosis with pulmonary involvement in North Andaman. *Indian J Med Res* 102:9–12.
Sehgal SC, Vijayachari P, Sugunan AP, Umapathi T. 2003. Field application of Leptolateral flow for rapid diagnosis of leptospirosis. *J Med Microbiol* 52:897–901.
Sehgal SC, Vijayachari P, Smythe LD, Norris M, Symonds M, et al. 2000. Lai-like leptospira from the Andaman Islands. *Indian J Med Res* 112:135–139.
Sharma S, Vijayachari P, Sugunan AP, Sehgal SC. 2003. Leptospiral carrier state and seroprevalence among animal population—A cross-sectional sample survey in Andaman and Nicobar Islands. *Epidemiol Infect* 131:985–989.
Singh SS, Vijayachari P, Sinha A, Sugunan AP, Rasheed MA, et al. 1999. Clinico epidemiological study of hospitalized cases of severe leptospirosis. *Indian J Med Res* 109:94–99.
Smits HL, Ananyina YV, Chereshsky A, Dancel L, Lai-A-Fat RF, et al. 1999. International multicenter evaluation of the clinical utility of a dipstick assay for detection of *Leptospira*-specific immunoglobulin M antibodies in human serum specimens. *J Clin Microbiol* 37:2904–2909.
Smits HL, Hartskeerl RA, Terpstra WJ. 2000. International multi-centre evaluation of a dipstick assay for human leptospirosis. *Trop Med Int Health* 5:124–128.
Speelman P. 1998. Leptospirosis. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, et al., eds., *Harrison's Principles of Internal Medicine*, 14 ed., pp. 1055–1057. New York: McGraw-Hill.

Stimson AM. 1907. Note on an organism found in yellow-fever tissue. *Public Health Reports* 22:541.

Sumathi G, Shivakumar S, Chinari Pradeep KS. 1997. MSAT: A screening test for leptospirosis. *Indian J Med Microbiol* =15:84.

Taylor RJ, Goyle AN. 1931. *Leptospirosis in the Andamans*. *Supplement to* Indian Journal of Medical Research. Memoir no. 20. Indian Research Fund Association. Calcutta: Thacker, Spink & Co.

Vijayachari P, Hartskeerl RA, Sharma S, Natarajaseenivasan K, Roy S, et al. 2004. A unique strain of leptospira isolated from a patient with pulmonary haemorrhages in the Andaman islands: A proposal of serovar portblairi of serogroup sehgali. *Epidemiol Infect* 132:663–673.

Vijayachari P, Sugunan AP, Sehgal SC. 2002. Evaluation of Lepto Dri Dot as a rapid test for the diagnosis of leptospirosis. *Epidemiol Infect* 129:617–621.

World Health Organization (WHO). 2003. *Human Leptospirosis: Guidance for Diagnosis, Surveillance, and Control*. Geneva: WHO.

Wuthiekanun V, Chierakul W, Limmathurotsakul D, Smythe LD, Symonds ML, et al. 2007. Optimization of Culture of *Leptospira* from Humans with Leptospirosis. *J Clin Microbiol* 45:1363–1365.

Zhang Y, Dai B. 1992. Detection of Leptospiral DNA in the serum of 175 patients with early leptospirosis by polymerase chain reaction. *Hua Xi Yi Ke Da Xue Xue Bao* 23:256–260.

National Institute of Communicable Disease (NICD). 2006. *Guidelines for Prevention and Control of Leptospirosis*. Geneva: World Health Organization. Accessed February 16, 2013, at http://www.scribd.com/doc/40377780/Communicable-Diseases-Guidelines-for-Prevention-and-Control-Leptospirosis.

Chapter 8
Dengue

Introduction

Dengue is caused by the dengue virus (DENV), a member of the *Flavivirus* genus of the *Flaviviridae* family. This family consists of enveloped, positive-stranded RNA viruses. The dengue viruses are comprised of four distinct serotypes, DENV1 through DENV4, which are mainly transmitted to humans through the bites of two mosquito species, *Aedes aegypti* and *Aedes albopictus*. The female *Aedes* (*Stegomyia*) mosquito transmits the dengue virus from person to person in the domestic environment. It can also be transmitted via infected blood products and through organ donation (Stramer et al., 2009; Wilder-Smith et al., 2009). Mother-to-child transmission (vertical transmission) during pregnancy or at birth has also been reported by Wiwanitkit (2010). Some other person-to-person modes of transmission have also been reported, but these are very unusual (Chen and Wilson, 2010). The origin of the dengue infection is still unclear. An epidemic of "knee fever" was described in Cairo, Egypt, in 1779 (Thongcharoen and Jatanasen, 1993). The name *dengue* is actually derived from the Swahili word *Ki denga pepo*, meaning a sudden seizure by a demon. The term *break bone fever* was coined during an epidemic in Philadelphia in the United States in 1780 (Ananthanarayan and Paniker, 2000). Outbreaks have occurred in the continental United States in 1780, in Hawaii in 1903, and in Greece during 1927 and1928. The clinical presentation of dengue fever resembles illness caused by chikungunya and O'nyong-nyong viruses (Ananthanarayan and Paniker, 2000). Over the years, the disease has been given several names: break bone fever, dandy fever, Korean hemorrhagic fever, Thai hemorrhagic fever, Philippine hemorrhagic fever, knee fever, 7-day fever, and Dhaka fever (Thongcharoen and Jatanasen, 1993).

In 2009, the World Health Organization (WHO) classified dengue fever into two groups: uncomplicated and severe (Whitehorn and Farrar, 2010). This completely replaced the 1997 WHO classification that divided dengue into undifferentiated fever,

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

dengue fever, and dengue hemorrhagic fever (Ranjit and Kissoon, 2010). Dengue hemorrhagic fever was further subdivided into grades I to IV, although the previous classification is still widely used. The dengue virus causes a wide range of diseases in humans, from the acute febrile illness dengue fever to life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Dengue fever is a self-limited illness, which is characterized by fever, headache, retro-orbital pain, myalgia, arthralgia, and rash, whereas DHF is marked by increased vascular permeability (resulting leakage of plasma), thrombocytopenia, and hemorrhagic manifestations. DSS occurs as a result of the leakage of fluid into the interstitial spaces and results in shock, which without appropriate treatment may lead to death. Over the past several decades, dengue spread throughout tropical and subtropical regions worldwide, with an annual estimation of 100 million infections and tens of millions of cases. DHF/DSS is one of the leading causes of pediatric hospitalization in Southeast Asia, where the syndrome first emerged 50 years ago, and it has become endemic to many Latin American countries over the last 25 years.

Magnitude

Global Situation

The socioeconomic impact of World War II resulted in increased spread of dengue globally. About 2.5 billion people, or 40 percent, of the world's population, live in areas where there is a risk of dengue transmission (Figure 8.1). WHO currently estimates there may be 50 million dengue cases that occur worldwide every year. Dengue spreads to

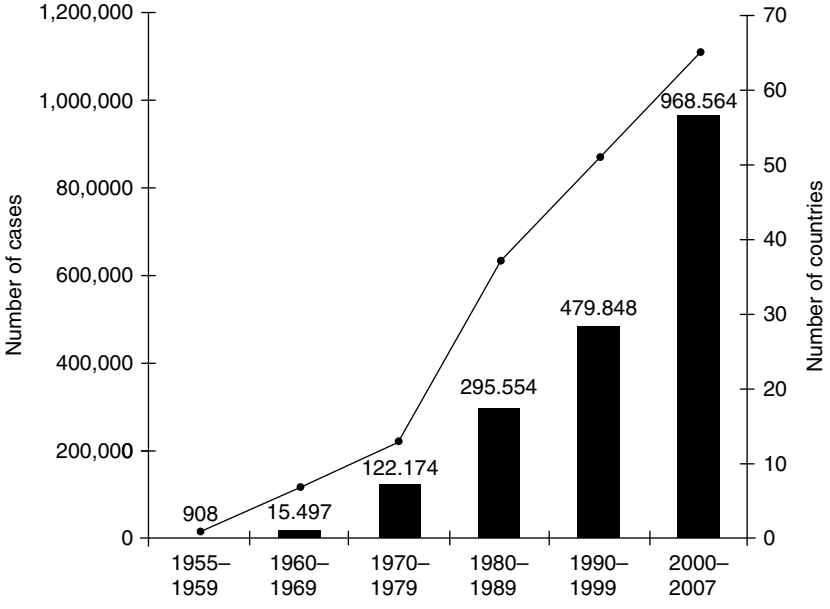

Figure 8.1. Reported cases of dengue fever and dengue hemorrhagic fever and the number of countries reporting dengue. World Health Organization (WHO). 2013. Global alert and response. Accessed March 25, 2013, at http://www.who.int/csr/disease/dengue/impact/en/index.html.

more than 100 countries in Asia, Pacific, the Americas, Africa, and the Caribbean. In 2007 alone, there were more than 890,000 reported cases of dengue in the Americas, out of which 26,000 cases diagnosed were of DHF. The disease is endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia, and the Western Pacific. Southeast Asia and the Western Pacific areas are the most seriously affected. Before 1970, only nine countries had experienced DHF epidemics, a number that had increased more than fourfold by 1995. In 2007, Venezuela, alone, reported more than 80,000 cases, including more than 6,000 cases of DHF.

Google dengue trends provides the daily updates of current dengue fever activity for 10 countries, which enables earlier detection of outbreaks and epidemics and public health officials to mobilize outbreak containment measures in a timely manner (Chan et al., 2011). Recent outbreaks of dengue fever have been reported from many countries in Central and South America and Mexico, as well as various regions in Southeast Asia; dengue is endemic in these areas and should always be considered in the differential diagnosis of acute febrile illness, especially in travelers returning from that area (Communicable Diseases Communiqué, 2012). Figure 8.2 shows countries or areas that are at risk of dengue infection.

India

In India, the first outbreak of dengue was recorded in 1812. Serological surveillance was carried out for the first time in 1954, which indicated that serotypes 1 and 2 (DEN-1 and DEN-2, respectively) were widespread (Smithburn et al., 1954). An epidemic of double-peak fever occurred for the first time in India in Kolkata between July 1963 and March 1964 (Pavri et al., 1964). DENV-2 strains were isolated from patients with severe hemorrhagic manifestations during the first peak of fever. In India, dengue/DHF has been restricted to the urban and the semi-urban areas of the country (Kalra et al. 1968; Yadav and Narasimham, 1992). However, over the years, large-scale developmental activities, such as the rapid growth of the transport system through railways and roads networks, industrial and building activities, provision of safe drinking water, electricity, overall improvement in civic amenities and socioeconomic conditions of rural masses, have resulted in the establishment and proliferation of the *A. aegypti* mosquito in urban and rural areas alike (Chusak and Andjaparidze, 1996; Katyal et al., 1997). The large-scale outbreaks of dengue/DHF were also reported in capital Delhi and in Faridabad and Hissar districts of Haryana and Ludhiana city in Punjab in 1996, (Gill et al., 1997).

Agent Factors

The causative agent of dengue fever is a single-strand of RNA and the positive strand belongs to the genus *Flavivirus* of the family *Flaviviridae*. This genus also includes the West Nile virus (WNV), tick-borne encephalitis (TBE) virus, yellow fever virus, and several other viruses causing encephalitis. Dengue virus causes a wide range of diseases in humans, from a self-limited dengue fever to life-threatening DHF or DSS. DENV appears as four antigenically different serotypic strains designated as DENV-1, DENV-2, DENV-3 and DENV-4, which exhibit 60 to 80 percent homology to each other. Because of this antigenic variation, primary infection with a particular serotype induces lifelong protection against the infecting serotype, but it provides only a short cross-protective immunity against the

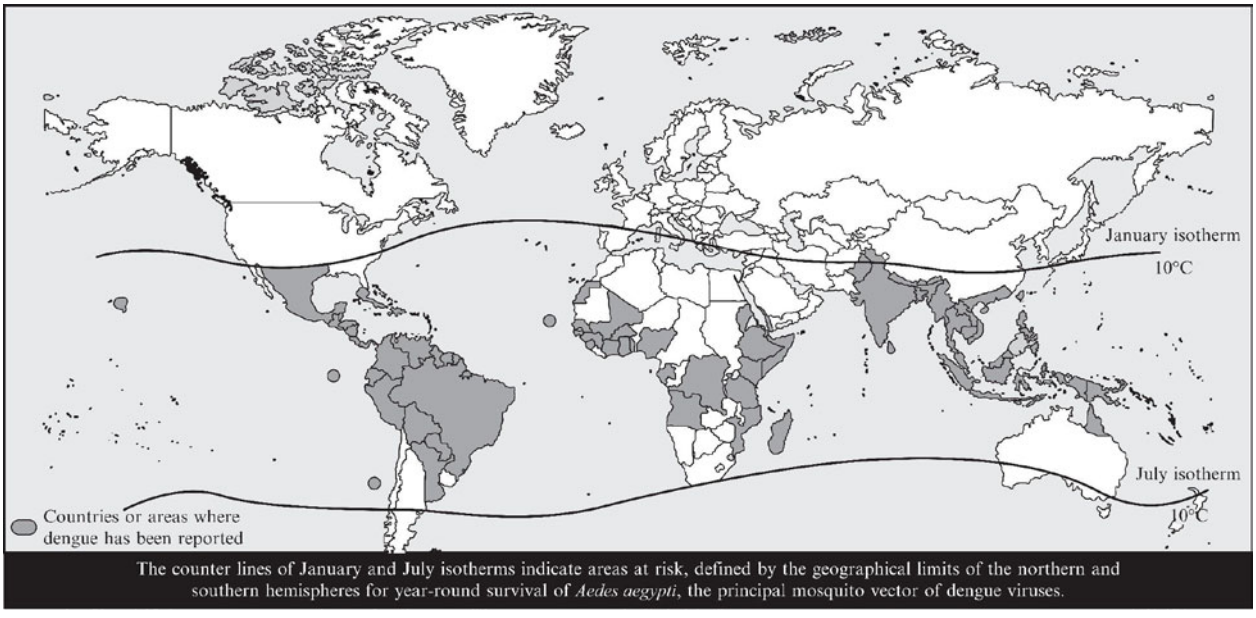

Figure 8.2. Countries or areas at risk of dengue infection. World Health Organization (WHO). 2010. Dengue. Accessed February 16, 2013, at http://www.who.int/topics/dengue/en/.

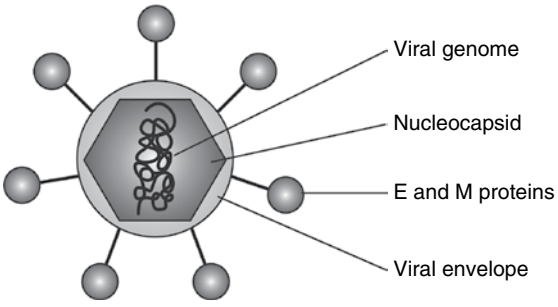

Figure 8.3. Dengue virus structure. E, envelope; M, membrane.

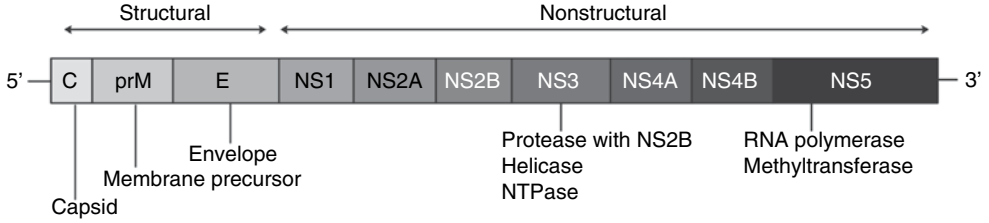

Figure 8.4. Dengue virus genome structure with the structural and nonstructural genes. C, capsid; E, envelope; M, membrane; NS, nonstructural; NTPase, nucleoside triphosphatase; prM, membrane precursor.

other existing subtypes. The primary infection mostly causes minor disease, but secondary infections have been reported to cause severe diseases (DHF or DSS) in both children and adults, which is probably as a result of the antibody-dependent enhancement.

The Dengue Virus Structure

Dengue is a roughly spherical 50-nm virus, enveloped with a lipid bilayer membrane, derived from the host cell (Figure 8.3). There are approximately 180 identical copies of the envelope (E) protein that get attached to the surface of a viral membrane by a short transmembrane segment. The other protein is designated as membrane (M) protein, and it spans through the lipid bilayer along with the E protein. Inside the virion is nucleocapsid, made up of the viral genome and capsid (C) proteins. The viral genome consists of about 11,000 bases encoding a single large polyprotein, which is subsequently cleaved into several structural and nonstructural (NS) mature peptides. The polyprotein is divided into three structural proteins (C, prM, E); seven NS proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5); and short noncoding regions on both the 5' and 3' end (Figure 8.4). The structural proteins include the C protein, the E glycoprotein, and the M protein, itself derived by furine-mediated cleavage from a prM precursor. The glycoprotein E is responsible for the attachment of the virion to receptor, mediates fusion of the virus envelope with the target cell membrane, and bears the virus neutralization epitopes. Along with the E glycoprotein, one NS protein, NS1, has been associated with a role in protective immunity. NS3 is a protease and a helicase, whereas NS5 is the RNA polymerase carrying out viral RNA replication.

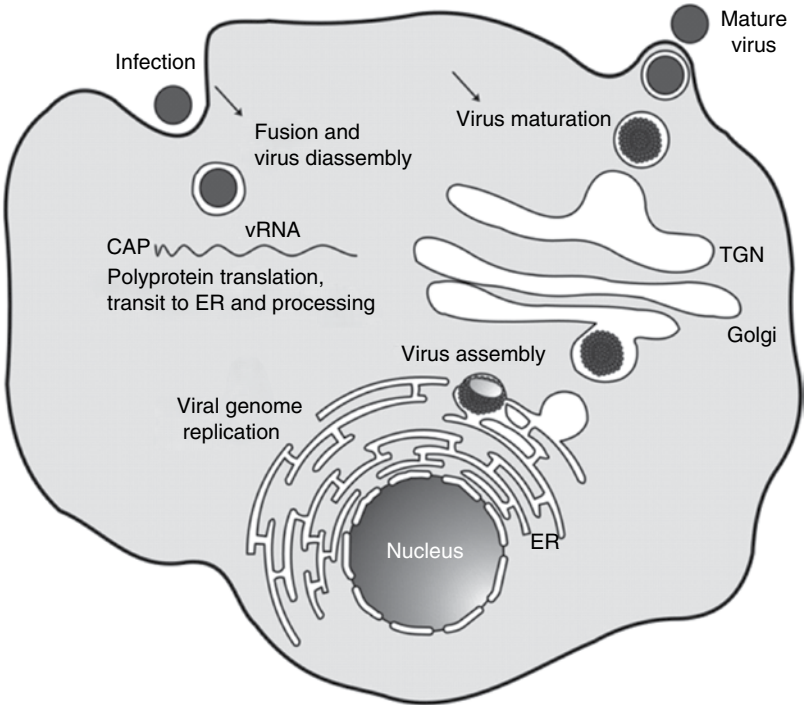

Figure 8.5. Dengue virus life cycle. ER, endoplasmic reticulum; TGN, Trans Golgi Network.

The Life Cycle of Dengue Virus

The replication cycle of DENV starts with the infection of a susceptible host cell (Figure 8.5). In vitro, DENV can infect numerous human cells, including dendritic cells (DCs), monocytes/macrophages, B cells, T cells, endothelial cells, hepatocytes, and neuronal cells, as well as a wide number of cell lines used for viral propagation (Anderson, 2003). But in vivo, the primary targets of the virion are the cells of the mononuclear phagocyte lineage (monocytes, macrophages, and DCs). The virus gets entry inside the body through binding between viral proteins and membrane proteins on the Langerhans cells of the skin (Martina et al., 2009), specifically the C-type lectins called DC-SIGN, mannose receptor, and CLEC5A (Rodenhuis-Zybert, 2010). DC-SIGN, which is a nonspecific receptor for foreign material on dendritic cells, seems to be the main point of entry (Guzman et al., 2010). The dendritic cells then moves to the nearest lymph node and meanwhile, the virus genome is replicated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins and synthesizes the copy of viral RNA. Immature virus particles are further transported to the Golgi apparatus, where some of the proteins receive necessary sugar chains (glycoproteins). These matured new viral particles now bud on the surface of the infected cell and release by exocytosis, and then they are capable of entering other white blood cells, such as monocytes and macrophages (Rodenhuis-Zybert, 2010).

Cellular Tropism Binding and Entry

Cellular tropism of dengue virus is determined by the stages of adsorption and entry. Generally DENV gains entry to its target cell through a cell surface receptor via a process called receptor-mediated endocytosis (RME), although its entry via direct fusion with the plasma membrane has also been reported (Hase et al., 1989; Lim and Ng, 1999). DENV can also bind to the Fc receptors (antibody-binding receptors) on macrophages, monocytes, and other cells when they are previously coated with antibody. The antibody thereby enhances the infectivity of these viruses by promoting its uptake into these target cells. RME of DENV involves two or more receptors: a ubiquitous, lower-affinity receptor, such as DC-SIGN, that initially captures the virus at the cell surface, increasing the local concentration, and a high-affinity receptor, which is less common and mediates the internalization of the virion. DC-SIGN can mediate infection of all four serotypes of DENV (Tan et al., 1996), and the ectopic expression of DC-SIGN also confers permissiveness to infection on normally nonpermissive cell lines (Navarro-Sanchez et al., 2003; Tan et al., 1996). DC-SIGN interacts with the virus via carbohydrate moieties on E glycoprotein (Navarro-Sanchez et al., 2003), and the type of glycosylation that occurs in insect cells (i.e., specifically the addition of high-mannose glycans to residue N67) is required for DC-SIGN-mediated entry (Lozach et al., 2005, Pokidysheva et al., 2006) The virus uncoats intracellularly via a specific process. In the infectious form of the virus, the E protein lays flat on the surface of the virus, forming a smooth coat with icosahedral symmetry. However, when the virus is carried into the cell or lysosomes, the protein snaps into a different shape as a result of their acidic environment, assembling into trimeric spike. Several hydrophobic amino acids at the tip of this spike insert into the lysosomal membrane causing the virus membrane fusion with lysosome and releasing the viral RNA into the cell to start the infection.

Translation of the Dengue Virus Genome

The replication machinery of positive-stranded RNA viruses is not packaged into the viral particle, so once inside the cell, the viral genome must first undergo translation to generate the viral RNA replicase to establish a productive infection (Figure 8.5). Translation and replication of positive-strand viruses occurs in association with intracellular membranous structures (Salonen et al., 2005). Specifically, DENV is translated in association with endoplasmic reticulum (ER)-derived membranes.

The efficiency of initial translation of viral proteins may impact the cellular tropism of the virus. The different strains of DENV are differing in their abilities to replicate in various cell types. In one study, DENV-2 clinical isolates of different geographic origins and with limited passage in tissue culture were competent for replication in hamster kidney (BHK21) cells, but Nicaraguan strains did not replicate in human cell lines of myeloid origin (U937), human peripheral blood mononuclear cells, and primary human foreskin fibroblasts (HFF) (Diamond et al., 2000; Edgil et al., 2003).

In the infected cell, the viral RNA genome is translated by the host ribosomes. The entire genome is translated into a single polyprotein, and as a result, no temporal distinction exists in the translation of the different viral proteins, which is the special property of a flaviviral genome. Another important property of DENV genome is the structural genes are at the 5' end of the genome; hence the portions of the polyprotein containing the structural, not the catalytic, proteins are synthesized first with high efficiency. This may allow the production of more structural proteins, but

it decreases the efficiency of NS protein synthesis and the initiation of viral replication, which further contribute to the long latent period that precedes detection of *Flavivirus* replication. The resulting DENV polyprotein is cleaved by cellular and viral proteases at specific recognition sites co- and post-translationally into three structural (C, prM, and E) and seven NS (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins. Polyprotein processing proceeds via a combination of signal peptidases, the viral serine protease, and additional cellular proteases (Lindenbach and Rice, 2003). DENV has a small-sized RNA genome, which requires maximization of the coding capacity of genes; thus, many of the DENV structural and NS proteins serve multiple functions in the viral life cycle. Whereas definitive roles for several of the viral proteins have not yet been established, recent work has implicated a number of the DENV proteins in functions beyond vRNA synthesis and proteolysis, including inhibition of interferon (IFN)-mediated signal transduction, and organization of membranous structures.

The viral NS proteins then use this newly formed negative-sense RNA intermediate to replicate the positive-sense RNA genome, which then associates with capsid protein and is packaged into individual virions. Of all the viral NS proteins, the most extensively characterized are NS3, its cofactor NS2B, and NS5. NS3 is a multifunctional protein essential for *Flavivirus* replication; it harbors a number of catalytic domains, including a serine protease. The N-terminal third of this polypeptide forms a two component serine protease domain together with NS2B. This viral protease is responsible for the processing of viral polyprotein. The C-terminus of NS3 has three different enzymatic activities as nucleoside triphosphatase (NTPase), RNA triphosphatase (RTPase), and helicase functions required for vRNA synthesis. The RTPase activity is the first step of three sequential enzymatic reactions of RNA 5′ ^{7}mG capping, which is essential for viral translation and RNA stability (Gebhard et al., 2012). Additionally NS3 in DENV-2 has been shown to interact with human nuclear receptor binding protein, which modulates intracellular trafficking between the ER and the Golgi compartment, to impact its cellular distribution and to induce some of the membranous structures seen during *Flavivirus* infection (Chua et al., 2004).

The NS5 protein of DENV also serves two functions as the viral RNA-dependent RNA polymerase (Nomaguchi et al., 2003; Talavera et al., 2004) and as a methyltransferase (Egloff et al., 2002), which is another essential enzyme required in capping pathway. NS5 has also been demonstrated to induce transcription and translation of interleukin-8 (IL-8) via activation of CAAT/enhancer binding protein, which is a neutrophil chemoattractant, (Medin et al., 2005).

The NS1 glycoprotein of DENV is expressed in three forms: an ER-resident form that co-localizes with the viral replication complex, a membrane-anchored form, and a secreted form. The secreted form of the glycoprotein (sNS1) is also a dominant target of humoral immunity and hence plays a significant role in the pathogenesis of disease.

Other small hydrophobic proteins, NS2A, NS4A, and NS4B, are relatively less well characterized. Their hydrophobic nature and complementation analysis potentially implicates them in proper localization and arrangement of viral proteins and vRNA to sites of RNA synthesis and virion assembly. It had been demonstrated that NS4B and, to a lesser extent, NS2A and NS4A are capable of blocking IFN-mediated signal transduction (Munoz-Jordan et al., 2005). Although the three proteins in combination are most effective, NS4B alone is a potent inhibitor of interferon beta (IFN-β) and interferon gamma (IFN-γ) signaling.

Viral RNA Synthesis

Positive-stranded viruses use the same template for both translation and genomic replication; however, the two processes cannot occur simultaneously. After the translation of input strand, the virus switches to the production of vRNA, which involves a negative-strand template for further generation of the positive strand. Because the positive strand serves as both viral genome and mRNA, it is generally produced in excess of the negative strand, although the mechanism by which this asymmetric synthesis occurs has not yet been described.

For DENV, the ability of the vRNA to cyclize is implicated in the step of viral RNA synthesis. The 5' and 3' cyclization sequence (CS) domains mediate this circularization (Hahn et al., 1987), and disruption of base pairing between the two regions has been shown to compromise vRNA synthesis (Holden et al., 2006; Yocupicio-Monroy et al., 2003). In addition to their roles in viral translation, conserved structures and sequences in the 3' untranslated region (UTR) have been shown to regulate vRNA synthesis. Replication of all positive-stranded RNA viruses occurs in association with virus-induced intracellular membrane structures. DENV also induces such extensive rearrangements of intracellular membranes, called *replication complex* (RC). These RCs contain viral proteins, viral RNA, and host cell factors.

Assembly and Release of Virion

The subsequently formed immature virions are then assembled by budding of newly formed nucleocapsids into the lumen of the ER, thereby acquiring a lipid bilayer envelope with the structural proteins prM and E. The virions mature during transport through the acidic trans-Golgi network, where the prM proteins stabilize the E proteins to prevent conformational changes. Before the release of the virions from the host cell, the maturation process is completed when prM is cleaved into a soluble pr peptide and virion-associated M by the cellular protease furin. Outside the cell, the virus particles encounter a neutral pH, which promotes dissociation of the pr peptides from the virus particles and generates mature, infectious virions. At this point, the replication and maturation cycle repeats itself.

Host Factors

Age

During early epidemics, 95 percent of the reported cases were the children younger than 15 years of age. In subsequent outbreaks, an increasing number of cases have occurred in older age groups. After adolescence, the susceptibility to DSS steadily declines. This produces higher fatality rates for children, in comparison to adults (Thongcharoen and Jatanasen, 1993).

Sex

There is no significant difference in the overall sex distribution in cases of dengue infection. However, because of a slight increase in DSS cases among females, there is a higher case fatality rate among females. In most of the infectious diseases, morbidity and mortality is usually high among males, but dengue differs from other infections in this respect. Hence, epidemiological data from outbreaks of dengue infection should consider age, sex (to some extent), and severity of illness as mandatory parameters for analysis (Thongcharoen and Jatanasen, 1993).

Ethnic Groups

Persons of Chinese origin were more affected than other ethnic groups in Singapore, Malaysia, and Thailand (Thongcharoen and Jatanasen, 1993).

Occupation

Some persons as policemen, farmers, gardeners, and those involved in daytime outdoor occupations are more vulnerable in areas where *A. albopictus* is the highly prevalent vector. Whereas, in places predominated by *A. aegypti* mosquito, the disease occurs mostly in children and adult females who stay indoors during the daytime (Thongcharoen and Jatanasen, 1993).

Nutritional Status

Paradoxically, better nutritional status seems to increase the risk of DSS, which has rarely occurred in malnourished children (Thongcharoen and Jatanasen, 1993).

Immunity

The immune response to dengue infection is still conflicting. Infection with one serotype only provides lifelong immunity against the same serotype (homologous immunity), but it does not provide protection against other serotypes (cross-immunity). Thus, a person can easily be infected as many as four times, once with each serotype. Paradoxically, infection with one serotype may exacerbate subsequent infection with other serotypes.

Increased Travel and Trade

The frequency of epidemic disease has increased significantly in the past 20 years, which is likely a result of increased air travel, which provides the ideal mechanism for dengue viruses to be carried around the world into the areas where *A. aegypti* breeds. This rapid movement of travelers around the world is also an important reason that dengue infections may be detected in virtually any part of the world (Thongcharoen and Jatanasen, 1993).

Presence of Noncommunicable Diseases

An increased risk of DSS or death due to dengue infection has been observed in patients with history of asthma or anemia in the individual or family and family history of diabetes (Thongcharoen and Jatanasen, 1993).

History of **Flavivirus** *Infection*

So far DSS has not been reported in persons with a history of infection with other nondengue flaviviruses (Japanese encephalitis, yellow fever, and WNV). During the Vietnam War, US troops stationed in Southeast Asia were given 17-D strain of yellow fever vaccine, and the dengue infections acquired by US troops were mild.

Environmental Factors

Seasonal Distribution

In most of the Southeast Asian countries (except Indonesia) and in India during the southwest monsoon season, epidemics occur almost every year. The number of patients is correlated with the amount of rainfall. The outbreaks start in the May, reach their peaks in July and August, and decline in October. Dengue has an incubation period of 5 to 6 days but may vary from 3 to 10 days. After the Second World War, the socioeconomic disruptions changed the pattern of epidemics, and they have become more regular, resulting in worldwide spread of dengue viruses and capable vectors. An estimated 50 to 100 million cases of dengue fever and 500,000 cases of DHF occur worldwide, with 22,000 deaths (especially in children) each year (Malavige et al., 2004; Stephenson, 2005; WHO, 2011). Approximately 40 percent of the world's population (an estimated 2.5-3 billion people) in 112 tropical and subtropical countries is at risk for dengue infection; the exceptions are Europe and Antarctica, which do not experience dengue transmission.

Risk Factors

In urban areas, risk factors include high-population density, poor sanitation, and presence of a large number of overhead tanks, desert coolers, discarded buckets, tires, utensils, and water containers. In rural areas, the environment is conducive for mosquito breeding if large containers are used for collecting rainwater and are not cleaned periodically.

Reservoir

Principally dengue is a zoonotic disease, which is maintained in the sylvatic cycle, involving wild monkeys and jungle mosquitoes, quite similar to that for yellow fever in Africa (Ananthanarayan and Paniker, 2000). Depending on the type of strain it follows two different types of life cycles: sylvatic and epidemic. Sylvatic strains are antigenetically different from epidemic strains and mostly maintain transmission between nonhuman primates and forest dwelling *A. aegypti*. Humans are rarely infected by sylvatic strain. Epidemic DENV strains infect and replicate within humans and domesticated *Aedes* species (Pepin and Hanley, 2008). Epidemic dengue has become endemic in tropical urban slums where poor sanitation and free-standing water have allowed the number of mosquito vectors to rise (Hanley et al., 2008). High-vector density combined with high-population density in urban areas has allowed DENV to get a strong foothold in many of the world's overcrowded tropical cities (WHO, 2010). Of all the other vertebrate animals, only monkeys have shown high levels of dengue antibody titer, similar to that found in humans (Thongcharoen and Jatanasen, 1993). Man is the only vertebrate host, and the reservoir of the infection is both man and mosquito (Ananthanarayan and Paniker, 2000).

Mode of Transmission

The transmission cycle is man–mosquito–man. Dengue infection is transmitted by the bite of an infected female mosquito belonging to *Aedes* species. Transmission of DENV starts when an uninfected mosquito bites an infected host and takes up virus with its blood meal. The virus replicates in the midgut of the mosquito and then reaches its body cavity (hemocoel). From the hemocoel, it eventually makes its way to the salivary glands, from where it can infect other human hosts and the cycle continues (Hanley et al., 2008).

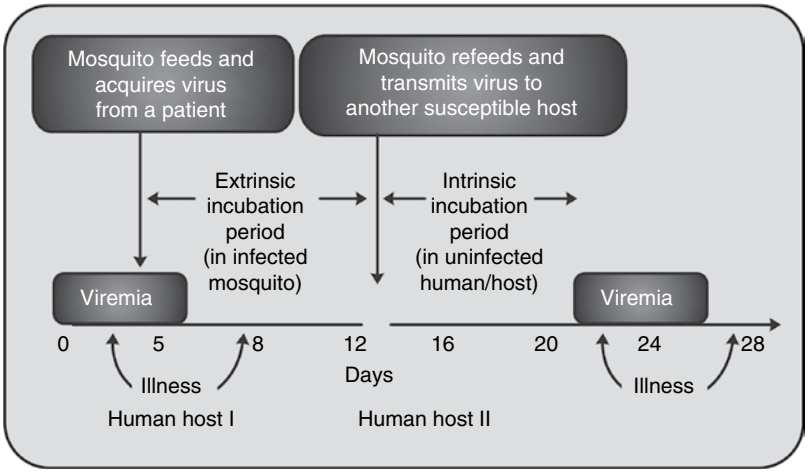

Figure 8.6. Transmission of dengue virus by *Aedes aegypti*.

It requires around 8 to 12 days for DENV to reach the salivary glands of a mosquito after the ingestion of an infected blood meal, depending on both viral and host factors (WHO, 2011). After this extrinsic incubation period, the virus can further be transmitted to other humans during subsequent probing or feeding (Figure 8.6). The extrinsic incubation period is influenced in part by some environmental conditions, especially ambient temperature. *A. aegypti* is one of the most efficient vectors for arbovirus transmission because it is highly anthropophilic, frequently bites several times before completing oogenesis, and thrives in proximity to humans. The transmission of a particular strain of dengue from vector to host is determined by how infective the virus is in the vector, the level of viral replication in tissues of the vector, and how easily the virus can disseminate from the midgut to the salivary glands (Hanley et al., 2008). Infectivity, replication, and dissemination are affected by both the genotype of the infecting virus as well as a variety of mosquito host factors that modulate DENV infection. Other factors that influence the dynamics of virus transmission include climatic factors, host pathogen interactions, and population immunological factors. Intrinsic incubation period starts with the human infection until the development of clinical symptoms of illness (*see* Figure 8.6). Vertical transmission (transovarial transmission) of DENV has also been demonstrated at the laboratory level but rarely in the field. Once the mosquito is infected, it remains infectious for its entire life span (Thongcharoen and Jatanasen, 1993). The life span of the mosquito is about 3 weeks under normal temperature and humidity.

In the post-monsoon period, the breeding of *Aedes* mosquitoes is at their peak, which is characterized by outbreaks of dengue and DHF.

Vector Biology

Vector Species

The various species of *Aedes* mosquito are known to transmit dengue infection; these include *A. aegypti*, *A. albopictus*, *Aedes vittatus*, *Aedes polynesiensis*, and *Aedes scutelloris*

complex. Among all of them, *A. aegypti* and *A. albopictus* are the most prevalent species responsible for dengue transmission.

Aedes Aegypti

In India, *A. aegypti* is the main vector for transmission of dengue infection (Thongcharoen and Jatanasen, 1993). However, in Africa, Central America, and South America, this is the principal vector of urban yellow fever disease. The origin of *A. aegypti* is believed to be from Africa, which is thought to spread along with travelers throughout the world. Urbanization plays a major role in the spread of this species. Urban ecological disturbances, including rapid growth of cities, population explosion, steady deterioration in standards of sanitation, and increase in number of water-retaining waste containers and debris, have amplified the population of *A. aegypti* in urban areas (Thongcharoen and Jatanasen, 1993).

Aedes Albopictus

A. albopictus is essentially an Asian mosquito species found to have spread to the United States in 1985 (Thongcharoen and Jatanasen, 1993). This species has been found in most states in the eastern half of the United States, in limited areas of Central and South America, Malaysia, and Myanmar.

Breeding Places

The female *Aedes* mosquito lays eggs in artificial accumulations of water (Figure 8.7) at various places in and around homes, schools, and workplaces. In Cuddalore (Tamil Nadu, India), breeding sites of the *Aedes* mosquito have also been discovered in shallow wells, which have been built because of a high water table (Thongcharoen and Jatanasen, 1993). In hot and humid tropical climates, water storage is necessary for frequent bathing and other household purposes. In these areas, unhygienic water storage habits of the local population also contribute to mosquito breeding. In Thailand, it has also been reported that the people staying in wooden houses store large amounts of water because they were scared of fire (Thongcharoen and Jatanasen, 1993), which also contributes to the emergence of new generations of *Aedes* mosquito.

Biting Time

The females are fearless biters, and they chiefly bite during the daytime. There are two distinct peak periods, which is constant throughout the year. The mid-morning peak occurs between 8 A.M. and 1 P.M., and the mid-afternoon peak is between 3 P.M. and 5 P.M. (Thongcharoen and Jatanasen, 1993).

Resting Habits

Aedes Aegypti

Mosquitoes of this species prefer to rest indoors in closets and dark places. In outdoors, crowded and poor urban localities are the main habitats of the species. The disease mostly occurs in children and adult females who stay indoors during the daytime—places where *A. aegypti* is the predominant vector (Thongcharoen and Jatanasen, 1993).

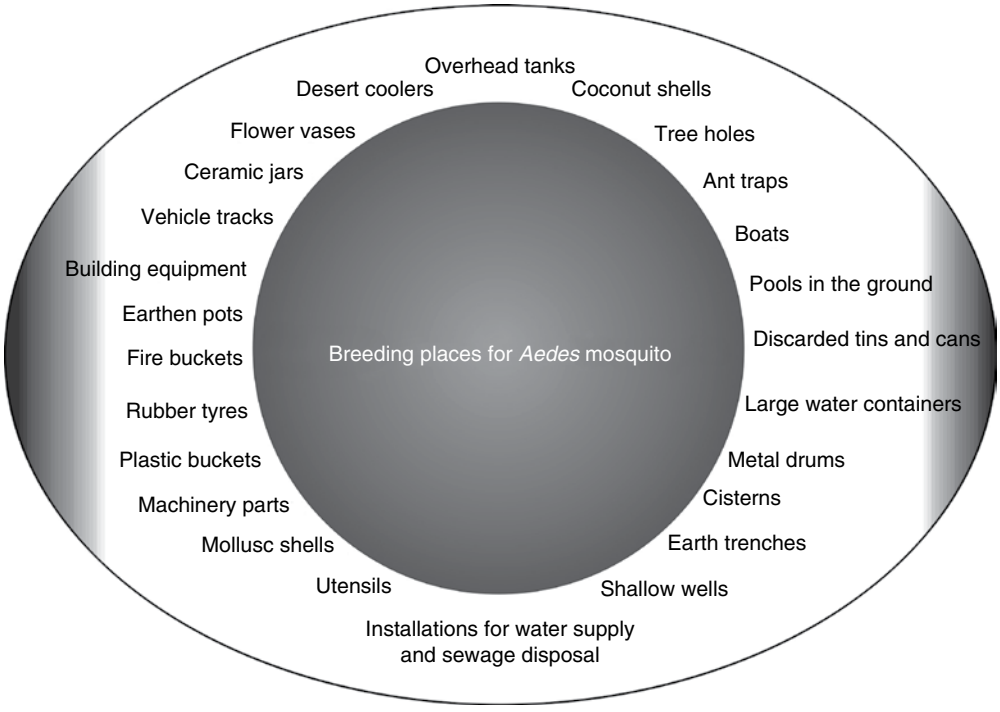

Figure 8.7. Breeding places for *Aedes* mosquito.

Aedes Albopictus

This species can also transmit DENVs, although its anthropophilic nature and its density in urban areas are not as great as that of *A. aegypti* (Amarasinghe et al., 2011). This is a peridomestic species that is found in boundaries of forests, agricultural areas, gardens around houses, and in plantations. *A. albopictus* also bites in the daytime; in areas where it is the predominant mosquito, persons with daytime outdoor occupations (policemen, farmers, gardeners) are more vulnerable (Thongcharoen and Jatanasen, 1993).

Displacement

A. albopictus is also a highly competent vector of several arboviruses, which has displaced *A. aegypti* from large areas of the southern United States within a few years (Rai, 1991). In Asia, *A. albopictus* and *A. aegypti* are generally not found in the same breeding sites. In India, *A. aegypti* displaced *A. albopictus* in urban areas, and the reverse occurred in rural areas (Gilotra et al., 1967). These populations coexist in an equilibrium state in gardens. *A. aegypti* and *Aedes bahamensis* infest different towns on Grand Bahama Island (Spielman and Feinsod, 1979). *A. bahamensis* is non-competent as a vector of a variety of arboviruses (Llewwellyn et al., 1970), which rarely feeds on vertebrate hosts. To displace a mosquito species, another species must compete for various available tropical resources, and interspecies competition must be keener as compared to intraspecies competition. The other factor responsible for replacement is sexual interaction and sexual

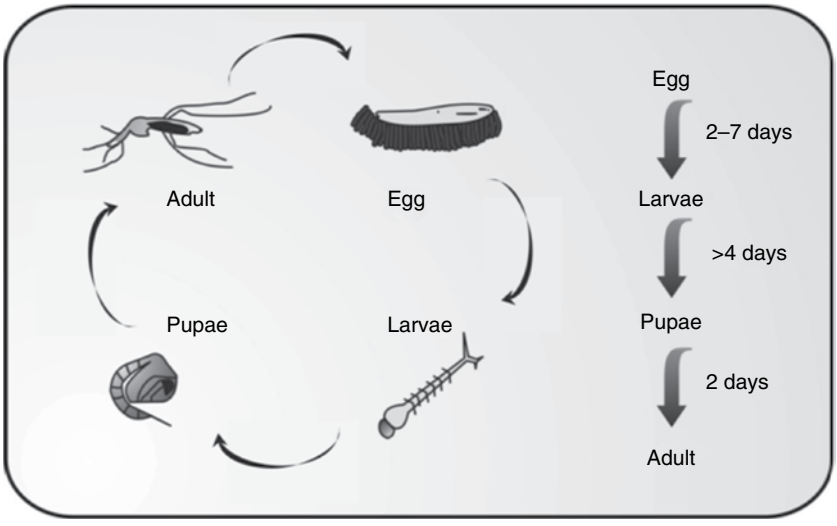

Figure 8.8. Life cycle of *Aedes aegypti* showing both the phases, an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults).

profligacy, which may help populations to perpetuate. Hypothetically, mating of male mosquitoes of one species with females of another will not effectively inseminate, and it impairs the fertility of those female mosquitoes. This hypothetical relationship has been termed the *Satyr effect* (Ribeiro and Spielman, 1986). The differential destruction of one species of mosquitoes by pathogens is also a factor that can cause asymmetry in population of different mosquito species (Beier and Craig, 1985). Pathogens are generally less virulent when parasitizing well-adapted hosts than when parasitizing alien hosts.

Life History of Aedes Mosquito

The adult male mosquitoes never bite and survive on plant juices, but the female mosquitoes require a blood meal after every 2 to 3 days for the development of their eggs. In hot and humid tropical areas, the gonotrophic cycle (time period between the blood meal and the laying of eggs) completes in about 48 hours. During unfavorable environmental conditions and severe winters, adult mosquitoes hibernate. Under appropriate favorable conditions, especially in terms of food supply and temperature, the *Aedes* mosquito completes its life cycle from an egg to the adult stage within 7 to 10 days. Hence, all water containers should be cleaned at least once in a week to reduce the risk of transmission of dengue syndrome.

Eggs

The eggs of *Aedes* mosquito are pale white in color and gradually turn into a black color within a short time. The female mosquito prefers laying the eggs on surfaces with a high degree of dark color, roughness, and water absorption. This specific habit of egg laying has been exploited for detecting the presence of *A. aegypti* in an area, by using oviposition traps (ovitraps). The cigar-shaped eggs (Figure 8.8) are laid singly, on the moist walls of the container. They do not possess lateral floats. The embryo develops within 2 to 5 days

(Thongcharoen and Jatanasen, 1993). The eggs can withstand desiccation for weeks to months. The ability of eggs to withstand desiccation helps in the transportation of *A. aegypti* in dried water containers. This phenomenon also hinders control measures because eggs from dried water containers can introduce mosquito infestation in places where they find water. When flooding occurs, the eggs hatch as a result of lowered oxygen tension in the floodwaters, which is a stimulus for egg hatching.

Larvae

The larvae hatch from the eggs and survive in the water for up 5 to 7 days. Because water is essential for the first 8 days during the life cycle of the *Aedes* mosquito, emptying and cleaning of the containers at least once in a week will greatly reduce the risk of dengue fever. The larva of *Aedes* mosquito is a freely-swimming creature, which swims with a distinct looping movement and feeds on algae, bacteria, and vegetable and organic matter. The larvae are almost vertically suspended in water with their heads downward (Thongcharoen and Jatanasen, 1993). They possess a siphon tube on the eighth abdominal segment, but they do not possess palmate hairs on abdominal segments.

Pupae

Pupae are the comma-shaped structures representing the resting stage in the life cycle of the mosquito. They do not feed and usually stay quiet on the water surface. If they get disturbed, they swim rapidly downward into the water. The pupal stage of *Aedes* mosquito lasts for 1 to 2 days (*see* Figure 8.8).

Adult Mosquito

On complete development, the pupal skin splits along the back, and the adult mosquito emerges. Adults rest for a while on the pupal skin or on the wall of the breeding site to allow their exoskeleton and wings to expand and harden and then fly away (Thongcharoen and Jatanasen, 1993). The adult mosquito has characteristic white stripes on the back and legs, giving it the name "tiger mosquito." Around 24 hours after the emergence, male and female both sexes can mate, and females can take a blood meal (Thongcharoen and Jatanasen, 1993). The *Aedes* mosquito can fly up to a limited distance of about one-quarter mile (400 m) but can spread for vast distances mechanically, by vehicles used by humans (Directorate of National Vector Borne Diseases Control Programme, 2008.). The life span of the female *Aedes* mosquito is about 3 weeks, which is further influenced by temperature and humidity, whereas the male mosquitoes usually have a shorter life span because they develop faster than females.

Clinical Features

Spectrum of Clinical Manifestations

Dengue has been described as a syndrome (i.e., a disease with a broad spectrum of clinical manifestations) that includes (Figure 8.9):

- Acute undifferentiated respiratory disease manifesting fever, coryza, pharyngeal inflammation with or without cough.
- An undifferentiated fever with or without symptoms of multiple system involvement.

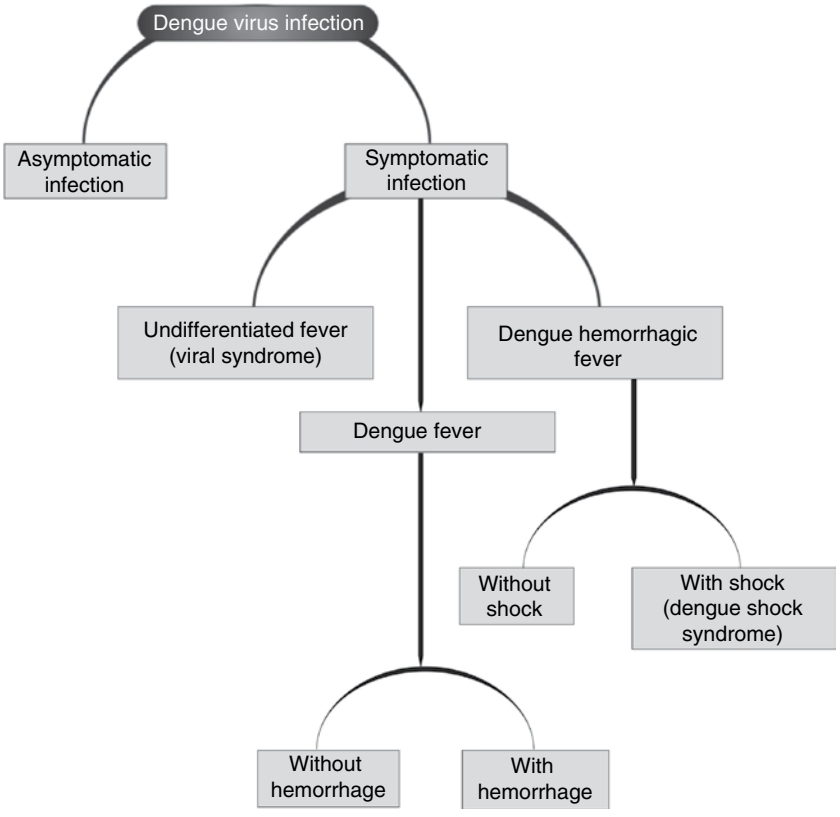

Figure 8.9. Clinical manifestations of dengue.

- Dengue fever, the most common manifestation in adults and older children. It can be further subdivided into DHF and DSS.
  (a) DHF: a dengue infection with hemorrhagic manifestations, but without shock.
  (b) DSS: a dengue hemorrhagic fever with shock (Halstead, 1966).

Among all the clinical manifestations of dengue syndrome, the mildest form is characterized by pharyngitis, mild rhinitis, cough, and mild gastrointestinal symptoms, which may be misdiagnosed as pharyngitis, influenza, or upper respiratory tract infection (Halstead, 1966).

Unusual Manifestations of Dengue Fever or Dengue Hemorrhagic Fever

- The clinical manifestations of disease might be reported as convulsions, spasticity, or altered consciousness. There is encephalopathy type involvement of the central nervous system (CNS), with absence of encephalitis and normal cerebrospinal fluid (CSF). The CNS involvement may also be associated with hepatic dysfunction, which might be iatrogenically induced by treatment with paracetamol or salicylates. This manifests as jaundice with an elevated levels of serum alanine amino transferase (ALT).

- DHF can lead to the hemolytic uremic syndrome and renal syndrome in patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency.
- Association of Reye's syndrome with dengue has already been reported in infants (Thongcharoen and Jatanasen, 1993).

Dengue Fever

Symptomatology

The classical dengue fever shows symptoms similar to that of other acute fevers of viral origin. Children usually have milder disease than adults. The classical symptoms include sudden onset of fever and chills, headache, and muscle and joint pains, which may be incapacitating and prevent all movements (thereby called break-bone fever). There may be the development of retro-orbital pain, particularly on eye movements or eye pressure and photophobia within 24 hours (Thongcharoen and Jatanasen, 1993). Other common symptoms include extreme weakness, anorexia, altered taste sensation, constipation, abdominal tenderness or colicky pain in abdomen, dragging pain in the inguinal region, sore throat, and general depression. There may be generalized enlargement of lymph nodes without enlargement of spleen and liver.

Fever

The body temperature in dengue is usually between 102.2 and 104° F (39 and 40° C), which is typically followed by a remission for few hours to 2 days and is called *biphasic fever* or *saddle back fever* (Ananthanarayan and Paniker, 2000). Fever lasts for about 5 days and rarely more than 7 days usually with complete recovery.

Skin Eruptions

In dengue fever, diffused, flushing, mottling, or fleeting pinpoint eruptions are seen on the face, neck, and chest during the early febrile phase. This is further followed by the development of a maculopapular or scarlatiniform rash on the trunk area, especially at third or fourth day of illness that spreads to the extremities and face. These rashes are rubelliform (measles-like) and appear in approximately 80 percent dengue cases during remission or the second febrile phase, which lasts for 1 to 2 days. Itching and hyperesthesia may also occur with this rash. The rash lasts for up to 2 hours to several days and may be followed by desquamation.

Convalescence

Convalescence may be protracted. During convalescence, the generalized rashes start fading and characteristic localized clusters of confluent petechiae may appear over the dorsum of the feet, legs, hands, and arms surrounded by the scattered pale areas of normal skin. Some patients may show scattered petechiae or bleeding from gums or nose.

Case Definitions for Dengue Fever

Suspect Case

Acute febrile illness of 2 to 7 days' duration, with two or more of the following: headache, backache, joint and muscle pain, pain behind eyes, hemorrhagic manifestations, leukopenia, and the presence or absence of skin rash.

Table 8.1. Diseases that resembles the different phases of dengue infection.

Conditions that Resembles Febrile Phase of Dengue	
Flu like syndromes	Influenza, measles, chikungunya, infectious mononucleosis, and HIV seroconversion illness
Illness with a rash	Rubella, measles, scarlet fever, meningococcal infection, chikungunya, and drug reactions
Diarrheal diseases	Rota virus and other enteric infections
Illness with other neurological manifestations	Meningo encephalitis and febrile seizures
Conditions that resembles critical phase of dengue infection	
Infectious	Acute gastroenteritis, malaria, leptospirosis, typhoid, viral hepatitis, acute HIV seroconversion illness, bacterial sepsis, and septic shock
Malignancies	Acute leukemia and other related malignancies
Other clinical pictures	Acute abdominal disorders as acute appendicitis, acute cholecystitis and perforated viscus, diabetic ketoacidosis, lactic acidosis, Leukopenia and thrombocytopenia with or without bleeding, platelet disorders, renal failure, respiratory distress, systemic lupus erythematosus

Probable Case

A suspect case of dengue fever, not responding to antimalarial drugs, blood slide negative for malarial parasite, high-vector density, and presence of confirmed case in the area are classified as probable cases.

Confirmed Case

A case can be confirmed if DENV has been isolated from patient's blood in the early phase of the disease in association with the positive serological test for immunoglobulin M (IgM) antibody in a single serum sample or fourfold difference in immunoglobulin G (IgG) antibodies in paired serum samples.

Differential Diagnosis of Dengue Fever

So many bacterial and rickettsial infections and a variety of viral infections, such as chikungunya fever, show symptoms similar to dengue (Table 8.1). It is therefore, impossible to diagnose mild dengue infection clinically, especially in sporadic cases.

Dengue Hemorrhagic Fever

DHF is a severe form of dengue fever, caused by infection with more than one serotype of DENV. Previous occurrence of classic dengue fever (infection with one serotype) increases the probability of DHF during subsequent infection with another serotype (National Institute of Communicable Diseases [NICD]/ WHO, 2006). The first infection probably sensitizes the patient, and the second appears to produce an immunological catastrophe. During the first few days, DHF resembles classical dengue fever. Headache, facial flushing, anorexia, vomiting and epigastric discomfort, tenderness at the right

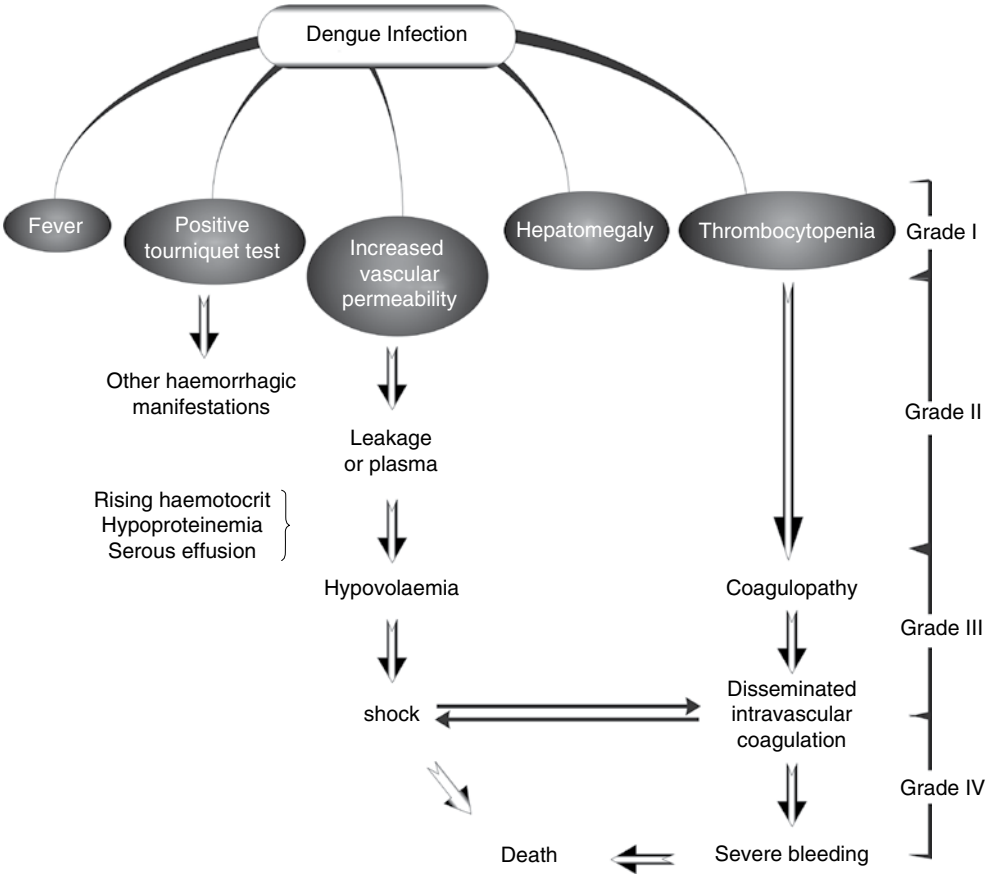

Figure 8.10. The clinical spectrum of dengue hemorrhagic fever.

costal margin, and generalized abdominal pain usually accompany sudden onset of high fever. The duration of DHF is between 7 and 10 days in most cases (WHO, 2012). Figure 8.10 explains the clinical spectrum of DHF.

Clinical Features of Dengue Hemorrhagic Fever

Fever

The temperature may reach 104 to 105.8° F (40–41° C), and febrile convulsions may occur in infants. The fever subsides after 2 to 7 days. The critical stage is reached 2 to 7 days after the fever subsides. After a rapid drop in temperature (usually accompanied by sweating, cold extremities and restlessness), varying degrees of circulatory disturbances may occur.

Skin Eruptions

Maculopapular (usually rubelliform) rash is less common as compared to classical dengue fever. Fine petechiae are commonly seen on the extremities, face, trunk, and axillae. There is always a tendency to bruise at the site of venepuncture.

Other Clinical Findings

Liver may be enlarged, soft, and tender. Generalized lymphadenopathy is seen in about 50 percent of patients. Sinus bradycardia may occur during convalescence. A characteristic confluent petechial rash may appear on the extremities. Scattered round areas of pale skin, just as in dengue fever, surround these petechiae.

Clinical Confirmation

Standard tourniquet test is used for clinical confirmation of DHF (Mayxay et al., 2011). After application of sphygmomanometer cuff to arm, the systolic and diastolic blood pressure is recorded. Then, the cuff is inflated so that the pressure is mid-way between the systolic and diastolic blood pressure. This pressure is maintained for 5 minutes. The cubital fossa is examined for petechiae. Presence of 20 petechiae (or more) per square inch indicates positive tourniquet test.

Clinical Laboratory Findings in Dengue Hemorrhagic Fever

DHF is characterized by the presence of thrombocytopenia, hemoconcentration, and plasma leakage. Thrombocytopenia (platelet count of 100,000 per cubic mm or less) may occur between the third and the eighth day of illness. Presence of pleural effusion on chest X-ray indicates evidence of plasma leakage in the pleural cavity. Plasma may also leak into the abdominal cavity, causing ascites (Srikiatkhachorn, 2009). Hemoconcentration is an increase in hematocrit by 20 percent or more is considered as the objective evidence of increased vascular permeability and leakage of plasma. It should be noted that the level of hematocrit may be affected by early volume replacement and by bleeding. The leukocyte count may be normal or show leukopenia with relative neutrophilia. Toward the end of the febrile phase there is a drop in the total number of white cells and in the number of polymorphonuclear cells. A relative lymphocytosis with more than 15 percent atypical lymphocytes is commonly observed toward the end of the febrile phase (critical stage) and at the early stage of shock. A transient mild albuminuria is sometimes observed. Occult blood is often found in the stool. In most cases, assays of coagulation and fibrinolytic factors show reductions in fibrinogen, prothrombin, factor VIII, factor XII, and antithrombin III. A reduction in antiplasmin (plasmin inhibitor) has also been observed in some cases. In severe cases showing marked liver dysfunction there is a reduction in the vitamin K-dependent prothrombin family, such as V, VII, IX, and X factors. Partial thromboplastin time and prothrombin time are generally prolonged in about one-half and one-third of DHF cases, respectively. In severe cases thrombin time is also prolonged. Serum complement levels are reduced. Hypoalbuminemia, hyponatremia (in severe cases), and slightly elevated serum ALT levels are other laboratory findings. Metabolic acidosis is frequently found in cases with prolonged shock. Blood urea nitrogen is elevated in the terminal stage of cases with prolonged shock.

Case Definitions for Dengue Hemorrhagic Fever

Suspect Case of Dengue Hemorrhagic Fever

Acute febrile illness of 2 to 7 days' duration, accompanied by severe headache, backache, joint and muscle pain, pain behind eyes, enlargement of liver, and bleeding tendencies (petechiae, purpura, ecchymosis, epistaxis, bleeding gums, hematemesis, melena).

Probable Case
A suspect case of DHF with:

- Presence of hemorrhagic tendencies as revealed by one or more of the following: positive tourniquet test (more than 20 petechiae per square inch), petechiae, ecchymoses or purpura.
- Bleeding from any site: buccal mucosa, gums, gastrointestinal tract, injection site, nose; hematemesis or passage of tarry stools (melena); or thrombocytopenia (platelet count of 100,000 or less per cubic mm).
- Evidence of plasma leakage: more than 20 percent rise in average hematocrit for age and sex, pleural effusion, ascites, and edema.

Confirmed Case
Probable case of DHF with thrombocytopenia (platelet count of 100,000 or less per cubic mm), evidence of hemoconcentration (hematocrit increased by 20 percent or more), or evidence of plasma leakage such as pleural effusion on chest radiography.

Grading the Severity of Dengue Hemorrhagic Fever

The severity of DHF can be graded depending on the type and extent of illness. In mild cases, there are minimal and transient changes in vital signs and the patient recovers spontaneously or after a brief period of treatment. In less severe cases, patients recover spontaneously or after a short duration of treatment. Severe cases reported acute abdominal pain shortly before the onset of shock. The patient becomes restless and circumoral cyanosis becomes prominent. The skin becomes cold, clammy, purplish, and blotchy. The major pathophysiological changes that determine the severity of DHF (and differentiate it from dengue fever) are plasma leakage and abnormal hemostasis as manifested by rising hematocrit value and moderate to marked thrombocytopenia. These two clinical laboratory findings are distinctive and constant findings in DHF. The severity of DHF has been classified into four grades (Table 8.2), based on two pathophysiological hallmarks shock and hemorrhagic manifestations (Thongcharoen and Jatanasen, 1993).

Table 8.2. Grades of severity of Dengue Hemorrhagic Fever.

Grade	Clinical Picture
Grade I	Fever accompanied by nonspecific constitutional symptoms. The only hemorrhagic manifestation is a positive tourniquet test.
Grade II	Patient with spontaneous bleeding, usually in the form of skin or other hemorrhages in addition to the manifestations in Grade I.
Grade III	Circulatory failure, manifested by rapid and weak pulse, narrowing of pulse pressure (20 mm Hg or less), or hypotension with a presence of cold clammy skin and restlessness
Grade IV	Profound shock with undetectable blood pressure and pulse.

Dengue Shock Syndrome

Symptomatology

DSS can occur both in children as well as in adults. It poses a greater fatality rates as compared to a bleeding disorder (Statler et al., 2008). The symptoms of DSS has been subdivided into two phases, early and advanced. The early signs of DSS are restlessness, cold clammy skin, rapid weak pulse, narrowing of pulse pressure, hypotension, whereas the warning signs are severe abdominal pain, protracted vomiting, marked change in body temperature (from fever to hypothermia), and change in mental status, such as irritability or obtundation. Hypovolemic shock is actually the result of the leakage of plasma into pleural or abdominal cavities, which, in turn, causes hypoproteinemia. The presence of metabolic acidosis and severe gastrointestinal bleeding in some cases indicates a poor prognosis. The degree of fall in platelet count and rise, in hematocrit level, are further correlated with severity of disease. These changes occur before the fever subsides and before the onset of shock. Some rare features also indicate grave prognosis, such as intracranial hemorrhage, metabolic and electrolyte disturbances, and hepatic failure.

Clinical Laboratory Tests in Dengue Shock Syndrome

- Platelet count, blood grouping, and cross matching.
- Serum electrolytes.
- Arterial blood gases.
- Prothrombin time and thrombin time.
- Liver function tests, including serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum proteins.

Differential Diagnosis of Dengue Hemorrhagic Fever and Dengue Shock Syndrome

During the early phase of febrile illness, the differential diagnosis should include a wide spectrum of viral and bacterial infections. The presence of thrombocytopenia along with concurrent hemoconcentration differentiates DHF/DSS from other conditions such as endotoxic shock.

Case Definitions

Suspect Case of Dengue Shock Syndrome
A case of DHF with circulatory failure (rapid and weak pulse), narrow pulse pressure, cold and clammy extremities, restlessness or lethargy, and altered sensorium.

Probable Case
A suspect case of DSS with one or more of the following: positive serological test and presence of a confirmed case of dengue in the area during the same period.

Confirmed Case
A probable case of DSS, confirmed by one of the following laboratory investigations:

1. Detection of dengue-specific IgM antibody in a single serum sample.
2. IgM sero-conversion.
3. Fourfold difference in IgG antibody titer in paired sera.

4. Virus isolation from plasma, serum, CSF, or autopsy tissue.
5. Detection of viral genomic sequence by polymerase chain reaction (PCR) in plasma, serum, CSF, or autopsy tissue.
6. Detection of viral antigen in autopsy tissue by immunohistochemistry or immunofluorescence or in serum samples by enzyme immunoassay (EIA).

The characteristic symptoms of dengue are sudden-onset fever, headache (typically located behind the eyes), muscle and joint pains, and a rash. The alternative name for dengue, break-bone fever, comes from the associated muscle and joint pains (Whitehorn and Farrar, 2010; Chen and Wilson, 2010). In 2009, WHO divided the course of dengue infection into three phases: febrile, critical, and recovery.

- The febrile phase (2 to 7 days): It involves high fever, often higher than 104° F (40° C), which is associated with generalized pain and a headache (Chen and Wilson, 2010). At this stage, a rash occurs in approximately 50 to 80 percent of those with symptoms (Chen and Wilson, 2010; Wolff and Johnson, 2009). It occurs in the first or second day of symptoms as flushed skin or later in the course of illness (Days 4 to 7), as a measles-like rash. Some petechiae (small red spots that do not disappear when the skin is pressed, caused by broken capillaries) can appear at this point (WHO, 2009), and some mild bleeding may occur from the mucous membranes of the mouth and nose. (Ranjit and Kissoon, 2010; Chen and Wilson, 2010). The fever itself is classically biphasic in nature, breaking and then returning for 1 to 2 days, although there is wide variation in how often this pattern actually happens (Knoop et al. 2010; Gould and Solomon, 2008).
- The critical phase (24–48 hours): In some people, the disease proceeds to a critical phase, which follows the resolution of the high fever and typically lasts 1 to 2 days (WHO, 2009). During this phase, there may be significant fluid accumulation in the chest and abdominal cavity as a result of increased capillary permeability and leakage. This leads to depletion of fluid from the circulation and decreases blood supply to vital organs (WHO, 2009). During this phase, organ dysfunction and severe bleeding, typically from the gastrointestinal tract, may occur (Ranjit and Kissoon, 2010; WHO, 2009) Shock (DSS) and hemorrhage (DHF) occur in less than 5 percent of all cases of dengue (Ranjit and Kissoon, 2010); however, those who have previously been infected with other serotypes of dengue virus (secondary infection) are at an increased risk (Ranjit and Kissoon, 2010; Rodenhuis-Zybert et al., 2010).
- The recovery phase (2 to 7 days): It occurs next, with resorption of the leaked fluid into the bloodstream (WHO, 2009). This usually lasts 2 to 3 days (Ranjit and Kissoon, 2010). The improvement is often striking, but there may be severe itching and a slow heart rate severe (Ranjit and Kissoon, 2010; WHO, 2009) During this stage, a fluid overload state may occur; if it affects the brain, it may cause a reduced level of consciousness or seizures (Ranjit and Kissoon, 2010).

Associated Problems

Dengue can occasionally affect several other body systems (WHO, 2009), either in isolation or along with the classic dengue symptoms. A decreased level of consciousness occurs in 0.5 to 6 percent of severe cases, which is attributable either to infection of the brain by the virus or indirectly as a result of impairment of vital organs, for example, the liver (Varatharaj, 2010; Gould and Solomon, 2008).

Other neurological disorders have been reported in the context of dengue, such as transverse myelitis and Guillain-Barré syndrome (Varatharaj, 2010). Infection of the heart and acute liver failure are among the rarer complications (Ranjit and Kissoon, 2010; WHO, 2009).

Laboratory Diagnosis

The definite diagnosis of dengue fever or DHF requires laboratory confirmation by serological tests. Once a person acquires dengue infection, the key to survival is early diagnosis, and DHF/DSS can be effectively managed by fluid replacement therapy. If diagnosed early, fatality rates can be kept below 1 percent (Thongcharoen and Jatanasen, 1993).

Sample Collection

During sample collection some information should be recorded as:

- Name and address of the institution sending the sample.
- Date of onset of illness
- Date of hospitalization.
- Provisional diagnosis.
- Brief clinical history and findings.
- Results of clinical laboratory investigations.

Samples for Serological Tests

For serological tests, 5 mL of blood is aseptically collected from the patient by venipuncture in a plain vial (without heparin). The first blood sample is collected 5 days after the onset of illness for detecting IgM antibodies. The second sample is collected at least 10 to 14 days after the first sample. Alternatively, the second sample may be collected at the time of death, should it occur. The collected blood sample is allowed to clot at room temperature. The serum is separated by centrifugation at 2000 RPM and collected in a clean dry vial.

Samples for Virus Isolation and Polymerase Chain Reaction Amplification Technique

For this purpose serum, plasma, and white blood cells (washed buffy coat) are to be collected within the first 5 days of illness. Autopsy tissues (liver, spleen, lymph nodes, and thymus) and mosquitoes collected in nature can also be used for virus isolation and PCR.

Labeling and Transport of Blood Samples

Specimen containers should be clearly labeled with the full name of the patient, identification number, and date of collection of the sample. Samples should be transported in an ice box at 35.6 to 46.4 °F (2–8 ° C) as soon as possible, to prevent heat exposure during transit. If transport to the laboratory is likely to be delayed by more than 24 hours, the serum should be separated and stored in a refrigerator. For virus isolation and PCR testings, the sample should always be stored in a frozen state.

Laboratory Tests

Hematological Tests

Two hematological tests, thrombocyte count and hematocrit, should be considered for lab diagnosis of dengue. The thrombocyte count of 100,000 platelets per cubic mm or fewer indicates thrombocytopenia and more than 20 percent rise in average hematocrit for age and sex indicates hemoconcentration.

Serological Tests

Immunoglobulin M Antibodies
The IgM antibodies appear about 5 days after the onset of symptoms and are detectable for 1 to 3 months thereafter by IgM capture enzyme-linked immunosorbent assay (ELISA) and rapid IgM strip test.

Immunoglobulin G Antibodies
IgG antibodies appear later than IgM antibodies and remain at high levels for 30 to 40 days before declining to levels found in past infection in primary dengue infection. These antibodies persist throughout the life of an individual. Fourfold or greater difference in IgG titer in paired serum samples obtained at an interval of 10 to 14 days is used for diagnosis of dengue. IgG antibodies are detected by ELISA and hemagglutination inhibition (HI) tests. IgG antibody detection is used in sero-epidemiological studies to determine the extent of silent infection and immunity levels in the population of a locality.

Isolation of Virus

Clinical material is inoculated in tissue culture, mosquitoes, or suckling mice. Further isolation is by using fluorescent antibody or HI test.

Demonstration of Viral Antigen/RNA

Specific monoclonal antibodies for dengue virus are used to demonstrate viral antigen by direct fluorescent antibody test. Viral RNA or genomic sequences can be detected in serum, CSF, or culture supernatant by PCR and gene sequencing.

Immune Response to Dengue Virus

Both humoral and cellular immunity are elicited in response to dengue and thereby important to the control of primary infection and to the prevention from future infections with the DENVs. Similar to other viral infections, the primary infection by DENV is a viremia, which presents the virus immediately to macrophages, the cells of the reticuloendothelial system, and the immune response. Infection with one DENV serotype results in immunity to that serotype only, the immune response to the primary serotype is cross-protective against other serotypes only during the first several months after infection (Sabin, 1952). It has been suggested that serotype-specific protection is because of neutralizing antibodies to DENV-specific memory T cells, or to both.

Innate Immune Response

In the early stage of acute DENV infection, innate immune response plays a key role in determining disease outcome, particularly during a primary infection. IFN-α and IFN-β, as well as IFN-γ has been shown to provide protection from DENV infection in vitro, but only when treatment precedes infection (Diamond et al., 2000; Ho et al., 2005). Among the main producers of IFN-γ are natural killer (NK) cells, which are likely to be important in clearing DENV during acute infections. Replication of DENV inside the macrophages and endothelial cells produce a double-stranded RNA intermediate, which induces IFN production. The IFN so produced is then released into the bloodstream soon after infection to limit the further replication of virus and stimulate the immune response. This IFN also causes the rapid onset of flulike symptoms characteristics of mild systemic disease.

Specific Immune Response

Cell Mediated

Cell-mediated immunity is also important in controlling primary infection. Apart from NK cells, T cells and macrophages are also activated by IFN and can respond to the cell surface antigens displayed on the infected cells. Immunity to these *Flaviviruses* is a double-edged sword. Inflammation resulted from the cell-mediated immune response can destroy tissue and promote hypersensitivity reactions such as delayed type hypersensitivity, formation of immune complexes with viral antigens and whole virion, and activation of complement. This can weaken and rupture the vasculature, leading to various hemorrhagic symptoms. Phenomena attributed to delayed hypersensitivity have been observed at different stages of dengue illnesses.

- The occurrence of delayed hypersensitivity has been postulated to explain the late appearing maculopapular rash seen in normal dengue fever (Halstead, 2008).
- Irritation cells seen frequently during the acute phase of DHF could represent an in vivo lymphoblast transformation to dengue antigen.
- Sabin, (1952) observed an erythematous response at the site of subcutaneous inoculation of DENVs in human volunteers. Skin reactions were noted 3 to 5 days after inoculation.

Skin biopsies from patients with dengue showed perivascular infiltration of mononuclear cells. The humans employed in these experiments presumably had not been sensitized to dengue antigen by previous infection experience. The observed reaction thus may be analogous to the induction of delayed hypersensitivity by small quantities of protein antigens. Such reactions precede the development of detectable circulating antibody and may be the result of the production of IgM.

Despite strong evidence that delayed hypersensitivity occurs in dengue infection, conventional delayed hypersensitivity is unlikely to mediate the alteration of host response in dengue if pathogenic unity of primary and secondary infection DHF is accepted. Transfer of sensitized lymphocytes could not occur in the former group. Further, the pathological findings in DHF are not consistent with those seen in delayed hypersensitivity states. If delayed hypersensitivity reactions can be altered in the direction of greater virulence by passively or endogenously acquired dengue antibody heterologous to the infecting virus, this might be a pathogenetic mechanism for both types of DHF.

Antibody Mediated

Circulating IgM is produced within 6 days of dengue infection, followed by class switching to IgG. The antibody blocks the spread of virus and subsequent infection of other tissues. In case of dengue infection, maternally acquired IgG antibody protects the infant if present at high concentration, but at low concentration it actually sensitizes. Polyclonal serum and monoclonal antibodies produced against E and prM proteins are capable of neutralizing DENV in vitro and, along with antibodies to NS1, providing protection in vivo (Johnson and Roehrig, 1999; Wills et al., 2004). Cross-reactive antibodies may contribute to the pathogenesis during DENV infection by targeting platelets for destruction (Oishi et al., 2003; Erfan et al., 2003). Anti-NS1 antibodies have also been demonstrated to bind and induced apoptosis in endothelial cells, as well as to induce secretion of proinflammatory cytokines and chemokines, implicating anti-NS1 antibodies as additional mediators of the increased vascular permeability seen in case of both DHF and DSS (Lin et al., 2003, 2005).

The secondary infection with a different strain of DENV places people at risk of DHF and DSS. The most widely accepted hypothesis behind this is an antibody–dependent enhancement (ADE) of dengue severity.

Antibody-Dependent Enhancement

After a person is infected with dengue, he or she develops an immune response both humoral and cell mediated to that dengue subtype. The humoral immune response produced specific antibodies to that subtype specific surface proteins that prevents the virus from binding to macrophage cells (the target cell that dengue viruses infect) and gaining entry. However, if another subtype of DENV infects the individual, the virus will activate the immune system to attack it, as if it was the first subtype. The immune system is tricked because the four subtypes have similar surface antigens. The antibodies bind to the surface proteins of this new subtype but are unable to inactivate the virus completely as being non-specific. The immune response thereby attracts numerous macrophages, which the virus proceeds to infect because it has not been inactivated. This situation is referred to as ADE of a viral infection. This makes the viral infection more acute because the body releases cytokines that cause the endothelial tissue to become permeable, which results DHF and fluid loss from the blood vessels. Goncalvez et al. (2007) demonstrated a significant increase of DENV-4 viremia titers in experimental monkeys that had been passively immunized with transferred dilutions of an antidengue humanized monoclonal antibody.

This phenomenon could be explained by one of the three possibilities:

1. A viral surface protein laced with antibodies against a virus of one serotype binds to a similar virus with a different serotype to neutralize the virus surface protein from attaching to the cell, but the antibody bound to virus also binds to the receptor of the cell, the Fc-region antibody receptor FcγR. This brings the virus into proximity to the virus-specific receptor, and the cell endocytoses the virus through the normal infection route.
2. A virus surface protein is attached to antibodies of a different serotype and can activate the classical pathway of the complement system. The complement component C1q then attached to the virus surface protein via the antibodies, which in turn binds C1q receptor found on cells and brings the virus and the cell close enough for a specific-virus receptor to bind the virus and beginning infection. This mechanism has not been shown specifically for the DENV infection, but is supposed to occur with Ebola virus infection in vitro (Takada et al., 2003).

3. When an antiviral antibody is present for a different serotype, it is unable to neutralize the virus and in turn is ingested into the cell as a subneutralized virus particle. These viruses are thereby phagocytosed as antigen-antibody complexes and attacked by macrophages to clear them from the circulation. Upon ingestion, the antibodies no longer even subneutralize the body because of the denaturing condition at the step for acidification of phagosome before fusion with lysosome; therefore, the virus remains active and begins its proliferation within the cell.

Clinical Management

Management of Dengue Fever

Clinical management of dengue infection is symptomatic, with bed rest and paracetamol (acetaminophen) for fever and pain. Some nonsteroidal anti-inflammatory drugs (NSAIDs) and salicylates, such as aspirin, are contraindicated because aspirin may cause gastritis, acidity, and its anticoagulant effects may aggravate the bleeding tendency associated with some dengue infections. Sponging helps in maintaining the body temperature below 102.2° F (39° C) (Thongcharoen and Jatanasen, 1993). Mild sedatives are indicated in case of severe pain. Hepatotoxic drugs should also be avoided. Oral fluids, such as fruit juices, rice-water, rice gruel, porridge, plain water, or oral rehydration solution, are recommended for preventing dehydration in patients with excessive sweating, vomiting, or diarrhea.

Management of Dengue Hemorrhagic Fever

On around the third day of illness, the patient generally starts feeling better and becomes febrile but still needs close clinical observation for development of complications, during which serial hematocrit values should be assessed for early recognition of DHF. If facilities for estimating hematocrit are not available, vital signs are to be monitored along with the maintenance of urinary output charts.

Indications for Hospitalization

Patients should be admitted to hospital in case the rise in hematocrit is 20 percent or more, a single hematocrit value is more than 40 percent, or if the platelet count is 50,000 per cubic mm or less. The clinical parameters for hospitalization include spontaneous bleeding from any site (minute spots on the skin suggesting bleeding within the skin, epistaxis, bleeding gums, hematemesis, passage of black tarry, stools), oliguria, circumoral cyanosis, restlessness, cool extremities, acute abdominal pain, abnormal behavior or drowsiness, and breathlessness. All patients with DHF ought to be admitted in a mosquito-free semi-intensive care unit because mosquitoes can transmit the virus from patients to other humans (Thongcharoen and Jatanasen, 1993). During the febrile phase, the clinical management of DHF is similar to that of classical dengue fever.

Intravenous Fluid Therapy

Intravenous fluid therapy (IFT) is recommended if there is a rise in hematocrit value, indicating the loss of plasma. Fluid therapy is actually dependent on the particular grade of the fever. In patients with Grade I and Grade II DHF, parenteral fluids can be given for a period of 12 to 24 hours (Figure 8.11), whereas 24 to 48 hours is recommended for

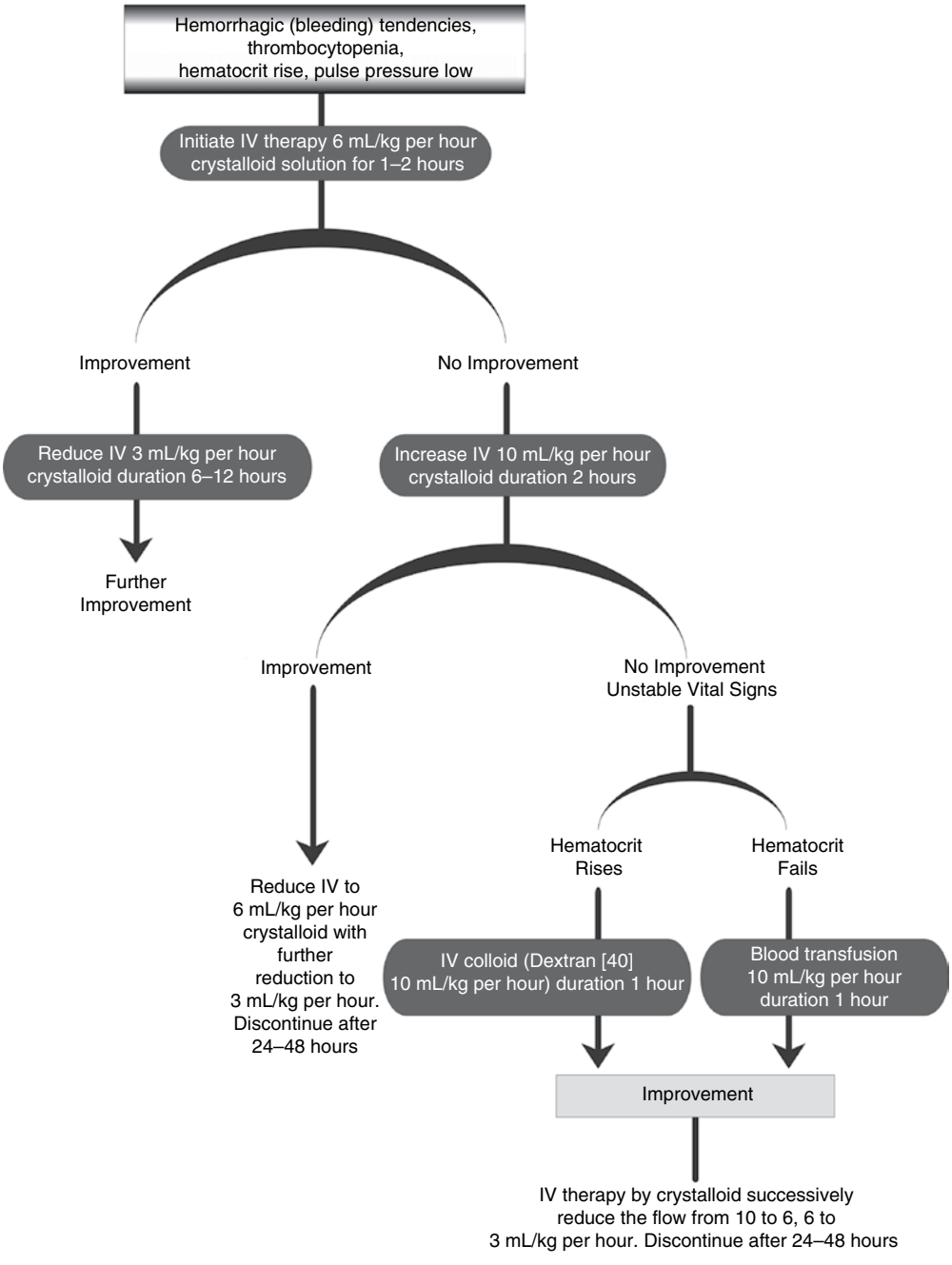

Figure 8.11. Volume replacement flow chart for patients with dengue hemorrhagic fever, Grades I and II.

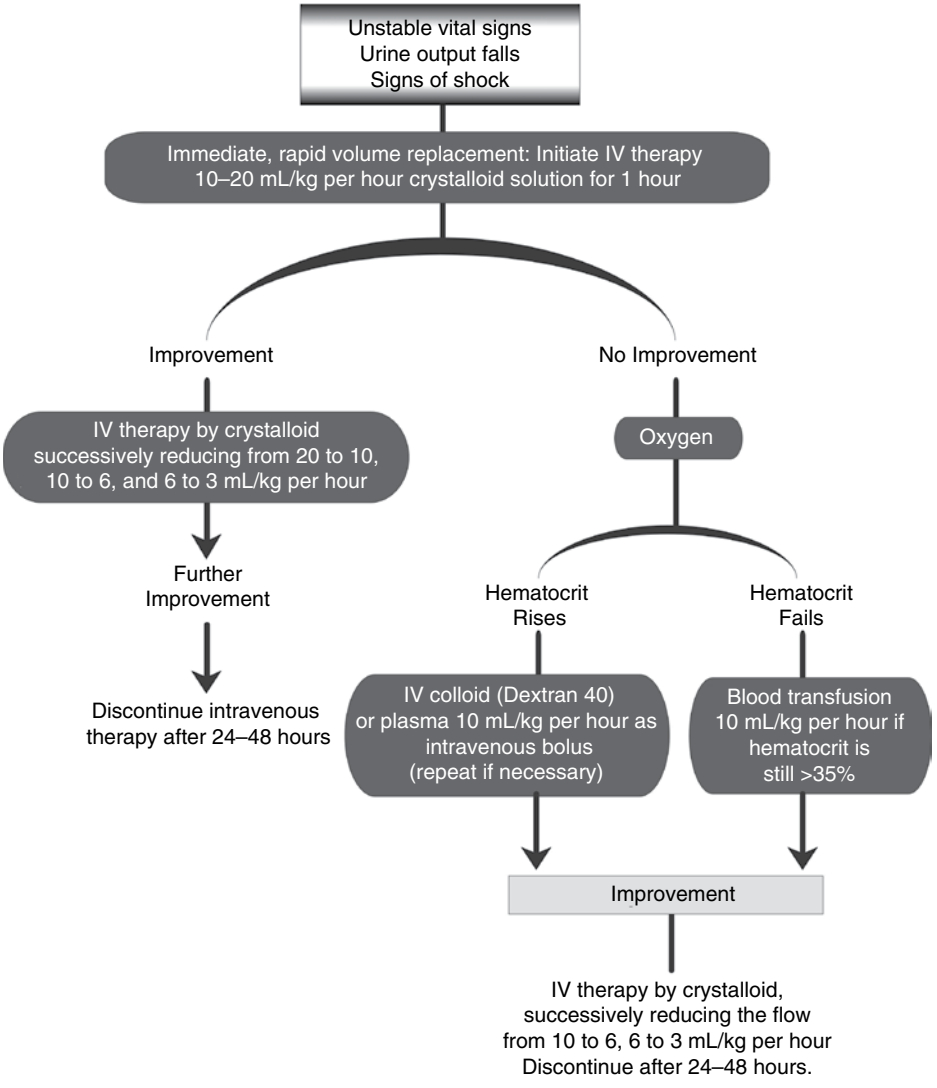

Figure 8.12. Volume replacement flow chart for patients with dengue hemorrhagic fever, Grades III and IV.

patients with Grade III and IV (Figure 8.12). The various intravenous fluids that could be given are (Thongcharoen and Jatanasen, 1993):

- 5% dextrose in Ringer's lactate solution (except in cases of acidosis hyperosmolar or Ringer's lactate solution should be avoided).
- 5% dextrose in Ringer's acetate solution.
- 5% dextrose in normal saline solution.

Improvement is considered when the hematocrit falls, pulse rate and blood pressure becomes stable with the rise in urine output; however, if hematocrit or pulse rate rises pulse pressure falls below 20 mm Hg(2.7 kPa) and urine output falls, that is no improvement. The other category is unstable vital signs, which is characterized by fall in urine output along with signs of shock.

For younger children, 5% dextrose in half-strength normal saline solution is recommended and 5% dextrose in one-third strength normal saline solution may be used for infants, if serum sodium levels are normal. Colloids are indicated in patients reporting massive leakage or profound shock and also in patients who have received a large volume of crystalloid. The commonly used colloids are Dextran 40 and plasma (WHO, 1999).

The fluid volume should be carefully charted on every 2 to 3 hours and administration rate should be adjusted throughout the period of plasma leakage (24–48 hours). The amount of fluid to be replaced should be the minimum amount that would be sufficient to maintain effective circulation during the period of leakage. The rate of fluid replacement may be roughly estimated by:

$$Fluid\ (mL/hr = Drops/min \times 3$$

To avoid fluid overload, vital signs and urine output should also be frequently monitored and the hematocrit values should be determined serially. Sometimes excessive fluid replacement leads to respiratory distress as a result of pleural effusion, pulmonary congestion/edema, and ascites when the extravasated plasma is reabsorbed during convalescent stage.

Management of Dengue Shock Syndrome

DSS is a severe medical emergency that warrants prompt and vigorous volume replacement and correction of electrolyte and acid-base disturbances. There is a risk of developing disseminated intravascular coagulation (DIC) as stagnant acidemic blood will promote or enhance DIC, which may lead to severe hemorrhage or irreversible shock. Thrombocytopenia with concurrent hemoconcentration occurs before the onset of shock. The physicians ought to note this fact, diagnose early, and treat accordingly, to decrease the severity of the illness. Unusual presentations, such as hepatic or renal failure require appropriate special treatment (Thongcharoen and Jatanasen, 1993).

Management of Shock

Parenteral Fluid Therapy

The plasma loss should be replaced immediately with intravenous fluids (5% dextrose in Ringer's acetate solution or in normal saline solution at the rate of 10–20 mL/kg body weight per hour). Glucose solution without saline is not able to restore the electrolyte balance and is, thereby, not recommended. The amount of fluid given should be always constantly monitored. During treatment, any evidence of swelling, breathlessness, or facial puffiness may indicate fluid overloading. Severely ill patients need correction of hyponatremia and metabolic acidosis; and simultaneous estimation of serum electrolytes and blood gases. Colloidal fluid (dextran in normal saline solution) should be given in case of profound shock with high hematocrit values, after the initial fluid replacement, at the rate of 10 to 20 mL/kg body weight per hour.

Blood Transfusion

Blood transfusion is recommended in cases with massive bleeding, profound or persistent shock, or DIC, even if the hematocrit values decline after initial fluid replacement. Fresh whole blood is preferable in patients with significant bleeding (usually hematemesis and melena). The volume of transfused blood should be such that the red blood cell (RBC) count becomes normal. Because it is difficult to estimate the degree of internal hemorrhage in the presence of hemoconcentration, fresh whole blood should always be given in smaller volumes at a time. Fresh-frozen plasma, concentrated platelets, and cryoprecipitates are indicated only in cases where consumptive coagulopathy causes significant bleeding. Exchange transfusion is indicated in Reye's syndrome (Casper et al., 1976).

Monitoring

Oxygen should be provided to all patients with hypotension or shock although the use of oxygen mask (or tent) might aggravate the apprehension of the patient. Some of the essential vital parameters such as temperature, pulse, respiration, and blood pressure ought to be monitored half-hourly till the shock is overcome. Hematocrit levels are to be determined every 2 hours for the first 6 hours and then every four hours thereafter, until the patient becomes stable. The fluid-balance sheet should be maintained, and the frequency and volume of urine output should also be recorded. The clinician should watch for signs of fluid overload or cardiac failure. These signs include respiratory distress with or without crepitations, rapid pulse, puffy eyes, restlessness, and sudden increase in size of the liver (Thongcharoen and Jatanasen, 1993).

Challenges in Management of Shock

There are challenges in managing shock; these include:

- Failure to recognize internal bleeding.
- Overtransfusion with crystalloid or plasma fluids, instead of fresh whole blood transfusion, in cases of internal bleeding.
- Corticosteroids are not useful in treatment of DSS.
- In case of hematemesis, insertion of gastric tube (for cold lavage) is hazardous and is not advisable.
- Heparin should be used with extreme caution.
- A drop in hematocrit should not be interpreted as a sign of internal hemorrhage during the period of reabsorption of extravasated plasma (Thongcharoen and Jatanasen, 1993).

Prognostic Indicators in Dengue Hemorrhagic Fever and Dengue Shock Syndrome

- There is no need for intravenous fluid therapy generally for more than 48 hours after the onset of leakage and or shock. Intravenous fluid should be discontinued when the hematocrit reading drops to around 40 percent with stable vital signs.
- A good urine flow is the indicator of sufficient circulating renal volume.
- Return of appetite and diuresis are recovery signs.
- Strong pulse and normal blood pressure and diuresis are the vital signs with good prognosis during the phase of reabsorption.

Reabsorption of Extravasated Plasma

Resorption of extravasated plasma occurs 1 to 2 days thereafter. If more fluid is infused during the period of reabsorption, this may cause hypervolemia, cardiac failure, and pulmonary edema. A further drop in hematocrit is an evidence of reabsorption if it occurs after intravenous fluid therapy has been stopped and clearing of pleural effusion and ascites has occurred. Therefore, a drop in hematocrit should not be interpreted as a sign of internal hemorrhage (Thongcharoen and Jatanasen, 1993).

Criteria for Discharging Patients

- Hematocrit should be stable.
- Platelet count should be more than 50,000 per cubic mm.
- Absence of respiratory distress due to pleural effusion.
- After at least 3 days hospitalization following recovery from shock.
- Visible clinical improvement on physician's perspective.
- Absence of fever for up to 24 hours without antipyretics.

Investigation of Outbreaks

Fundamentals

The dengue epidemic requires the presence of the vector mosquito *Aedes*, dengue virus, and a large number of susceptible human hosts (US Department of Health and Human Services/Centers for Disease Control, 2009). The principles for disease control are quite similar to that for other epidemic diseases. Health-care providers should be alerted to report increased and clustering of all dengue cases. Dengue should be considered as the possible etiology where influenza, rubella, or measles are suspected in a dengue-receptive area at a time and place where vector mosquito populations are abundant and active. Outbreaks may be explosive or progressive, depending on the various factors such as vector density, susceptibility, strain of DENV, level of immunity in the human population, and degree of human-vector contact. Factors that increase the risk of outbreaks are increasing human population especially in urban areas, increasing mosquito breeding sites with traditional methods of water storage, changing lifestyles such as use of water coolers, and availability of rapid modes of transport and population migration (Pham et al., 2011).

Investigation to Define the Case

Verification of Information

As soon as the first information is received, the validity of the information should be verified. The initial sources of information are epidemiological surveillance (considered as the early warning system), reports from curative facilities, media reports, and reports from community sources.

Preliminary Steps for Case Definition

The case definition includes all the common and uncommon signs and symptoms of mild, moderate, and severe forms of the disease; criteria for deciding suspect case, presumptive case, and confirmed case; and laboratory tests for confirming the disease.

Notification

Any sudden increase or clustering of cases or deaths due to DF/DHF should be notified immediately to the local health authorities, who in turn will inform the state and national health authorities about the outbreak and the action taken. All cases of dengue fever DHF should be enlisted and reported monthly basis to the concerning health authorities.

Case Finding

Case finding can be carried out by institutions and community-based surveys. Health-care providers should be alerted to report increased or clustering of dengue cases. There should be the line listing or recording of age, sex, complete address, and clinical details of suspected cases and reported to the local health authorities. An active search should also be carried out to detect suspected cases.

Early Application of Control Measures

The district health authorities should assess and provide the case management facilities for patients with DHF/DSS. The patients should be treated in nearby health centers or hospitals equipped with all the essential laboratory facilities (for platelet count and estimation of hematocrit values). On confirmation of dengue outbreak, precautionary measures should be taken in other high-risk areas in the neighborhood. After the control of the outbreak, the details (including report of the taken preventive measures) should also be recorded.

Surveillance

It is a mandatory prerequisite for monitoring the problem of dengue epidemic in a particular locality. Regular surveillance is essential for early detection of an outbreak and for initiating timely interventions for dengue prevention and control. Three types of surveillance should be considered for dengue epidemic including epidemiological, entomological, and serological. The staff of peripheral health centers should be vigilant about any increase or clustering of cases of acute febrile illnesses showing similarity to the case definition of dengue or DHF. Such situations should be considered critical and require local epidemiological and entomological investigations.

Epidemiological Surveillance

Epidemiological surveillance includes fever surveillance using standard case definitions for DF and DHF, notification of DF/DHF cases, and confirmation of clinical diagnosis in 5% of diagnosed cases using mandatory laboratory parameters. Health care personnel ought to be alerted to report clustering of acute febrile illness compatible with case definition of dengue/DHF.

Entomological Surveillance

Entomological surveillance is also important because it provides information on locally prevalent mosquito species, estimate of adult and larval mosquito populations, location of breeding sites, problem sites where control activities should be concentrated, timing

and choice of mosquito control activities, and effectiveness of ongoing control strategies. Increase in density of one species might indicate missed breeding sites, which can be thoroughly investigated.

Entomological surveillance should be carried out on a monthly basis during the pre-monsoon period and weekly or fortnightly during monsoon and post-monsoon periods. During outbreaks, it is essential to carry out daily surveillance in the affected and surrounding localities, which should be continued till the outbreak abates. In areas with outbreaks of dengue fever and DHF where *A. aegypti* is absent, or is scarce, special efforts should essentially be made to identify the local vectors so as to develop a strategy for vector surveillance. To check for the vector mosquito, necessary field investigations must be carried out in the patient's residential area. Subsequently, the species of all the collected mosquitoes are identified and calculate the vector density and their susceptibility to insecticides. Based on the life cycle stage of mosquito the entomological survey is of three types:

1. Mosquito egg surveys: An oviposition jar (a black container with a suitable substrate either paper or wood) is used for female mosquitoes to lay eggs. The ovitrap is useful for collecting mosquito eggs breeding in containers. The number of eggs on the substrate can easily be counted, which provides an estimate of the number of container-breeding mosquitoes that may hatch following the next rain and the number of adult females present within the sampling area.
2. Larval surveys: Larval surveys provide information on mosquito larvae density and effectiveness of prior larval control operations. Estimates of larval densities are obtained by using a standard one-pint white plastic dipper that collects water from small containers to large breeding habitats. A minimum of three to five dips should be completed at each site, and the number of mosquito larvae obtained per dip is counted. Large habitats require at least three to five dips and should be obtained from several sites within the habitat to best represent the resident mosquito population. The number of larvae per dip, the number of dip, and the stage of larval instars provide information regarding the emergence of the adult mosquitoes and finally the type of control measures to be employed. In breeding habitats, such as tree holes, tires, and crevices, it is difficult to use the standard one-pint dipper. In such situations, soup ladles, turkey basters, large syringes, or manual siphon pumps may be used for the collection of larval samples.

   Any water-holding container in and around human residents can serve as a breeding site for *Aedes* mosquitoes. In a given locality, at least 50 to 100 houses should be systematically searched for water-holding or wet containers lying in the domestic or peridomestic situation and examined thoroughly for the presence of mosquito larvae and pupa. In cases in which 25 consecutive houses are found negative for mosquito larvae, a different locality, at least 100 meters away from the previous one should also be surveyed. If they are found positive, the larvae are immediately collected for confirmation of the mosquito species. Vector control measures should be immediately initiated even if a single water container is found positive for *Aedes* larvae.
3. Adult vector surveys: Surveillance of adult mosquitoes helps in determining mosquito habitat, mosquito density, and their susceptibility to various insecticides generally used to eliminate mosquitoes. This includes the collection, transport, and study of mosquito index.

(a) Collection: Adult mosquitoes can be collected by aspirator, baited traps, light traps, gravid traps, or on human baits. Aspirator is a device that sucks mosquitoes into a collection tube or jar. Light trap can be stationed at fixed locations, uses a 25-w incandescent bulb to attract mosquitoes, whereas gravid traps are light-weight portable traps that use a dark plastic tray containing an organic-water mixture as the attractant. The trap mainly collects gravid female mosquitoes.

(b) Transport: Mosquitoes should be transported immediately to an entomological laboratory alive in "Barraud cages," wrapped with moistened lint or cloth. Raisins soaked in water or a cotton pledget soaked in 10% solution is kept inside the Barraud cage. If immediate transportation is not possible, the mosquitoes are identified, pooled by species, and stored in liquid nitrogen, refrigeration, or on dry ice. If cold storage facilities are not available, pooled mosquitoes are stored in transport medium and transported on wet ice. Once identified, female *Aedes* mosquitoes can be used for virus isolation.

(c) Adult vector indices:

- House density index: It represents the number of female mosquitoes per house or the number of female mosquitoes per house per unit of time.
- Biting index (or landing index): Biting (or landing) index is the total number of female mosquitoes taken at bait per unit of time. The collection should be carried out between 9 and 11 A.M.. Male mosquitoes should not be counted during the calculation of landing or biting rate.
- Net index: Number of mosquitoes caught per man-hour of collection using mosquito nets. For calculating net index, the time should be standardized, and this should be followed every time. Table 8.3 represents the correlation of mosquito index and risk of transmission.

Serological Surveillance

For serological surveillance, the clinical samples are to be collected from cases with fever of unknown origin and timely initiate the control measures. Sero-surveillance is particularly useful in identifying high-risk individuals and geographical areas, any unusual increase in disease transmission implying outbreaks of epidemics, introduction of new serological variants and their geographical distribution, and new strategies for the prevention and control of dengue epidemic.

Table 8.3. Epidemiological interpretation of vector indices.

Index	High Risk of Transmission	Low Risk of Transmission
House index	>10 percent	<1 percent
Breteau index	>50	<5
Biting rate	>2 per man-hour	<0.2 per man-hour

National Institute of Communicable Diseases (NICD)/World Health Organization (WHO). 2006. Training modules on epidemic prone communicable diseases: Dengue and dengue hemorrhagic fever of Integrated Diseases Surveillance Project (2004) Training module/material.

Prevention and Control

At present, the only method of controlling or preventing DENV transmission is to combat the vector mosquito population. In Asia and the Americas, *A. aegypti* breeds primarily in man-made containers, such as earthenware jars, metal drums, and concrete cisterns used for domestic water storage and discarded plastic food containers, used automobile tires and other items that collect rainwater. In Africa, the mosquito also breeds extensively in natural habitats such as tree holes and leaves that gather to form "cups" and catch water.

Preventive Measures

Insect Repellents

The active ingredients in repellents (usually a compound known as DEET) repel but do not kill mosquitoes. Most repellents are effective at a short distance away from the site of application (estimated to be about 4 cm). The neck, wrists, and ankles are usually mentioned as target areas for application, but all exposed skin is at risk of biting. When applied on the skin surface, the repellent effect may last from 15 minutes to 10 hours, depending on a number of factors, including climate and humidity, the formulation of the product, the concentration of the formulation, and species of the biting insect. Applying repellents on clothing extends their duration of effectiveness.

Insecticide Spray Cans

Insecticide spray cans are effective for an immediate knockdown and show killing effect on the mosquitoes depending on the range of the spray. However, there is hardly any residual effect. Spraying a room with an insecticide spray can help to free it from mosquitoes soon after the spray, but it will not keep the room mosquito free. Effectiveness of spray cans can be enhanced by combining it with a mosquito coil or mosquito net. Spraying is particularly useful in reducing the mosquito population and killing mosquitoes that are harboring indoors because killing of mosquitoes carrying DENV is better than just repelling it.

Mosquito Coils

These are actually insecticide vaporizers, usually having a synthetic pyrethroid as the active ingredient. Under usual conditions, one coil is effective for a normal bedroom for the whole night, but air circulation in well-ventilated rooms has a diluting effect on the insecticide of the coil because the coils tend to burn faster, which shortens the period of effectiveness. The smoke from coils deters the mosquitoes from entering the room while those inside are expelled out of the room. Although the allethrin group of chemicals is metabolized rapidly in mammals and there were no reports of accumulation of these compounds in animal tissues, they are highly biodegradable and disintegrate in sunlight. Nevertheless prolonged use is best avoided. Another fact is that the lighted mosquito coil can be a fire hazard and thereby need precaution.

Protective Clothing

Protective clothing is found to be effective, but their practicality is limited in hot and humid climate areas. Clothing is a good substrate for the application of repellents for certain insecticides such as synthetic pyrethroids, which could extend the effectiveness of the repellent.

Air-Conditioning

Air-conditioning is fairly effective in keeping mosquitoes and other flying insects out of a room. This is only effective when the room is closed to the surrounding air. Mosquitoes that are already in the room remain unless they are killed off by insecticides.

Information, Education, and Communication

To prevent and control of dengue infections, information, education, and communication (IEC) should include:

- The information for prevention of dengue infections because specific vaccine or specific treatment is not yet available.
- Information and awareness regarding high-risk localities, modes of transmission, preferred breeding sites, biting time, resting habits and resting places of the adult mosquito, and control of adult mosquitoes by insecticide spray.
- Regarding control of mosquito breeding by drying out water containers at least once a week and fitting lids and screens at all openings in water containers to deny access of the female mosquito to water.
- Humans should be protected by using full sleeved clothing, net screens on windows, mosquito repellent creams, etc.
- Involvement of the community in prevention of dengue infection.

Community Participation

Community participation is essential for obtaining the cooperation of the community members in IEC activities against DENV, in keeping the locality clean, for prevention of mosquito breeding, and during spraying of insecticides inside homes. It requires constant efforts for motivating the public to continue to perform source reduction over a long period, even in the absence of an epidemic. The prime requirement of an antidengue program is the ability to seek obscure breeding sites in and around human settlements, which may be construed as a violation of privacy. Antidengue programs are difficult to implement particularly in societies in which the use-and-throw culture provides alternative breeding sites that may be too small, numerous, and difficult to identify.

Intersectoral Coordination

In case of an outbreak, it is necessary for the health sector to coordinate control activities with other government sectors, such as sanitation, urban development, and education. For this purpose, an interdepartmental committee should be constituted, having representatives from the panchayats (village councils), the community, and nongovernmental organizations (NGOs) as members of this Committee.

Legal Measures

Legislation and health education are interdependent and help to promote each other. Most countries and local government bodies have already enacted laws for the control

and eradication of disease vectors, but these laws have not yet been adequately enforced owing to lack of local political support.

Potential Vaccine against Dengue

The Mahidol University in Thailand, has successfully developed a live attenuated, safe, tetravalent vaccine, in collaboration with WHO, which has shown promising results against all four serotypes of DENVs. This vaccine, which is undergoing clinical trials, should prevent dengue infection in the target population in dengue endemic areas (Hombach, 2007; Hirschler and Regan, 2012).

References

Amarasinghe A, Kuritsky JN, Letson GW, Margolis HS. Dengue virus infection in Africa. *Emerg Infect Dis*. Accessed March 25, 2013, at http://wwwnc.cdc.gov/eid/article/17/8/10-1515_article.htm.

Ananthanarayan R, Paniker CKJ. 2000. *Textbook of Microbiology*, 6th ed. Chennia: Orient Longman Limited.

Anderson R. 2003. Manipulation of cell surface macromolecules by flaviviruses. *Adv Virus Res* 59:229–274.

Beier JC, Craig GB Jr. 1985. Gregarine parasites on mosquitoes. *Integr Mosq Cont Meth* 2:167–184.

Casper JT, Varma RR, Lewis JD, Harrington GJ, Aster RH. 1976. Exchange transfusion in Reye's syndrome with saline-washed red blood cells. *Transfusion* 16:130–134.

Chan EH, Sahai V, Conrad C, Brownstein JS. 2011. Using web search query data to monitor dengue epidemics: A new model for neglected tropical disease surveillance. *PLoS Negl Trop Dis* 5:e1206.

Chen LH, Wilson ME. 2010. Dengue and chikungunya infections in travelers. *Curr Opin Infect Dis* 23:438–444.

Chua JN, Ng MML, Chow VTK. 2004. The non-structural 3 (NS3) protein of dengue virus type 2 interacts with human nuclear receptor binding protein and is associated with alterations in membrane structure. *Virus Res* 102:151–163.

Chusak P, Andjaparidze AG. 1996. Dengue haemorrhagic fever prevention and control activities in South-East Asia Region. *WHO/SEARO Dengue Bulletin* 20:24–30.

Communicable Diseases Communiqué. 2012. Dengue fever. *National Institute for Communicable Diseases* 11:9.

Diamond MS, Edgil D, Roberts TG, Lu B, Harris E. 2000. Infection of human cells by dengue virus is modulated by different cell types and viral strains. *J Virol* 74: 7814–7823.

Directorate of National Vector Borne Diseases Control Programme. 2008. *Guidelines for Clinical Management of Dengue Fever, Dengue Haemorrhagic Fever and Dengue Shock Syndrome*. AccessedMarch25,2013,athttp://www.docstoc.com/docs/70010602/Guidelines-for-Clinical-Management-of-DFDHFDSS-NVBDCP--dengue-fever.

Edgil D, Diamond MS, Holden KL, Paranjape SM, Harris E. 2003. Translation efficiency determines differences in cellular infection among dengue virus type 2 strains. *Virology* 317: 275–290.

Egloff MP, Benarroch D, Selisko B, Romette JL, Canard B. 2002. An RNA cap (nucleoside-2'-O-)-methyltransferase in the flavivirus RNA polymerase NS5: Crystal structure and functional characterization. *EMBO J* 21:2757–2768.

Erfan E, Sadikin M, Prijanti AR, Soebandrio A. 2003. Detection of anti-thrombocyte autoantibody in sera of dengue hemorrhagic fever patients. *J Clin Virol* 28:S65–S65.

Gebhard LG, Kaufman SB, Gamarnik AV. 2012. Novel ATP-independent RNA annealing activity of the Dengue virus NS3 helicase. *PLoS One* 7:e36244.
Gill KS, Bora D, Bhardwaj M, Bandyopadhayay S, Kumar K, et al. 1997. Dengue/DHF outbreak in Ludhiana city (Punjab), India. *WHO/SEARO Dengue Bulletin* 21:47–51.
Gilotra SK, Rozeboom LE, Bhattacharya NC. 1967. Observations on possible competitive displacement between populations of *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse in Calcutta. *Bulletin of the World Health Organization* 37:437–446.
Goncalvez AP, Engle RE, St Claire M, Purcell RH, Lai CJ. 2007. Monoclonal antibody-mediated enhancement of dengue virus infection *in vitro* and *in vivo* and strategies for prevention. *Proc Natl Acad Sci USA* 104:9422–9427.
Gould EA, Solomon T. 2008. Pathogenic flaviviruses. *Lancet* 371:500–509.
Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, et al. 2010. Dengue: A continuing global threat. *Nat Rev Microbiol* 8:S7–S16.
Halstead SB. 1966. Mosquito-borne hemorrhagic fevers of South and Southeast Asia. *Bull World Health Organ* 35:3–15.
Halstead SB. 2008. *Dengue (Tropical Medicine: Science and Practice)*, pp. 1–10. River Edge, NJ: Imperial College Press.
Hahn CS, Hahn, YS, Rice CM, Lee E, Dalgarno et al. 1987. Conserved elements in the 3' untranslated region of flavivirus RNAs and potential cyclization sequences. *J Mol Biol* 198(1):33–41.
Hanley KA, Nelson JT, Schirtzinger EE, Whitehead SS, Hanson CT. 2008.Superior infectivity for mosquito vectors contributes to competitive displacement among strains of dengue virus. *BMC Ecology* 8 February, 3:1.
Hase T, Summers PL, Eckels KH. 1989. Flavivirus entry into cultured mosquito cells and human peripheral blood monocytes. *Arch Virol* 104:129–143.
Hirschler B, Regan J. 2012. World's first dengue vaccine beats three virus strains. Accessed March 25, 2013, at http://www.reuters.com/article/2012/07/25/us-sanofi-dengue-idUSBRE86O07E20120725.
Ho LJ, Hung LF, Weng CY, Wu WL, Chou P, et al. 2005. Dengue virus type 2 antagonizes IFN-α but not IFN-γ antiviral effect via down-regulating Tyk2-STAT signaling in the human dendritic cell. *J Immunol* 174:8163–8172.
Holden KL, Stein DA, Pierson TC, Ahmed AA, Clyde K, et al. 2006. Inhibition of dengue virus translation and RNA synthesis by a morpholino oligomer targeted to the top of the terminal 3' stem-loop structure. *Virology* 344:439–452.
Hombach J. 2007. Vaccines against dengue: A review of current candidate vaccines at advanced development stages. *Pan Am J Public Health* 21:254–260.
Johnson AJ, Roehrig JT. 1993. New mouse model for dengue virus vaccine testing. *J Virol* 73:783–786.
Kalra NL, Wattal BL, Raghavan NGS. 1985. Distribution pattern of *Aedes (Stegomyia) aegypti* in India: Some ecological considerations. *Bull Ind Soc Mal Comm Dis* 5:307–334.
Katyal R, Gill KS, Kumar K. 1997. Breeding of *Aedes aegypti* and its impact on dengue/DHF in rural areas. *Dengue Bulletin* 21:93–95.
Knoop KJ, Stack LB, Storrow A, Thurman RJ. 2010. Tropical medicine. In: *Atlas of Emergency Medicine*, 3rd ed., pp. 658–659 New York: McGraw-Hill Professional.
Lim HY, Ng ML. 1999. A different mode of entry by dengue-2 neutralisation escape mutant virus. *Arch Virol* 144:989–995.
Lin CF, Chiu SC, Hsiao YL, Wan SW, Lei HY, et al. 2005. Expression of cytokine, chemokine, and adhesion molecules during endothelial cell activation induced by antibodies against dengue virus nonstructural protein 1. *J Immunol* 174:395–403.
Lin CF, Lei HY, Shiau AL, Liu CC, Liu HS, et al. 2003. Antibodies from dengue patient sera cross-react with endothelial cells and induce damage. *J Med Virol* 69:82–90.
Lindenbach BD, Rice CM. 2003. Molecular biology of flaviviruses. *Adv Virus Res* 59:23–61.
Llewwellyn CH, Spielman A, Frothingham TE. 1970. Survival of arboviruses in *Aedes albonotatus*: A peridomestic Bahamian mosquito. *Proc Soc Exp Biol Med* 133:551–559.

Lozach PY, Burleigh L, Staropoli I, Navarro-Sanchez E, Harriague J, et al. 2005. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *J Biol Chem* 280:23698–23708.

Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. 2004. Dengue viral infections. *Postgrad Med J* 80, 588–601.

Martina BE, Koraka P, Osterhaus AD. 2009. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev* 22:564–581.

Mayxay M, Phetsouvanh R, Moore CE, Chansamouth V, Vongsouvath M, et al. 2011. Predictive diagnostic value of the tourniquet test for the diagnosis of dengue infection in adults. *Trop Med Int Health* 16:127–133.

Medin CL, Fitzgerald KA, Rothman AL. 2005. Dengue virus nonstructural protein NS5 induces interleukin-8 transcription and secretion. *J Virol* 79:11053–11061.

Munoz-Jordan JL, Laurent-Rolle M, Ashour J, Martinez-Sobrido L, Ashok M, et al. 2005. Inhibition of alpha/beta interferon signaling by the NS4B protein of flaviviruses. *J Virol* 79:8004–8013.

National Institute of Communicable Diseases (NICD)/World Health Organization (WHO). 2006. Training modules on epidemic prone communicable diseases: Dengue and dengue hemorrhagic fever of Integrated Diseases Surveillance Project (2004) Training module/material.

Navarro-Sanchez E, Altmeyer R, Amara A, Schwartz O, Fieschi F, et al. 2003. Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep* 4:723–728.

Nomaguchi M, Ackermann M, Yon C, You S, Padmanabhan R. 2003. De novo synthesis of negative-strand RNA by dengue virus RNA-dependent RNA polymerase in vitro: nucleotide, primer, and template parameters. *J Virol* 77:8831–8842.

Oishi K, Inoue S, Cinco M, Dimaano EM, Alera MTP, et al. 2003. Correlation between increased platelet-associated IgG and thrombocytopenia in secondary dengue virus infections. *J Med Virol* 71:259–264.

Pavri KM, Banerjee G, Anderson CR, Aikat BK. 1964. Virological and serological studies of cases of haemorrhagic fever in Calcutta. *Indian J Med Res* 52:692–697.

Pepin KM, Hanley KA. 2008. Density-dependent competitive suppression of sylvatic dengue virus by endemic dengue virus in cultured mosquito cells. *Vector Borne Zoonotic Diseases* 8(6):821–828.

Pham HV, Doan HTM, Phan TTT, Minh NNT. 2011. Ecological factors associated with dengue fever in a central highlands Province, Vietnam. *BMC Infectious Diseases* 11:172.

Pokidysheva E, Zhang Y, Battisti AJ, Bator-Kelly CM, Chipman PR, et al. 2006. Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. *Cell* 124:485–493.

Rai KS. *Aedes albopictus* in the Americas. *Ann Rev Entomol* 1991;36:459–484.

Ranjit S, Kissoon N. 2010. Dengue hemorrhagic fever and shock syndromes. *Pediatr Crit Care Med* 12:90–100.

Ribeiro JMC, Spielman A. 1986 The satyr effect: A model predicting parapaty and species extinction. *Am Nat* 128:513–528.

Rodenhuis-Zybert IA, Wilschut J, Smit JM. 2010. Dengue virus life cycle: Viral and host factors modulating infectivity. *Cell Mol Life Sci* 67:2773–2786.

Sabin AB. 1952. Research on dengue during World War II. *Am J Trop Med Hyg* 1:30–50.

Salonen A, Ahola T, Kaariainen L. 2005. Viral RNA replication in association with cellular membranes. *Curr Top Microbiol Immunol* 285:139–173.

Smithburn KC, Kerr JA, Gatne PB. 1954. Neutralizing antibodies against certain viruses in the sear of residents of India. *J Immunol* 72:248—257.

Spielman A, Feinsod FM. 1979. Differential distribution of peridomestic Aedes mosquitoes on Grand Bahama Island. *Trans Roy Soc Trop Med Hyg* 73:381–384.

Srikiatkhachorn A. 2009. Plasma leakage in dengue haemorrhagic fever. *Thromb Haemost* 102:1042–1049.

Statler J, Mammen M, Lyons A, Sun W. 2008. Sonographic findings of healthy volunteers infected with dengue virus. *J Clin Ultrasound* 36:413–417.

Stephenson JR. 2005. Understanding dengue pathogenesis: Implications for vaccine design. *Bull World Health Organ* 83:308–314.

Stramer SL, Hollinger FB, Katz LM, Kleinman S, Metzel PS, et al. 2009. Emerging infectious disease agents and their potential threat to transfusion safety. *Transfusion* 49:S1–S29.

Takada A, Feldmann H, Ksiazek TG, Kawaoka Y. 2003. Antibody-dependent enhancement of Ebola virus infection. *Journal of Virology* 77:7539–7544.

Talavera D, Castillo AM, Dominguez MC, Gutierrez AE, Meza I. 2004. IL8 release, tight junction and cytoskeleton dynamic reorganization conducive to permeability increase are induced by dengue virus infection of microvascular endothelial monolayers. *J Gen Virol* 85:1801–1813.

Tan BH, Fu J, Sugrue RJ, Yap EH, Chan YC, Tan YH. 1996. Recombinant dengue type 1 virus NS5 protein expressed in *Escherichia coli* exhibits RNA-dependent RNA polymerase activity. *Virology* 216:317–325.

Thongcharoen P, Jatanasen S. 1993. Epidemiology of dengue and dengue haemorrhagic fever. In: Thongcharoen P, ed., *Monograph on Dengue/Dengue Haemorrhagic Fever*, pp. 1–8. New Delhi: World Health Organization.

US Department of Health and Human Services/Centers for Disease Control and Prevention (CDC). 2009. Dengue and dengue hemorrhagic fever: Information for health care practitioners. Accessed March 25, 2013, at http://www.cdc.gov/dengue/resources/Dengue&DHF%20Information%20for%20Health%20Care%20Practitioners_2009.pdf.

Varatharaj A. 2010. Encephalitis in the clinical spectrum of dengue infection. *Neurol India* 58:585–591.

Whitehorn J, Farrar J. 2010. Dengue. *Br Med Bull* 95:161–73.

Wilder-Smith A, Chen LH, Massad E, Wilson ME. 2009. Threat of dengue to blood safety in dengue-endemic countries. *Emerg Infect Dis* 15:8–11.

Wills BA, Oragui EE, Dung NM, Loan HT, Chau NV, et al. 2004. Size and charge characteristics of the protein leak in dengue shock syndrome. *J Infect Dis* 190:810–818.

Wiwanitkit V. 2010. Unusual mode of transmission of dengue. *Journal of Infection in Developing Countries* 4:51–54.

Wolff K, Johnson RA. 2009. Viral infections of skin and mucosa. In: *Fitzpatrick's Color Atlas and Synopsis of Clinical Dermatology*, 6th ed., pp. 810–812. New York: McGraw-Hill Medical.

World Health Organization (WHO). 1999. *Guidelines for Treatment of Dengue Fever/Dengue Haemorrhagic Fever in Small Hospitals*. New Delhi: SEARO.

World Health Organization (WHO). 2009. *Dengue: Guideline for Diagnosis, Treatment, Prevention and Control*. Geneva: WHO.

World Health Organization (WHO). 2010. Dengue. Accessed February 16, 2013, at http://www.who.int/topics/dengue/en/.

World Health Organization (WHO). 2011. Impact of dengue. Accessed February 16, 2013, at http://www.who.int/csr/disease/dengue/impact/en/.

World Health Organization (WHO). 2012. *Global Strategy for Dengue Prevention and Control 2012–2020*. Accessed March 25, 2013, at http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf.

World Health Organization (WHO). 2013. Global alert and response. Accessed March 25, 2013, at http://www.who.int/csr/disease/dengue/impact/en/index.html.

Yadav RL, Narasimham MVVL. 1992. Dengue/dengue haemorrhagic fever and its control in India. *Dengue Newsletter* 17:3–8.

Yocupicio-Monroy RME, Medina F, Reyes-del Valle J, del Angel RM. 2003. Cellular proteins from human monocytes bind to dengue 4 virus minus-strand 3′ untranslated region RNA. *J Virol* 77:3067–3076.

Chapter 9

Japanese Encephalitis

History

Japanese encephalitis (JE) is a disease of public health importance because of its epidemic potential and high case fatality rate. It is mosquito-borne zoonotic disease primarily occurs in rural agricultural areas where mosquitoes proliferate in close association with pigs and other animal reservoirs. The epidemic of the disease has been reported in peri-urban and urban areas where similar conditions may exist. In temperate climates, epidemics occur mostly in post-monsoon months. Japanese encephalitis virus (JEV) is one of 66 belongs to the JE serocomplex, composed of several flaviviruses, including Alfuy, Koutango, Kokobera, Kunjin, Murray Valley encephalitis, JE, Stratford, West Nile, St. Louis encephalitis, and Usutu virus. Usutu virus is an African mosquito-borne flavivirus that emerged in 2002 and has since then appeared in many European countries, presenting even further surveillance and transmission challenges (Vazquez et al., 2011).

The disease was first recognized in Japan in 1871 and was formerly named Japanese B encephalitis to differentiate it from encephalitis A (also called *encephalitis lethargica* or *von Economo's disease*), which was prevalent that time. The causative viral agent was first isolated in Japan during an outbreak in 1935 (Ananthanarayan and Paniker, 2001) from the brain of a patient with fatal encephalitis and mosquito transmission was demonstrated in 1938. In 1924, an epidemic occurred in Japan during which, approximately 4,000 people died, and nearly 2,500 people died in South Korea in 1949. Since late 1960s, JE has virtually disappeared in Japan (20 cases annually) and is declining in China (<10,000 cases annually). However, it remains a major problem in northern Thailand with an attack rate of 10 to 20 per 100,000 people annually. In temperate areas, epidemics mostly occur in post-monsoon months. JE epidemic now occurs mainly in India, Nepal, China, Thailand, and Vietnam and sporadically in Indonesia, Singapore, and Malaysia. JE also may occur with a lower frequency in Japan, Taiwan, Singapore, Hong Kong, and

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

eastern Russia. In endemic areas, the disease primarily affects the children between the ages 2 and 15, whereas, in nonendemic areas, it affects all the age groups, children, and older adults being the predominant victims.

Magnitude of the Problem

Asia

JEV is endemic throughout the temperate and tropical regions of Asia and parts of the Western Pacific region. It is prevalent in China, Southeast Asia, and south Asia including India, Korea, Japan, Bangladesh, Sri Lanka, Burma, Thailand, Vietnam, Cambodia, Laos, Singapore, Malaysia, Philippines, Taiwan, and Indonesia (Figure 9.1). China has high enzootic transmission where human infection is particularly common in children younger than 5 years of age. Southeast Asia has an intermittent transmission, which affects children younger than 15 years of age. In south Asia, transmission is sporadic, and persons up to 50 years of age are susceptible (World Health Organization [WHO], 2010). In temperate areas (including China, Japan, South Korea, Nepal, northern Vietnam, and northern India), most JE cases occur over a period of several months when the weather is warmest, especially when the monsoons begin or are associated with heavy rainfall (Endy and Nisalak, 2002; Mackenzie et al., 2007; Arai et al., 2008). In tropical areas (including Cambodia, Indonesia, southern Vietnam, and southern Thailand), there is the year-round transmission of JE virus, and most of the cases may be observed during the rainy season (Ompusunggu et al., 2008).

The increase in incidence of JE in India and Southeast Asia is attributed to extensive paddy cultivation (where vector mosquitoes breed) and extensive rearing of pigs, which are the "amplifier" hosts for JE virus. The disease is rare in other parts of the world but may be associated with travelers returning from endemic countries.

India

Almost every year, some undiagnosed illnesses, invade India and unfailingly claim thousands of lives; viral encephalitis is among those that are a major global emerging public health problem. Although many encephalitis outbreaks have been reported in India since 1955, several have remained undiagnosed (Saxena et al., 2009). JE infection is widespread in India and is particularly high in southern states including Andhra Pradesh, Tamil Nadu, and various parts of Karnataka and Kerala (Samuel et al., 2009). There was an outbreak of JE in Vellore (Tamil Nadu) in 1955. In subsequent years, cases were reported mainly during October and November in Tamil Nadu and Andhra Pradesh. Mainly children (i.e., nonimmune population) were affected, indicating the epidemic nature of the disease. The disease was confined to southeastern India until 1973, when it caused an outbreak in West Bengal. The epidemic affected adults also indicating that the virus was freshly introduced into the area. Cases mainly occurred in June and October and the mortality rates approached 50 percent. Since 1976, there have been sporadic outbreaks in various parts of India, except the northwestern states (Ananthanarayan and Paniker, 2001).

Apart from southern part, the first major JE epidemic occurred in Gorakhpur (Uttar Pradesh) in 1978 and reported 1,002 cases and 297 deaths (Mathur et al., 1982). Afterward,

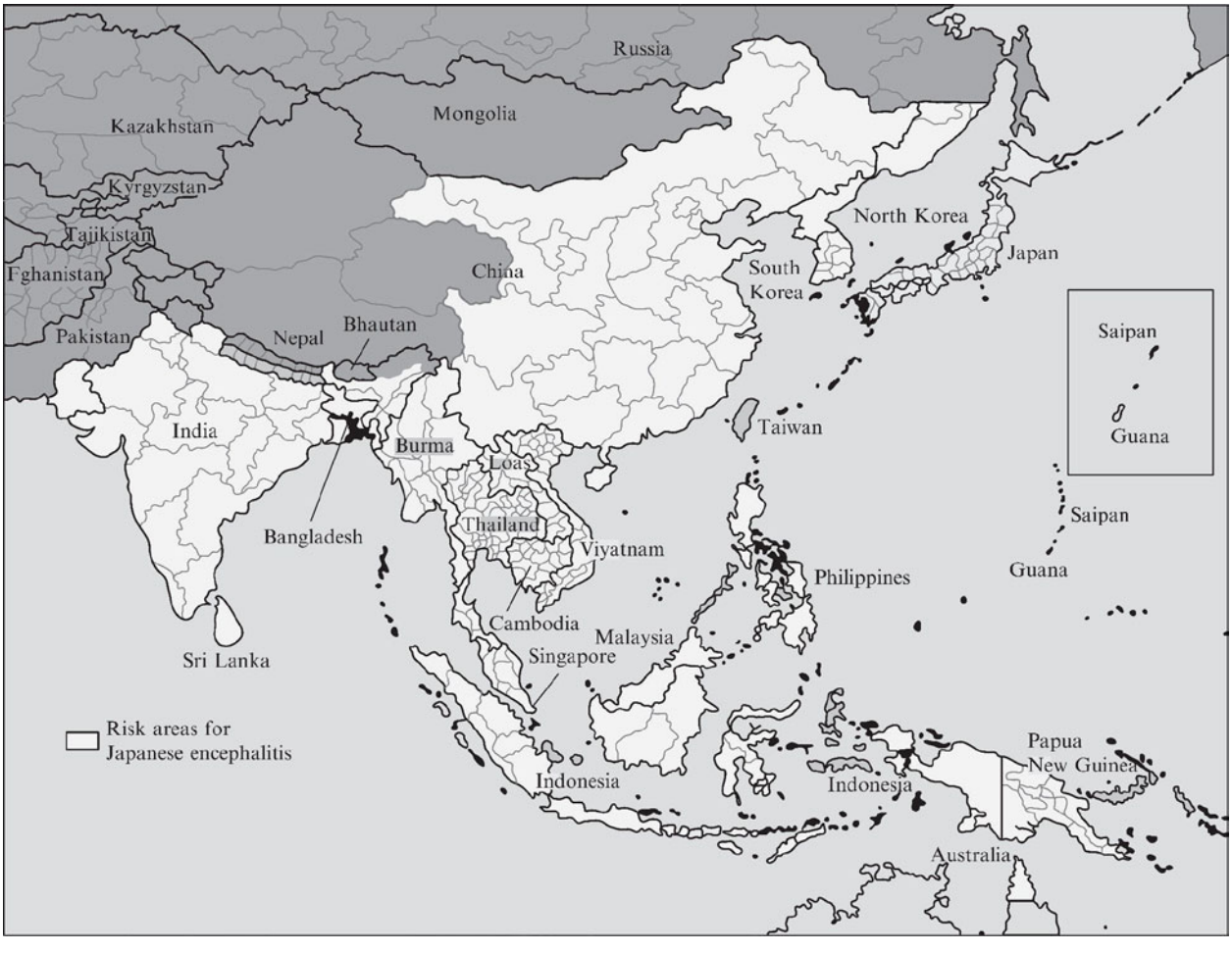

Figure 9.1. Worldwide magnitude of Japanese encephalitis disease.

many outbreaks were reported in Gorakhpur, but in intensity and magnitude, the 2005 epidemic surpassed all previous reported epidemic outbreaks in the country affecting 6,061 cases with 1,500 deaths followed by another outbreak in 2006 with 2,320 cases and 528 deaths. Similarly in 2007, JE cases in Uttar Pradesh reported 3,024 cases and 645 deaths (Samuel et al., 2009).

Epidemiology

Agent Factors

The causative agent of JE is a RNA flavivirus from the family *Togaviridae*, which is antigenically related to other arthropod-borne viruses causing West Nile fever, dengue (Ananthanarayan and Paniker, 2001), and St. Louis encephalitis virus. There are four typical variants of JEV depending on the location (Figure 9.2); among all, type II genotype appears to have the greatest spread.

The virus has a positive sense single stranded RNA molecule surrounded by a protein capsid. This positive sense genome is almost similar to the cellular messenger RNA molecule except it lacks the poly-adenylated (poly-A) tail, which allows the virus to use cellular apparatus for the synthesis of both structural and nonstructural proteins, during viral replication. The viral RNA also contains a long open reading frame (ORF) encoding the viral polyprotein with 5' and 3' untranslated regions (UTRs), which will subsequently self-assemble into complete JE virion particle. The outer envelope of JEV is formed by envelope (E) protein and is the protective antigen, which is responsible for the entry of the virus to the inside of the cell. The genome encodes for several nonstructural (NS) proteins as NS1, NS2a, NS2b, NS3, N4a, NS4b, and NS5 (Figure 9.3). JEV infects the lumen of the endoplasmic reticulum (ER) and rapidly accumulates substantial amounts of viral proteins (He, 2006).

Environmental Factors

Environmental factors include physical, chemical, biological, social, economic, cultural, and political factors. Several socioeconomic factors such as education and employment can affect an individual's ability and opportunity to make healthy choices. Physical,

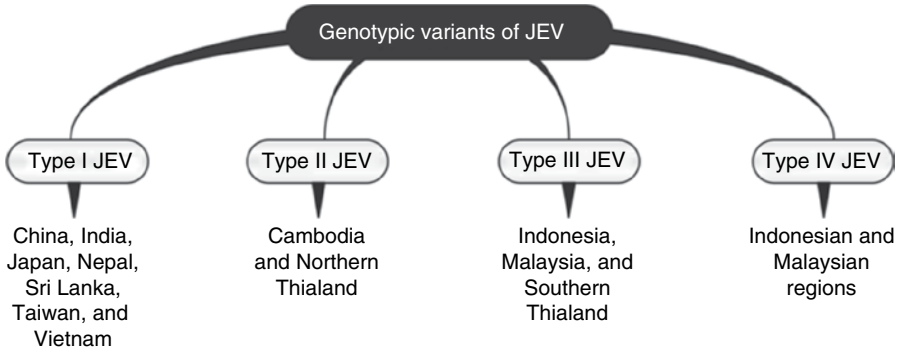

Figure 9.2. Genotypic variants of Japanese encephalitis virus.

chemical, and biological factors affect the safety of air in terms of quality and safety, availability of pure and safe water, soil and food by, for example, chemical pollution and waste disposal methods. Apart from these some larger scale environmental disruptions, such as human-induced climate changes and ozone depletion, can also present major health implications. In the temperate zones, a seasonal peak is observed in summer and autumn (Ananthanarayan and Paniker, 2001), but in the tropical zone including India, outbreaks occur during the monsoon and post-monsoon periods with a high-vector population density (National Institute of Communicable Diseases (NICD)/World Health Organization (WHO), 2006).

Host Factors

JE exhibits iceberg phenomenon because all the individuals bitten by infected female mosquitoes do not develop the disease. Human cases of JE may show scattered distribution. There is about 500 to 1,000 cover or subclinical infections for every clinical case (Ananthanarayan and Paniker, 2001). Human infection appears to be correlated with living in proximity with reservoir animals, particularly pigs (NICD/WHO, 2006). Risk indicators of JE mainly are children younger than age 15 years or the elderly (Rao et al., 2000), particularly in areas where JE has become endemic; although both sexes are affected, males outnumber females (Kaur et al., 2002); season (Kanojia et al., 2003;

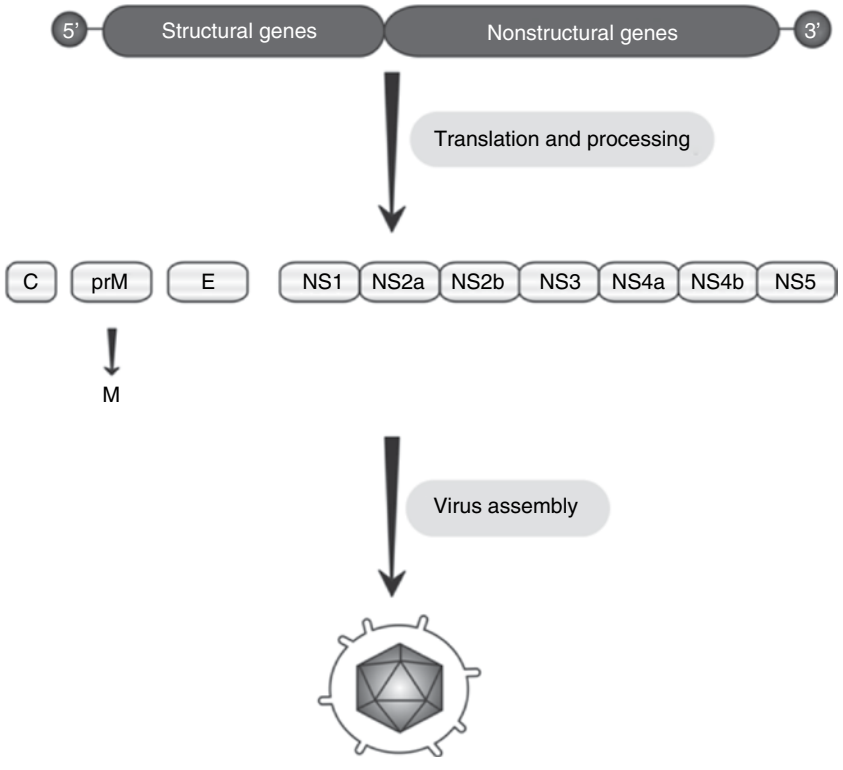

Figure 9.3. Genomic structure of Japanese encephalitis virus.

Murty et al., 2002); socioeconomic status (Bu'Lock, 1986; Potula et al., 2003); ethnicity (Kamala et al., 1989); outdoor occupation and relatively less clothing worn by males. These risk factors increase their risk of exposure to mosquito bites. Although the real *risk* factors are unknown because a database for real risk factors is not available. In fact, in most of the diseases, these proxies confound the risk factors. Hence factors associated with risk indicators could be suspected as risk factors of these diseases. Another important factor is to detect what protects the larger part of population from the disease and to detect what does not protect in the smaller part.

Reservoir

Viral infection is maintained in enzootic cycles between birds and pigs. Water birds (herons and egrets) are the main reservoir for disseminating the JE virus, whereas pigs are the amplifier hosts. Pigs usually do not show signs of infection other than abortion and stillbirth, but they show continuous viremia, which allows the transmission of the virus to a human via mosquitoes. Humans and other large vertebrates including horses are not considered as efficient amplifying hosts and are, therefore "dead-end" hosts for the JEV. Natural infection indicated by the presence of antibodies to JEV and bird-to-bird transmission by female mosquitoes (*Culex tritaeniorhynchus*) has been demonstrated in water birds, particularly those belonging to family *Ardeidae* (i.e., pond heron [*Ardeola grayii*] and cattle egret [*Bubulcus ibis*]) (NICD/WHO, 2006). Other birds such as ducks, pigeons, and sparrows may also be involved (Benenson, 1995). Other than pigs, vertebrate hosts include cattle and buffaloes. Though cattle and buffaloes are not natural hosts of JEV, they simply act as "mosquito attractants." Cattle and pigs do not manifest the signs and symptoms of the disease. The horse is the only domestic animal known to manifest signs of encephalitis following infection (Benenson, 1995).

Transmission

The transmission cycle of JEV involves mosquitoes, vertebrate animals, and water birds. Mosquitoes prefer to bite outdoors, and the risk of infection is greatest in the evening and night when mosquitoes are extremely active. Adult female mosquitoes need blood to develop their eggs, and therefore, become infected with the virus while feeding on the blood of an infected animal, which is followed by the multiplication of the virus inside the salivary glands of infected female mosquitoes. The virus is principally transmitted by mosquitoes belonging to the *C. tritaeniorhynchus* and *Culex vishnui* groups, which breed particularly in flooded rice fields. JEV was also reported in *Culex bitaeniorhynchus* in the Republic of Korea (Takhampunya et al., 2011). *Culex* mosquitoes are zoophilic in nature and prefer blood of animals and wild birds over human blood (NICD/WHO, 2006; Benenson, 1995). JE epidemics usually coincide with monsoons and the post-monsoon period with an extremely high-vector density. Female mosquitoes get infected while feeding on a viremic host and can transmit JEV to another host after an extrinsic incubation period of 9 to 12 days. The mosquitoes remain infected throughout their life (NICD/WHO, 2006). Transmission is maintained mainly by water birds and pigs because the virus can multiply to a high level in their blood, which increases the chances of mosquitoes being infected while feeding on them. The life cycle is either the bird–mosquito–bird or the pig–mosquito–pig cycle. Transmission may also occur by bird to pigs via mosquitoes and vice versa. In countries where JE is endemic, 100 percent

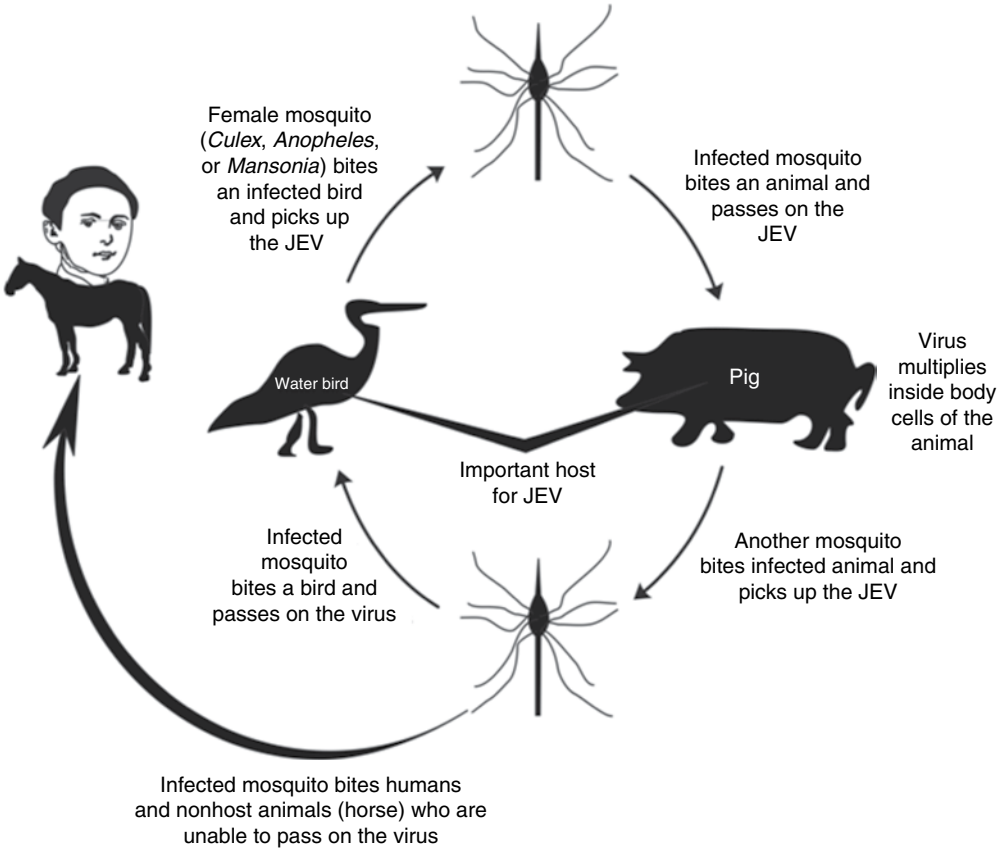

Figure 9.4. Transmission cycle of Japanese encephalitis virus. JEV, Japanese encephalitis virus.

infection of pigs has been reported. In India, the pig population is relatively less probably because of the low pork-eating population; therefore, cattle mainly act as amplifiers of infection (Benenson, 1995) (Figure 9.4). Because cattle do not develop viremia, they do not transmit the virus, and the high cattle-to-pig ratio in India probably limits human infection (Ananthanarayan and Paniker, 2001). Infection in human and horses is an accidental dead-end event (because they cannot pass the virus to mosquitoes), which occurs when the number of infected mosquitoes reaches a high density. The disease can be transmitted to humans by the bite of an infected female mosquito with an intrinsic incubation periods ranging from 5 to 15 days (NICD/WHO, 2006; Benenson, 1995). Man-to-man transmission has not yet been reported probably because viremia does not occur in humans after the onset of illness. In horses also the viremia is rarely seen for long periods. Hence horse, cattle, and man are the rare sources for infecting vector mosquitoes. In enzootic areas, spill over of zoonotic infection to humans is probably the result of the density of absolute number of infected vector mosquitoes, more opportunities for man-mosquito contact, life span of the vector, climatic factors that favor vector activity, extensive paddy cultivation, large-scale pig rearing, and possible role of amplifying hosts (Benenson, 1995).

Vector Biology

JEV has been isolated from the following mosquito species:

- *Culex*: species include *tritaeniorhyncus*, *bitaeniorhyncus*, *gelidus*, *vishnui*, *pseudovishnui*, *whimorei*, and *epidesmus*.
- *Anopheles*: species include *barbirostris*, *hyreanus*, and *subpictus*.
- *Mansonia*: species *annulifera* (Benenson, 1995).

Life Cycle of Mosquitoes

For JE, the time between mosquito's blood meal and laying of eggs (the gonotrophic cycle) is about 48 hours particularly in hot and humid areas. The time duration of the life cycle (from the egg stage to adult) is 7 to 10 days, with an optimum range of 7 to 14 days, under favorable conditions of temperature and food supply. Mosquito eggs are generally about one millimeter in size, but the shape varies depending on the specific genera of mosquito. Female mosquito lay about 100 to 300 eggs per cycle, and there are around 12 egg-laying cycles in the life span of a female mosquito. The egg stages persist 1 to 2 days and convert into larvae, which are elongated, free-swimming creatures with darting movements that feed on algae, bacteria, and vegetable or organic matter. Larval body is divided into head, thorax, and abdomen, and the stage lasts 5 to 7 days whereas pupae stage is a comma-shaped, with a large cephalothorax and narrow abdomen and lasts 1 to 2 days. The adult male mosquito is smaller and slender as compared to the female, feeds on plant juices, and lives in the vegetation near water bodies. Both male and female become sexually mature within 24 hours after hatching. The male mosquito dies after mating whereas the life span of the female mosquito is about 21 days under favorable environmental conditions (NICD/WHO, 2006).

Clinical Features

Most of the human JE cases are asymptomatic, or the virus may cause a nonspecific febrile illness. Symptomatic neuro-invasive disease occurs in less than 1 percent of JEV infections (Halstead and Jacobson, 2008). It has been reported that the ratio of asymptomatic JEV infection to symptomatic infection in the range from 25 to 1000:1 (Jani and Kunha, 2012). In neurological patients, the disease is usually severe with a high case fatality rate and causes the most serious clinical disease observed among all the arthropod-borne viruses. Encephalomyelitis is completely established soon after onset of symptoms. Hence an appropriate clinical detection requires a high degree of alertness, awareness, and high index of clinical suspicion on the part of the physician. The disease course has three phases:

1. Prodromal phase: This phase does not involve the nervous system and is characterized by abrupt onset of continuous high fever, headache, malaise, and vomiting which lasts 1 to 2 days.
2. Acute encephalitis phase: In most of the cases with JE, the infection is mild with no overt clinical symptoms or the disease may simply present as mild fever with headache. Some patients present signs of meningeal irritation with rigid neck. About 1 to 6

days after onset, convulsions, altered sensorium (lethargy, stupor, somnolence, irritability, apathy, or loss of consciousness), generalized paresis, hypertonia, and loss of coordination may also be observed with high fever (100.4– 105.26° F; 38–40.7° C). The patient develops difficulty of speech and other neurological deficiencies including ocular palsies, hemiplegia, tremors, and ataxia. Hypertonia, hyperreflexia (including the presence of pathologic reflexes), and weakness are common. Approximately less than 10 percent of the patients develop papilledema, and 33 percent have cranial nerve findings. Patients also develop extrapyramidal signs quite similar to Parkinson's disease including mask-like facies, tremor, rigidity, and choreoathetoid movements.

3. Recovery phase: This phase is characterized by complete or partial recovery of neurological defect. Body temperature returns to normal, but convalescence may be prolonged over many weeks, months, or years depending on the severity of the disease and the immune status of the infected host (Ananthanarayan and Paniker, 2001; Benenson, 1995).

Case Definitions

Case definitions for JE include:

Suspect Case

Acute febrile illness with headache, meningeal signs, stupor, disorientation, coma, tremors, generalized paresis, hypertonia, and loss of coordination.

Probable Case

A suspected clinical illness (clinical illness is characterized by a febrile illness of variable severity associated with neurological symptoms ranging from headache to aseptic meningitis or encephalitis) with any one of the following: elevated and stable JE-specific antibody titer in serum or presence of JE-specific immunoglobulin M (IgM) antibodies in serum.

Confirmed Case

A probable case of JE along with the following laboratory evidence: isolation of JE virus, genome, or antigen in tissues, blood or other body fluid sample from patient by immunochemistry, immunofluorescence, or polymerase chain reaction (PCR), or detection of JE virus-specific IgM antibodies in cerebrospinal fluid (CSF), or at least a fourfold rise in JE virus specific antibody titer in paired (acute and convalescent) sera (NICD/WHO, 2006).

Differential Diagnosis

It is quite difficult to differentiate JE from encephalitis occurs as a result of other causes. Similar to other viral and bacterial diseases, JEV causes febrile illness of variable severity ranging from headache to meningitis or encephalitis (NICD/WHO, 2006). Some clinical manifestations of Chandipura virus encephalitis in India mimics that of JE disease because

Table 9.1. Distinguishing features of Chandipura virus encephalitis and Japanese encephalitis.

Distinguishing Feature	Chandipura Virus Encephalitis	Japanese Encephalitis
Incubation period	1–2 days	Ranges from 5 to 15 days
Fever	Mild to moderate fever (100.94–103.1° F; 38.3–39.5° C)	High-grade fever (100.4–105.26° F; 38–40.7° C)
Gastrointestinal symptoms	Might be present	Absent
Neurological defect	In 20 percent of cases during acute stage and rare after recovery	Residual neurological deficit in up to 50 percent of survivors
Onset of encephalitis	Within hours of onset of symptoms	1–6 days after onset
Effect of anticonvulsants	Usually refractory to drugs	Usually controlled by drugs
Increased intracranial tension	Rapidly occurs and is common	Rare
Recovery	Rapid recovery within 1 week	Slow recovery over months or years
Death if untreated	Occurs within 2 days	Occurs in about 9 days
Vector and its control	Vector is female sand flies, which can easily be controlled because they do not fly by choice and hop within 50 yards (45 m) of their breeding places	Vector is female *Culex* mosquitoes, which are difficult to control because they can fly up to 6.83 miles (11 km) from their breeding places
Occurrence of outbreaks	1– 2 days after onset of monsoon	At least 6 to 7 weeks after onset of monsoon

in both the diseases, encephalitis is fully established soon after the onset of symptoms and rapid clinical detection of JE requires a high degree of alertness, awareness, and high index of clinical suspicion on the part of the physician diagnosing the disease. Table 9.1 shows some of the distinguishing features of Chandipura virus encephalitis and JE.

In tropical Asian regions the serologic cross-reactivity among other viruses, specifically dengue and West Nile virus, may hinder the accurate diagnostic evaluation of JE. Some noninfectious conditions such as central nervous system (CNS) lupus erythematosus, nonmetastatic CNS tumors, and cerebrovascular diseases also should be considered in the differential diagnosis of JE. Apart from this, JE should also be differentiated from other viral (Nipah virus infection, California encephalitis, enterovirus infection, herpes simplex infection, and dengue fever) and bacterial diseases (pyogenic focal brain abscess, tuberculous meningitis, mycoplasma meningitis, ehrlichiosis, typhoid fever, tuberculosis, and Rocky Mountain spotted fever, etc).

Laboratory Diagnosis

Specimens

Sample specimens for the diagnosis of JE include serum (both acute and convalescent phase), blood clot, and autopsied brain tissue (in case of postmortem diagnosis). JEV can also be isolated from disease vectors. During an epidemic outbreak, preference for sample collection should be given from suspected cases over contacts and animal reservoirs (NICD/WHO, 2006).

Blood and Serum

To isolate JE virus, at least 5 mL of venous blood should be collected aseptically from the suspected cases within 4 days of the onset of illness and 5 days after onset of illness for the detection of JE-specific IgM antibodies. This is further followed by the collection of a second convalescent sample at least 10 to 14 days after the first sample to examine seroconversion or fourfold rise in antibody titer. All collected blood samples are then kept at room temperature for at least 15 minutes to separate the serum, which is then transferred to a screw-capped sterile bottle. The bottle is properly sealed with an adhesive tape and labeled, mentioning the patient's name, identification number, and its date of collection. The collected serum sample should be refrigerated always in case the transportation to the referral laboratory is likely to be delayed.

Cerebrospinal Fluid

CSF samples should also be collected to confirm the diagnosis. CSF is collected aseptically in sterile screw-capped bottles, which are then sealed and labeled as with serum samples.

Autopsied Brain Tissue

This has been considered as the best sample for the isolation of JEV. For such purposes, the brain tissues should be collected within hours of the patient's death during the first 2 weeks of illness. Small tissue pieces are obtained from cerebral cortex, cerebellum, basal nuclei, and brain stem and then immersed in at least 2 mL (depending on the size of tissue pieces) of virus transport medium (VTM) in sterile screw-capped bottles. If VTM is not available, glycerol-saline or nutrient broth medium may be used. Autopsied brain tissues are then transported to the laboratory after prior intimation (NICD/WHO, 2006).

Virus Isolation from Vectors

During an epidemic outbreak, mosquitoes are collected from indoors and outdoors, which provides information on the mosquito species responsible for transmission of JE in the particular locality. Adult mosquitoes can be collected using aspirator, baited traps, biting collection, and light traps. If immediate transportation to the laboratory is feasible, these mosquitoes should be rapidly transported alive in "Barraud cages," wrapped with moistened cloth. Inside the Barraud cage, raisins soaked in water or a soaked cotton pledget should be kept. If immediate transportation is not possible, the mosquito species should be identified, pooled, and stored in liquid nitrogen, refrigeration, or on dry ice. In case, cold-storage facilities are not available, pooled mosquitoes should be stored in transport medium and transported on wet ice (NICD/WHO, 2006).

Labeling and Transport

Each sample should be properly labeled including, name of the patient, age, sex, address, areas visited by the patient in the last 1 month, date of onset of illness, clinical findings, and date of collection of the specimen. Samples should be transported immediately to the designated JE laboratory on wet ice in a large thermos flask, an ice box, or vaccine carrier. Samples for PCR diagnosis should be transported on dry ice (NICD/WHO, 2006).

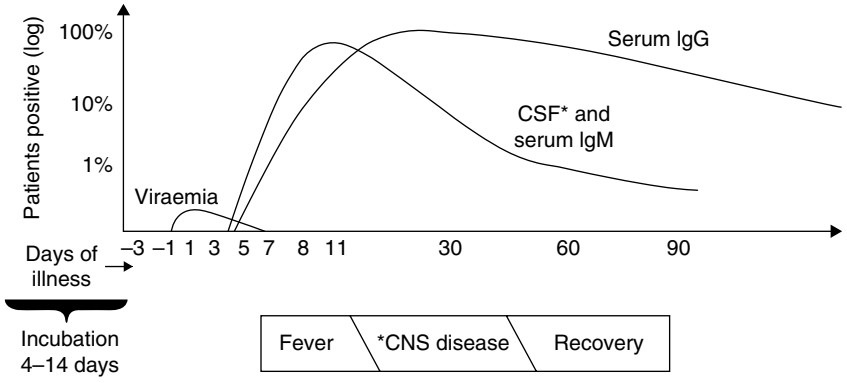

Figure 9.5. Schematic antibody responses in Japanese encephalitis infection. CNS, central nervous system; CSF, cerebrospinal fluid.

Serological Diagnosis

The accurate diagnosis of JE is based on evidences of a diagnostic rise or depletion JEV-specific antibody titers, taken during the acute and convalescent phases of the disease. Virus-specific antibodies are usually detectable 7 to 10 days after the onset of illness (Brunette et al., 2010). Viremia is usually just for a brief period in humans and neutralizing antibodies are present by the time distinctive clinical symptoms are recognized (Figure 9.5). Serological diagnosis mainly involves the detection of IgM or immunoglobulin G (IgG) antibodies to JEV in collected serum or CSF specimens. IgM antibodies to JEV start appearing after the first week of onset of symptoms and remain detectable for up to 3 months depending on the immune reaction. Fourfold rise in IgG antibody titer in paired sera (which is obtained at an interval of 10 days) is considered confirmatory diagnosis. Detection of IgG antibodies indicates previous infection and are, therefore, useful for sero-epidemiological studies, which determine silent infection and immunity levels in the local population. IgM capture enzyme-linked immunosorbent assay (ELISA) is used for detecting IgM antibodies against JEV and Hemagglutinin Inhibition (HI) test is used to demonstrate fourfold rise in IgG antibody titer.

Isolation of Virus

The JEV can be isolated using embryonated eggs, infant mice, or cell culture especially from CSF samples, occasionally from peripheral blood (within 3 to 4 days after the onset of symptoms) or autopsied brain tissue (NICD/WHO, 2006).

Detection of Viral Antigen or Viral RNA

Viral antigen can be detected by ELISA immunofluorescent assay and neutralization test in autopsied brain tissue whereas viral RNA can be detected by PCR (Benenson, 1995; Sapkal et al., 2007).

IgM Capture Enzyme-Linked Immunosorbent Assay

The CSF and sera collected from suspected encephalitis patients can be tested by IgM capture ELISA for JE. IgM antibodies against JE can be detected by standard ELISA

(Thakre et al., 2002). In this technique IgM antibodies from patient CSF and sera are captured on antihuman IgM-coated wells, and JE antigen is then added overnight at 39.2° F (4° C). The captured antigens are probed with biotin-labeled flaviviruses cross-reactive monoclonal antibody and avidin horse-radish peroxidase. Hydrogen peroxide and O-phenylene diamine (OPD) are used as substrate and chromogen, respectively. Reaction can be terminated using 4 N H_2SO_4 and read the absorbance at 492 nm. The test also uses known strong and weak positive samples as positive controls, and negative samples for JEV IgM are used as negative controls.

Immunoflourescene Test

Antibodies to JEV infections are usually detected by indirect immunofluorescence (IF) assay using virus-specific antibodies on acetone-fixed smears. Acetone treatment was reported to damage certain epitopes on JEV glycoprotein. Kabilan (2001) developed a modified fluorescent technique to adopt quick paraformaldehyde fixation followed by a short detergent treatment of cells in suspension for identification of JEV-infected brain cells of laboratory-reared *Toxorhynchites splendens* mosquito larvae using virus-specific antibodies. JEV-positive cells can be detected by the presence of a well-defined intracellular immunofluorescence staining against unstained uninfected antibody-treated cells. These stained cell suspensions can be stored for up to 4 weeks, allowing analysis at convenience.

Viral Neutralization Assay

An in vitro neutralization test can be performed by incubating twofold serially diluted JEV-specific monoclonal or polyclonal antibodies with 100 $TCID_{50}$ (tissue culture infective dose) of the test virus for 1 hour at 98.6° F (37° C) with 5 percent carbon dioxide (Sapkal et al., 2007). The test virus–antibody mixture can then be added on a preformed monolayer of cells in 96-welled microtiter plate and incubated at 98.6° F (37° C) with 5 percent carbon dioxide for 72 hours. Controls may include samples without antibody and with normal nonimmune serum. Neutralization titer of MAbs is then expressed as the reciprocal of the dilution at which 50 percent of virus added was neutralized (Gore et al., 1990).

RNA Isolation, Real Time-Polymerase Chain Reaction, and Sequencing

JE viral RNA can be isolated by a real time-polymerase chain reaction (RT-PCR) technique and PCR amplification can be carried out by denaturing the DNA followed by cycling and extension.

Other Investigations

In JE, the patient's peripheral blood shows neutrophilia and pleocytosis. Blood sugar level is normal or raised. In the acute encephalitic stage, CSF is clear showing normal glucose levels, but levels of proteins are mildly elevated (NICD/WHO, 2006).

Case Management

Clinical management of JE is symptomatic. To reduce morbidity and mortality, the first important thing is identifying early warning signs and accordingly refers the patient to health facility, and this also involves educating the health workers about the first line of

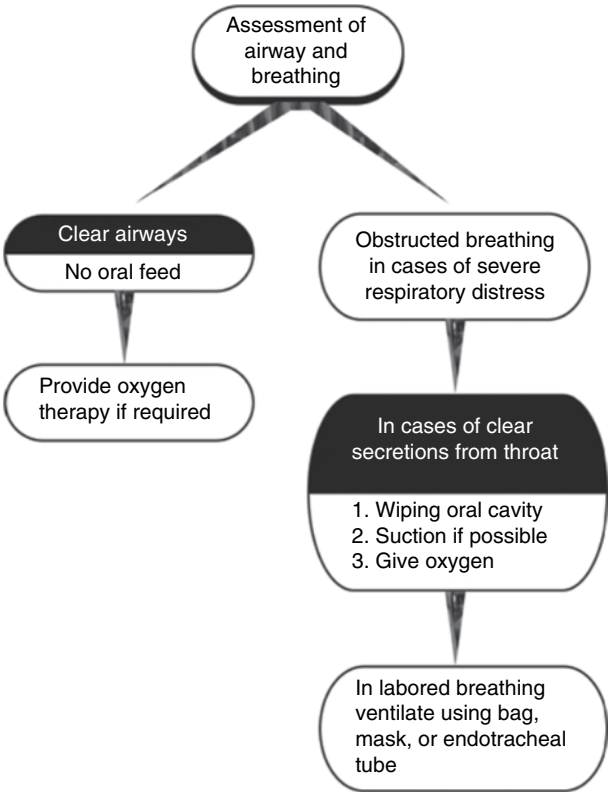

Figure 9.6. Management of airway and breathing.

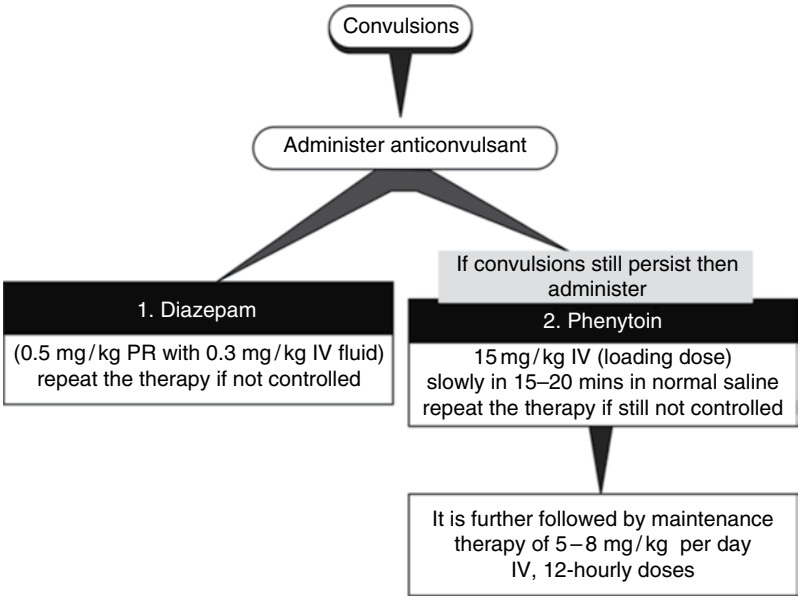

Figure 9.7. Management of convulsions. PR, per rectal.

management at the grass-roots level. At the health facility, other causes of CNS affliction, such as meningitis or cerebral malaria, should also be excluded, which requires specific treatment. The treatment further depends on the condition in which patient is admitted in the health facility. Management of airway and breathing is to be done as per Figure 9.6 and convulsions can be managed as per Figure 9.7.

Management at Primary-Care Level

Primary management comprises assessment of vital signs, provision of basic life support (maintain airway, resuscitate manually; *see* Figure9.6), immediate intravenous alimentation, and nursing care. Intravenous administration of dextrose saline, 5% dextrose, or Ringer's lactate should be done to maintain circulation (Figure 9.8). The fluid therapy is restricted to 70 percent of the total daily fluid requirement. All patients with signs of encephalitis should be immediately hospitalized, without waiting for the results of sero-

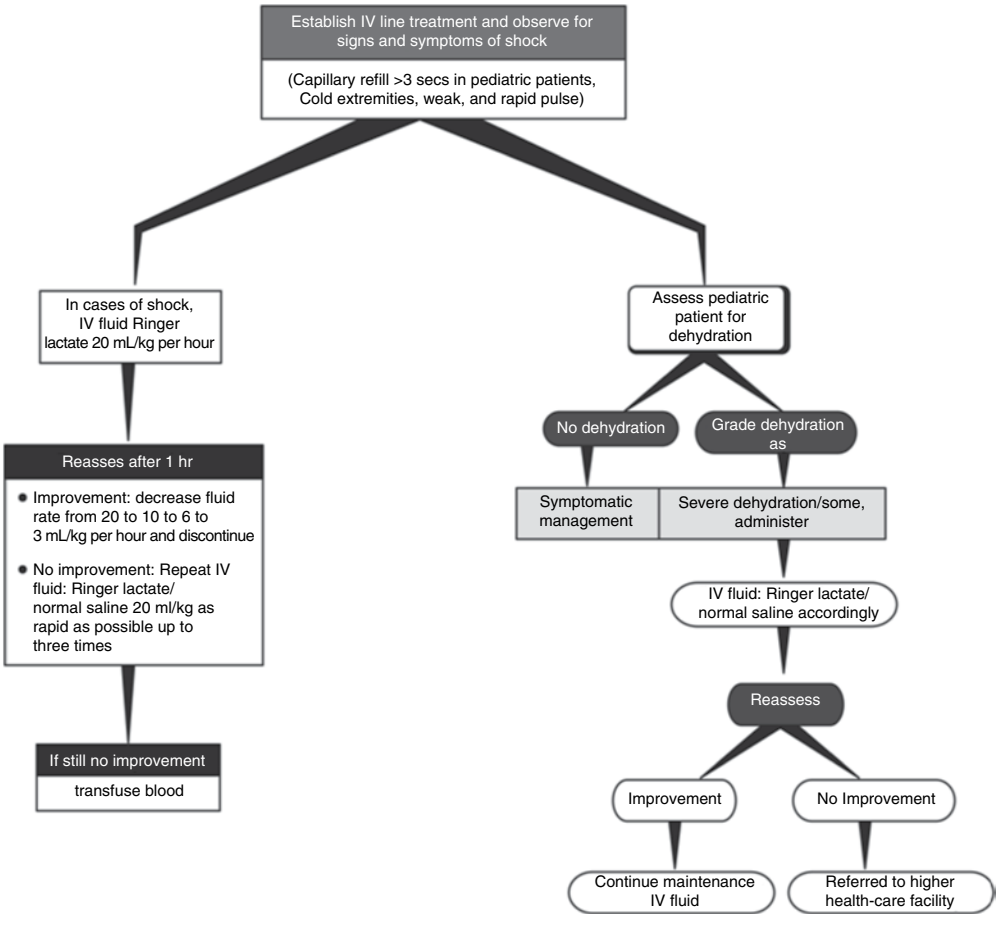

Figure 9.8. Management of circulation. IV, intravenous.

logical tests (NICD/WHO, 2006). If required, the patient ought to be transferred to a higher-grade health facility with a detailed referral note; basic life support should be maintained during transferring the patient.

Management at Secondary-Care Level

Secondary-level care comprises immediate hospitalization, history and clinical examination of the patient, and provision of basic life support (maintenance of airway, resuscitation, intravenous alimentation, and nursing care). Signs of shock strongly suggest immediate initiation of treatment depending on the stage of shock. To begin treatment, 20% mannitol is given intravenously (dose is 2–5 ml/kg of body weight), every 4 to 6 hours to reduce intracranial tension. However, in cases of pulmonary edema and fluid overload, mannitol administration is contraindicated. All required relevant laboratory investigations should be carried out, including hemoglobin estimation and total and differential leukocyte count. Acute convulsions should be controlled by administering injection diazepam (dose is 0.25–0.5 mg/kg of body weight) (NICD/WHO, 2006).

Management at a Tertiary-Care Level

If the patient has a more critical condition and requires ventilatory support, he or she should be referred to tertiary-care hospital, which is to be accompanied by continued basic life support and a detailed referral slip.

Follow-Up

After the acute episode, the provider should follow-up with the patient for detecting residual neurological deficits. The patient may be treated using physiotherapy, speech therapy, or special support, depending on the type of neurological deficit (NICD/WHO, 2006).

Prevention and Control

Currently there is no specific drug for treatment of JE. The promising strategy for the prevention of JE is the early identification of disease, isolation of cases from contact with vector mosquitoes, vaccination together with efficient mosquito control, protection of the amplifying hosts, and preventing humans from being bitten by mosquitos. Thus, the prevention and control of this disease has been a challenging issue.

Human Vaccination

A vaccine is available for high-risk groups, including children in an endemic area, military men, physicians, and nurses. Currently three vaccine types are available for preventing JE: inactivated vaccine derived from mouse brain cells; cell culture-derived inactivated vaccine; and cell culture-derived live attenuated vaccines. At the international level, the only currently used vaccine is formalin-inactivated vaccine (JE-Vax). It can be produced using mouse brain tissue. The development of a vaccine that would induce rapid protection in people and animals is still an important issue. Some new

generation safe chimeric viruses, which are unable to infect mosquitoes, are currently ongoing in the developmental and preclinical studies; these would be a major step in the production of live-attenuated vaccine that is safer for the environment and would minimize the risk for vaccine-born outbreaks.

Live Attenuated Vaccine

Live-attenuated JEV vaccine has some advantages because only a small dose is required, and it produces secretory immunoglobulin A (IgA) against the viral agent resulting is longer immune response in recipients. One live-attenuated JE vaccine is available and is used in China. It is derived from SA14-14-2 strain. This requires a lower dose in vaccination, low cost for production, and induces long-lasting immune response in immunized children. This strain, SA 14-14-2 of JEV, has a small plaque size and good growth and is currently used to produce live attenuated vaccine in China (Tsai, 2000). Its small plaque size phenotype is influenced by the burst size and short incubation period.

DNA Vaccine

Plasmid DNA vaccine against JEV can be constructed, which are capable of synthesizing JEV premembrane (prM) and envelope (E) proteins. Both forms of E protein generate JEV-neutralizing antibodies, which provide significant protection against the lethal JEV as observed in mice. The E protein of JEV is also an interesting target for vaccine development because it contains antigenic determinants for hemagglutination and neutralization. Mutations in the E gene are also responsible for significant alterations in the biological functions of the virus and may affect its virulence (Richman et al., 2002).

Containment in Pigs

Pigs, the amplifier hosts for JE, have been slaughtered as an important containment measure. Approximately one million pigs were slaughtered during an epidemic in Malaysia in 1999 (Ananthanarayan and Paniker, 2001). Piggeries should be at least 2.5 to 3 miles (4 to 5 km) away from human settlements; this can also reduce the risk of human-vector contact. Additionally, vaccine against JEV for pig vaccination is also available and is useful in reducing the number of susceptible amplifying hosts. However, a large number of newborn pigs need to be immunized each year, and the vaccine only confers a limited duration of protection (NICD/WHO, 2006).

Vector Control

An alternative approach for controlling JE is the reduction in mosquito vector population. JEV can cause a long-lasting viremic phase in its amplifying hosts. In JE, the viremic phase in pigs last for 28 days, whereas in infected birds it last for 4 to 5 days with a high virus titer (approximately 105 pfu/mL as reported in a house sparrow; Yuill, 1969). This long-lasting viremic phase in amplifying hosts provides an opportunity for the susceptible mosquito vectors to become infected while feeding on them. Therefore, the transmission of the virus can be blocked by reducing the number of mosquito vector populations, which will lead to the reduction of new cases of infected patients, especially in the endemic areas. Although many chemical insecticides have been used in controlling mosquito population, WHO has recommended the use of *Bacillus thuringiensis var. israelensis* (Bti)

as an agent for biological control of mosquitoes since 1978 (Arata et al., 1978). Bti produces proteinaceous parasporal crystals during sporulation, composed of multiple proteins that are lethal to larval mosquitoes (Goldberg and Margalit, 1977) and to blackflies. Another bacterial agent that can be used as microbial insecticides is a spore-forming bacillus, *Bacillus sphaericus*. Some strains of this bacterium produce a toxin that is lethal when ingested by filter-feeding mosquito larvae. Several attempts have been made to produce formulations of microbial insecticides, *Bacillus sphaericus* and Bti. Microbial insecticides have been suggested as substitutes for chemical insecticides because they have a narrow and specific spectrum of activities that enable them to kill only certain insect species, but their use is limited because most microbes show a narrow spectrum of activity enabling them to kill only certain insect species. Moreover, they have low persistence in the environment and require precise application practices because many of these pathogens are specific to young insect larval stages or are sensitive to irradiation.

Surveillance

Clinical surveillance helps in early diagnosis of JE and prompts treatment of JEV-infected cases. Epidemiological surveillance in JE-prone areas proved to be useful for monitoring vector behavior and vector density. Serological surveillance should be done periodically to determine the antibody titers in humans, reservoir animals, and birds. Periodic training of health personnel should also be a part of JE control activities. Veterinary surveillance efforts can also be expanded for the epidemiologic monitoring of potentially infected goats as sentinel animals (Yang et al., 2007). According to WHO surveillance standards for field-based testing for JEV-acute CSF samples have been shown to be more useful than acute serum samples.

References

Ananthanarayan R, Paniker CKJ. 2001. *Textbook of Microbiology*, Chennai: Orient Longman.

Arai S, Matsunage Y, Takasaki T, Tanaka-Taya K, Taniguchi K, et al. 2008. Japanese encephalitis: surveillance and elimination effort in Japan from 1982 to 2004. *Jpn J Infect Dis* 61:333–338.

Arata AA, Chapman HC, Cupello JM, Davidson EW, Laird M, et al. 1978. Status of biocontrol in medical entomology. *Nature (London)* 276:669.

Benenson, AS, ed. 1995. *Control of Communicable Diseases Manual*. American Public Health Assoc, Washington, DC: American Public Health Association.

Brunette GW, Kozarsky PE, Magill AJ, Shlim DR. 2009. CDC health information for international travel 2010. Atlanta: Centers for Disease Control and Prevention.

Bu'Lock FA. 1986. Japanese B virus encephalitis in India-a growing problem. *Q J Med* 60:825–836.

Endy TP, Nisalak A. 2002. Japanese encephalitis virus: ecology and epidemiology. *Curr Top Microbiol Immunol* 26:11–48.

Goldberg LJ, Margalit J. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles* serget II, *Uranotaenia unquiculata*, *Culex univitattus, Aedes aegypti*, and *Culex pipiens*. *Mosq News* 37:355–358.

Gore MM, Gupta AK, Ayachit VM, Athawale SS, Ghosh SN, et al. 1990. Selection of a neutralization escape variant strain of Japanese encephalitis virus using monoclonal antibody. *Ind J Med Res* 91A:231–233.

Halstead S, Jacobson J. 2008. Japanese encephalitis vaccines. In: Plotkin S, Orenstein W, Offit P, eds., *Vaccines*, 5th ed., pp. 311–352. Philadelphia: Saunders Elsevier.

He B. 2006. Viruses, endoplasmic reticulum stress, and interferon responses. *Cell Death Differ* 13:393–403.

Jani AA, Kunha BA. 2012. Japanese encephalitis treatment and management. Drugs, disease and procedures. Accessed February 17, 2013, at http://emedicine.medscape.com/article/233802-treatment.

Kabilan L. 2001. A modified immunofluorescence assay for detection of Japanese encephalitis virus-infected cells. *Acta Virol* 45:253–255.

Kamala CS, Rao MV, George S, Prasanna NY. 1989. Japanese encephalitis in children in Bellary Karnataka. *Indian Pediatr* 26:445–452.

Kanojia PC, Shetty PS, Geevarghese GA. 2003. Long-term study on vector abundance and seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, Uttar Pradesh. *Indian J Med Res* 117:104–110.

Kaur R, Agarwal CS, Das D. 2002. An investigation into the JE epidemic of 2000 in Upper Assam—A perspective study. *J Commun Dis* 34:135–145.

Mackenzie JS, Williams DT, Smith DW. 2007. Japanese encephalitis virus: The geographic distribution, incidence, and spread of a virus with a propensity to emerge in new areas. In: Tabor E, ed., *Perspectives in Medical Virology: Emerging Viruses in Human Populations*, pp. 201–268. Amsterdam: Elsevier.

Mathur A, Chaturvedi UC, Tandon HO, Agarwal AK, Mathur GP, et al. 1982. Japanese encephalitis epidemic in Uttar Pradesh, India during 1978. *Indian J Med Res* 75,:161–169.

Murty US, SatyaKumar DV, Sriram K, Rao KM, Singh TG, et al. 2002. Seasonal prevalence of *Culex vishnui* subgroup, the major vectors of Japanese encephalitis virus in an endemic district of Andhra Pradesh, India. *J Am Mosq Control Assoc* 18:290–293.

National Institute of Communicable Diseases (NICD)/World Health Organization (WHO). 2006. Guidelines for surveillance of acute encephalitis syndrome (with special reference to Japanese encephalitis). Accessed March 25, 2013, at http://nvbdcp.gov.in/Doc/AES%20guidelines.pdf.

Ompusunggu S, Hills SL, Maha MS, Moniaga VA, Susilarini NK, et al. 2008. Confirmation of Japanese encephalitis as an endemic human disease through sentinel surveillance in Indonesia. *Am J Trop Med Hyg* 79:963–970.

Potula R, Badrinath S, Srinivasan S. 2003. Japanese encephalitis in and around Pondicherry, South India: a clinical appraisal and prognostic indicators for the outcome. *J Trop Pediatr* 49:48–53.

Rao JS, Misra SP, Patanayak SK, Rao TV, Das Gupta RK, et al. 2000. Japanese Encephalitis epidemic in Anantapur district, Andhra Pradesh (October–November, 1999). *J Commun Dis* 32:306–312.

Richman DD, Whitley RJ, Hayden FG. 2002. Flaviviruses. In: Monath TP, Tsi TF, eds., *Clinical Virology*, 2nd ed., 857–873. Washington, DC: ASM Press.

Samuel PP, Ayanar K, Kannan M, Thenmozhi V, Paramasivan R, et al. 2009. Sero-entomological investigations on Japanese encephalitis outbreak in Gorakhpur division, Uttar Pradesh, India. *Indian J Med Res* 129:329–332.

Sapkal GN, Wairagkar NS, Ayachit VM, Bondre VP, Gore MM. 2007. Detection and isolation of Japanese Encephalitis virus from blood clots collected during the acute phase of infection. *Am J Trop Med Hyg* 77:1139–1145.

Saxena SK, Mishra N, Saxena R, Singh M, Mathur A. 2009. Trend of Japanese encephalitis in North India: evidence from thirty-eight acute encephalitis cases and appraisal of niceties. *J Infect Dev Ctries* 3:517–530.

Takhampunya R, Kim HC, Tippayachai B, Kengluecha A, Klein TA, et al. 2011. Emergence of Japanese encephalitis virus genotype V in the Republic of Korea. *Virology Journal* 8:449. doi:10.1186/1743-422X-8-449.

Thakare JP, Rao TLG, Padbidri VS. 2002. Prevalence of West Nile virus infection in India. *Southeast Asian J Trop Med Public Health* 33:801–805.

Tsai TF. 2000. New initiative for the clonal of Japanese encephalitis by vaccination: Minutes of WHO/CVI meeting, Bangkok, Thailand. 13–15 October 1998. *Vaccine* 18:1–25.

Vazquez A, Jimenez-Clavero M, Franco L, Donoso-Mantke O, Sambri V, et al. 2011. Usutu virus: Potential risk of human disease in Europe. *Eurosurveillance* 16(31). Accessed February 17, 2013, at http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19935.

World Health Organization (WHO). 2010. South-East Asia conference on epidemiology, New Delhi, 8–10 March.

Yang DK, Kweon CH, Kim BH, Hwang IJ, Kang MI, et al. 2007. The seroprevalence of Japanese encephalitis virus in goats raised in Korea. *J Vet Sci* 8:197–199.

Yuill TM. 1969. Mosquitoes for drawing blood from small reptiles. *Trans Roy Soc Trop Med Hyg* 63:407–408.

Chapter 10
Chikungunya Fever

Introduction

Chikungunya was first described by Marion Robinson and W. H. R. Lumsden in 1955 (Robinson, 1955), following an outbreak in 1952 on the Makonde Plateau, along the border between Mozambique and Tanganyika (Tanzania). The name is derived from the Makonde language word spoken by an ethnic group in southeast Tanzania and northern Mozambique, which means "that which bends up" (in reference to the stooped posture developed as a result of the arthritic symptoms). According to the initial 1955 report regarding the epidemiology of the disease, the term *chikungunya* is derived from the Makonde root verb *kungunyala*, meaning to dry up or become contorted. Subsequent authors apparently overlooked the references to the Makonde language and assumed that the term derived from Swahili, the lingua franca of the region. The erroneous attribution of the term as a Swahili word has been repeated in numerous print sources. Other common spellings and forms of the term include "Chicken guinea," "Chicken gunaya," and "Chickengunya." During the 2005–2007 explosive epidemics on the Indian Ocean islands and in India, anecdotal cases of deaths, encephalitis, and neonatal infections associated with Chikungunya were reported. Until recently, Chikungunya fever attracted only minor interest in the medical community and did not evoke the fear associated with other arboviruses, such as dengue and West Nile virus. The recent resurgence of Chikungunya fever has drawn global attention because of its explosive onset, rapid spread, high morbidity, and myriad clinical manifestations (Pialoux et al., 2007; Powers and Logue, 2007; Chevillon et al., 2008; Simon et al. 2008; Sudeep and Parashar, 2008). From 2006 onward, Chikungunya fever has emerged as an important disease in returning travelers, even in nonendemic areas. Indeed, travelers have emerged as sentinels, transporters, and transmitters of the disease (Pialoux et al., 2007; Simon et al. 2008). The social and economic impact of Chikungunya fever has also been tremendous, especially in India (Rishnamoorthy et al., 2009).

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Epidemiology

Global

The Chikungunya virus is probably maintained in nature by transmission between jungle primates (Markoff, 2005). The disease displays a striking epidemiological profile with major epidemics appearing and disappearing cyclically, usually with inter epidemic period of 7 to 8 years and sometimes as long as 20 years (World Health Organization [WHO], 2008). Currently, Chikungunya is a major arboviral disease in urban parts of Africa and Asia. The known geographic distribution of the virus includes large parts of sub-Saharan Africa, Southeast Asia including Indonesia, Philippines, and India, as well as islands in the southwest Indian Ocean (Peters Sherif and Zakioverview, 2005; Figure 10.1). Other affected regions include Mauritius and Seychelles in the Indian Ocean. Imported cases have been reported by European countries such as France, Germany, Italy, Norway, and Switzerland (Carey, 1971; Powers et al., 2001; Krastinova et al., 2006).

India

The virus was first isolated in India from Kolkata in 1963 (Shah et al., 1964). In the mid sixties outbreaks resembling Chikungunya was reported from various parts of India including Vellore, Kolkata, and different parts of Maharashtra (WHO, 2008). The last outbreak of Chikungunya virus infection was reported in 1971. There has been no active or passive surveillance of Chikungunya and, therefore, it appeared that the virus had disappeared from the country (Pavri, 1986). Since 2005, however, there have been several reports of outbreaks from widespread parts of the country and the re-emergence of the virus has been confirmed (Ravi, 2006; Chhabra et al., 2008). In an outbreak, 151 districts of eight states of India reported Chikungunya fever as of October 2006; this outbreak affected Andhra Pradesh, Andaman and Nicobar Islands, Tamil Nadu, Karnataka, Maharashtra, Gujarat, Madhya Pradesh, Kerala, and Delhi. More than 1.25 million cases have been reported from the country with 752,245 cases from Karnataka and 258 998 from Maharashtra. In some areas, attack rates have reached up to 45 percent (WHO, 2008). Table 10.1 shows the chronological order of documented outbreaks of Chikungunya virus.

The Chikungunya Virus

The etiological agent of Chikungunya fever is an alphavirus of the family *Togaviridae*. The viral genome consists of a linear, positive-sense, single-stranded RNA molecule of approximately 11.8-kb long. The nucleic acid core is surrounded by a capsid of 60- to 70-nm diameter and a phospholipid envelope (Pialoux et al., 2007; Powers and Logue, 2007; Simon et al., 2008). In thin sections, the virus shows a roughly spherical shape with a diameter of 42 nm composed of a 25- to 30-nm core. Chikungunya virus isolates have three lineages each with distinct genotypic and antigenic characteristics. These include the West-African; the East, Central, Southern African (ECSA) phylogroups that have contributed to epidemics in Africa; and the Asian phylogroup. Phylogenic analysis based on partial sequences of NS4 and E1 genes showed that isolates from India during the present epidemic and the isolates from the ongoing Indian Ocean outbreak represent a

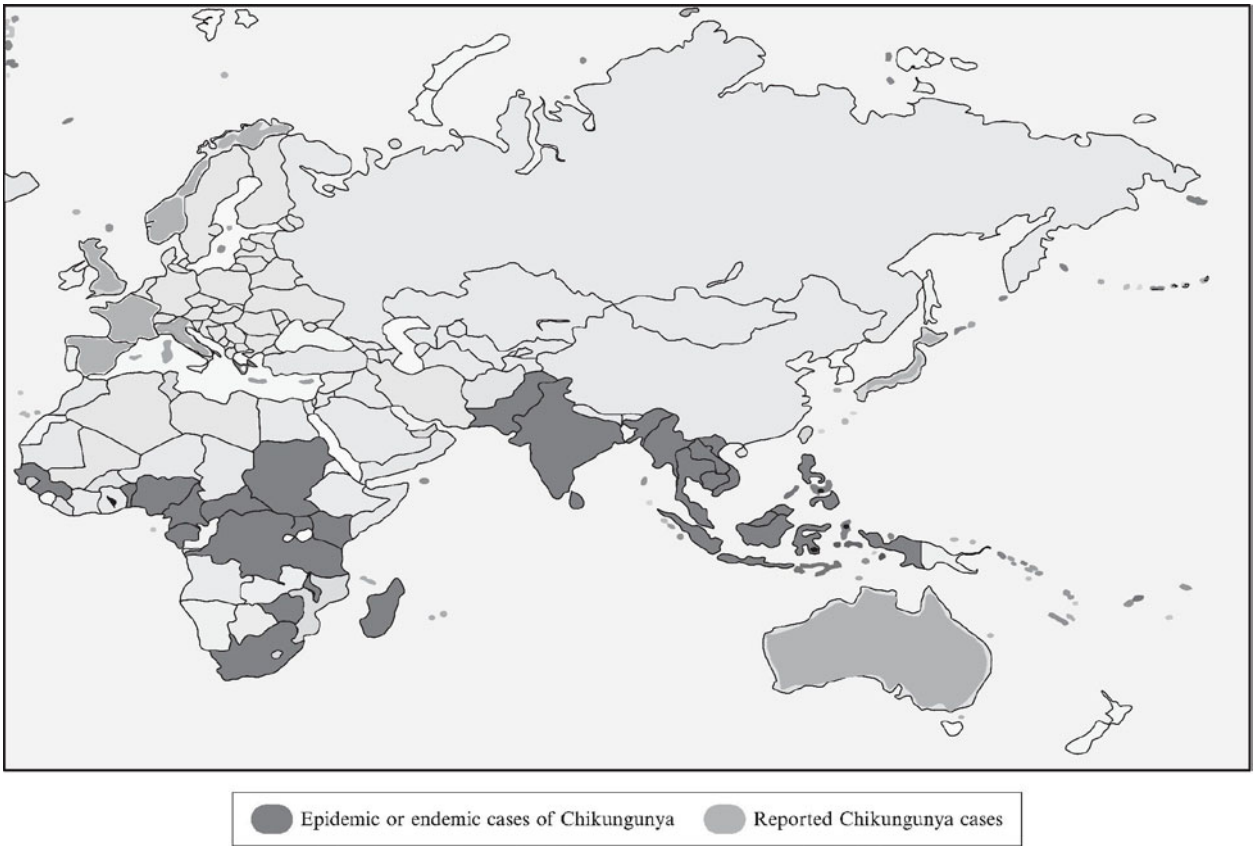

Figure 10.1. Worldwide distribution of chikungunya virus in 2010. Both dark gray and light gray indicate countries where cases of chikungunya fever have been documented, and dark gray indicates countries where chikungunya virus has been endemic or epidemic.
Source: Modified from Schwartz O, Albert ML. 2010. Biology and pathogenesis of chikungunya virus. *Nat Rev Microbiol* 8:491–500.

Table 10.1. Chronological order of documented outbreaks of Chikungunya virus.

Year of Outbreak(s)	Country	Genotype
1952	Tanzania	Central/East African
1958, 1971, 1985	Uganda	Central/East African
1960, 1999–2000	DRC	Central/East African
1960, 1962–1964, 1988, 1991–1993, 1995	Thailand	Asian
1961, 1963	Zimbabwe	Central/East African
1963	Cambodia	Asian
1963–1965, 1973, 2006	India	Asian & Central/East African
1965, 1967	Vietnam	Asian
1965–1969, 1998–1999, 2006	Malaysia	Asian
1966, 1982, 1996–1997	Senegal	West African
1967, 2006	*Taiwan**	Asian and Central/East African
1969, 1974–1975, 1980	Nigeria	West African
1970	South Africa	Central/East African
1970	Kenya	Central/East African
1973	Burma	Asian
1973, 1980, 1983–1984, 1998–1999, 2000–2005	Indonesia	Asian
1980–1982	Burundi	Central/East African
1982	Gabon	Central/East African
1983	Pakistan	Asian
1985–1986	Philippines/United States	Asian
1987–1989	Malawi	Central/East African
1990–2006	*Australia**	Asian and Central/East African
1992	Guinea	West African
2003	Timor	Asian
1999–2000	CAR	Central/East African
2004, 2005	Kenya	Central/East African
2005, 2006	Comoros	Central/East African
2005, 2006	Reunion	Central/East African
2005	Seychelles	Central/East African
2005, 2006	Mauritius	Central/East African
2006	Mayotte	Central/East African
2006	Madagascar	Central/East African
2006	Cameroon	Central/East African
2006	*Canada**	Central/East African
2006	*Hong Kong**	Central/East African
2006	*United Kingdom**	Central/East African
2006	*Belgium**	Central/East African
2006	*Czech Republic**	Central/East African
2006	*Germany**	Central/East African

(*Continued*)

Table 10.1. (*Continued*)

Year of Outbreak(s)	Country	Genotype
2006	*Norway**	Central/East African
2006	*Switzerland**	Central/East African
2006	*France**	Central/East African
2006	*Italy**	Central/East African
2006	*Corsica**	Central/East African
2006	*Sri Lanka**	Central/East African
2006	*Singapore**	Central/East African
2006	*United States**	Central/East African
2007	*Spain**	Central/East African
2007	*Japan**	Central/East African
2007	*Gabon**	Central/East African

Countries in the italics represent cases involving individuals from those countries, but infected elsewhere and do not represent epidemics nor endemicity in those countries.

distinct clade within the ECSA phylogroup (Mohan, 2006; Yergolkar et al.,2006; Lakshmipathy and Dhanasekaran 2006; Paramasivan et al., 2009), whereas all previous isolates from India (1963–1973) were Asian genotype (Shah et al., 1964; Jadhav et al.,1965; Jupp and McIntosh,1988; Rodrigues et al., 1972; Padbidri and Gnaneswar, 1979; Mourya et al., 2001).

The Genomic Structure of Chikungunya Virus

In cases of Chikungunya virus, the nonstructural (NS) proteins that are required for viral replication are encoded in the 5′ two-thirds of the genome whereas the structural genes are collinear with the 3′ one-third. The structural proteins are produced by translation of mRNA that is generated from an internal, subgenomic promoter immediately downstream of the NS open reading frame. The 5′ end of the genome has a 7-methylguanosine cap and the 3′ end is polyadenylated; thus Chikungunya virus genome resembles eukaryotic mRNAs as it possesses 5′ cap structures and 3′ poly A tail. This has not yet specifically been investigated for Chikungunya virus, but it was documented with other related alphaviruses that the 5′ end is capped with a 7-methylguanosine. For Chikungunya virus (e.g., S27 strain), the 5′-nontranslatable regions (NTR) are composed of 76 nucleotide, whereas the 3′-NTR is composed of 526 nucleotides.

The structural proteins are generated by translation of a subgenomic mRNA, which produce a polyprotein to produce a capsid protein, two major envelope surface glycoproteins E1 and E2 (Figure 10.2) as well as two small peptides, E3 and 6K (Simizu et al., 1984; Weaver et al., 2005). E1 and E2 both are modified post-translationally in the endoplasmic reticulum and Golgi apparatus before being transported to the plasma membrane (Schlesinger and Schlesinger, 1986) where they maintain a close association with each other, forming a trimeric heterodimer spike structure (Anthony and Brown, 1991; Paredes et al., 1993)

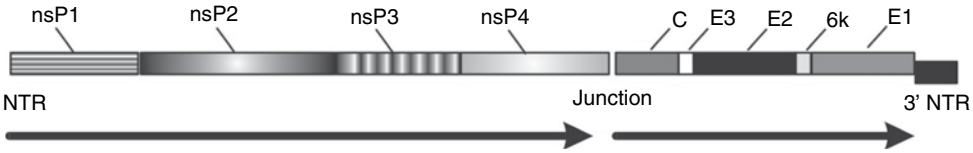

Figure 10.2. Genomic organization of the Chikungunya virus genome. (Ross strain, GenBank accession no. AF490259). The four nonstructural proteins (nsP1–nsP4) are translated as a single polyprotein directly from the positive-sense RNA genome. The structural proteins (C, E3, E2, 6 K, and E1) are translated from a subgenomic RNA (26S) transcribed from a separate promoter within the nontranslated (NTR) junction region. 5′ and 3′ NTR regions flank the coding region.

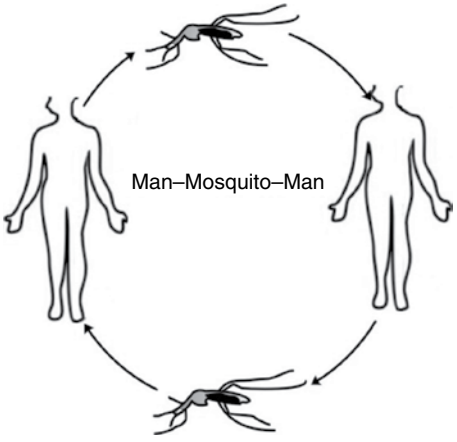

Figure 10.3. Natural cycle of Chikungunya virus.

Life Cycle of the Chikungunya Virus

The natural cycle of the virus is human–mosquito–human (Figure 10.3). Some evidence suggests the existence of epizootic cycles that maintain the virus during the interepidemic period (Mohan, 2006; Chevillon et al., 2008; Simon et al., 2008; Sudeep and Parashar, 2008). During epidemics, human beings serve as the Chikungunya virus reservoirs, whereas during interepidemic periods the reservoirs are several vertebrates, such as monkeys, rodents and birds.

Unlike dengue virus, there is no evidence for transovarial transmission of Chikungunya virus in mosquitoes. Variations in the geographical strains of *Aedes* mosquitoes regarding their susceptibility to infection and ability to transmit the virus may be crucial factors in determining endemicity of Chikungunya virus in a given region (Mohan, 2006; Powers and Logue, 2007; Chevillon et al., 2008; Simon et al., 2008; Sudeep and Parashar, 2008). The virus is transmitted from human to human by the bites of infected female mosquitoes. Most commonly, the mosquitoes involved are *Aedes aegypti* and *Aedes albopictus* two species that can also transmit other mosquito-borne viruses, including dengue. These mosquitoes can be found biting throughout daylight hours, although there may be peaks of activity in the early morning and late afternoon. Both species are found biting outdoors, but *A. aegypti* will also readily feed indoors. Unlike in Africa, where the virus

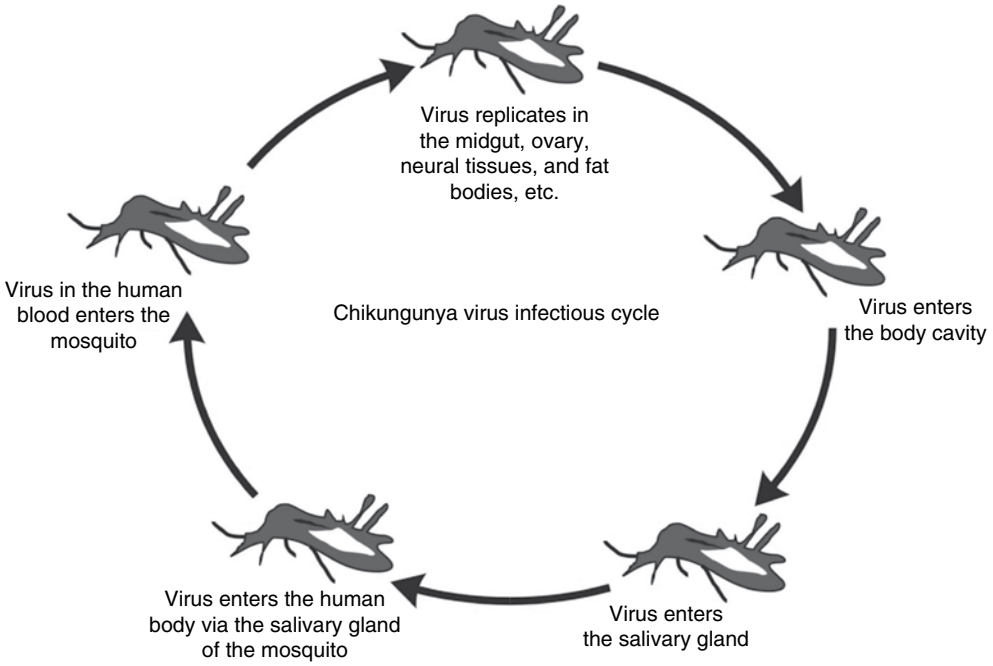

Figure 10.4. Infectious cycle of Chikungunya virus.

is maintained in a sylvatic cycle, Chikungunya in Asia has been an urban disease, typically found in dengue-endemic areas and transmitted largely by *A. aegypti* mosquitoes. However, the predominant *Aedes* spp. in locations such as Rúunion Island, where chikungunya emerged in 2005, was *A. albopictus* (Reiter et al., 2006). The spread of Chikungunya into rural areas during the later stages of outbreaks in India further confirmed the potential of *A. albopictus* mosquitoes in transmitting Chikungunya virus (Kumar et al., 2008). These changes were concurrent with the emergence of a strain having an alanine to valine substitution at codon 226 (A226V) of the envelope 1 (E1) gene in Rúunion Island (Santosh et al., 2008) and India (Schuffenecker et al., 2006). This type of mutation is known to increase the transmissibility of the virus by *A. albopictus* mosquitoes (Tsetsarkin et al., 2007). Vertical maternal-fetal transmission has also been documented in pregnant women affected by Chikungunya fever (Robillard et al., 2006)

Initial Stage of Chikungunya Virus Infection

Chikungunya virus enters the human body via the salivary glands of the mosquito (Figure 10.4). When an infected mosquito bites, it introduces the virus into the bloodstream of the host. On entering the bloodstream, the virus combines immediately with the permissive cells, especially the cells of the nose, throat, and mouth.

Host–Chikungunya Virus Interactions and Entry of Viral RNA by Clathrin-Dependent Endocytic Pathway

Chikungunya virus attaches the host cell via cell surface glycoproteins. The E1 and E2 glycoproteins form heterodimers, which associate as trimeric spikes on the viral surface.

Enveloped viruses use membrane-bound receptor(s) for entry into specific target host cells (Smith et al., 1995). Primary peripheral blood mononuclear cells, including primary CD4+ T lymphocytes, primary CD14+ monocytes, and dendritic cells, were reported to be refractory to Chikungunya virus binding and infection (Sourisseau et al., 2007). Surprisingly, primary macrophages were highly sensitive to Chikungunya virus and showed cytopathic effect following infection, whereas most of the blood-derived cells lack Chikungunya virus cell surface receptor(s) except macrophages and platelets.

Cellular requirements for entry into their target cells differ among alphaviruses. Cholesterol represents one of the key constituents of small, dynamic, sterol- and sphingolipid-enriched domains on the plasma membrane, which are called lipid rafts and compartmentalize all the cellular processes. Cholesterol-enriched domains also play an important role during entry of Chikungunya virus particles in human cells. Hence cholesterol depletion of the target cell membrane significantly reduced (up to 65 percent) infection of the cells. On the basis of RNA interference strategies Sourisseau et al. (2007) suggested that the clathrin-dependent endocytic pathway mediates the entry of Chikungunya virus into human cells.

Replication of Viral RNA

The endosome carrying the Chikungunya virus migrates to the cytoplasm and then finally to the nucleus. On entering the nucleus, the virus starts depositing its genetic material and starts replicating the genome. Minus-strand (only detected at an early stage of infection) and plus-strand RNAs (synthesized at a constant rate throughout the remainder of the infection cycle) are both transcribed under the control of nonstructural proteins (nsPs) of virion. NS proteins of Chikungunya virus (e.g., S27 strain) are encoded by an open reading frame (ORF) of 7424 nucleotide initiated by an ATG at position 77/79 from the 5′ cap site of the 49S NS RNA and terminated by a TAG at position 7499/7501. This ORF encodes a polyprotein precursor of 2474 amino acids termed nsP123 that, on proteolysis produces the different nsP. On the basis of available data for related alphaviruses, after synthesis and maturation, the nsP123 precursor complexed with the free nsP4 protein and host cell proteins to act as a minus-RNA strand replicase, which catalyze the synthesis of the negative-strand RNA (Barton et al., 1991). In Chikungunya virus, (e.g., S27 strain) the nsP4 protein is 611 amino acids long, which together with nsP1, catalyze the initiation of the negative-strand RNA synthesis. This protein is also involved in methylation and capping of the positive strand RNA (Mi et al., 1989). The nsP3 protein also participates in the transcription of negative strands in the early phase of infection. The nsP3 RNA replicase of Chikungunya virus (e.g., S27 strain) is 530 amino acids long and has two distinct domains, a N-terminal, which is conserved among alphaviruses, and a C-terminal, which varies significantly both in sequence and length among alphaviruses.

The fully cleaved precursor complexed with host cell proteins acts as a plus-strand RNA replicase to amplify the full-length subgenomic (26S) positive-strand mRNA using the negative-strand RNA as a template (Shirako and Strauss, 1994). Both steps are regulated by helicase and proteinase functions of nsP2 protein whose proteolytic cleavage (of precursor polypeptide to yield the mature NS proteins) plays an important role in the viral replication process (Figure 10.5). For Chikungunya virus, the ORF encodes a polyprotein precursor of 1244 residues, which processed cotranslationally and post-translationally into structural proteins C, PE2, and E1, and a small peptide termed 6K (that may act as a signal sequence for the translocation of E1).

Assembly, Budding, and Maturation

Once the nucleocapsid protein is released from the nascent polypeptide chain, an N-terminal signal sequence leads to the insertion of glycoprotein PE2 into the endoplasmic reticulum. The PE2 precursor is 65 Kda protein (Simizu et al., 1984). After being transported to trans-Golgi, the PE2-E1 heterodimer moves to the cell surface; during this movement, PE2 is cleaved by a cellular furin or furinlike proteinase to form E2 and E3 (De Curtis and Simons, 1988). The E2 protein (43 Kda) is 423 amino acids long, whereas E3 (11Kda) is 64 amino acids long, which is not associated with virions, but is released from infected cells (Simizu et al., 1984). The exact function of E3 protein in Chikungunya virus is not clearly identified but it could be involved in proper folding and disulfide bond formation during viral envelope spike formation (Parrott et al., 2009). Assembly of the virion starts with nucleocapsid assembly in the cytoplasm. The capsid protein forms RNA-bound dimmers, a total of 120 copies of these dimers further oligomerize to form the nucleocapsid of Chikungunya virus (Perera et al., 2001). Nucleocapsid complexes assemble in the cell cytoplasm and diffuse freely to the plasma membrane.

Like other alpha viruses, Chikungunya virus buds through the cell membrane. Virions acquire a lipid bilayer envelope containing the virus-encoded E1 and E2 glycoproteins. The mature envelope glycoprotein spikes are composed of trimers of E1 and E2. The nucleocapsid, E1, and E2 glycoproteins are phosphorylated (Liu et al., 1996; Waite et al., 1974); these phosphorylations play a role in the assembly or postassembly steps. The mature virus particles were also observed within endosomal vesicles (*see* Figure 10.5), probably during the propagation of the virus to neighboring cells. The envelope of the mature alphavirus particle must be derived from a segment of the plasma membrane from which all host cell proteins are excluded. After the complete assembly, the newly formed viral particle releases from the infected cell through budding and enters the blood.

When a mosquito bites a viremic host or a human who has been infected, the virus is transmitted to it, and it gets replicated in the midgut, ovary, neural tissues, and fat of the insect. The virus reproduces there and migrates to the salivary glands of the mosquito. When this infected mosquito bites someone, it transfers the life cycle of Chikungunya virus onto the other person making another viremic host.

Clinical Features

The symptoms of Chikungunya fever include 102.2 ° F (39 ° C) fever, a petechial or maculopapular rash involving the limbs and trunk, and arthralgia or arthritis affecting multiple joints, which can be debilitating. The nonspecific symptoms of Chikungunya can include headache, conjunctivitis, slight photophobia, and partial taste loss (Anand, 2012). In the present epidemic in the state of Andhra Pradesh in India, high fever and crippling joint pain are the prevalent complaint. Fever typically lasts for 2 days and abruptly comes down; however, joint pain, intense headache, insomnia, and an extreme degree of prostration lasts for a variable period, usually for about 5 to 7 days. Patients have complained of joint pains for much longer time periods depending on their age. Younger patients recover within 5 to 15 days, whereas middle-aged people recover in 30 to 75 days. Recovery is longer for the elderly. The severity of the disease as well as its duration is less in younger patients and pregnant women.

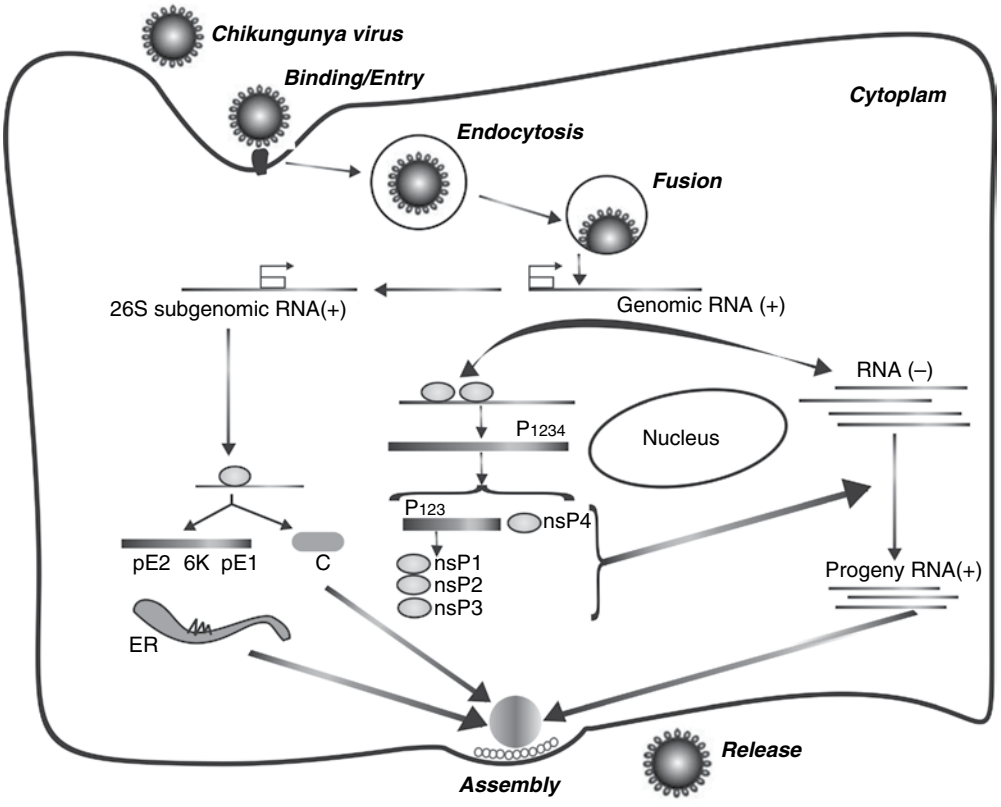

Figure 10.5. Replication cycle of Chikungunya virus.

Acute Stage of Infection

Chikungunya fever affects all age groups and equally affects both the genders. The incubation period ranges from 3 to 12 days (usually 3–7 days) (Mohan, 2006; Pialoux et al., 2007; Powers and Logue, 2007; Chevillon et al., 2008; Simon et al., 2008; Sudeep and Parashar, 2008). In susceptible populations, the attack rates are as high as 40 to 85 percent. The acute phase of the Chikungunya fever lasts for 3 to 10 days, but the convalescent phase can usually last from weeks to months with accompanying joint pain, swelling, and tenderness, which can sometimes last for even a year or more. The frequency of major symptoms in different series is shown in Table 10.2. The Réunion island outbreak identified a severe form of the disease in some patients that required management in the intensive care units for supporting at least one vital function. Some deaths were also reported (Ledrans et al., 2007).

General Manifestations

In the acute stage, the onset is usually abrupt and sudden with high-grade fever (usually 102–105°F; 39–40.5° C), severe arthralgias, myalgias, and skin rash. Headache, throat discomfort, abdominal pain, and constipation may also be present. Conjunctival

Table 10.2. Frequency of clinical manifestations in different series.

Symptoms	Andhra Pradesh (India) Outbreaks, Jan–Sept 2006 (%)	Réunion Island Outbreaks 2005–2006 (%)	Malaysian Outbreak, 1998 (%)
Fever	100	100	100
Arthralgia/arthritis	98	100	78
Skin rashes	Not reported	39	50
Headache, spinal pain	Not reported	Not reported	50/50
Myalgia	Not reported	60	50
Number of cases reported in the series	876	504	51

Adapted from World Health Organization (WHO). 2009. Guidelines for prevention and control of Chikungunya fever, 2009. Accessed February 17, 2013, at http://www.wpro.who.int/mvp/topics/ntd/Chikungunya_WHO_SEARO.pdf.

suffusion, persistent conjunctivitis, cervical pain, or sometimes generalized lymphadenopathy may be visualized.

Mucocutaneous Manifestations

Several mucocutaneous manifestations, such as morbilliform eruption, scaling, macular erythema, intertrigo, hypermelanosis, xerosis, excoriated papules, urticaria, and petechial spots, have been described in patients with Chikungunya fever (Bandyopadhyay and Ghosh, 2008; Inamadar et al., 2008).

Bone Manifestations

The Chikungunya viral polyarthropathic infection involves the small joints of the hand, wrist, and ankles and the larger joints, such as knee (Figure 10.6) and shoulder; more than 10 joint groups may be involved with the typical Chikungunya infection (Pialoux et al., 2007; Powers and Logue, 2007; Simon et al., 2007, 2008; Chevillon et al., 2008; Sudeep and Parashar, 2008). Axial involvement is common, and the joints are swollen with asymmetric involvement. Acute tenosynovitis is frequently present. Sometimes, atypical features such as Baker's cyst may be present. There may be the involvement of sternoclavicular and temporomandibular joints hips are relatively spared (Borgherini et al., 2007; Simon et al., 2007, 2008). Swollen tender joints and crippling arthritis can be seen in almost all patients. The pain may be severe enough to immobilize the patient and interfere with sleeping in the night. Joint pain and backache may worsen with movement. Radiological findings are usually normal, and biological inflammatory markers may be normal or moderately elevated (Fourie and Morrison, 1979; Kennedy et al., 1980).

Effect on Pregnancy

Chikungunya fever has a direct impact on pregnancy with a higher risk of abortion in the first trimester. In the last trimester, it can also be transmitted from mother-to-child (Pialoux et al., 2007; Powers and Logue, 2007; Chevillon et al., 2008; Simon et al., 2008; Sudeep and Parashar, 2008). In the Réunion Islands outbreak, three out of nine

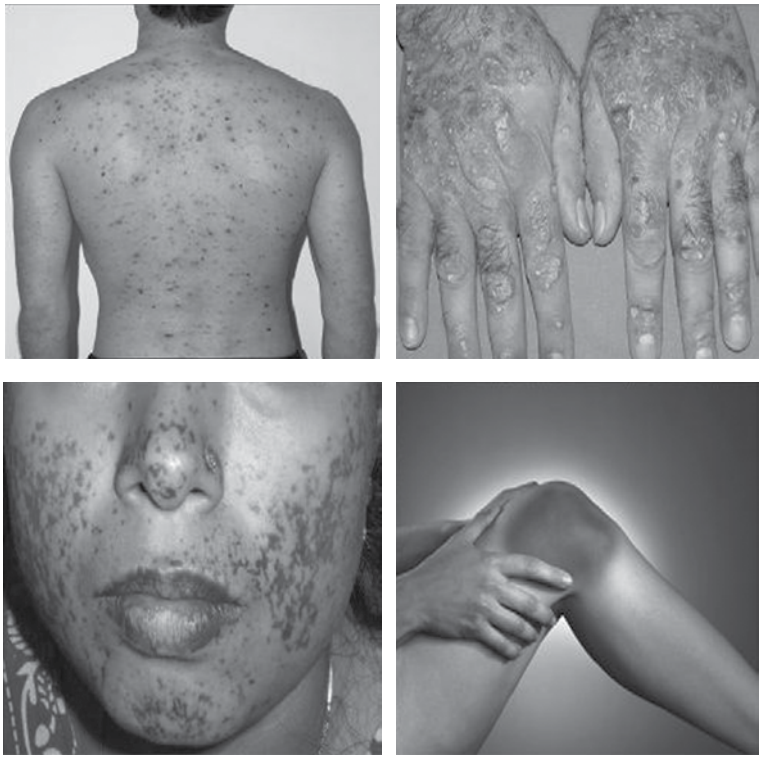

Figure 10.6. Chikungunya-associated rheumatism. (Severe relapse with polyarthritis and multiple tenosynovitis of wrists and fingers, hypertrophic tenosynovitis of one ankle, and swelling in the knee of a patient infected with Chikungunya virus.)

miscarriages were reported before 22 weeks of gestation as a result of the Chikungunya virus infection which was confirmed by positive reverse transcription-polymerase chain reaction (RT-PCR) in amniotic fluid.

Other Manifestations

So many hemorrhagic, neurological, and ocular manifestations of fulminant hepatitis reported during the recent epidemic of Chikungunya infection as reported by Economopoulou et al. (2009). There are some other uncommon manifestations, such as myocarditis after acute febrile illness (Obeyesekere and Hermon, 1972, 1973) and mixed cryoglobulinemia in the first months of the disease onset.

Course of the Acute Infection

In the acute phase, the fever is usually of short duration and usually resolves in 3 to 4 days. Some of the patients present a biphasic pattern of fever along with a febrile phase of 4 to 6 days that are followed by a fever-free period of a few days, which is again sometimes associated with the recurrence of fever (usually 101–102° F; 38.3–38.8° C) that may last a few days.

Chronic Stage of Infection

In most of the patients, the joint pains resolve in 1 to 3 weeks. However, the arthritis can persist in about 33 percent of patients for 4 months, 15 percent for 20 months, and in 12 percent for 3 to 5 years (Fourie and Morrison, 1979; Kennedy et al., 1980; Brighton et al., 1983). The chronic stage is characterized by unpredictable relapses, which include fever sensation, asthenia, exacerbation of arthralgias, and stiffness. Affected patients usually manifest inflammatory polyarthritis, severe subacute tenosynovitis/bursitis (consequently nerve tunnel syndromes) in hands, wrists, and exacerbation of pain on movement in previously injured joints (Simon et al., 2007, 2008). Older individuals and those having underlying rheumatic and traumatic joint disorders appear to be vulnerable for the chronic stage of the disease. In some patients, rheumatoid arthritis may occur following Chikungunya fever, suggesting that the viral infection may also have a role in the initiation or unmasking of rheumatoid arthritis (Bouquillard and Combe, 2009).

Chikungunya Fever in Neonates

Mothers afflicted with Chikungunya fever in the perinatal period (–4 days to +1 days) can transmit Chikungunya fever to neonates by vertical transmission (Sebastian et al., 2009). Intrapartum transmission may also be possible, and the transmission cannot be prevented by caesarean section (Gérardin et al., 2008; Simon et al., 2008; Sebastian et al., 2009). Neonatal Chikungunya fever is associated with fever, poor feeding, pain, distal edema, various skin manifestations, seizures, meningoencephalitis, and echocardiographic abnormalities in the newborn.

Chikungunya Fever in Infants

Chikungunya fever in infants manifests with certain differences. Fever is commonly present, which is associated with some constitutional symptoms, such as lethargy or irritability and excessive cry. Acrocyanosis may be prominent followed by symmetrical superficial vesicobullous lesions, erythematous asymmetrical macules, and patches, which later progressed to morbiliform rashes. The face and oral cavity are usually spared (Sebastian et al., 2009; Valamparampil et al., 2009).

Chikungunya Fever in Children

The symptoms of Chikungunya fever in children resemble symptoms that are observed in adults but with some important differences (Sebastian et al., 2009). Common clinical manifestations include abrupt onset of high-grade fever, skin rashes, minor hemorrhagic manifestations, arthralgia/arthritis, lymphadenopathy, conjunctival injection, swelling of eyelids, and pharyngitis. Rare clinical features include neurological manifestations including seizures, altered level of consciousness, blindness resulting from retrobulbar neuritis, acute flaccid paralysis, and rheumatological manifestations. Pediatric patients may also experience febrile seizures, vomiting, abdominal pain, and constipation (Lewthwaite et al., 2009).

Cardinal Features

The demographic characteristics and clinical presentation of Chikungunya fever reported during the current epidemic from India are listed in Table 10.3. Majority of the patients had presented with fever, arthralgias, arthritis, and mucocutaneous manifestations.

Table 10.3. Clinical presentation of Chikungunya fever reported during the epidemic from India (2006–2009).

Variable	Study (reference)				
	Mohan 2006 (n = 1226)*	Lakshmi et al., 2008 (n = 296)	Suryawanshi et al., 2009 (n = 405)	Kannan et al., 2009 (n = 354)	Bandyopadhyay et al., 2009 (n = 321)
Period of study	January 2006–July 2009*	March–December 2006	July–September 2006	2007	August–December 2007
Place of study	Tirupati, Andhra Pradesh	Hyderabad, Andhra Pradesh	Nagpur, Maharashtra	Four severely affected districts of Kerala (Pathanamthitta,Idukki, Kottayam, and Thrissur)	Nine districts of West Bengal
Method of diagnosis Age	IgM antibodies positive (n = 914) Mean age ± SD (years) = 38.4 ± 18.2	RT-PCR (n = 144): RT-LAMP (n = 20) Most affected age group = 31–40 years (34%)	IgM antibodies positive (n = 87) Mean age ± SD (years) = 26 ± 11.7†	Community based survey Most affected age group = 16–35 years (31%)	IgM antibodies positive Most affected age group = 31–50 years (43%)
Male:Female	1.1:1	1:1.6	2.3:1*	1.2:1	2:1
Symptoms‡					
Fever	100	100	100	100	100§
Headache	64	31	56	98	70§
Chills	30	ND	ND	ND	55§
Arthralgias	98	100	100	99	96§
Myalgias	96	ND	ND	99	80§
Photophobia	11	ND	ND	ND	25§
Nausea	42	ND	ND	83	38§
Vomiting	16	4	ND	11	6§
Eye pain	ND	08	ND	12	ND

Physical signs‡					
Conjunctival suffusion	74	ND	ND	8	ND
Painful swollen joints	70	40	24	ND	16§
Lymphadenopathy	4	ND	4	ND	65§
Skin rash	8	28	16	81	2§
Oral ulcers	ND	ND	10	18	ND
Altered consciousness	01	ND	3	ND	ND
Bleeding manifestations	2	ND	01	01	4§
Others	High-grade fever (>104° F, >40° C) (24%); fulminant hepatitis (2%)	Back pain 42%; shoulder pain 19%; difficulty in walking 6%; polyarthritis 12%	Transverse myelitis ($n=05$): acute inflammatory demyelinating polyneuropathy ($n=2$)	Edema (61.3%); distaste (84.4%)	Cough 05%; diarrhea 4%; abdominal pain 3%; chest pain 3%; edema of legs 3%‡

IgM, immunoglobulin M; ND, not described; RT-LAMP, real-time loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction.
*Data updated from Mohan, 2006; †Described for confirmed cases; ‡Percentage positive; §Described in 100 serologically confirmed patients.

Table 10.4. Neurological manifestations reported from an epidemic in India, 2006.

Name of Investigator	Total Number of Patients (*n*)	City and State	Manifestations along with the Number of Patients
Wadia (2007)	359	Kota, Rajasthan	Encephalitis (*n* = 175), neuropathy (*n* = 129), myelitis (*n* = 69), entrapment neuropathy (*n* = 34), muscle injury(*n* = 34)
Chandak et al. (2009)	300	Nagpur, Maharashtra	Encephalitis (*n* = 27) predominantly demyelinating type, myelopathy (*n* = 7), peripheral neuropathy (*n* = 7), myeloneuropathy (*n* = 7), myopathy (*n* = 1)
Tandale et al. (2009)	99	Ahmedabad, Gujrat and Pune, Maharashtra	Encephalitis (*n* = 57), encephalopathy (*n* = 42), myelopathy (*n* = 14), myeloneuropathy (*n* = 12)

Rare Manifestations

In addition to the aforementioned clinical manifestations, several other rare manifestations have also been reported in publications from India, and these include neurological manifestations, ocular manifestations, hemorrhagic manifestations, etc.

Neurological Manifestations

Neurovirulence and neuroinvasiveness, which are common to other alphavirus infections, are not identical to Chikungunya fever. Table 10.4 shows some of the neurological manifestations that were reported in India during the 2006 epidemic of Chikungunya.

Ocular Manifestations

Nodular episcleritis, acute iridocyclitis, uveitis, and neuro-retinitis have been reported as unusual ocular manifestations of Chikungunya fever (Mittal et al., 2007; Mahendradas et al., 2008; Mahesh et al., 2009).

Hemorrhagic Manifestations

In contrast to the dengue fever, hemorrhagic manifestations are uncommon in Chikungunya fever. If present, they are mild and more frequently encountered in Asian patients compared with African patients. These manifestations include epistaxis, bleeding from the gums, subconjunctival bleeding, and petechial or purpuric rash.

Others

Sudden sensorineural hearing loss (Bhavana et al., 2008) and hypokalemic periodic paralysis (Rampal et al., 2007) are other rare manifestations that have been reported in patients suffered from Chikungunya fever. Severe systemic disorders with multiple organ system involvement have also been observed during the 2006 epidemic in India (Tandale et al., 2009).

Laboratory Diagnosis

WHO (2009) developed a series of laboratory diagnosis of Chikungunya, which include RT-PCR, some serological assays such as IgM capture enzyme-linked immunosorbent assay (MAC–ELISA), and isolation of the infectious agent.

Collection and Transportation of Samples

To diagnose the disease correctly, proper collection, processing, storage, and transportation of the specimens is important.

Collection of Samples for Isolation and Molecular Diagnosis

SAMPLE

Serum, plasma, or sometimes whole blood (in heparinized tube) can be taken aseptically in a clean sterilized container. Samples should be collected within first 5 days of illness. To collect serum aseptically collect 4 to 5 mL of venous blood in a tube or vial, allow the collected blood to clot at room temperature, centrifuge at 2000 RPM to separate serum. Collect the serum in a clean dry vial, use adhesive tape marked with pencil, indelible ink, or a typewritten self-adhesive label to identify the container. The name of the patient, identification number, and date of collection must be indicated on the label.

Collection of Samples for Serology

SAMPLE

Blood in plain vial or serum can be taken aseptically. For serological diagnosis, two samples at different time intervals must be taken. The first sample should be taken 5 days after the onset of illness for immunoglobulin M (IgM) detection because these primary antibodies appear at this time. Then the second sample is taken at least 7 to 14 days after the first sample, or in the event of a fatality, at the time of death.

Other Types of Specimen for Laboratory Investigation

SPECIMENS

Cerebrospinal fluid (CSF) in meningo-encephalitis cases, synovial fluid in arthritis with effusion, and autopsy tissues as the liver, spleen, lymph nodes, and thymus are possible specimens for testing.

Transportation of Samples

Specimens should be transported immediately to the laboratory using an ice box (35.6–46.4° F; 2–8° C). Whole blood should not be frozen because hemolysis may interfere with serological diagnosis. If there is a delay of more than 24 hours, specimens can immediately be submitted to the laboratory; the serum should be separated and stored at refrigerated temperature. For the isolation, and molecular diagnosis the samples should always be stored and transported in frozen conditions.

Laboratory Tests

Three major laboratory tests are used for the diagnosis of Chikungunya fever including virus isolation, molecular technique of polymerase chain reaction (PCR), and serological tests. Specimen is usually blood or serum, but in neurological cases with meningo-encephalitic feature specimen is CSF.

Virus Isolation

The most definitive test is 2 to 5 mL of whole blood collected during the first week of illness in a commercial heparinized tube and transported on ice to the laboratory.

The Chikungunya virus can produce cytopathic effects in a variety of cell lines, including BHK-21, HeLa, and Vero cells. The cytopathic effects must be confirmed by Chikungunya-specific antiserum. The results can appear within 1 to 2 weeks. Virus isolation must only be carried in laboratories that are Biosafety Level-3 to reduce the risk of viral transmission.

Reverse Transcription-Polymerase Chain Reaction

RT-PCR technique is used for the diagnosis of Chikungunya virus. It uses nested primer pairs amplifying specific components of three structural gene regions; capsid (C), envelope E-2, and part of Envelope E1. PCR results can be available in 1 to 2 days. A specimen for PCR is exactly similar to the one for virus isolation (i.e., heparinized whole blood).

Serological Diagnosis

For serological diagnosis, 10 to15 mL of whole blood is collected from patient. The blood specimen transports at 39.2° F (4° C) immediately to the laboratory. If testing is not possible immediately, the serum is separated and then stored and transported under frozen environment. An acute phase serum must be collected immediately after the onset of illness, and the convalescent phase serum is collected after 10 to 14 days. Serological diagnosis can be made by the demonstrating a fourfold rise in antibody titer in acute and convalescent sera or by demonstrating IgM antibodies specific for Chikungunya virus. A commonly used test is the MAC-ELISA. Results of MAC-ELISA can be available within 2 to 3 days. MAC-ELISA may show cross-reaction with other flavivirus antibodies such as o'nyong-nyong and Semliki Forest viruses. Although these cross-reacting viruses are relatively rare in Southeast Asia and further could be differentially diagnosed by neutralization tests and hemagglutination inhibition assay (HIA).

Interpretation of Results

Sero-diagnosis usually demonstrates a fourfold increase in Chikungunya immunoglobulin G (IgG) titer between the acute and convalescent phase sera. However, getting paired sera is practically not possible. Alternatively, when paired sera cannot be collected, the demonstration of IgM antibodies specific for Chikungunya virus in acute-phase sera is used. A positive virus culture supplemented with neutralization is taken as the positive control for the presence of Chikungunya virus. Positive PCR result for E1 and C genome either singly or together from the specimen (serum, CSF, etc.) also constitutes a positive evidence of Chikungunya virus infection.

Differential Dignosis

Chikungunya fever has to be differentiated from dengue fever, which has the potential for considerably worse outcomes, including death. The two diseases can often be seen simultaneously in the same patient. Observations from previous outbreaks in Thailand and India have characterized the principal features distinguishing Chikungunya from dengue fever. In the former, shock or severe hemorrhage is not observed. The onset is acute, and

Table 10.5. Major manifestations that differentiate chikungunya from dengue.

Distinguishing Features	Chikungunya Fever	Dengue Fever
Clinical Signs and Symptoms		
Onset of fever of 104° F (40° C)	Acute	Gradual
Duration of fever	1–2 days	5–7 days
Maculopapular rash	Frequent	Rare
Presence of shock and severe hemorrhage	Rare	Common
Arthralgia	Frequent and lasting over a month	Infrequent and shorter duration
Laboratory Parameters		
Leucopenia	Frequent	Infrequent
Thrombocytopenia	Infrequent	Frequent

the duration of fever is much shorter in Chikungunya fever. In Chikungunya fever, maculopapular rash is more frequent than in dengue fever (Table 10.5).

Some diseases that can also be considered in the differential diagnosis are:

1. **Leptospirosis**: Severe myalgia localized to calf muscles with conjunctival congestion or subconjunctival hemorrhage with or without oliguria or jaundice in a person with history of contact to contaminated water might suggest leptospirosis.
2. **Malaria**: Periodicity of fever and alteration of consciousness or seizures should prompt diagnosis for malaria. Hence in the early stage when rashes are absent, malaria has to be ruled out.
3. **Meningitis**: High fever with neck stiffness or alteration of consciousness should prompt a thought about meningitis. All cases of meningoencephalitis during an outbreak of Chikungunya fever must be suspected to have Chikungunya infection.
4. **Rheumatic fever**: This is more common in children and presents with migratory polyarthritis predominantly affecting the large joints. Modified Jones criteria should be the basis for diagnosis. Raised antistreptolysin O (ASO) titer and a history of recurrent sore throat are other points to be noted.

Apart from these with the presence of rashes, measles or German measles need to be ruled out.

Differential diagnosis with other arthropod-borne viruses of the alphavirus genus (Ross River, Barmah Forest, O'nyong nyong, Sindbis, and Mayaro viruses) is difficult, but these are comparatively rare.

Clinical Management

Clinical management of Chikungunya fever could be done at two stages:

1. Acute stage of the illness.
2. Sequelae.

Principles Involved in Clinical Management

- Because there is no specific antiviral drug against Chikungunya virus, the treatment is entirely symptomatic. Paracetamol is the drug of choice with the use of other analgesics if it does not provide relief. Steroids are usually not recommended during the acute stage of the disease because of the adverse effects. Aspirin is preferably avoided for fear of gastrointestinal and the other side effects like Reye's syndrome.
- Mild exercise and physiotherapy are recommended in recovering persons.
- Treatment should be started in all suspect cases without waiting for serological or viral confirmation.
- During an epidemic, all cases should not be subjected for virologic or serologic investigations.
- All suspected cases should be kept under mosquito nets during the febrile period.
- Communities in the affected areas should be educated and trained about the mosquito control measures.

Guiding Principles for Managing Acute Stage

During the acute stage, clinical management of Chikungunya fever can be done at four levels: domiciliary, at the primary level or point of first contact, at the secondary level, and at the tertiary level.

Domiciliary (Home Based)

All cases of fever should be cared in the patient's own homes as.

1. Adequate rest in a warm environment and avoid damp surroundings. Heat may increase or worsen joint pain and is, therefore, best to avoid during the acute stage.
2. Avoid exertion and recommend mild exercises and physiotherapy in recovering persons.
3. Cold compresses may help in reducing joint damage.
4. Consume plenty of water with electrolytes. If possible, ensure a measured urine output of more than a liter in 24 hours.
5. Take paracetamol tablets during periods of fever (up to two 500-mg tablets four times daily), in persons with no pre-existing liver or kidney disease.
6. Avoid self-medication with aspirin or other pain killers.

If fever persists for more than 5 days and there is intractable pain, postural dizziness, cold extremities, decreased urine output, any bleeding under the skin or through any orifice and nonstop vomiting, then the patient should immediately be admitted in hospital.

At the Point of First Contact

All fever cases must be seen by a medical officer and differential diagnoses of dengue fever, leptospirosis, malaria, and other illnesses excluded by history, clinical examination, and basic laboratory investigations. All patients should be assessed for dehydration, and proper rehydration therapy (preferably oral) instituted quickly.

Severe dehydration is characterized by any two of these signs:

1. Abnormal sensorium, excessive sleepiness or lethargy.
2. Sunken eyes.
3. Poor fluid intake.
4. Dry, parched tongue.
5. Reduced skin turgor (slow skin pinch taking more than 2 seconds to retract).

Mild or moderate dehydration is also characterized by any two of these signs:

1. Restlessness or irritability.
2. Sunken eyes.
3. Dry tongue.
4. Excessive thirst.
5. Slow skin pinch (less than 2 seconds to retract).

Blood samples should be collected for total leucocyte count and platelet count. The total leucocyte count is usually on the lower side (below 5000 cells/mm^3). If it is more than 10,000 per mm^3, the possibility of leptospirosis has to be considered. A low platelet count (below 50,000 per mm^3) should alert the possibility of dengue fever. The peripheral smear has to be examined for malarial parasite as well, and if positive, treatment started as per national guidelines. Treat symptomatically (1 g of paracetamol three to four times a day for fever, headache, and pain, antihistamines for itching). Paracetamol must be used with caution in persons with pre-existing underlying serious illnesses. Tepid sponging can also be suggested.

If the case has already been treated with paracetamol or other analgesics, start one of the nonsteroidal anti-inflammatory drugs (NSAIDS) per standard recommendations. Monitor for any adverse side effects of NSAIDS. Cutaneous manifestations can be managed with topical or systemic drugs.

The persons having hemodynamic instability (frequent syncopal attacks, hypotension with a systolic blood pressure less than 100 mm Hg or a pulse pressure less than 30 mm Hg), oliguria (urine output less than 500 mL in 24 hours), altered sensorium, or bleeding manifestations, refer immediately to a higher health-care center. Mild forms of exercise and physiotherapy is recommended in recovering persons. Patients may be encouraged to walk, use their hands for eating, writing and regular isotonic exercises. Cold compresses may be suggested depending on the response. Exposure to warm environments (morning and evening sun) may be suggested as the acute phase subsides.

At the Secondary Level (District Hospital)

All fever cases with joint or skin manifestations must be evaluated by a physician. The patient should be assessed for dehydration and proper rehydration therapy instituted, preferably by the oral route. Blood samples for serology (IgM–ELISA) should be collected; blood test for IgG may also be done as an alternative method, which is essentially been followed by a second sample after 2 to 4 weeks. The infected person should be examined for renal failure (urine output, serum creatinine, serum sodium and potassium), hepatic insufficiency (serum aminotransferases, bilirubin), cardiac illness (electrocardiogram [ECG]), malaria (peripheral smear study), and thrombocytopenia.

CSF should be examined in cases in which meningitis is suspected. If the case has already been treated with paracetamol or other analgesics, a NSAID should be started. Cutaneous manifestations can be treated with topical or systemic drugs.

In cases of a patient who is pregnant or reporting oliguria/anuria, refractory hypotension, bleeding disorders, altered sensorium, meningo-encephalitis, persistent fever of more than 1 week's duration, or elderly (older than 60 years) and infants (younger than 1 year of age), cases should be referred to a higher level health-care center.

At the Tertiary Care-Level (Teaching Hospital Situations, Infectious Diseases Specialists, or Advanced Care Centers)

In cases referred to tertiary care center all aforementioned processes and test have been completed, and blood samples should be collected for serology/PCR/genetic studies as early as possible. If facilities are available, the possibility of other rheumatic diseases such as rheumatoid arthritis (with the criteria for rheumatoid arthritis diagnosis being fulfilled) and rheumatic fever (with modified Jones' criteria) should be considered. In unusual cases, NSAIDs therapy should be started. CURB-65 is a clinical prediction rule that has been validated for predicting mortality in community-acquired pneumonia and infection of any site. The score is an acronym for each of the risk factors measured. Each risk factor scores one point, for a maximum score of 5:

1. Confusion.
2. Urea greater than 7 mmol/L (blood urea nitrogen >19).
3. Respiratory rate of 30 breaths per minute or greater.
4. Blood pressure less than 90 systolic or diastolic blood pressure 60 or less.
5. Age 65 or older.

Serious complications such as bleeding disorders with blood components could be treated by platelet transfusions; in case of bleeding with platelet counts of less than 50,000 cells/mm^3, fresh-frozen plasma, or vitamin K injections, acute renal failure with dialysis, contractures, and deformities with physiotherapy/surgery, cutaneous manifestations with topical or systemic drugs, and neuropsychiatric problems can be managed with special care and suitable drugs. Patients with myopericarditis or meningo-encephalitis may require intensive care with regular monitoring, inotropic support, ventilation, etc. In cases with ophthalmic complications, standard practice guidelines may be obtained from the ophthalmologists. In cases of arthralgia, hydroxychloroquine 200 mg orally once daily or chloroquine phosphate 300 mg orally per day for a period of 4 weeks (but only after the peripheral blood smear examination at least twice to rule out malaria) should be used. If only one IgG test has been done previously, a second blood sample should be drawn after a gap of 2 to 4 weeks. The disability of the patient should be assessed and appropriate rehabilitative procedures assessed.

Management of Osteoarticular Problems

The osteoarticular problems observed with Chikungunya fever usually subside in 1 to 2 weeks. In approximately 20 percent of cases, they disappear after a gap of few weeks. In less than 10 percent of cases, they tend to persist for months. In about 10 percent of cases, the swelling disappears and the pain subsides, but reappears with every other febrile

illness for many months. Each time the same joints get swollen with mild effusion and symptoms persist for a week or two after the fever subsides. Management of osteoarticular manifestations should follow the general guidelines given previously. Because an immunologic etiology is suspected in chronic cases, a short course of steroids may be useful to manage chronic cases. Even though, NSAIDS produce symptomatic relief in the majority of individuals, patients should be carefully managed to avoid renal, gastrointestinal, cardiac, and bone marrow toxicity. Cold compresses have been reported to lessen the joint symptoms. As discussed previously, timely and appropriate physiotherapeutic nonweight-bearing exercises will help patients with contractures and deformities (e.g., slowly touching the occiput [back of the head)] with the palm, slow ankle exercises, pulley assisted exercises, milder forms of yoga, etc.) In cases with severe and disabling contractures, surgery may be suggested. Although, the management plans may be finalized in major hospitals, the follow-up and long-term care can only be done at a domiciliary or primary health center level.

Management of Neurological Problems

Various neurologic sequelae can occur with persistent Chikungunya fever. Approximately 40 percent of patients will complain various neurological symptoms, but hardly 10 percent will have persistent manifestations. Peripheral neuropathy in association with a predominant sensory component is the most common (5–8 percent). Paresthesias, pins and needles sensations, crawling sensation, and disturbing neuralgias have also been described by the patients in isolation or in combination. Worsening or precipitation of entrapment syndromes such as carpal tunnel syndrome has been reported in many patients with rarer motor neuropathies. Occasional cases of ascending polyneuritis have been observed as a postinfective phenomenon, as usually seen with many viral illnesses. Antineuralgic drugs (Amitriptyline, Carbamazepine, Gabapentin, and Pregnable) may be used in standard doses in cases with disturbing neuropathies. Ocular involvement, during the acute phase of the disease in less than 0.5 percent of cases as described previously, may lead to defective vision and painful eye in a small percentage. Progressive defects in vision resulting from uveitis or retinitis may warrant treatment with steroids.

Management of Dermatological Problems

After the acute phase, the skin manifestations of Chikungunya fever subside and rarely require long-term care. However, worsening of psoriatic and atopic lesions may require specific management by a qualified skin specialist. Hyperpigmentation and papular eruptions could be managed with zinc oxide cream or calamine lotion. Persistent non-healing ulcers are rare. Scrotal and aphthous-like ulcers on the skin and intertriginous areas may be managed by saline compresses, and topical or systemic antibiotics in cases with secondary infection.

Management of Psychosomatic Problems

Neuropsychiatric problems have also been observed in up to 15 percent of Chikungunya cases, which are more common in persons with premorbid disorders, and those having family history of mood disorders. The emotional and psychosocial issues need individual counseling and have to be considered in the social context of the patient and community.

Most of the patients have inadequate information regarding Chikungunya fever, and thereby psychosocial support and reassurance may solve some of the problems associated with the disease. As an appropriate plan for community support, occupational and social rehabilitation may hold the key for success.

Investigation of Outbreaks

Like other endemic diseases transmitted by mosquitoes, patients with Chikungunya should also be thoroughly investigated by a monitory committee, which includes public health laboratory staff, skilled persons in surveillance and epidemiology of infectious diseases, and environmental health and safety and officials responsible for parks and recreations. The peripheral health staff should also be alerted to report an increase of clustering of acute febrile illness compatible with the standardized case definition of Chikungunya fever.

Case Definition

Although case diagnosis of Chikungunya can only be made by laboratory means, it should be suspected when the epidemic occurs with the characteristic triad of fever, rash, and joint manifestations. This case definition of Chikungunya is adapted from that proposed by the European Centre for Disease Control.

- **Clinical criteria**: Acute onset of fever >101.3° F (38.5° C) and severe arthralgia or arthritis does not explained by other medical conditions.
- **Epidemiological criteria**: Recently residing or having visited epidemic areas, having reported transmission within 15 days prior to the onset of symptoms.
- **Laboratory criteria**: At least one of the following tests in the acute phase:
  1. Confirmation by virus isolation.
  2. Presence of viral RNA by RT-PCR.

Surveillance and Outbreak Response

Presence of virus-specific IgM antibodies in a single serum sample collected in acute or convalescent stage, and fourfold increase in IgG values in samples collected at least 3 weeks apart. On this basis, cases are to be categorized as:

- **Possible case**: A patient meeting clinical criteria.
- **Probable case**: A patient meeting both the clinical and epidemiological criteria.
- **Confirmed case**: A patient meeting the laboratory criteria irrespective of the clinical presentation. During an epidemic, all patients need not be subjected to confirmatory tests. An epidemiological link may be enough. Clinical management as of now does not differ between a probable case and a confirmed case.

Notification and Reporting

Chikungunya is not a disease that requires notification to public health officials; however, depending on its spread, countries in the region may make it mandatory for the primary health centers, other clinics, and hospitals, both in the public and private sectors to notify

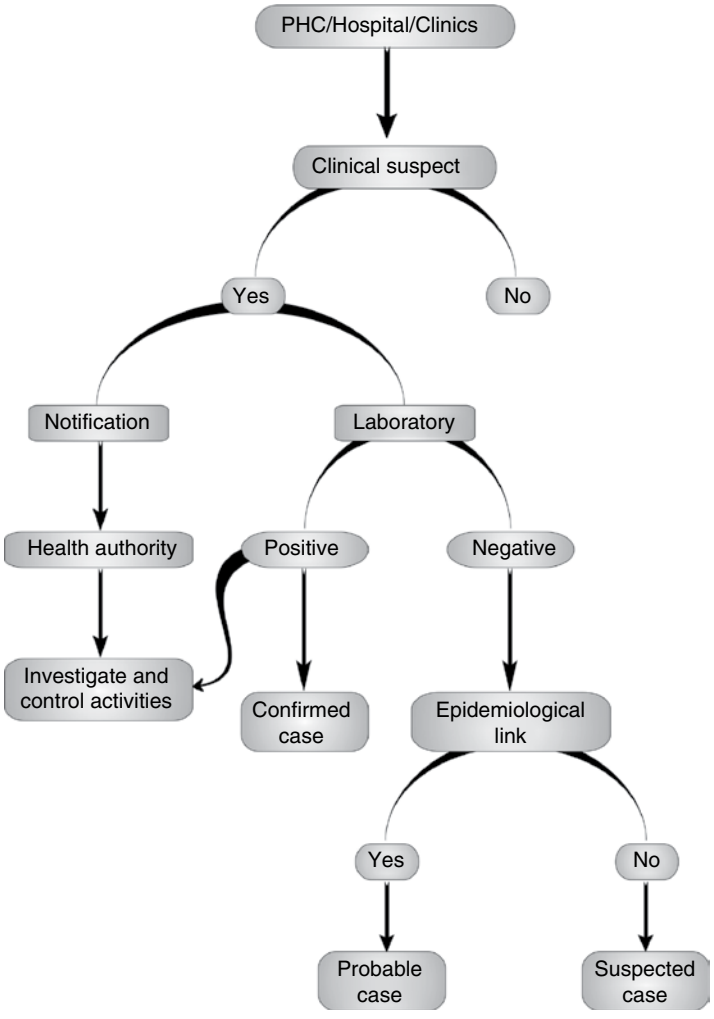

Figure 10.7. Scheme for the notification of a suspected outbreak of Chikungunya and response. PHC, primary health center.

suspected cases to authorities. A positive test for Chikungunya may emerge from a laboratory, in which case the laboratory should report it to the nearest district or civic authorities (Figure 10.7). Occurrence of clustering of cases compatible with the definition of a suspected case should indicate a possible outbreak, and this should be reported immediately to the nearest health authority.

Treatment

There is no specific treatment for Chikungunya, although some reports suggested mild relief from Chikungunya through an ayurvedic and homeopathic medicine (Chikungunya Fever Guide, n.d.).

Ayurvedic Treatment

Recently it was discovered that Triphala, an ayurvedic composition of three fruits, Harada (haritaki), Amla (amalaki), and Behada (bibhitaki), is a good medicine for Chikungunya. Sunflower seeds (powdered) can also be taken along with honey, which is a good supplement prescribed by ayurvedic doctors in Cochin and India. This mixture must be taken three times a day along with regular medicines to achieve relief from joint pains, which is a major after effect of this illness. Drinking lots of water will also help in reducing the pain and sufferings from this disease.

Homeopathic Treatment

Homeopathy is effective for the treatment for Chikungunya. On diagnosis of Chikungunya disease, homeopathic treatment should be started as early as possible. Homeopathic treatment can also be given along with conventional allopathic treatment if desired. Conventional treatment consists of only symptomatic treatment of fever, stiffness, and joint pain of this viral illness, whereas homeopathic medicines give better and comparatively long-lasting relief from the disease. Rhus Tox, Eupatorium Perf, Bryonia, and Arnica are the few homeopathic remedies that help in Chikungunya, but more accurate and effective remedies can be chosen by a homeopathic doctor according to the clinical picture of that particular case. Homeopathy works effectively in all viral diseases. Homeopathic pills are simply chewed (dissolved) on tongue and are sweet in taste. Homeopathic medicines do not cause gastric irritation and are safe on kidneys and liver even when taken for a long duration.

In epidemics, prevention of Chikungunya can be achieved by administering homeopathic "genus epidemicus." Genus epidemicus is a homeopathic remedy that is chosen as a preventive remedy for that particular epidemic in that particular locality. Homeopathic medicines are equally effective for various post-Chikungunya complaints such as weakness, stiffness in joints, and muscles pain. Infected persons should be protected from further mosquito exposure (staying indoors or under a mosquito net during the first few days of illness) so that they can not contribute to the transmission cycle.

Prevention and Control

Prevention and control are entirely dependent on taking steps to avoid mosquito bites and elimination of mosquito breeding sites.

Avoiding Mosquito Bites

1. Wear full sleeve clothes and long dresses to cover the limbs.
2. Use mosquito coils, repellents, and electric vapor mats during the daytime.
3. Use mosquito nets to protect babies, old people, and others, who may rest during the day. The effectiveness of such nets can be improved by treating them with permethrin (pyrethroid insecticide). Curtains can also be treated with insecticide and hung at windows or doorways to repel or kill mosquitoes.
4. Mosquitoes become infected when they bite people who are sick with Chikungunya. Mosquito nets and mosquito coils will effectively prevent mosquitoes from biting sick people.

Preventing Mosquito Breeding

The *Aedes* mosquitoes that transmit chikungunya breed in a wide variety of man-made containers, which are common around human dwellings. These containers collect rain water and include discarded tires, flowerpots, old oil drums, animal water troughs, water storage vessels, and plastic food containers. These breeding sites can be eliminated by

1. Draining water from coolers, tanks, barrels, drums and buckets, etc.
2. Emptying coolers when, not in use.
3. Removing mosquitoes from all house objects (e.g. plant saucers, which have collected water).
4. Cooperating with the public health authorities in applying antimosquito measures.

Role of Local Health Authority

Local health authorities are advised to be proactive if outbreaks occur in nearby states or provinces or if the outbreaks have occurred in previous years. The preparatory steps should always be initiated 2 to 3 months in advance of the onset of the rainy season. The district health committee or civic health authorities should call and conduct monthly meetings to develop an action plan. The entomological team should carefully monitor vector density in domestic and peridomestic areas on a monthly basis and inform the authorities of the trend. Rapid response teams should be placed to conduct epidemiological investigations.

The community representatives, nongovernmental organizations (NGOs), and others should hold meetings for social mobilization in an attempt to eliminate the breeding sites, keep the surroundings clean, and maintaining the basic hygienic conditions. It is the responsibility of local health administrator to assess the available resources and plan to meet additional manpower and material resources (insecticides and equipments) required for fogging, ultra low volume (ULV) spraying, and distribution and application of larvicides. Information, education, and communication IEC) materials should be prepared and distributed on time for the reduction of mosquito breeding sites and to minimize contact between mosquitoes and human beings. During an outbreak, daily reports should be prepared and reported based on the number of cases. All deaths reportedly attributed to Chikungunya should be thoroughly investigated. The media should also prove to be helpful in portraying correct information regarding the epidemic on a regular basis.

Community Action

The community plays a major role in keeping the environment clean, eliminating vector breeding sites, and minimizing human contact with the vector. Social mobilization for these outcomes is the key to the containment of a Chikungunya outbreak. These activities need to be done at the individual (household) level and at the institutional level, such as in schools, universities, hospitals, and other establishments.

At the Household Level

Aedes mosquitoes bite during the daytime, between dawn and dusk, only. Adult mosquitoes can be rendered ineffective by using commercially available pyrethroid-based aerosols for spraying. They are considered quite safe for use in the living areas provided

that the food items are well covered or removed from the area that is being sprayed. Bedrooms including the closets, bathrooms, and kitchens can be fumigated. The spray should be used for a few seconds and the room closed for 15 to 20 minutes. The timing of the spray should coincide with the peak biting time of the mosquito (i.e., early morning or late afternoon). Alternatively, mosquito coils, electric mats, and vaporizers should be used at the household level.

All members of the family should wear clothes that cover extremities and use commercially available insect repellents during the daytime on exposed parts of the body. Infants and others should sleep under the bed nets during the daytime. Any member of the family suspected of Chikungunya should essentially rest under bed nets during the viremic phase, which is usually the first 4 days of illness.

At School Level

Schoolchildren should be taught about all the features of Chikungunya fever, including what it is, how it spreads, the role of mosquitoes, where and how they breed/rest, and how they can be controlled. Weeds and tall grasses in the school premises should be cut down because adult mosquitoes look for these shady places to rest during the hot daylight hours.

Role of Public Health Authorities

Public health authorities play an important role in the prevention of Chikungunya fever:

1. National program for the prevention and control of vector-borne diseases should be strengthened and implemented efficiently with multisectoral coordination.
2. Legislations for elimination of domestic and peridomestic mosquito sites should be enforced effectively.
3. Communities must be made aware of the disease and their active cooperation in prevention and control measures elicited.
4. Minimize transmission of infection by vector surveillance and control.

Integrated Vector Management

Integrated vector management (IVM) is a process for managing vector populations in such a way so as to reduce or interrupt transmission of disease. It consists of vector surveillance and vector control through better knowledge of the characteristics of vector biology, disease transmission, morbidity, and a range of interventions and collaborations of all related parties. It also requires the community to increase their participation in vector surveillance, vector control, and in developing and implementing regulations concerning healthy public policy and environment.

The five key elements of IVM include:

1. Advocacy, social mobilization, and legislation.
2. Collaboration within the health sector and with other sectors.
3. Integrated approach.
4. Evidence-based decision making.
5. Capacity building.

Chemoprophylaxis

Pracetamol is commonly used medication to relieve symptoms of fever and joint pain. Chloroquine is suggested as a possible treatment for the symptoms associated with Chikungunya and as an anti-inflammatory agent to combat the arthritis associated with Chikungunya virus. A University of Malaya study found that for arthritis-like symptoms that are not relieved by aspirin and NSAIDs, chloroquine phosphate (250 mg/day) has produced promising results.

Immunoprophylaxis

As far as immunoprophylaxis are concerned, there is no currently available vaccine. A Phase II vaccine trial, sponsored by the US government and published in the *American Journal of Tropical Medicine and Hygiene* in 2000, used a live, attenuated virus, developing viral resistance in 98 percent of those tested after 28 days and 85 percent still showed resistance after 1 year (Edelman et al., 2000). A DNA vaccine is being tested for Chikungunya virus. The vaccine cassette was designed based on Chikungunya virus capsid- and envelope-specific consensus sequences with several modifications, including codon optimization, RNA optimization, the addition of a Kozak sequence, and a substituted immunoglobulin E leader sequence. These constructs have induced humoral and cellular immune responses in mice (Muthumani et al., 2008).

A virus-like particle based vaccine has protected monkeys from Chikungunya virus infection, and passive immunization from these monkeys protected immunodeficient mice against exposure to a dose of virus that would otherwise be lethal, demonstrating that the humoral response was highly protective (Akahata et al.,2010).

References

Akahata W, Yang ZY, Andersen H, Sun S, Holdway HA, et al. 2010. A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection. *Nat Med* 16:334–338.

Anand V. 2012. After effects of chikungunya fever. Only my health editorial. Accessed February 17, 2013, at http://www.onlymyhealth.com/after-effects-chikungunya-fever-1300445384.

Anthony RP, Brown DT. 1991. Protein-protein interactions in an alphavirus membrane. *J Virol* 65:1187–1194.

Bandyopadhyay D, Ghosh SK. 2008. Mucocutaneous features of Chikungunya fever: A study from an outbreak in West Bengal, India. *Int J Dermatol* 47:1148–1152.

Bandyopadhyay B, Pramanik N, De R, Mukherjee D, Mukherjee H, et al. 2009. Chikungunya in West Bengal, India. *Trop Doct* 39:59–60.

Barton DJ, Sawicki SG, Sawicki DL. 1991. Solubilization and immunoprecipitation of alphavirus replication complexes. *J Virol* 65(3):1496–1506.

Bhavana K, Tyagi I, Kapila RK. 2008. Chikungunya virus induced sudden sensorineural hearing loss. *Int J Pediatr Otorhinolaryngol* 72:257–259.

Borgherini G, Poubeau P, Staikowsky F, Lory M, Le Moullec N, et al. 2007. Outbreak of chikungunya on Reunion Island: Early clinical and laboratory features in 157 adult patients. *Clin Infect Dis* 44:1401–1407.

Bouquillard E, Combe B. 2009. Rheumatoid arthritis after Chikungunya fever: A prospective follow-up study of 21 cases. *Ann Rheum Dis* 68:1505–1506.

Brighton SW, Prozesky OW, de la Harpe AL. 1983. Chikungunya virus infection. A retrospective study of 107 cases. *S Afr Med J* 63:313–315.

Carey DE. 1971. Chikungunya and dengue: A case of mistaken identity. *J Hist Med Allied Sci* 26:243–262.

Chandak NH, Kashyap RS, Kabra D, Karandikar P, Saha SS, et al. 2009. Neurological complications of Chikungunya virus infection. *Neurol India* 57:177–180.

Chevillon C, Briant L, Renaud F, Devaux C. 2008. The Chikungunya threat: An ecological and evolutionary perspective. *Trends Microbiol* 16:80–88.

Chhabra M, Mittal V, Bhattacharya D, Rana UVS, Lal S. 2008. Chikungunya fever: A re-emerging viral infection. *Ind J Med Microbiol* 26:5–12.

Chikungunya Fever Guide, n.d. Accessed March 25, 2013, at http://www.chikungunya.in/index.shtml.

De Curtis I, Simons K. 1988. Dissection of Semliki Forest virus glycoprotein delivery from the trans-Golgi network to the cell surface in permeabilized BHK cells. *Proc Natl Acad Sci USA* 85:8052–8056.

Economopoulou A, Dominguez M, Helynck B, Sissoko D, Wichmann O, et al. 2009. Atypical Chikungunya virus infections: Clinical manifestations, mortality and risk factors for severe disease during the 2005–2006 outbreak on Réunion. *Epidemiol Infect* 137:534–541.

Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, et al. 2000. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. *Am J Trop Med Hyg* 62:681–685.

Fourie ED, Morrison JG. 1979. Rheumatoid arthritic syndrome after chikungunya fever. *S Afr Med J* 56:130–132.

Gérardin P, Barau G, Michault A, Bintner M, Randrianaivo H, et al. 2008. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Réunion. *PLoS Med* 5:e060.

Inamadar AC, Palit A, Sampagavi VV, Raghunath S, Deshmukh NS. 2008. Cutaneous manifestations of chikungunya fever: Observations made during a recent outbreak in south India. *Int J Dermatol* 47:154–159.

Jadhav M, Namboodripad M, Carman RH, Carey DE, Myers RM. 1965. Chikungunya disease in infants and children in Vellore: a report of clinical and haematological features of virologically proved cases. *Indian J Med Res* 53:764–766.

Jupp PG, McIntosh BM. 1988. Chikungunya virus disease. In: Monath TP, ed., *The Arboviruses: Epidemiology and Ecology*, vol II:137–157. Boca Raton: CRC Press.

Kannan M, Rajendran R, Sunish IP, Balasubramaniam R, Arunachalam N, et al. 2009. A study on chikungunya outbreak during 2007 in Kerala, south India. *Indian J Med Res* 129:311–315.

Kennedy AC, Fleming J, Solomon L. 1980. Chikungunya viral arthropathy: A clinical description. *J Rheumatol* 7:231–236.

Krastinova E, Quatresous I, Tarantola A. 2006.0Imported cases of Chikungunya in metropolitan France. *Eurosurveillance* 11(34). Accessed February 17, 2013, at http://www.eurosurveillance.org/viewarticle.aspx?articleid=3030.

Kumar NP, Joseph R, Kamaraj T, Jambulingam P. 2008. A226V mutation in virus during the 2007 chikungunya outbreak in Kerala, Ind. *J Gen Virol* 89:1945–1948.

Lakshmi V, Neeraja M, Subbalaxmi MV, Parida MM, Dash PK, et al. 2008. Clinical features and molecular diagnosis of Chikungunya fever from South India. *Clin Infect Dis* 46:1436–1442.

Lakshmipathy DT, Dhanasekaran D. 2008. Molecular epidemiology of Chikungunya virus in Vellore district, Tamilnadu, India in 2006. *East Afr J Public Health* 5:122–125.

Ledrans M, Quatresous I, Renault P, Pierre V. 2007. Outbreak of chikungunya in the French Territories, 2006: lessons learned. *Euro Surveill* 12(36):3262.

Lewthwaite P, Vasanthapuram R, Osborne JC, Begum A, Plank JL, et al. 2009. Chikungunya virus and central nervous system infections in children, India. *Emerg Infect Dis* 15:329–331.

Liu LN, Lee H, Hernandez R, Brown DT. 1996. Mutations in the endo domain of Sindbis virus glycoprotein E2 block phosphorylation, reorientation of the endo domain, and nucleocapsid binding. *Virology* 222:236–246.

Mahendradas P, Ranganna SK, Shetty R, Balu R, Narayana KM, et al. 2008. Ocular manifestations associated with chikungunya. *Ophthalmology* 115:287–291.
Mahesh G, Giridhar A, Shedbele A, Kumar R, Saikumar SJ. 2009. A case of bilateral presumed chikungunya neuroretinitis. *Indian J Ophthalmol* 57:148–150.
Markoff L. 2005. Alphaviruses. In: Mandell GL, Bennet JE and Dolin R eds. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease*, 6th ed., pp. 1913–1919. Philadelphia: Elsevier Churchill Livingstone.
Mi S, Durbin R, Huang HV, Rice CM, Stollar V. 1989. Association of the Sindbis virus RNA methyltransferase activity with the nonstructural protein nsP1. *Virology* 170:385–391.
Mittal A, Mittal S, Bharathi JM, Ramakrishnan R, Sathe PS. 2007. Uveitis during outbreak of Chikungunya fever. *Ophthalmology* 114:1798.
Mohan A. 2006. Chikungunya fever strikes in Andhra Pradesh. *Natl Med J India* 19:240.
Mourya DT, Thakare JR, Gokhale MD, Powers AM, Hundekar SL, et al. 2001. Isolation of chikungunya virus from *Aedes aegypti* mosquitoes collected in the town of Yawat, Pune District, Maharashtra State, India. *Acta Virol* 45:305–309.
Muthumani K, Lankaraman KM, Laddy DJ, Sundaram SG, Chung CW, et al. 2008. Immunogenicity of novel consensus-based DNA vaccines against Chikungunya virus. *Vaccine* 26:5128–5134.
Obeyesekere I, Hermon Y. 1972. Myocarditis and cardiomyopathy after arbovirus infections (dengue and chikungunya fever). *Br Heart J* 34:821–827.
Obeyesekere I, Hermon Y. 1973 Arbovirus heart disease: Myocarditis and cardiomyopathy following dengue and chikungunya fever-a follow-up study. *Am Heart J* 85:186–194.
Padbidri VS, Gnaneswar TT. 1979. Epidemiological investigations of chikungunya epidemic at Barsi, Maharashtra state, India. *J Hyg Epidemiol Microbiol Immunol* 23:445–451.
Paramasivan R, Philip Samuel P, Thenmozhi V, Rajendran R, Victor Jerald Leo S, et al. 2009. Chikungunya virus isolated in Lakshadweep islands in the Indian Ocean: Evidence of the Central/East African genotype. *Jpn J Infect Dis* 62:67–69.
Paredes AM, Brown DT, Rothnagel R, Chiu W, Schoepp RJ, et al. 1993. Three-dimensional structure of a membrane-containing virus. *Proc Natl Acad Sci USA* 90:9095–9099.
Parrott MM, Sitarski SA, Arnold RJ, Picton LK, Hill RB, et al. 2009. Role of conserved cysteines in the alphavirus E3 protein. *J Virol* 83:2584–2591.
Pavri K. Disappearance of Chikungunya virus from India and South East Asia. *Trans R Soc Trop Med Hyg* 1986;80:491.
Perera R, Owen KE, Tellinghuisen TL, Gorbalenya AE, Kuhn RJ. 2001. Alphavirus nucleocapsid protein contains a putative coiled coil alpha-helix important for core assembly. *J Virol 001*;75:1–10.
Peters Sherif CJ, Zakioverview R. 2005. Overview of viral haemorrhagic fevers. In: Guerrant RL, Walker DH, Weller PF eds., *Tropical Infectious Diseases: Principles, Pathogens & Practice*, 2nd ed., 726–733. Philadelphia: Elsevier Churchill Livingstone.
Pialoux G, Gaüzère BA, Jauréguiberry S, Strobel M. 2007. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis* 7:319–327.
Powers AM, Logue CH. 2007. Changing patterns of chikungunya virus: Re-emergence of a zoonotic arbovirus. *J Gen Virol* 88:2363–2377.
Powers AM, Brault AC, Shirako Y, Strauss EG, Kang W, et al. 2001. Evolutionary relationships and systematics of the alphaviruses. *J Virol* 75:10118–10131.
Rampal, Sharda M, Meena H. 2007. Hypokalemic paralysis following Chikungunya fever. *J Assoc Physicians India* 55:598.
Ravi V. 2006. Re-emergence of Chikungunya virus in India. *Indian Journal Medical Microbiology* 24:83–84.
Reiter P, Fontenille D, Paupy C. 2006. *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? *Lancet Infect Dis* 6:463–464.
Rishnamoorthy K, Harichandrakumar KT, Krishna Kumari A, Das LK. 2009. Burden of chikungunya in India: estimates of disability adjusted life years (DALY) lost in 2006 epidemic. *J Vector Borne Dis* 46:26–35.

Robillard PY, Boumahni B, Gérardin P, Michault A, Fourmaintraux A, et al. 2006. Vertical maternal fetal transmission of the chikungunya virus. Ten cases among 84 pregnant women. *Presse Med* 35:785–788.

Robinson MC. 1955. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952–53. I. Clinical features. *Trans R Soc Trop Med Hyg* 49(1):28–32.

Rodrigues FM, Patankar MR, Banerjee K, Bhatt PN, Goverdhan MK, et al. 1972. Etiology of the 1965 epidemic of febrile illness in Nagpur city, Maharashtra State, India. *Bull World Health Organ* 46:173–179.

Santhosh SR, Das PK, Parida MM, Khan MM, Tiwari M, et al. 2008. Comparative full genome analysis revealed E1 A226V shift in 2007 Indian chikungunya virus isolates. *Virus Res* 135:36–41.

Schlesinger M, Schlesinger S. 1986. Formation and assembly of alphavirus glycoproteins. In: Schelsinger S, Schlesinger MJ, eds., *The Togaviridae and Flaviviridae*, 121–148. New York: Plenum Publishing.

Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, et al. 2006. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* 3:e263.

Schwartz O, Albert ML. 2010. Biology and pathogenesis of chikungunya virus. *Nat Rev Microbiol* 8:491–500.

Sebastian MR, Lodha R, Kabra SK. 2009. Chikungunya infection in children. *Indian J Pediatr* 76:185–189.

Shah KV, Gibbs CJ Jr, Banerjee G. 1964. Virological investigation of the epidemic of haemorrhagic fever in Calcutta: Isolation of three strains of Chikungunya virus. *Indian J Med Res* 52:676–683.

Shirako Y, Strauss JH. 1994. Regulation of Sindbis virus RNA replication: uncleaved P123 and nsP4 function in minus-strand RNA synthesis, whereas cleaved products from P123 are required for efficient plus-strand RNA synthesis. *J Virol* 68:1874–1885.

Simizu B, Yamamoto K, Hashimoto K, Ogata T. 1984. Structural proteins of Chikungunya virus. *J Virol* 51:254–258.

Simon F, Parola P, Grandadam M, Fourcade S, Oliver M, et al. 2007. Chikungunya infection: An emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. *Medicine (Baltimore)* 86:123–137.

Simon F, Savini H, Parola P. 2008. Chikungunya: A paradigm of emergence and globalization of vector-borne diseases. *Med Clin North Am* 92:1323–1343.

Smith TJ, Cheng RH, Olson NH, Peterson P, Chase E, et al. 1995. Putative receptor binding sites on alpha viruses as visualized by cryoelectron microscopy. *Proc Natl Acad Sci USA* 92:10648–10652.

Sourisseau M, Schilte C, Casartelli N, Trouillet C, Guivel-Benhassine F, et al. 2007. Characterization of reemerging chikungunya virus. *PLoS Pathog* 3:e89.

Sudeep AB, Parashar D. 2008. Chikungunya: an overview. *J Biosci* 33:443–449.

Suryawanshi SD, Dube AH, Khadse RK, Jalgaonkar SV, Sathe PS, et al. 2009. Clinical profile of chikungunya fever in patients in a tertiary care centre in Maharashtra, India. *Indian J Med Res* 129:438–441.

Tandale BV, Sathe PS, Arankalle VA, Wadia RS, Kulkarni R, et al. 2009. Systemic involvements and fatalities during Chikungunya epidemic in India, 2006. *J Clin Virol* 46:145–149.

Tsetsarkin KA, Vanlandingham DL, McGee CE, Higgs S. 2007. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog* 3:e201.

Valamparampil JJ, Chirakkarot S, Letha S, Jayakumar C, Gopinathan KM. 2009. Clinical profile of Chikungunya in infants. *Indian J Pediatr* 76;151–155.

Wadia RS. 2007. A neurotropic virus (Chikungunya) and a neuropathic amino acid (homocysteine). *Ann Indian Acad Neurol* 10:198–213.

Waite MR, Lubin M, Jones KJ, Bose HR. 1974. Phosphorylated proteins of Sindbis virus. *J Virol* 13:244–246.

Weaver SC, Frey TK, Huang HV, Kinney RM, Rice CM. 2005. Togaviridae. In: Faquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, eds., *Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses*. Amsterdam: Academic Press, 2005:999–1008.

World Health Organization (WHO). 2008. Guidelines on clinical management of Chikungunya fever, 2008. Accessed February 17, 2013, at http://www.wpro.who.int/mvp/topics/ntd/Clinical_Mgnt_Chikungunya_WHO_SEARO.pdf.

World Health Organization (WHO). 2009. Guidelines for prevention and control of Chikungunya fever, 2009. Accessed February 17, 2013, at http://www.wpro.who.int/mvp/topics/ntd/Chikungunya_WHO_SEARO.pdf.

Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep AB, et al. 2006. Chikungunya outbreaks caused by African genotype, India. *Emerg Infect Dis* 12:1580–1583.

Chapter 11
West Nile Fever

Epidemiology

According to the phylogenetic analysis West Nile virus (WNV) had been emerged as a distinct virus around 1,000 years ago (Galli et al., 2004), which developed into two distinct lineages (Lanciotti et al., 1999; Platonov et al., 2001; Scherret et al., 2001). Lineage 1 and its other profiles are the source of the epidemic transmission in Africa and throughout the world. Lineage 2 was considered to be responsible for an African zoonosis, but in 2008, it started appearing in horses in Europe, The first known outbreak affected 18 animals in Hungary in 2008 (Christy, 2010). In 2010, lineage 1 was detected in a mare and her aborted fetus, which was new because up until this point only lineage 2 had been associated with infection in horses and humans in South Africa (Venter et al., 2011). In 2007, a killer whale in Texas died of the disease caused by WNV, and that broadened the known host range of the virus to also include cetaceans (St. Leger et al., 2011).

In 2009, the Centers for Disease Control and Prevention (CDC) reported that WNV had been one of the possible causes of Alexander the Great's early death, which was based on reports of avian deaths before his illness period (Marr and Calisher, 2009). The virion was first isolated from a feverish 37-year-old woman at Omogo in the West Nile district of Uganda in 1937 while working on a yellow fever virus (Smithburn et al., 1940). In 1939, a series of serological surveys reported antibodies against WNV in central Africa ranging from 1.4 (Congo) to 46.4 percent (White Nile region, Sudan). It was further subsequently identified in Egypt in 1942 and in India in 1953. In Egypt, a serological survey performed in 1950 reported that 90 percent patients older than 40 years of age had WNV antibodies. The ecology was characterized in 1953 based on studies in Egypt (Work et al., 1953) and Israel (Bernkopf et al., 1953). The virus became recognized as a causative agent of severe human meningoencephalitis in elderly patients during an outbreak in Israel in 1957. The disease was first noted in horses in Egypt and France in the early 1960s and further found to be widespread in southern Europe, southwest Asia, and Australia.

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

WNV was first reported in the Western hemisphere in 1999 when it caused encephalitis in humans, dogs, cats, and horses, which subsequently spread in the United States. In America, the outbreak began in the New York City area and was later observed in New Jersey and Connecticut; without any clear evidence, the virus is believed to have entered an infected bird or mosquito in this outbreak (Calisher, 2000). The virus isolated from US patients was closely related to a lineage 1 strain found in Israel in 1998. After the North American cases of 1999, the virus was then reported throughout the United States, Canada, Mexico, the Caribbean, and Central America in humans, equines, and many birds. The *Macaca sylvanus* (Barbary Macaque) was the first nonhuman primate infected with WNV (Hogan, 2008). High mortality rates were reported in the US and Israeli strains in infected avian populations. The presence of dead birds especially belonging to the family *Corvidae* was considered as an early indicator of the arrival of the virus.

Global Scenario

West Nile fever been reported from Africa, Europe, the Middle East, west and central Asia, Oceania and most recently, North America.

Recent Outbreaks

In chronological order, recent outbreaks of WNV encephalitis in humans have occurred in Algeria (1994), Romania (1996–1997), the Czech Republic (1997), Congo (1998), Russia (1999), the United States (1999–2009), Canada (1999–2007), and Greece (2010); see Table 11.1 for US and Canadian numbers during this period.

There were 417 confirmed WNV cases in Israel in the year 2000, with 326 patients hospitalized and 33 dead (Weinberger et al., 2001). Most of the patients showed

Table 11.1. Recent outbreaks of West Nile Virus infection in United States and Canada.

	United States (1999–2009)		Canada (1999–2007)	
Outbreak Year	No. of cases reported	Death	No. of cases reported	Death
1999–2001	149	18	—	1
2002	4,156*	284	416	10
2003	9,862	264†	1,494	14
2004	2,539	100	26	2
2005	3,000	119	239	12
2006	4,269	177	127	Death not reported
2007	3,623‡	124	445	2
2008	236	02	—	—

*13 cases were contracted through blood transfusion.
†30 percent patients reported meningitis and encephalitis.
‡1,227 cases reported neuro-invasive disease with 117 fatal cases.
Adapted from List of West Nile virus outbreaks. n.d. Wikipedia. Accessed March 25, 2013, at http://en.wikipedia.org/wiki/List_of_West_Nile_virus_outbreaks.

encephalitis (57.9 percent), whereas others reported febrile disease (24.4 percent) and meningitis (15.9 percent) (Chowers et al., 2001). Apart from Israel, 500 cases of WNV were reported in Romania with a 10 percent fatality rate in 1996 and 1997 with 34 confirmed cases with 3 fatalities in the year 2010. In the same year, northern Greece also reported 261 WNV cases with 34 fatalities. A more recent example of West Nile fever was reported in Washington state in the United States in August 2012; (Wire, 2012). Another case was reported from Brazos County in Texas of a female affected with the virus (West, 2012). The United States was badly affected by mosquito-borne WNV in 2012, with 241 cases of the disease, including four deaths, nationwide (Wire, 2012)

The Etiological Agent

WNV is actually part of Japanese encephalitis (JE) serocomplex of viruses, which mainly infects birds belonging to the family *Flaviviridae*. This group contains a number of viruses associated with human encephalitis, JE, St. Louis encephalitis (SLE), Murray Valley encephalitis, and Kunjin (a subtype of WNV). Antigenically all the flaviviruses are closely related and show serologic cross-reactions in the diagnostic laboratory. Members of the JE complex are closely related, often needing specialized tests (e.g., virus neutralization assays) to differentiate the infecting flavivirus (Roehrig, 1999). Humans acquire infection through the bite of an infected mosquito.

Genetically WNV can be divided into two lineages (Platonov et al., 2001; Lanciotti et al., 1999; Scherret et al., 2001).

- **Lineage 1**: These viruses have been found to be associated with clinical human encephalitis. This lineage was the major cause of human outbreak in South Africa in 1974. Hence, these viruses have been isolated from Africa, India, Europe, Asia, and North America. In addition, Kunjin virus, a subtype of lineage 1 WNVs co-circulates in Australia with a second encephalitis virus member of the JE virus complex, Murray Valley encephalitis virus (Scherret et al., 2001).
- **Lineage 2**: They are maintained in enzootic foci in Africa and have not been found to be associated with clinical human encephalitis.

Structure of West Nile Virus

Cryoelectron microscopy revealed that WNV is a 45 to 50 nm virion (Figure 11.1), which is covered with a relatively smooth protein surface. Its structure is quite similar to the dengue virus (described in Chapter 8) because both belong to the genus *Flavivirus* within the family *Flaviviridae*.

The genetic material of WNV is a positive-sense, single strand of RNA of around 11,000 to 12,000 nucleotides long. This RNA genome encodes for three structural proteins and seven nonstructural proteins. The RNA strand is surrounded by a nucleocapsid core (30–35 nm) formed from 12 kDa protein blocks and exhibits icosahedral symmetry (Figure 11.2), the capsid is again surrounded by a host-derived envelope that has been modified by the insertion of two integral membrane glycoproteins, E (53 kDa) and prM (18-20 kDa).

At the end of virus maturation, the prM protein is cleaved to M protein (of 8 kDa) by a cellular protease, which is then incorporated into the mature virion. The nonstructural

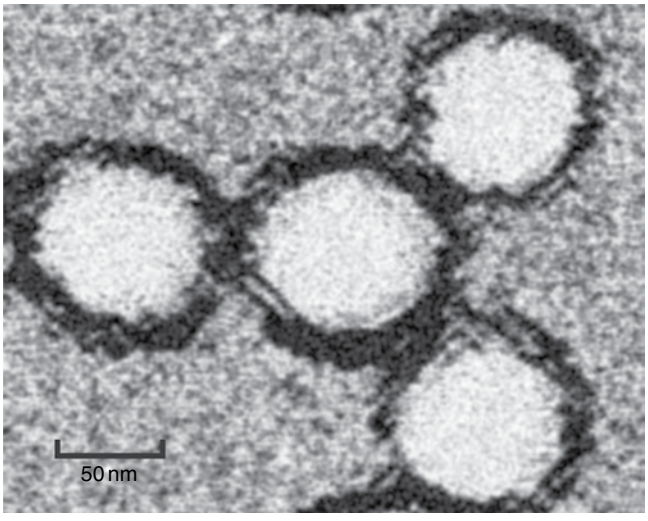

Figure 11.1. West Nile virus structure by cryoelectron microscopy.

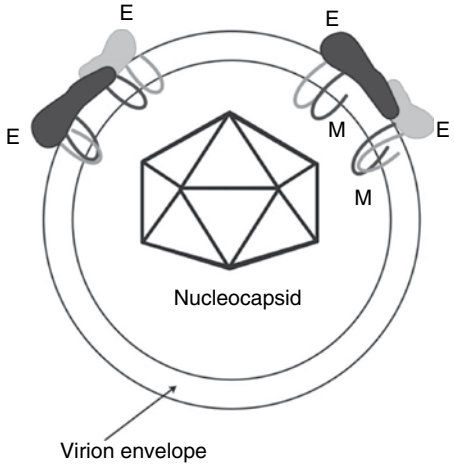

Figure 11.2. The structure of West Nile Virus is an icosahedral nucleocapsid that encloses the virion RNA. The virion has an envelope derived from the host cell membranes. E-glycoprotein (E), an integral membrane protein, is arranged as homodimers (head-to-tail) and associates with the other integral membrane proteins, prM protein (in immature virions).

proteins encoded by the genome are NS1, NS2a, NS2a, NS3, NS4a, NS4b, and NS5 (Figure 11.3), and they make up the intracellular replication machinery of the virus. Immunologically E-glycoprotein is the most important structural protein, which is the viral hemagglutinin and mediates virus-host cell binding. Most of the virus neutralizing antibodies are elicited by this E-glycoprotein.

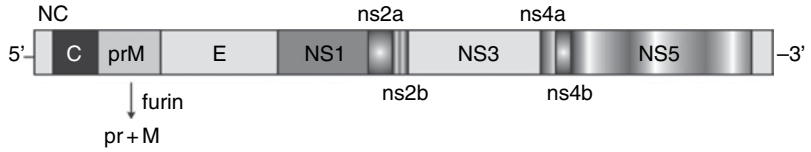

Figure 11.3. Genomic structure of West Nile virus. The virus genome is 11,000 to 12,000 nucleotides long. The 5' and 3' both the ends contain noncoding (NC) regions. The genome encodes 10 proteins, 3 of which are structural proteins (C, M, and E), and 7 of which are nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). The M protein is initially synthesized as a precursor (prM) protein, which is then processed to pr + M protein late during the virus maturation by a convertase enzyme (furin).

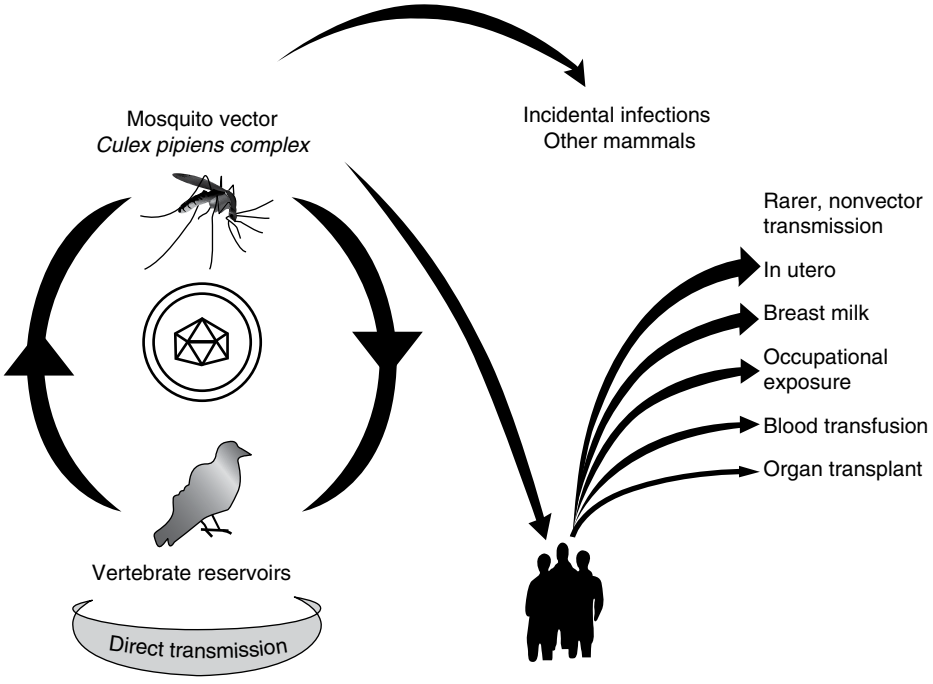

Figure 11.4. An enzootic mosquito–bird–mosquito cycle of West Nile virus. Humans and other mammals serve as dead-end hosts, which do not sufficiently amplify virus for mosquito transmission, although they may transmit or acquire virus in utero, through breast milk, via blood transfusion or organ transplantation, or through occupational exposure.

Life Cycle of West Nile Virus

WNV is actually maintained in an enzootic mosquito–bird–mosquito cycle (Figure 11.4). In the United States, the mosquito (*Culex pipiens*) is the primary vector for WNV infection that commonly breeds in urban areas and prefers to feed on birds. These mosquitoes become infected by feeding on birds that are infected with the virus. The infected birds may or may not become ill. These birds are also the vectors or intermediate carriers of the

virus, which is important for the virus transmission cycle. The birds are also considered amplifying hosts, developing sufficient viral levels to transmit the infection to other biting mosquitoes, which further infects other birds (in the Western hemisphere, the American robin and the American crow are the most common carriers) and also humans. The main route of human infection is through the bite of an infected mosquito. From infected birds, the virus may circulate in the blood of mosquitoes for a few days and eventually enters the mosquito's salivary glands, and then subsequently may be injected into humans and animals (Peterson et al., 2003). In mammals, the virus multiplies slowly (i.e., usually does not develop high viremia during the initial phase of infection), and it is believed that mosquitoes biting infected mammals do not ingest sufficient virus to become infected (Taylor et al., 1953) making mammals so-called dead-end infections. Hence human beings and other mammals serve as dead-end hosts because they do not sufficiently amplify virus for mosquito transmission, although they may transmit or acquire the virus.

WNV is not transmitted from person to person through touching, kissing, or other contact. However, there is evidence of WNV transplacental (mother-to-child) transmission, through breastfeeding and occasionally through occupational exposure. Therefore, pregnant mothers should be aware of the presence of WNV in their area and take appropriate precautions. WNV has also been evidenced to be transmitted through blood transfusions and organ transplants, although the current blood supply is now tested for the presence of the WNV. Immunocompromised persons (e.g., patient with a disease or patients undergoing chemotherapy) and people aged 50 or older represent the highest risk group for serious WNV infection.

In Europe mosquitoes (*C. pipiens*) existed in two populations: one that bites birds and one that bites humans. In North America, 40 percent of *C. pipiens* were found to be hybrids of the two types that bite both birds and humans, providing a vector for WNV. Mosquito saliva plays a potential role in the course of WNV disease (Schneider et al., 2006, 2007; Styer et al., 2006). While sucking the blood, mosquitoes inoculate their saliva into the skin, which is considered a pharmacologic cocktail of secreted molecules, made up of principally proteins that can affect vascular constriction, blood coagulation, platelet aggregation, inflammation, and immunity. Mosquito saliva alters the host immune response, which can be advantageous to a virus (Limesand et al., 2003; Wanasen et al., 2004). This specific modulation of the immune response occurs during the early phases of viral infection (Schneider et al., 2004), and mosquito feeding can exacerbate WNV infection, which further leads to higher viremia and can develop into a more severe forms of the disease.

Among birds, crows are most vulnerable to become infected by WNV, and they are often killed by it. More than 200 species of birds have been found to be infected by the virus, and in New York, the common dust-colored house sparrow is probably a principal bird reservoir. Sparrows can harbor the virus for 5 days or more at levels high enough to infect mosquitoes that bite them. The infected mosquitoes then transmit the virus when they bite and suck blood from people and animals and, in the process, inject the virus into them.

Pathogenesis

WNV starts replication at the site of inoculation and then spreads to lymph nodes and the bloodstream (Diamond et al., 2003). From the lymph nodes, the virus starts penetrating the central nervous system, which is a result of the stimulation of toll-like

receptors and increased levels of tumor necrosis factor-α; both increase the permeability of the blood-brain barrier (Wang et al., 2004). WNV can directly infect neurons, especially in deep nuclei and gray matter of the brain, brainstem, and spinal cord (Ceccaldi et al., 2004; Guarner et al., 2004; Kleinschmidt-DeMasters et al., 2004). It causes destruction of bystander nerve cells collaterally, which may contribute to paralysis (Darman et al., 2004). Viral activation of the immune system catalyses tissue damage, which may also contribute to pathologic changes as appear in some patients infected with WNV (Leis and Stokic, 2005). Although most of the WNV infections are nonfatal and can be cleared by the host immune response mechanisms, the virus may persist in some vertebrate hosts for a longer time periods (Kuno, 2001).

Clinical Features

Most of the persons infected with WNV usually do not develop any clinical signs or symptoms after acquiring it. In Northern Hemisphere outbreaks, an estimated 80 percent of people infected with WNV never developed symptoms attributable to the infection. Approximately 20 percent of infected people develop symptoms referred to as West Nile fever. The incubation period for WNV infection is about 2 to 14 days (Figure 11.5), although some patients who are immunosuppressed reported longer incubation period depending on their immune status. Once a patient recovers from the WNV infection, the immunity is considered to be life long.

WNV has been found to be associated with three major clinical categories of infection as asymptomatic, West Nile fever, and West Nile meningoencephalitis.

- **Asymptomatic**: Most of persons infected with the WNV are asymptomatic because symptoms are seen in only about 20 percent of infected patients (Mostashari et al., 2001). The recovery in these persons is self-limited, which is probably because of the healthy immune status of the host or the person might have cross-reacting antibodies or cytotoxic T cells against WNV antigens.
- **West Nile fever**: It is the usual presentation of West Nile viral infection. It is a self-limited febrile illness, which is indistinguishable from dengue fever and other viral

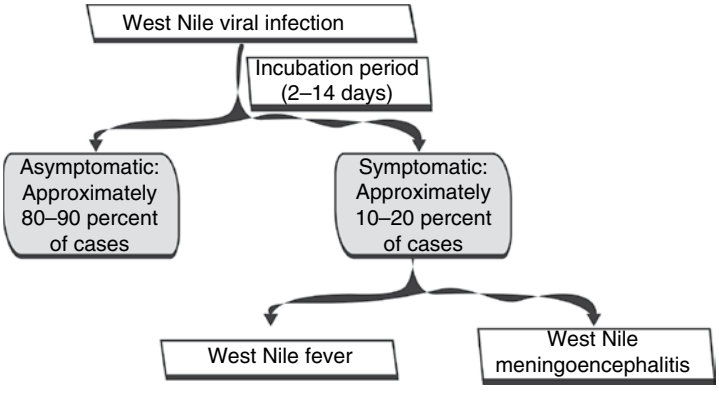

Figure 11.5. Clinical categories of West Nile viral infection.

grippes. West Nile Fever occurs much more frequently than West Nile meningoencephalitis. This febrile stage has an incubation period of 2 to 8 days followed by flulike symptoms such as fever, headache, chills, myalgia, malaise, diarrhea, vomiting, fatigue, diaphoresis (excessive sweating), weakness, lymphadenopathy (swelling of lymph nodes), drowsiness, pain in the joints, etc. Sometimes gastrointestinal symptoms and a transient macular rash on the trunk have also been reported (Watson et al., 2004). The rash is typically maculopapular, involves the chest, back and arms, and generally lasts for less than 1 week, which is accompanied by dysesthesia and pruritus (Ferguson et al., 2005). Symptoms are generally resolved within 7 to 10 days, although fatigue can persist for some weeks and association lymphadenopathy up to 2 months.

- **West Nile meningoencephalitis**: When the central nervous system (CNS) is involved, clinical syndrome ranging from febrile headache to aseptic meningitis to encephalitis, which are usually indistinguishable from similar syndromes caused by other viruses. This is the more severe form of the disease, which can be life-threatening and is called West Nile encephalitis or West Nile meningitis depending on the involved organ. Neuro-invasive disease develops in less than 1 percent of individuals infected with WNV; despite that the number of patients that reported neuro-invasive disease is higher because neuro-invasive disease is more likely to be reported than West Nile fever or asymptomatic infections (Mostashari et al., 2001). Headache is a prominent feature of West Nile fever, meningitis, or encephalitis, and therefore, it is not a useful indicator of neuro-invasive disease. West Nile meningitis usually involves fever, headache, stiff neck, and pleocytosis. West Nile encephalitis is the most severe form of neuro-invasive West Nile viral disease, which also involves fever and headache, but is characterizes by mild alteration of consciousness resulting lethargy that may further progress to confusion or coma. The risk for encephalitis increases with age and is high among organ transplant recipients (Kumar et al., 2004).

Other Clinical Manifestations

Tremor, myoclonus, and parkinsonian features such as rigidity, postural instability, bradykinesia (Pepperell et al., 2003; Sejvar et al., 2003), seizures, cerebellar ataxia (Kanagarajan et al., 2003), and optic neuritis are some other neurologic manifestations found to be associated with WNV infection. WNV infrequently induces other forms of weakness, including brachial plexopathy, radiculopathy, and a predominantly demyelinating peripheral neuropathy similar to Guillain-Barré syndrome (Ahmed et al., 2000; Park et al., 2003).

Another less common (than meningitis or encephalitis) manifestation is West Nile poliomyelitis, which is a flaccid paralysis syndrome associated with WNV infection. This syndrome is generally characterized by the acute onset of asymmetric limb weakness or paralysis in the absence of sensory loss. The paralysis can occur in the absence of fever, headache, or other common symptoms associated with WNV infection. Involvement of respiratory muscles leads to acute respiratory failure (Sejvar et al., 2005).

Apart from neurological manifestations, some ocular manifestations have also been reported following WNV infection, and these include chorioretinitis, vitritis (Bains et al., 2003), iridocyclitis (Hershberger et al., 2003), occlusive vasculitis (Kaiser et al., 2003), and uveitis (Kuchtey et al., 2003).

Laboratory Diagnosis

Physical Examination

Signs of WNV infection are similar to those of other viral infections without any specific findings on physical examination except approximately half of patients infected with WNV may have a rash.

Tests that may be performed to diagnose WNV infection include:

1. **Complete blood count (CBC) and cerebrospinal fluid (CSF) examination**: In examining blood count, the total leukocyte counts (TLC) in peripheral blood are mostly normal or elevated. CSF samples obtained from patients with signs of CNS involvement typically shows a pleocytosis with a predominance of lymphocytes as well as an elevated protein concentration, although in 37 to 45 percent of cases, neutrophils predominate (Tyler et al., 2006).
2. **Head computerized tomography (CT) scan and magnetic resonance imaging (MRI) scan**: CT of the brain typically does not show any evidence of acute disease (Nash et al., 2001) In approximately one-third of patients showing change in their mental behavior as a result of WNV infection, magnetic MRI shows enhancement of the leptomeninges, the periventricular areas, or both in some cases. Hyperintensity on T2-weighed MRIs may be seen in regions such as the basal ganglia, thalami, caudate nuclei, brainstem, and spinal cord (Petropoulou et al., 2005).
3. **Electroencephalography (EEG)**: EEF can be done for patients with meningitis or encephalitis and typically shows generalized, continuous slowing, prominently in the frontal or temporal regions (Gandelman-Marton et al., 2003). Electrodiagnostic studies of the patients of WNV infection with acute flaccid paralysis show normal sensory nerve action potentials (SNAPs) with compound motor action potentials (CMAPs) varying between normal and markedly decreased, depending on the degree of paralysis (Li et al., 2003).

Virus Isolation

WNV can be isolated to detect viral antigen or nucleic acid from CSF, tissue blood, or other body fluids. To propagate WNV, live cell cultures or suckling mice can be used, and isolation must be performed in a laboratory that is Biosafety Level-3. Culture is less sensitive and time consuming as compared to other diagnostic techniques and is not useful for routine screening purposes and primarily remains a research tool. CSF or brain tissue culture in a human being provides a low yield of the virus, which can further be increased using nucleic acid amplification techniques such as real-time polymerase chain reaction (PCR).

Nucleic Acid Amplification Tests

Nucleic acid amplification tests (NAAT) play an important role in avian and mosquito surveillance programs (Huang et al., 2001). These techniques include real-time reverse transcription polymerase chain reaction (RT-PCR) and nucleic acid sequence-based amplification (NASBA), both of these techniques are able to detect around 50 viral RNA copies/mL (Busch et al., 2005). PCR is a sensitive technique for the identification of WNV in brain tissue, but it is not useful for detecting virus in spinal fluid. NAATs are not

routinely recommended in immunocompetent hosts and may be diagnostically used for the identification of WNV infection in patients who are immunocompromised and those at high risk for CNS involvement. Some cases documented positive NAAT in transplantation patients and patients with hematological malignancies. In them, viremia persists for a longer time period along with the delay in detectable neutralizing antibody (Hiatt et al., 2003).

Serological Tests

In patients with suspected meningitis, encephalitis, or acute flaccid paralysis, CSF examination and serological testing should be done for the detection of IgM antibody (Table 11.2). Serological tests considered being the standard test for the diagnosis of WNV

Table 11.2. West Nile virus laboratory serological and nucleic acid diagnostic tests.

Name and Type of Test	Duration of the test	Interpretation
HI: This is for the detection of IgM and IgG flavivirus antibodies. Two serum samples are taken from each patient, a sample from the acute phase and a convalescent sample taken 2 to 3 weeks later. HI test determines the presence of antibodies and documents seroconversions.	This test takes approximately 48 hours optimum	Positive result indicates flavivirus infection. This is not specific for WNV and will cross-react with antibodies against other members of the flavivirus family.
WNV IgM ELISA: This detects the presence of WNV antibodies in serum and CSF.	Different formats exist that differ in the time required to perform the assay. Commercial kits (e.g., Focus, PanBio IgM ELISA) can be completed in 4–5 hours. The CDC IgM ELISA takes 48 hours to obtain the results.	Positive results generally suggest recent WNV infection (persistence of IgM antibodies can be a factor in test interpretation). IgM antibody in CSF strongly suggests CNS infection.
Flavivirus IgG ELISA: To detect the presence of IgG flavivirus antibodies. Two serum samples are taken, a sample from the acute phase and a convalescent sample taken 2 to 3 weeks later. IgG ELISA determines the presence of antibodies and can document seroconversions.	Paired (acute and convalescent) serum samples taken 2 to 3 weeks apart. The test takes approximately 1 day	Positive result indicates flavivirus infection. This test is also not specific for WNV and is extremely cross-reactive with other flavivirus antibodies
PRNT: Tests for the presence of WNV neutralizing antibodies and is a confirmatory serologic test.	This test takes at least 7 days	Confirms current WNV infection by detecting the presence of specific WNV neutralizing antibodies in convalescent sera
RT-PCR: A less frequently used test to demonstrate the presence of WNV genome in patient samples such as cerebrospinal fluid, blood, and brain tissue.	This test takes approximately 24 hours.	Confirms WNV

CNS, central nervous system; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition; IgG, immunoglobulin G; IgM, immunoglobulin M; PRNT, plaque reduction neutralization test; RT-PCR, reverse transcriptase-polymerase chain reaction; WNV, West Nile virus.

infection. In cases of acute infection WNV antibodies can be detect and can be confirmed by demonstrating a fourfold increase in antibody titer between acute and convalescent serum samples. There is variability in the appearance of detectable WNV antibody because serial testing of asymptomatic blood donors reported immunoglobulin M (IgM) antibody approximately 9 days and immunoglobulin G (IgG) antibody approximately 2 weeks following viremia (Hayes et al., 2005). Because IgM antibody does not cross the blood-brain barrier, presence of IgM in CSF strongly suggests infection of the CNS. For the detection of IgM antibody to WNV, IgM antibody capture enzyme-linked immunosorbent assay is used, which is simple, sensitive, and applicable to serum and CSF samples. IgM antibody to WNV may persist for 6 months or longer. Because most infected persons are asymptomatic, in endemic areas residents may have detectable IgM antibody from previous WNV infection, which is unrelated to their current clinical illness (Roehrig et al., 2003). Other important serological tests include indirect immunofluorescence (IFA) and hemagglutination inhibition (HI) test (*see* Table 11.2), which are also useful in detecting WNV infection. Although WNV antibodies can cross-react serologically with members of the Japanese encephalitis virus serogroup and other related flaviviruses, which include SLE, Japanese encephalitis, yellow fever, and dengue. Hence in cases of diagnostic uncertainty, plaque reduction neutralization testing (PRNT) may be used to discriminate between the different flaviviruses, which can help distinguish false-positive results of MAC-ELISA or other assays, as well as serologic cross-reactions among the flaviviruses.

Clinical Management

Most of the people who contract WNV infection are asymptomatic, therefore, they do not require special medical treatment; most people with mild symptoms can care for themselves at home. Medical management of severe illness involves supportive and may include hospitalization, intravenous fluids, respiratory support (ventilator treatment), and prevention of secondary infections. Patients with severe meningeal symptoms require pain control for headaches, antiemetic therapy, and rehydration to control associated nausea and vomiting. Patients with severe encephalitis should be observed for development of elevated intracranial pressure and seizures, and patients with encephalitis or paralysis must be monitored for inability to protect the airway. Acute neuromuscular respiratory failure may develop rapidly, particularly in patients with prominent bulbar signs; these patients required immediate prolonged ventilatory support (Leis et al., 2003).

Antibiotic Therapy

In in vitro conditions, ribavirin and alpha interferon therapy found to be effective against the virus; however, a patient treated with both agents did not recover completely (Jordan et al.,2000; Weiss et al., 2001). Broad-spectrum antibiotic therapy should also be supplemented to eliminate secondary and opportunistic infections that probably occur following WNV infection.

Passive Immunization

WNV-specific immunoglobulin and antisense gene-targeted compounds have been considered as specific treatments for WNV disease but only before cerebral infection.

The precise timing of WNV infection in a human being is usually undetermined, and most of the individuals do not seek treatment before severe illness set in, hence the administration of antibodies (passive immunization) may provide limited use therapeutically. As prophylaxis, however, antibodies could prove useful for individuals at high risk of infection (Roehrig et al., 2001). Nonspecific immunoglobulin and plasmapheresis should be considered for patients with Guillain-Barré syndrome but are not indicated for patients with paralysis because of damage of anterior horn cells (Sejvar et al., 2003).

Vaccination

A variety of WNV vaccines are in various stages of testing. Because of the low incidence of disease in humans, and the sporadic nature of most outbreaks, it is difficult to target human populations for vaccination and to assess the economic feasibility of a human vaccine. An equine vaccine has been in use since 2001 and was licensed by the US Department of Agriculture in 2003. This vaccine is a formalin-inactivated WNV and was found to prevent development of viremia in 94 percent of immunized horses (Ng et al., 2003). Killed vaccines, although safe, may have to be administered in multiple doses to elicit and sustain an immune response because the manufacturer of the commercially available vaccine recommends annual revaccination. A live attenuated WNV/dengue virus serotype 4 chimera showed a strong neutralizing antibody response and prevented viremia in monkeys challenged with WNV (Pletnev et al., 2003). Additional vaccines include recombinant DNA vaccines expressing the prM and E (Davis et al., 2001) or capsid proteins and a recombinant E protein subunit vaccine (Wang et al., 2001)

Investigation of Outbreaks

WNV should be strongly considered in patients that have the onset of unexplained febrile illness, encephalitis or meningitis, or flaccid paralysis during mosquito season. Evidence of WNV enzootic activity or other human cases, either locally or in a region where the patient has traveled, should raise the index of suspicion. Year-round transmission is possible in temperate climates

Case Description

A case of WNV is nonspecific, self-limited, febrile illness caused by infection with WNV, a mosquito-borne *Flavivirus*. Clinical disease generally occurs 2 to 6 days (range, 2–14 days) following the bite of an infected mosquito. Typical cases are characterized by the acute onset of fever, headache, arthralgias, myalgias, and fatigue. Maculopapular rash and lymphadenopathy generally are observed in less than 20 percent of cases; illness typically lasts 2 to 7 days. Laboratory and surveillance criteria of WNV infections involve the study of all the surveillance types, such as mosquitoes, sentinel species, avian, human, and veterinary (Table 11.3).

There is a little difference in the chronic form of the disease in humans with CNS involvement, which can be assessed as possible, probable, confirmed, and non-case of West Nile encephalitis (Figure 11.6).

Table 11.3. West Nile virus Laboratory and surveillance case criteria.

Surveillance Type	Laboratory-Confirmed WNV Infection	Laboratory-Probable WNV Infection
Mosquito	WNV isolation: Presence of virus established by at least two of the following techniques: 1. Positive RT-PCR test for WNV RNA with validation by a. repeated positive test using different primers, b. positive PCR result using another system (e.g., TaqMan), or c. virus isolation. 2. Detection of WNV antigen (e.g., IFA, EIA, VecTestTM) validated by a. inhibition test (for ELISA), b. RT-PCR, or c. virus isolation	1. Positive RT-PCR test for WNV RNA in a single test 2. Antigen detection not validated by another procedure
Sentinel species	1. WNV isolation, RNA detection, or antigen detection as described for mosquitoes, 2. Seroconversion to WNV in serially collected serum specimens, by plaque-reduction neutralization* 3. Detection of IgM antibody to WNV, validated by demonstration of neutralizing antibody to WNV*	1. Detection of IgM antibody to WNV 2. Seroconversion to WNV in serially collected serum specimens, strongly reactive by EIA or IFA
Avian mortality	WNV isolation, RNA detection, or antigen detection similar to as described for mosquitoes	1. Positive RT-PCR test for WNV RNA in a single test 2. Antigen detection not validated by another procedure
Human and Veterinary (non avian)	**In West Nile fever** 1. Fourfold or greater change in WNV-specific serum antibody titer 2. Isolation of WNV from or demonstration of specific WNV antigen or genomic sequences in tissue, blood, CSF, or other bodily fluid or 3. WNV-specific IgM antibodies first demonstrated in serum by antibody-capture enzyme immunoassay and further confirmed by demonstration of WNV-specific serum neutralizing antibodies in the same or a later specimen.	**In West Nile fever** WNV-specific serum IgM antibodies detected initially by antibody-capture enzyme immunoassay with the unavailability of the results of a confirmatory test for WNV-specific serum neutralizing antibodies in the same or a later specimen.
	In West Nile meningitis/encephalitis An encephalitis or meningitis case occurring during a period when arboviral transmission is likely, and with the following supportive serology: 1. A single or stable (less than or equal to twofold change) but elevated titer of virus-specific serum antibodies; or 2. Serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus specific serum IgG antibodies in the same or a later specimen	**In West Nile meningitis/encephalitis** An encephalitis or meningitis case that is laboratory confirmed.

CSF, cerebrospinal fluid; EIA, enzyme immunoassays; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescent assay; IgM, immunoglobulin M; PCR, polymerase chain reaction; PRNT, plaque reduction neutralization test; RT-PCR, reverse transcription-polymerase chain reaction; SLE, St. Louis encephalitis; WNV, West Nile virus.
*SLE virus infection should be ruled out by cross-neutralization, criterion for PRNT positive is a 90 percent neutralization titer of at least 1:10, and fourfold greater titer compared to other flaviviruses such as SLE.

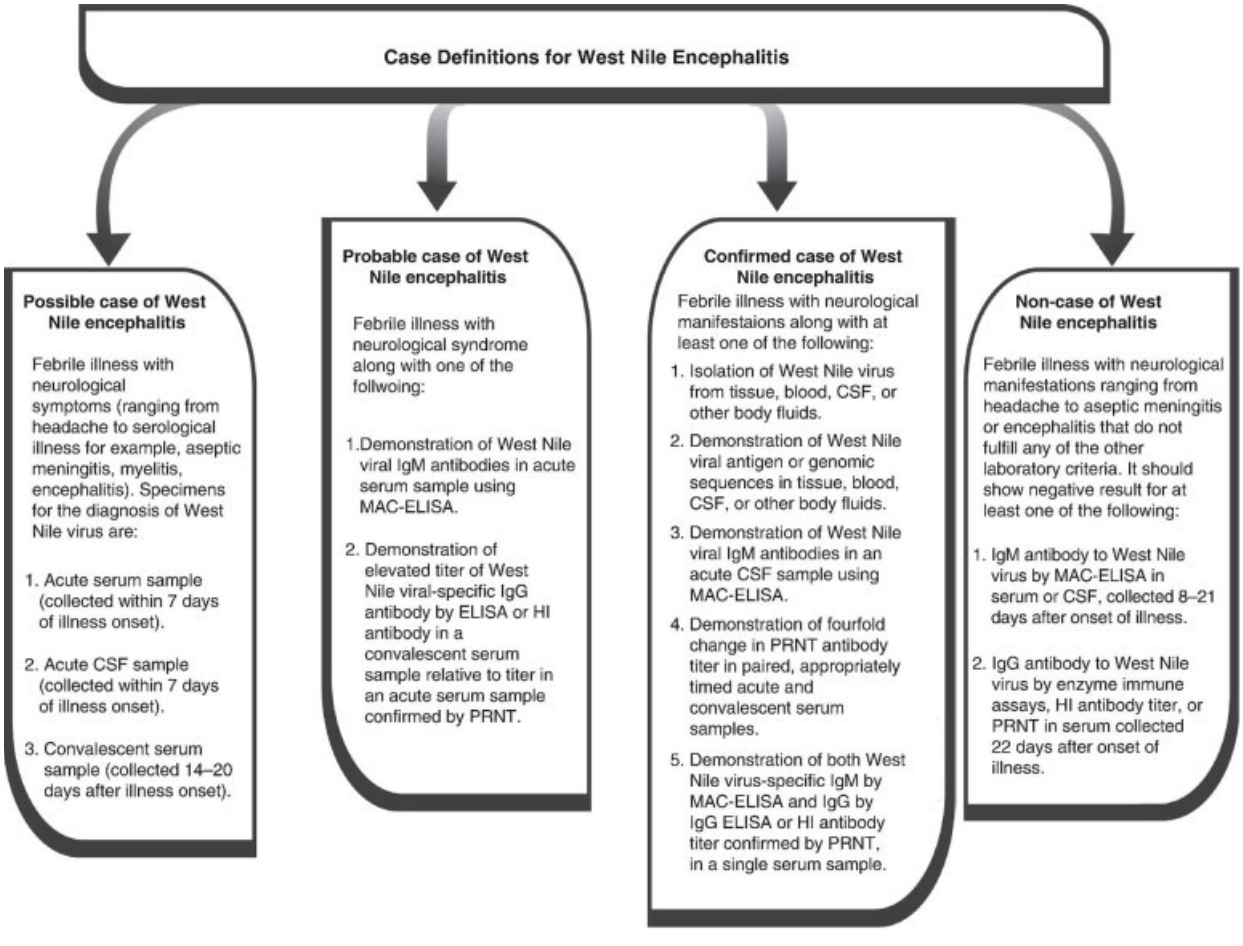

Figure 11.6. Case definitions for West Nile encephalitis in human beings. CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition; IgG, immunoglobulin G; IgM, immunoglobulin M; PRNT, plaque reduction neutralization testing.

Prevention and Control

Prevention and control of WNV diseases can be achieved through comprehensive, integrated mosquito management program using sound integrated pest management (IPM) principles. IPM-based systems employ a variety of physical, mechanical, cultural, biological, and educational measures, singly or in appropriate combination, to achieve the desired pest population control. Integrated mosquito management programs particularly designed to minimize risk of WNV transmission and prevent infections in both humans and domestic animals should include the following components.

Surveillance

Effective mosquito control should start with a sustained, consistent surveillance program that targets pest and vector species, identifies and maps their immature habitats by season, and documents the need for control. The surveillance methodologies used by mosquito control agencies are strictly depending on the stage of mosquito and the virulence of viral strain as.

Larval Mosquito Surveillance

This type of surveillance involves sampling of a wide range of aquatic habitats for the presence vector species during their developmental stages. This requires a team of trained inspectors to collect larval specimens on a regular basis from known larval habitats and to perform systematic surveillance for new sources. This surveillance type requires properly trained mosquito identification specialists that can separate nuisance and vector mosquito species, design effective mosquito control programs targeting these populations, and avoid managing habitats that support benign species.

Adult Mosquito Surveillance

This surveillance monitors species of adult mosquitoes in a particular geographical area. Various methods are available for the effective collecting of a variety of mosquito species. Pigeon-baited traps could be employed to measure host-seeking *Culex* mosquitoes that amplify SLE and WNV. For collecting day-active mosquitoes like *Aedes albopictus*, sample sizes may be enhanced by using carbon-dioxde–baited CDC miniature light traps during daylight hours or by using alternative trap configurations (e.g., Fay trap or traps using counterflow geometry.

Virus Surveillance

The purpose of virus surveillance is to determine the prevalence of WNV in the mosquito population, which can be expressed as the number of WNV-positive mosquito pools of a given species collected at a defined location and time period. The number of positive pools does not simply provide an exact index of virus prevalence in the vector population, it also provides the proportion of the mosquito population carrying the virus. This should be expressed as the infection rate (IR), which is an estimated number of infected individual mosquitoes per 1,000 specimens tested. This is a useful index of virus prevalence. The IR can be calculated by

$$\text{Infection rate} \rightarrow \frac{\text{No. of positive pools}}{\text{Total No. of specimens tested}} \times 1{,}000$$

Elevated IRs sustained over several weeks or in populations of opportunistic blood-feeders acting as bridge vectors are indicators of increased WNV transmission risk.

Source Reduction

Source reduction can be done by the alteration or elimination of mosquito larval habitat breeding. This is the most effective and economical method of providing long-term mosquito control in many habitats. Source reduction can be done by many integrated activities, such as proper disposal of used tires and cleaning of rain gutters, bird baths, and unused swimming pools by individual property owners and extensive regional water-management projects conducted by mosquito control agencies on state or federal lands. Source reduction activities can be achieved through proper sanitation and water management.

Sanitation

The by-products of human activities are the major contributor in creating mosquito breeding habitats. Sanitation, such as tire removal, stream restoration, catch-basin cleaning, and container removal, is a major part of all integrated vector management programs. The sanitation problems include neglect, oversight, or lack of information on the part of property owners. To overcome these sanitation problems, videos, slide shows, and fact sheets should be distributed at press briefings, fairs, schools, and other public areas.

Water Management

Water management for mosquito control is conducted in fresh and saltwater breeding habitats. Water-management programs can be achieved through:

1. **Impoundment management**: These are mosquito-producing marshes around which dikes are constructed, thereby allowing water to stand or to be pumped onto the marsh surface from the adjacent estuary. This eliminates mosquito oviposition sites on the impounded marsh and effectively reduces their populations.
2. **Open marsh water management (OMWM)**: OMWM is a technique whereby mosquito-producing locations on the marsh surface are connected to deep-water habitat (e.g., tidal creeks, deep ditches) with shallow ditches. Mosquito broods are controlled without pesticide use by allowing larvivorous fish access to mosquito-producing depressions. OMWM can also include establishing or improving a hydrological connection between the marsh and estuary, providing natural resource enhancement and mosquito control benefits. For this purpose, the shallow ditching (approximately 3 feet or less in depth) is environmentally more acceptable than deep ditching.

Chemical Control

When source reduction and water management are not feasible or have failed as a result of unanticipated problems, or when surveillance indicates the presence of infected adult mosquito, insecticide chemicals could be used directing against either the immature or the adult stage of the mosquito life cycle. Chemicals used by mosquito control agencies must comply with state and federal requirements.

Larviciding

The application of chemicals to kill mosquito larvae or pupae by ground or aerial treatments is termed *larviciding*. This is more effective and target-specific than adulticiding methods but less permanent than source reduction. The objective of larviciding is to control the immature stages at the breeding habitat before adult populations have had a chance to disperse and to maintain populations at levels at which the risk of arbovirus transmission is minimal. Several materials in various formulations are labeled for mosquito larviciding, including the organophosphate temephos (Abate); several biological larvicides such as *Bacillus thuringiensis israelensis* (Bti, a bacterial larvicide) and *Bacillus sphaericus*; methoprene, an insect growth regulator (e.g., Altosid); several larvicidal oils (e.g., petroleum-based Golden Bear and mineral-based Bonide) and monomolecular surface films (e.g., Agnique, Arosurf); and in some limited habitats diflubenzuron (e.g., Dimilin, a chitin synthesis inhibitor). Use of larvicides includes smaller area (as larvae are concentrated) than adulticides. Larvicide material used for this purpose should be highly specific for mosquitoes, minimizing the impacts on nontarget organisms.

Adulticiding

Adulticiding is the application of pesticides to kill adult mosquitoes. This is also an important component of any integrated mosquito management program. It is the *only* practical control technique available when surveillance data indicate the necessity to reduce the density of adult mosquito populations to lower the risk of WNV transmission. Mosquito adulticides are applied as an ultra-low-volume (ULV) spray in which small amounts of insecticide are dispersed either by truck-mounted equipment or from fixed-wing or rotary aircraft. Adulticides used for mosquito control include several organophosphates such as malathion and naled. Some natural pyrethrins and synthetic pyrethroids (permethrin, resmethrin, and sumithrin) can also be used as an adulticide.

Resistance Management

Integrated vector management programs should also include a resistance management component to prevent the development of insecticide resistance in vector populations. Ideally, this should include annual monitoring of the status of resistance in the target populations especially to:

- Provide baseline data for program planning and pesticide selection before the start of control operations.
- Detect resistance at an early stage so that timely management can be implemented.
- Continuously monitor the effect of control strategies on resistance. Monitoring techniques should include:
  (a) **Management by Moderation**: by preventing onset of resistance through
    1. Using dosages not lower than the defined rate to avoid genetic selection.
    2. Using less frequent applications.
    3. Using chemicals of short environmental persistence to prevent drug resistance.
    4. Avoiding slow-release formulations.
    5. Avoiding the use of the same class of insecticide to control both adults and immature stages.

6. Leaving certain generations, population segments, or areas untreated.
7. Establishing high pest mosquito densities or action thresholds prior to insecticide application.
8. Alternation of biorational larvicides and insect growth regulators annually or at longer intervals.

(b) **Management by continued suppression**: a strategy used in areas of high value (e.g., heavy tourist areas) or where arthropod vectors of disease must be kept at low densities. This can be achieved by the application of dosages within defined rates but sufficiently high to be lethal to susceptible and to heterozygous-resistant individuals.

(c) **Management by multiple attack**: achieving control through the action of several different and independent processes such that selection for any one of them would be below that required for the development of resistance. This strategy involves the use of insecticides with different modes of action in mixtures or in rotations.

Biological Control

Biological control is the process of using biological organisms or their by-products to control pest population. It is a highly host-specific control and virtually without non-target effects. Larvivorous fishes are the most extensively used bio-control agents for mosquitoes. Predaceous fish, typically *Gambusia* or other species, occur naturally in many aquatic habitats and can be placed in permanent or semi-permanent water bodies where mosquito larvae occur. Other bio-control agents are the predaceous mosquito *Toxorhynchites*, predacious copepods, the parasitic nematode *Romanomermis*, and the fungus *Lagenidium giganteum*. Bio-control is an important tool and will play a larger role in mosquito control in the future but will likely be effective only as part of an integrated approach.

Health Education and Public Information

The health education and public information programs are to inform the public about WNV, promote the adoption of preventive behaviors that reduce disease risk, and gain public support for control measures. These programs include the use of print materials (posters, brochures, fact sheets), electronic information (Web sites), presentations (health experts or peers speaking to community groups), and the media.

Key West Nile Virus Prevention Messages

- Address multiple levels at which prevention can occur: personal protection (use of repellent on skin and clothing, use of protective clothing, awareness of prime mosquito-biting hours); household protection (eliminating mosquito breeding sites, repairing/installing screens); and community protection (reporting indicators as dead birds, advocating for organized mosquito abatement, participating in community mobilization).
- Use of DEET-based repellents on skin and clothing is the backbone of personal protection.
- Emphasize the feasibility of actions that can lower an individual's WNV risk through personal protection measures.

- The hours from dusk until dawn are prime mosquito-biting hours and that protecting oneself through repellent use during these hours is important, with the option of remaining indoors.

Selected Best Practices

Targeted Prevention
Some groups require specific targeting as:

- Persons over age 50: US surveillance data indicate that persons over age 50 are at higher risk for severe disease and death resulting from a WNV infection. Hence activities should be identified in areas where older adults may be exposed to mosquito bites (e.g., jogging, golf, gardening).
- Persons with outdoor exposure: The persons engaged in extensive outdoor work or recreational activities are at greater risk of being bitten by WNV-infected mosquitoes. Opportunities should be developed to inform people who engage in outdoor activities about WNV and encourage them to use of repellent and protective clothing.
- Homeless persons: Application of repellents with DEET or permethrin to clothing may be most appropriate for this group of people.
- Persons who live in residences lacking window screens: The absence of intact window and door screens is a likely risk factor for exposure to mosquito bites.

Partnerships with Media and the Community
Cultivate relationships with the media. Obtain media training for at least one member of staff, and designate that individual as the organization's spokesperson.

Community Mobilization and Community Outreach:
A community task force that includes civic, business, health, and environmental concerns can be valuable in achieving buy-in from various segments of society and in developing a common message against WNV.

Guidelines for a Phased Response to West Nile Virus Surveillance Data

The primary goal is to minimize the health impact of the WNV in humans, as well as in domestic and zoo animals. These programs have two major objectives:

1. To control nuisance mosquitoes.
2. To control vector mosquitoes that can transmit pathogens.

The recommended phased response to WNV surveillance data is shown in Table 11.4. Local and regional characteristics may alter the risk level at which specific actions must be taken.

The public health action should depend on interpreting the best available surveillance data in an area, in light of these general guidelines. In addition, the following factors should be considered when translating these guidelines into a plan of action:

- Current weather and predicted climate anomalies.
- Quality, availability, and timeliness of surveillance data.

Table 11.4. Suggested guidelines for phased response to West Nile virus surveillance data.

Risk Category	Probability of Human Outbreak	Definition	Recommended Response
0	None	Off-season, adult vectors inactive because of inappropriate climate.	Develop WNV response plan. Secure surveillance and control resources necessary to enable emergency response. Initiate community outreach and public education programs. Conduct audience research to develop education and community involvement. Contact community partners.
1	Remote	Spring, summer, or fall areas anticipating WNV epizootic based on previous WNV activity in the region, no current surveillance findings indicating WNV epizootic activity in the area.	Response as in category 0, plus: conduct entomological survey (inventory and map mosquito populations, monitor larval and adult mosquito density); initiate source reduction; use larvicides at specific sources identified by entomologic survey and targeted at likely amplifying and bridge vector species; maintain avian mortality, vector and virus surveillance; expand community outreach and public education programs focused on risk potential and personal protection and emphasizing residential source reduction; maintain surveillance (avian mortality, mosquito density/IR, human encephalitis/meningitis and equine illness).
2	Low	Summer or fall, areas with limited or sporadic WNV epizootic activity in birds or mosquitoes. No positives prior to August.	Response as in category 1, plus: increase larval control, source reduction, and public education emphasizing personal protection measures, particularly among the elderly; enhance human surveillance and activities to further quantify epizootic activity (e.g., mosquito trapping and testing); implement adulticide applications if vector populations exceed locally established threshold levels, emphasizing areas where surveillance indicates potential for human risk to increase.
3	Moderate	Spring, summer, or fall; areas with initial confirmation of epizootic WNV in birds before August; a horse or a human case, or sustained WNV activity in birds or mosquitoes.	Response as in category 2, plus: intensify adult mosquito control in areas where surveillance indicates human risk; initiate adult mosquito control if not already in progress; initiate visible activities in community to increase attention to WNV transmission risk (through speaker, social marketing efforts, community mobilization for source reduction, etc.); work with collaborators to reduce risks to elderly (e.g., screen repair).

(*Continued*)

Table 11.4. (*Continued*)

Risk Category	Probability of Human Outbreak	Definition	Recommended Response
4	High	Spring, summer, or fall; quantitative measures indicating WNV epizootic activity at a level suggesting high risk of human infection (e.g., high dead bird densities in early summer, sustained high mosquito infection rates, multiple positive mosquito species, horse or mammal cases indicating escalating epizootic transmission, or a human case and high levels of epizootic activity). Areas with early season positive.	Response as in category 3, plus: expand public information program to include TV, radio, and newspapers (use of repellents, personal protection, continued source reduction, risk communication about adult mosquito control); increase visibility of public messages, engage key local partners (e.g., government officials, religious leaders) to discuss WNV; intensify and expand active surveillance for human cases; intensify adult mosquito control program, repeating applications in areas of high risk or human cases; surveillance indicators where WNV epidemic activity has occurred in the past.

IR, infection rate; WNV, West Nile virus.

- Feasibility of the planned prevention and control activities, given existing budgets and infrastructure.
- Public acceptance of the planned prevention and control strategies.
- Expected future duration of WNV transmission.
- Other ongoing mosquito control activities, such as nuisance mosquito control or vector mosquito control for the established arboviral encephalitis infections.

References

Ahmed S, Libman R, Wesson K, Ahmed F, Einberg K. 2000. Guillain-Barre syndrome: an unusal presentation of West Nile virus infection. *Neurology* 55:144–146.

Bains HS, Jampol LM, Caughron MC, Parnell JR. 2003. Vitritis and chorioretinitis in a patient with West Nile virus infection. *Arch Ophthalmol* 121:205–207.

Bernkopf H, Levine S, Nerson R. 1953. Isolation of West Nile virus in Israel. *J Infect Dis* 93:207–218.

Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, et al. 2005. Screening the blood supply for West Nile Virus RNA by nucleic acid amplification testing. *N Engl J Med* 353:460–467.

Calisher CH. 2000. West Nile virus in the New World: Appearance, persistence, and adaptation to a new econiche-an opportunity taken. *Viral Immunol* 13:411–414.

Ceccaldi PE, Lucas M, Despres P. 2004. New insights on the neuropathology of West Nile virus. *FEMS Microbiol Lett* 233:1–6.

Chowers MY, Lang R, Nassar F, Ben-David D, Giladi M, et al. 2001. Clinical characteristics of the West Nile fever outbreak, Israel. 2000. *Emerg Infect Dis* 7:675–678.

Christy W. 2010. Different West Nile Virus genetic lineage evolving? The Horse online edition. Accessed February 19, 2013, at http://www.thehorse.com/articles/24992/different-west-nile-virus-genetic-lineage-evolving.

Darman J, Backovic S, Dike S, Maragakis NJ, Krishnan C, et al. 2004. Viral-induced spinal motor neuron death is non-cellautonomous and involves glutamate excitotoxicity. *J Neurosci* 24:7566–7575.

Davis BS, Chang GJ, Cropp B, Roehrig JT, Martin DA, et al. 2001. West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses *in vitro* a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. *J Virol* 75:4040–4047.

Diamond MS, Shrestha B, Mehlhop E, Sitati E, Engle M. 2003. Innate and adaptive immune responses determine protection against disseminated infection by West Nile encephalitis virus. *Viral Immunol* 16:259–278.

Ferguson DD, Gershman K, LeBailly A, Petersen LR. 2005. Characteristics of the rash associated with West Nile virus fever. *Clin Infect Dis* 41:1204–1207.

Galli M, Bernini F, Zehender G. 2004. Alexander the Great and West Nile Virus encephalitis. *Emerging Infect Dis* 10:1328–1333.

Gandelman-Marton R, Kimiagar I, Itzhaki A, Klein C, Theitler J, et al. 2003. Electroencephalography findings in adult patients with West Nile virus-associated meningitis and meningoencephalitis. *Clin Infect Dis* 37:1573–1578.

Guarner J, Shieh WJ, Hunter S, Paddock CD, Morken T. et al. 2004. Clinicopathologic study and laboratory diagnosis of 23 cases with West Nile virus encephalomyelitis. *Hum Pathol* 35:983–990.

Hayes EB, Sejvar JJ, Zaki SR, Lanciotti RS, Bode AV, et al. 2005. Virology, pathology, and clinical manifestations of West Nile virus disease. *Emerg Infect Dis* 11:1174–1179.

Hershberger VS, Augsburger JJ, Hutchins RK, Miller SA, Horwitz JA, et al. 2003. Chorioretinal lesions in nonfatal cases of West Nile virus infection. *Ophthalmology* 110:1732–1736.

Hiatt B, Desjardin L, Carter T, Gingrich R, Thompson C, et al. 2003. A fatal case of West Nile virus infection in a bone marrow transplant recipient. *Clin Infect Dis* 37:129–131.

Hogan, CM. 2008. Barbary macaque: *Macaca sylvanus*. GlobalTwitcher.com. Accessed February 19, 2013, at http://globaltwitcher.auderis.se/gt2/artspec_information.asp?thingid=31757.

Huang C, Slater B, Campbell W, Howard J, White D. 2001. Detection of arboviral RNA directly from mosquito homogenates by reverse-transcription-polymerase chain reaction. *J Virol Methods* 94:121–128.

Jordan I, Briese T, Fischer N, Lau JY, Lipkin WI. 2000. Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. *J Infect Dis* 182:1214–1217.

Kaiser PK, Lee MS, Martin DA. 2003. Occlusive vasculitis in a patient with concomitant West Nile virus infection. *Am J Ophthalmol* 136:928–930.

Kanagarajan K, Ganesh S, Alakhras M, Go ES, Recco RA, et al. 2003. West Nile virus infection presenting as cerebellar ataxia and fever: case report. *South Med J.* 96:600–601.

Kleinschmidt-DeMasters BK, Marder BA, Levi ME, Laird SP, McNutt JT, et al. 2004. Naturally acquired West Nile virus encephalomyelitis in transplant recipients: Clinical, laboratory, diagnostic, and neuropathological features. *Arch Neurol* 61:1210–1220.

Kuchtey RW, Kosmorsky GS, Martin D, Lee MS. 2003. Uveitis associated with West Nile virus infection. *Arch Ophthalmol* 12:1648–1649.

Kumar D, Prasad GV, Zaltzman J, Levy GA, Humar A. 2004. Community acquired West Nile virus infection in solid-organ transplant recipients. *Transplantation* 77:399–402.

Kuno G. 2001. Persistence of arboviruses and antiviral antibodies in vertebrate hosts: its occurrence and impacts. *Rev Med Virol* 11:165–190.

Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, et al. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286:2333–2337.

Leis AA, Stokic DS, Webb RM, Slavinski SA, Fratkin J. 2003. Clinical spectrum of muscle weakness in human West Nile virus infection. *Muscle Nerve* 28:302–308.

Leis AA, Stokic DS. 2005. Neuromuscular manifestations of human West Nile virus infection. *Curr Treat Options Neurol* 7:15–22.

Li J, Loeb JA, Shy ME, Shah AK, Tselis AC, et al. 2003. Asymmetric flaccid paralysis: A neuromuscular presentation of West Nile virus infection. *Ann Neurol* 53:703–710.

Limesand KH, Higgs S, Pearson LD, Beaty BJ. 2003. Effect of mosquito salivary gland treatment on vesicular stomatitis New Jersey virus replication and interferon alpha/beta expression in vitro. *J Med Entomol* 40:199–205.

List of West Nile virus outbreaks. n.d. Wikipedia. Accessed March 25, 2013, at http://en.wikipedia.org/wiki/List_of_West_Nile_virus_outbreaks.

Marr JS, Calisher CH. 2009. Alexander the Great and West Nile Virus encephalitis. Centers for Disease Control and Prevention. Accessed February 19, 2013, at http://wwwnc.cdc.gov/eid/article/9/12/03-0288_article.htm.

Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, et al. 2001. Epidemic West Nile encephalitis, New York, 1999: Results of a household-based seroepidemiological survey. *Lancet* 358:261–264.

Nash D, Mostashari F, Fine A, Miller J, O'Leary D, et al. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med* 344:1807–1814.

Ng T, Hathaway D, Jennings N, Champ D, Chiang YW, et al. 2003. Equine vaccine for West Nile virus. *Dev Biol* 114:221–227.

Park M, Hui JS, Bartt RE. 2003. Acute anterior radiculitis associated with West Nile virus infection. *J Neurol Neurosurg Psychiatry* 74:823–825.

Pepperell C, Rau N, Krajden S, Kern R. 2003. West Nile virus infection in 2002: morbidity and mortality among patients admitted to hospital in southcentral Ontario. *CMAJ* 168:1399–1405.

Peterson LR, Marphin AA, Gubler DJ. 2003. West Nile virus. *JAMA* 290:524–528.

Petropoulou KA, Gordon SM, Prayson RA, Ruggierri PM. et al. 2005. West Nile virus meningoencephalitis: MR imaging findings. *Am J Neuroradiol* 26:1986–1995.

Platonov AE, Shipulin GA, Shipulina OY, Tyutyunnik EN, Frolochkina TI, et al. 2001. Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. *Emerg Infect Dis* 7:128–132.

Pletnev AG, Claire MS, Eikins R, Speicher J, Murphy BR, et al. 2003. Molecularly engineered live-attenuated chimeric West Nile/dengue virus vaccines protect rhesus monkeys from West Nile virus. *Virology* 314:190–195.

Roehrig JT. 1999. Arboviruses. In: Specter S, Hodinka RL, Young SA, eds., Clinical Virology Manual, 3rd ed., pp. 356–373. Washington, American Society for Microbiology.

Roehrig JT, Nash D, Maldin B, Labowitz A, Martin DA, et al. 2003. Persistence of virus-reactive serum immunoglobulin m antibody in confirmed west nile virus encephalitis cases. *Emerg Infect Dis* 9:376–379.

Roehrig JT, Staudinger LA, Hunt AR, Mathews JH, Blair CD. 2001. Antibody prophylaxis and therapy for flavivirus encephalitis infections. *Ann N Y Acad Sci* 951:286–297.

Scherret JH, Poidinger M, Mackenzie JS, Broom AK, Deubel V, et al. 2001. The relationships between West Nile and Kunjin viruses. *Emerg Infect Dis* 7:697–705.

Schneider BS, McGee CE, Jordan JM, Stevenson HL, Soong L, et al. 2007. Prior exposure to uninfected mosquitoes enhances morality in naturally-transmitted West Nile virus infection. *PLoS ONE* 2(11):e1171.

Schneider BS, Soong L, Girard YA, Campbell G, Mason P, et al. 2006. Potentiation of West Nile encephalitis by mosquito feeding. *Viral Immunol* 19:74–82.

Schneider BS, Soong L, Zeidner NS, Higgs S. 2004. *Aedes aegypti* salivary gland extracts modulate anti-viral and TH1/TH2 cytokine responses to Sindbis virus infection. *Viral Immunol* 17:565–473.

Sejvar JJ, Leis AA, Stokic DS, Van Gerpen JA, Martin AA, et al. 2003a. Acute flaccid paralysis and West Nile virus infection. *Emerg Infect Dis* 9:788–793.

Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Pape J, et al. 2005. West Nile virus-associated flaccid paralysis. *Emerg Infect Dis* 11:1021–1027.

Sejvar JJ, Haddad MB, Tierney BC, Campbell GL, Marfin AA, et al. 2003b. Neurologic manifestations and outcome of West Nile virus infection. *JAMA* 290:511–515.
Smithburn KC, Hughes TP, Burke AW, Paul JH. 1940. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med* 20:471–492.
St Leger J, Wu G, Anderson M, Dalton L, Nilson E, et al. 2011. West Nile virus infection in killer whale, Texas, USA, 2007. *Emerg Infect Dis* 17:1531–1533.
Styer LM, Bernard KA, Kramer LD. 2006. Enhanced early West Nile virus infection in young chickens infected by mosquito bite: Effect of viral dose. *Am J Trop Med Hyg* 75:337–345.
Taylor RM, Hurlbut HS, Dressler HR, Spangler EW, Thrasher D. 1953. Isolation of West Nile virus from Culex mosquitoes. *J Egypt Med Assoc* 36:199–208.
Tyler KL, Pape J, Goody RJ, Corkill M, Kleinschmidt-DeMasters BK. 2006. CSF findings in 250 patients with serologically confirmed West Nile virus meningitis and encephalitis. *Neurology* 66:361–365.
Venter M, Human S, van Niekerk S, Williams J, Van Eeden C, et al. 2011. Fatal neurologic disease and abortion in mare infected with lineage 1 West Nile virus, South Africa. *Emerg Infect Dis* 17:1534–1536.
Wanasen N, Nussenzveig RH, Champagne DE, Soong L, Higgs S. 2004. Differential modulation of murine host immune response by salivary gland extracts from the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*. *Med Vet Entomol* 18(2):191–199.
Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, et al. 2004. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med* 10:1366–1373.
Wang T, John F. Anderson JF, Magnarelli LA, Wong SJ, et al. 2001. Immunization of mice against West Nile virus with recombinant envelope protein. *J. Immunol* 167:5273–5277.
Watson JT, Pertel PE, Jones RC, Siston AM, Paul WS, et al. 2004. Clinical characteristics and functional outcomes of West Nile Fever. *Ann Intern Med* 141:360–365.
Weinberger M, Pitlik SD, Gandacu D, Lang R, Nassar F, et al. 2001. West Nile fever outbreak, Israel, 2000: Epidemiologic aspects. *Emerg Infect Dis* 7(4). Accessed March 25, 2013, at http://wwwnc.cdc.gov/eid/article/7/4/01-7416_article.htm.
Weiss D, Carr D, Kellachan J, Tan C, Phillips M, et al. 2001. Clinical findings of West Nile virus infection in hospitalized patients, New York and New Jersey. *Emerg Infect Dis* 7:654–658.
West B. 2012. West Nile strike again; second case of West Nile confirmed in Brazos County. The eagle.com. Accessed February 19, 2013, at http://www.theeagle.com/news/local/article_29f717aa-fcbb-55ae-af6f-1d89fb50cebf.html.
Wire F. 2012. West Nile virus hits US, four dead. First post. Accessed February 19, 2013, at http://www.firstpost.com/fwire/west-nile-virus-hits-us-four-dead-405172.html.
Work TH, Hurlbut HS, Taylor RM. 1953. Isolation of West Nile virus from hooded crow and rock pigeon in the Nile delta. *Proc Soc Exp Biol Med* 84:719–722.

Chapter 12
Chandipura Virus Encephalitis

Epidemiology

Global

Chandipura virus (CHPV) can infect many mammalian species in different places throughout the world, but human cases have only been reported in India. Retrospective analysis revealed that this virus is highly prevalent in India (Bhatt and Rodrigues, 1967), Sri Lanka (Peiris et al., 1993), and Africa in Nigeria and Senegal (Fontenille et al. 1994; Traore-Lamizana et al. 2001). Figure 12.1 illustrates the worldwide geographical distribution of the CHPV.

India

CHPV was first isolated in the National Institute of Virology (NIV) in Pune, India, from serum samples of patients, collected during an outbreak of febrile illness in the Nagpur area of Maharashtra state, India in 1965. During investigating the outbreak, serum samples from two human patients of febrile illness were negative for dengue and Chikungunya viruses inoculated in BSC-1 cells, and surprisingly both the samples produced cytopathic effects. The recovered agent was filterable and identified as a new virus designated as Chandipura virus because of the name of the locality from where it was isolated (Bhatt and Rodrigues, 1967). Further, the same entity was also isolated from sand flies during a routine entomological investigation in Aurangabad in 1967 (Dhanda et al., 1970) and subsequently, from serum sample of a child suffering from encephalopathy in Jabalpur, Madhya Pradesh (Rodrigues et al., 1983). Apart from these sporadic cases in the states of Maharashtra and Madhya Pradesh, the CHPV did not gain any public health importance until 2003, when NIV in Pune investigated CHPV associated with a large encephalitis

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Figure 12.1. Worldwide distribution of Chandipura virus.

outbreak in children in many districts of Andhra Pradesh and Maharashtra, India (Rao et al., 2004).

Afterward many viral encephalitis outbreaks were found to be associated with CHPV; these include 2003 (Maharashtra, and Andhra Pradesh), 2004 (Gujarat), 2005 and 2007 (Maharashtra, and Andhra Pradesh) (Rao et al., 2004; Chadha et al., 2005). Some evidence suggests the existence of CHPV for more than 50 years, although CHPV has recently attained the status of an important emerging pathogen of public health. Figure 12.2 shows the distribution of Chandipura virus in various Indian states.

In 2010, an outbreak in Kheda, Vadodara, and Panchmahal district of Gujarat state killed 17 people (DNA correspondent, 2010); sand flies were considered to be responsible because they inhabit cracks in walls or home parts made up of sand or mud.

Investigation of an outbreak of acute encephalitis case in Nagpur region, Maharashtra, in 2007 among hospitalized children younger than 15 years of age, recorded total 78 cases of acute encephalitis. They tested serum and cerebrospinal fluid (CSF) for immunoglobulin M (IgM) antibodies against CHPV and Japanese encephalitis virus (JEV) and for CHPV RNA by reverse transcription-polymerase chain reaction (RT-PCR). Virus isolations were also attempted using rhabdomyosarcoma cell line. In this outbreak, the case fatality ratio was 43.6 percent, and the male to female ratio was 1:1.2; CHPV was confirmed in 39 cases (Table 12.1).

The Chandipura Virus

Chandipura virus belongs to the genus *Vesiculovirus* placed in the order of *Mononegavirales* of *Rhabdoviridae* family (Figure 12.3). Viruses of this family are designated as rhabdoviruses because of their rod-shaped and the typical bullet-shaped morphology. CHPV shows similarity in genetic makeup, polypeptide composition, life cycle, protein, and nucleic acid composition (as having single stranded RNA genome of negative sense) to vesicular stomatitis virus. Hence CHPV was included within the genus *Vesiculovirus* and

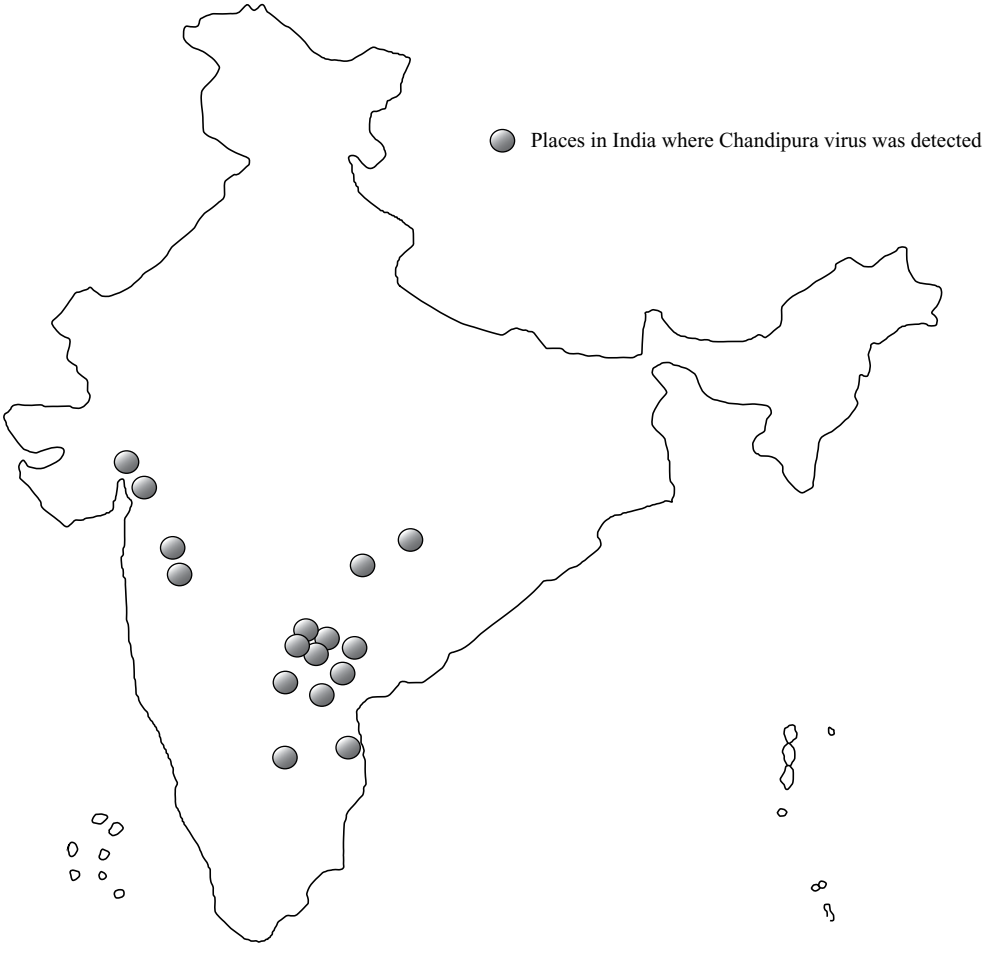

Figure 12.2. Geographic distribution of Chandipura virus in India.

Table 12.1. District-wise distribution of Chandipura virus encephalitis cases and with those of unknown etiology in the state of Maharashtra, India.

District of Maharashtra state (India)	Chandipura virus encephalitis ($n = 39$)	Unknown etiology ($n = 39$)
Bhandara	12 (30.8%)	11 (28.2%)
Wardha	11 (28.2%)	6 (15.4%)
Nagpur	4 (10.2%)	9 (23.1%)
Gondia	6 (15.4%)	5 (12.8%)
Chandrapur	2 (5.1%)	7 (17.9%)
Gadchiroli	4 (10.2%)	1 (2.6%)

n = total number of investigated cases

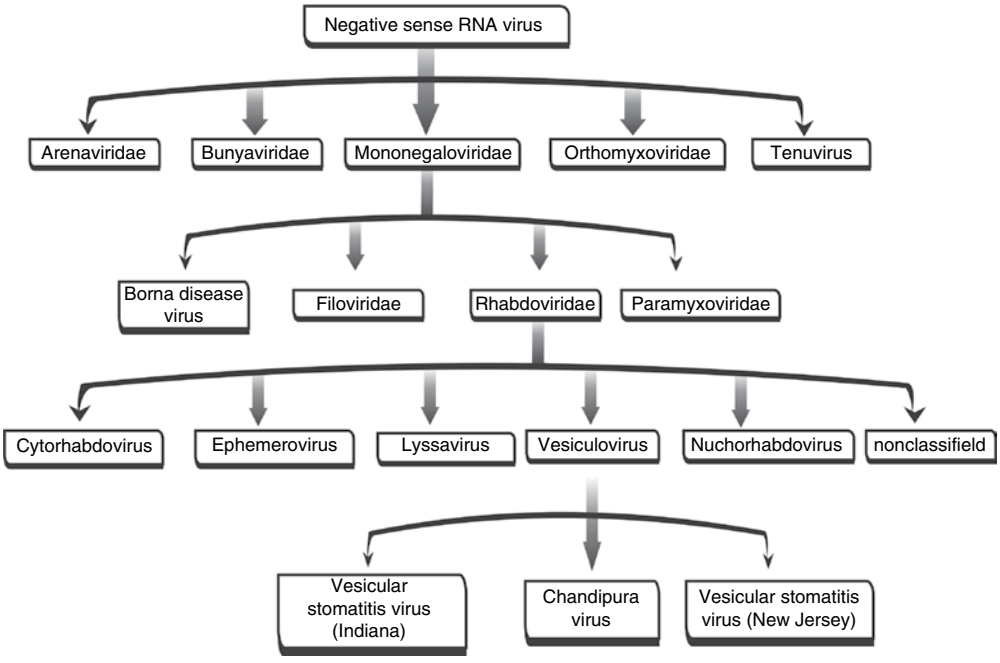

Figure 12.3. Classification of Chandipura virus.

is placed between vesicular stomatitis virus, New Jersey (VSVNJ) and vesicular stomatitis virus, Indiana (VSVI) (Rose and Whitt, 2001).

Structure and Genome Organization

CHPV is an enveloped RNA virus with an approximate genome length of 11 kb. It is 150- to 165-nm long and 50- to 65-nm wide showing distinct surface projections of 9 to 11 nm in size and stain-filled canal at the base of the virus particle (Figure 12.4).

The lipoprotein envelope of CHPV encloses a helical ribonucleoparticle (RNP) with a nonsegmented single-stranded negative sense RNA (Figure 12.5). Viral genome codes for five monocistronic mRNAs, which then undergo translation to generate five polypeptides, namely, nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and large protein (L).

CHPV genome RNA comprises of a 49 nucleotide leader sequence (l), which is followed by five transcriptional units coding for viral polypeptides separated by intragenic spacer regions and a short nontranscribed 46 nucleotide trailer sequence (t) (Figure 12.6) arranged in the order 3' l-N-P-MG-L-t 5'.

The Nucleocapsid Protein

N is 422 amino acids long polypeptide of 49 kDa coded by N gene. It is the most abundant viral protein in infected cells. It binds with the nascent leader RNA thereby encapsidating RNA genome in a manner just like histone-mediated enwrapping of a DNA molecule into a nucleosome structure (*see* Figure 12.5). This encapsidated genome is used

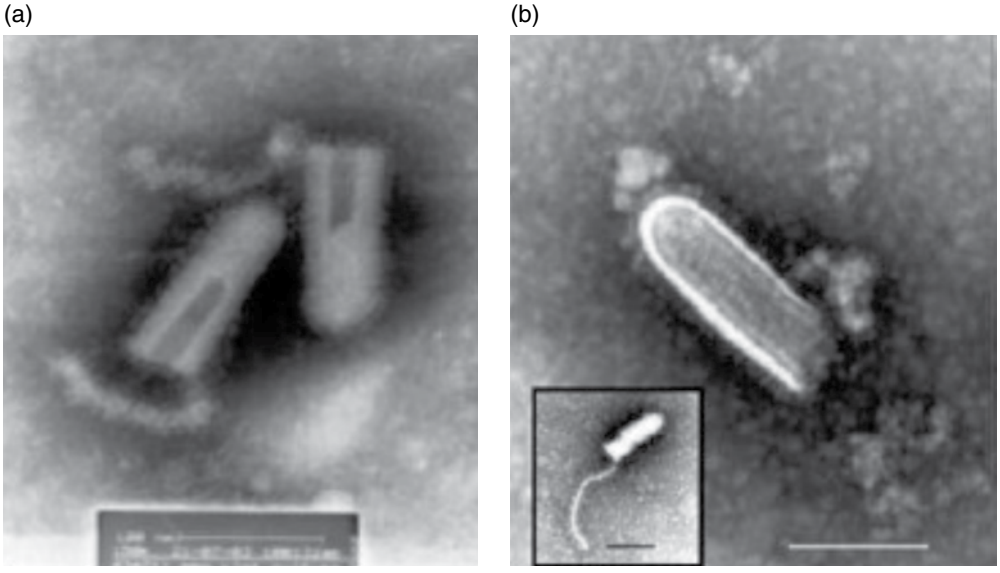

Figure 12.4. Transmission electron micrographs of primary Chandipura virus isolates from tissue culture. Bar is equal 100 nm in both micrographs. (a) Two negative-stained virus particles showing the stain-filled canals and basal attachments. (b) Negative-stained Chandipura virus particle showing typical vesiculovirus morphology, including the internal ribonucleoprotein coil. Inset shows a virus particle with a released helical ribonucleoprotein coil.
Source: Rao BL, Basu A, Wairagkar NS, Gore MM, Arankalle VA, et al. 2004. A large outbreak of acute encephalitis with high case fatality rate in children in Andhra Pradesh, India in 2003 associated with Chandipura virus. *Lancet* 364:869–874.

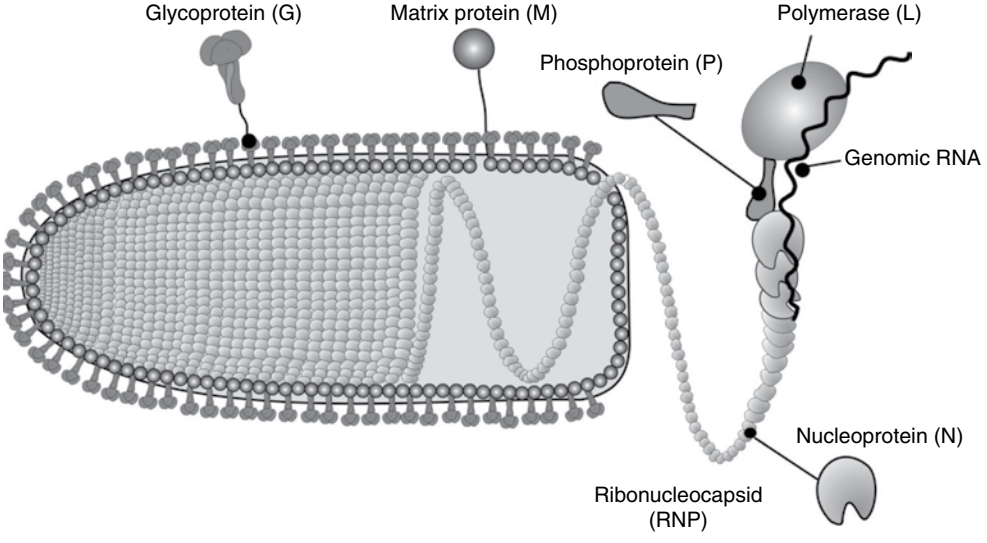

Figure 12.5. Bullet-shaped structure of Chandipura virus.

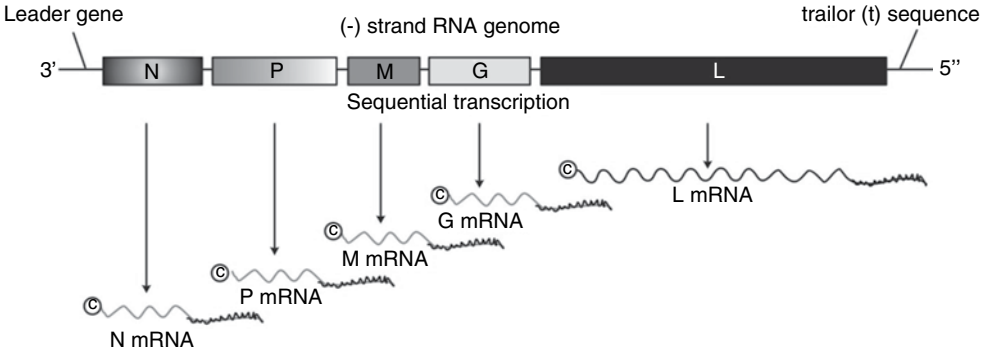

Figure 12.6. Genomic organization of a Chandipura virus.

by viral polymerase as a template during transcription and replication (Banerjee, 1987a, 1987b). The N protein protects the viral genome from host ribonucleases and plays regulatory roles in the transition from transcription to replication in the virion life cycle; hence it is designated as the transcription-replication switch (Majumdar et al., 2004; Bhattacharya et al., 2006).

Phospoprotein

P is 294 amino acids long polypeptide of 29 kDa coded by P gene. P together with the L proteins constitutes the viral RNA dependent RNA polymerase (RdRp), where L is the catalytic subunit, an activator of viral transcription and P is the cofactor.

Matrix Protein

M protein is a 229 amino acid long polypeptide of 26.3 kDa. It lies in the inner bilayer of the membrane and acts as a bridge between the encapsidated genomic RNA (called the *nucleocapsid*) and the outer membrane envelope. M protein also interacts with and recruits mature nucleocapsid particles to the membrane during viral assembly and budding. Matrix (M) protein of vesicular stomatitis virus (VSV) functions in virus assembly (also known as VSV-M) was shown to stop host transcription by RNA polymerase I and II (Ahmed and Lyles, 1998). This M protein mediated inhibition of host gene expression constitutes in the suppression of cellular interferon response (Enninga et al., 2002).

Glycoprotein

G is a 59.5 kDa protein that protrudes externally from the outer membrane and acts as a major antigenic determinant (Basak et al., 2007) and therefore, elicits an antibody response. It is the sole spike protein of CHPV that contributing in the absorption, assembly, and finally budding of newly formed viral particle. VSV G exists as a trimer (Zagouras and Rose, 1993), which reversibly adopts three distinct conformational states:

1. The native state present on the virus surface which is stable above pH 7 (Clague et al., 1990).
2. The activated state that fuses with target membrane (Durrer et al., 1995).
3. A fusion inactive post fusion state that is stable under low pH condition (Yao et al., 2003).

G of CHPV was found to be an ideal candidate for the vaccine development (Venkateshwarlu and Arankalle, 2009). In this study CHPV, G was expressed in baculovirus expression system and the purified protein was further evaluated for its immunogenicity in mice. The encouraging result obtained in this study is a testimonial for its potential as candidate vaccine

The Large Protein

L encodes for the largest viral protein of 2092 amino acids of 238.5 kDa (Marriott, 2005). Together with P, it constitutes viral transcriptase. It persists the catalytic activity of RNA polymerization, capping, and polyadenylation. L shows the protein kinase activity and also associated with cellular translation elongation factors (Banerjee, 1987a, 1987b; Das et al. 1998).

Ribonucleoprotein Particle

RNP particle is composed of core nucleocapsid (Figure 12.7) and associated viral RdRp subunits. RNP is infectious by itself because it contains all the enzymatic activity necessary for the synthesis of RNA and could produce progeny viral particles when introduced artificially within the cell (Thornton et al., 1983).

Life Cycle of Chandipura Virus

The CHPV completes its life cycle in a cytosolic manner within an infected cell (Banerjee 1987a) (*see* Figure 12.10). The life cycle can be divided into several steps, as adsorption of viral particle, penetration of virus into the host cell, uncoating and release of core RNP into the cytosol from late endosomal vesicles, transcription of the viral genome by viral polymerase, translation of viral mRNA, post-translational modifications of viral proteins, the replication of viral genome, assembly of progeny particles, and finally budding and release of mature virion particles.

Adsorption, Penetration, and Uncoating

The Life cycle of CHPV starts with the adsorption of the infectious viral particle to a receptor on the surface of host cell. The specific receptors, which mediate this adsorption process, are difficult to identify because of broader host range and the binding properties of rhabdo viral particles. The binding appears to be pH dependent, which is probably the result

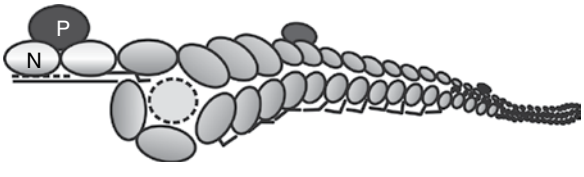

Figure 12.7. A proposed structure of Chandipura virus genome RNA encapsidated with nucleocapsid protein. Nucleocapsid protein binds to viral RNA to enclose it in a disclike structure. The disclike structures stack on each other to generate a helical assembly to form core nucleocapsid. Int region, intergenic region; L, large protein; N, nucleoprotein; P, phosphoprotein.
Source: Modified from Basak S, Mondal A, Polley S, Mukhopadhyay S, Chattopadhyay D. 2007. Reviewing Chandipura: a vesiculovirus in human epidemics. *Biosci Rep* 27(4–5):275–298.

of the pH-induced conformational changes in the G (Fredericksen and Whitt, 1998). This binding is inhibited by binding inhibitor, which was resistant to protease and neuraminidase, but it could be inactivated by phospholipase C, suggesting phospholipid helps in the binding process. After getting adsorbed the virions are endocytosed through a clathrin-dependent pathway typical of receptor-mediated endocytosis mechanism (Matlin et al., 1982). Because of the reduction in the endocytic pH, the envelope of the endocytosed virion get fused with the endosomal membrane, catalyzed by the G of the virion resulting the release of the RNP core into the host cell cytoplasm (Matlin et al., 1982), which is further followed by the dissociation of M protein from the RNP core. Both of these processes of membrane fusion and M protein dissociation contribute the uncoating event of rhabdoviruses.

Transcription and Translation

After uncoating, the viral genome undergoes primary transcription, which occurs in the absence of protein synthesis, (Davis and Wertz, 1982). The transcription process is catalyzed by the L-P3 (P protein trimer) polymerase complex of the virion (transcriptase), and genomic RNA complexed with the N protein serves as a template. The order of transcription has been determined on the basis of in vitro transcriptional mapping analysis using ultraviolet radiation, which shows polar transcription indicating polymerase activity starts at 3' end of the genome, that is, at the beginning of leader gene (Ball and White, 1976). Transcription process yield five monocistronic m-RNAs, which utilize host translational machinery to generate viral proteins.

Viral polymerase composed of L protein and phosphorylated form of P protein transcribes the genomic RNA with progressive attenuation at each intergenic region (Int Region) to synthesize leader RNA and five capped and poly-adenylated mRNA (Figure 12.8). The polymerase remains associated with N-RNA while transcribing the genomic template and reinitiates the synthesis of downstream genes after termination.

Replication

Replication process starts by a read-through mechanism in which the same polymerase read through the termination signals present at the Int Region to synthesize an exact complement of negative sense RNA genome (*see* Figure 12.8). The replicase consists of L–(N–P) complex, and in this, P is uphosphorylated. N is recruited on nascent genome RNA to protect it from cellular RNase action whereas unphosphorylated form of P is recruited to leader RNA to modulate polymerase activity during replication to bring about antitermination (*see* Figure 12.8). The genomic RNA always remain encapsidated by N and progressive encapsidation of nascent genomic RNA during its synthesis is necessary for replication because it protects replication product from cellular RNases (Banerjee 1987a; Barr et al., 2002).

Encapsidation of Genomic RNA

During encapsidation, N monomer self-assembly to form oligomer that binds to leader RNA. P associates with N to keep N in a monomeric form. This monomer N is capable of recognizing specific sequence present on leader chain (stem loop structure) in the nucleation step (I) and is recruited to the viral RNA with concomitant release of P (Figure 12.9). Subsequently, additional N molecules associates with RNA bound N monomers in the elongation phase (II). N polymerization mediates a conformational change, and thus, generates broad specificity within RNA binding interface of N protein and allows for progressive encapsidation enclosing RNA into a helical conformation.

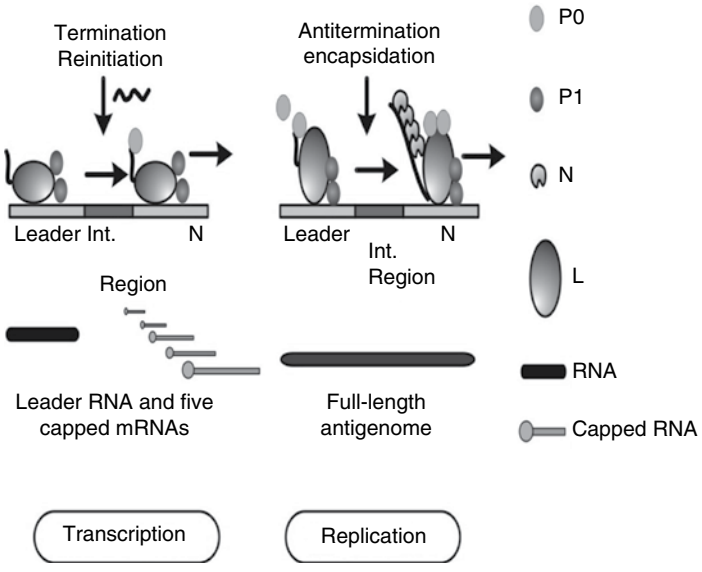

Figure 12.8. Synthesis of Chandipura viral RNA.

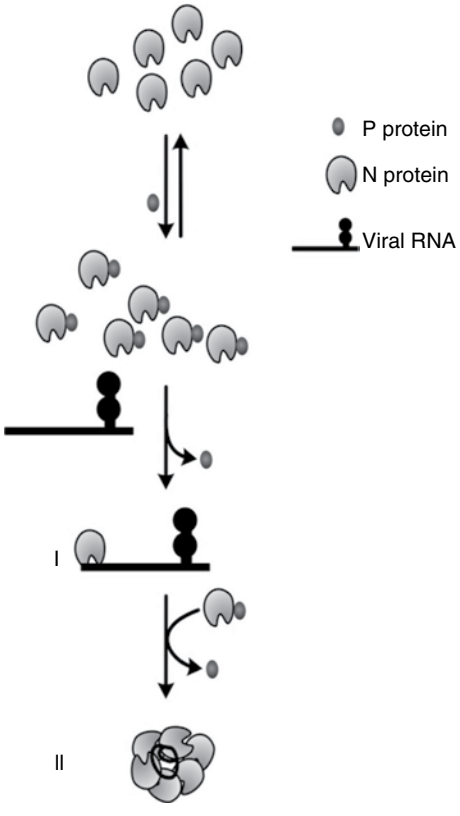

Figure 12.9. Encapsidation of Chandipura virus RNA by nucleocapsid (N).

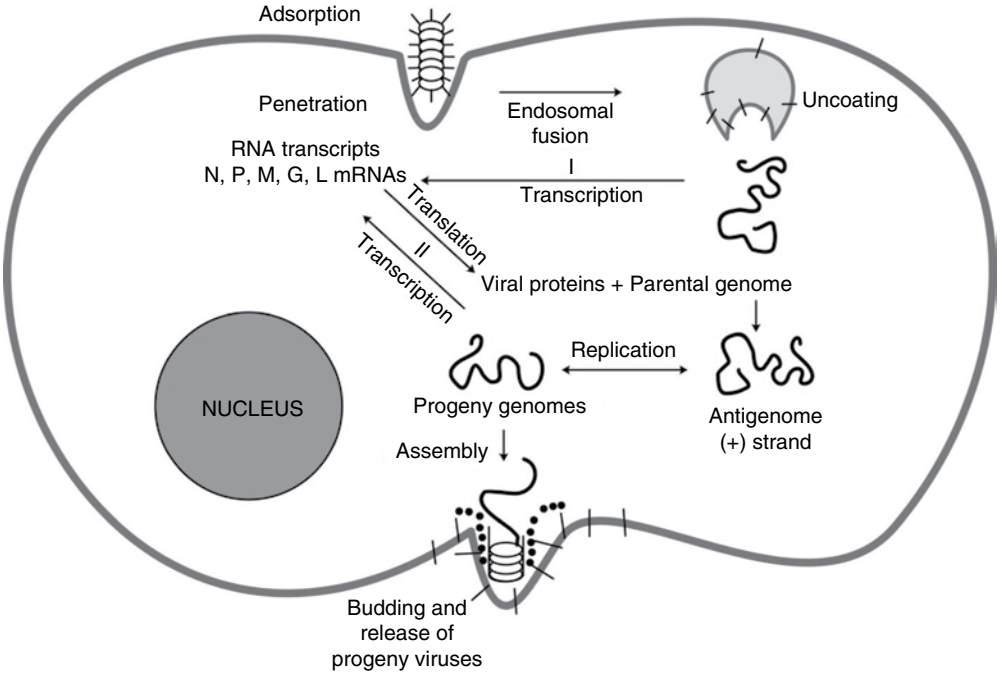

Figure 12.10. Life cycle of Chandipura virus. The steps involved are virus adsorption and penetration by endocytosis, fusion of the envelope with endosomal membranes, release of parental genome into the cytoplasm, primary (I) transcription, genome replication to produce nucleocapsids containing antigenomes (**+** strand) and progeny genomes, secondary transcription(II), and assembly by budding from host plasma membrane. G, glycoprotein; L, large protein; M, matrix protein; N, nucleoprotein; P, phosphoprotein.

Thus during replicative mode, the polymerase switches to copy entire genomic template into an exact polycistronic complement, which then acts as replication intermediate for the production of more copies of negative sense RNA upon further rounds of replication. These progeny-negative sense RNAs are also undergoing transcription, called *secondary transcription* (Figure 12.10).

Assembly and Budding

Assembly and budding of CHPV takes place at the plasma membrane. After the encapsidation of RNA genome, some cytoplasmic M protein may associate with the newly formed RNPs. G in the plasma membrane forms favorable sites (i.e., microdomains) for the initiation of budding and RNP condensation by M protein (Jayakar et al., 2004). These budding favorable sites must be formed soon after delivery of G to the cell surface because virus budding commences as soon as 2 to 3 hours post-infection.

Following association of M protein with RNPs another soluble form of M protein get deposited beneath the plasma membrane, which then interacts with the nucleocapsid forming condensed tightly coiled structures. This condensation of RNPs occurs at regions

of the plasma membrane highly concentrated with the G resulting in the formation of bud site. This interaction finally induces evagination of the membrane in which envelopment of the underlying condensed RNP core occurs via recruitment of both soluble and membrane-associated M protein into the condensing skeleton. Thus, condensation of RNPs by M protein results in the formation of bullet-shaped protrusions extending from the plasma membrane (*see* Figure 12.10).

Clinical Features

The clinical picture of patients infected with CHPV is not very clear. It varies from high-grade fever of short duration, vomiting, altered sensorium, generalized convulsions, decerebrate posture leading to Grade IV coma to acute encephalitis or encephalopathy, and death within a few hours to 48 hours of hospitalization (Rao et al., 2004). During an outbreak of acute encephalitis syndrome (AES) among children from Nagpur division, Maharashtra, India, a total 78 cases were investigated of children younger than 15 years of age to confirm the etiology of Chandipura encephalitis and also to describe clinico-epidemiological features of the disease (Table 12.2) (Gurav et al., 2010). In this study, the recorded case fatality ratio was 43.6 percent in which the male-to-female ratio was 1:1.2; Chandipura was confirmed in 39 cases.

Neurological manifestations included abnormal plantar reflex, abnormal deep tendon reflexes, sluggish pupillary reflex, hypertonia, and hypotonia. Convulsions were a generalized type with up-rolling of eyeballs. Other manifestations included tachycardia, severe breathlessness, tachypnea, crepitations, hemorrhages, urinary incontinence, anemia, leucocytosis, and raised blood urea. No statistically significant difference has been observed in clinical manifestations among confirmed Chandipura cases and cases with unknown etiology.

Table 12.2. Clinical features of Chandipura virus encephalitis cases compared with those of unknown etiology.

Clinical Features	Chandipura Virus Encephalitis, $N=38$	Unknown etiology, $N=38$
Fever less then 3 days at admission	32 (84.2)	34 (89.5)
Altered sensorium	13 (34.2)	18 (47.4)
Seizures	29 (76.3)	20 (52.6)
Skin rash	4 (10.5)	0
Gastrointestinal symptoms	9 (23.7)	8 (21.1)
Bleeding tendencies	4 (10.5)	2 (2.7)
Abnormal pupils	5 (13.6)	3 (7.9)
Shock	6 (15.8)	4 (10.5)
Headache	9 (23.7)	2 (5.3)

Figures in parentheses are percentages.

Laboratory Diagnosis

Because CHPV does not present any specific clinical picture, it is not possible to diagnose the disease simply on the basis of physical examination. To diagnose Chandipura viral encephalitis cases, some serological, molecular, and tissue culture-based methods are available, which have been suggested by the National Institute of Virus (NIV) in Pune, India.

Antigen Detection Testing

The Chandipura viral antigen can be detected in various samples, such as blood, cerebrospinal fluid (CSF), and brain tissue of diseased child using enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA). The advantages of these antigen-detection techniques are rapidity (because results can be available within hours of receipt of the specimen in the laboratory) and lack of requirement for viral viability in the specimen, allowing greater flexibility in the handling and transport of specimens. This technique is particularly useful for CSF specimens.

Genome Detection

The RT-PCR technique is used for the detection of CHPV RNA. Real time RT-PCR is technical advancement over conventional RT-PCR, and this method combines PCR chemistry with the fluorescent probe based detection of amplified product in the same reaction tube. In comparison with conventional RT-PCR, the fluorogenic assay present many advantages. In addition to its higher sensitivity, this technique is rapid, allowing several samples to be processed in few hours. The assay is a closed system in which the tube is never opened post-amplification and thus eliminates the possibility of cross-contamination. In this technique, a fluorescent signal is generated as PCR takes place. The assays are run on highly specialized automated instruments that include optical systems to excite the fluorescent dyes and detect fluorescent emissions. The combination of amplification and signal detection markedly reduces the time required for nucleic acid detection and greatly reduces the likelihood of contamination. Many laboratories have developed their own "home-brew" PCR assays.

In a RT-PCR assay, viral RNA can be isolated from the clinical specimens using the Trizol LS reagent (Life Technologies, Inc., Gaithersburg, MD). cDNA is then prepared with the help of reverse transcriptase further perform a two-step DNA amplification with 35 cycles each of denaturation at 120.2° F (94° C) for 1 minute are then subjected to electrophoresis on 2% agarose gels. The primers which can be used are CHAND-G-F2, 425–445: 5′-GTC TTG TGG TTA TGC TTC TGT-3′; CHAND-G-R5, 750–771: 5′-TTC CGT TCC GAC CGC AAT AACT-3′; CHAND-G-F5, 541–560: 5′-GAG AAT GCG ACC AGT CTT AT-3′; and CHAND-G-R6, 724–744: 5′-TGC AAG TTC GAG ACC TTC CAT-3′ (Chadha et al., 2005). The expected size of the nested PCR product is 204 base pairs. Negative controls should also be included in all PCR assays. Pre-amplification and post-amplification should be conducted on the different floors of the laboratory. These PCR products are further purified and subsequently sequenced. Finally, phylogenetic analysis can be done based on the partial G gene sequences. Recently Cherian et al. (2012), disclosed the complete genomic sequences of four isolates from 2003 to 2007 epidemics based on phylogeny, motif search, homology modeling, and epitope prediction methods.

Serological Tests

Serological examination should also be performed using IgM and IgG ELISA, complement fixation , and neutralization tests.

Enzyme-Linked Immunosorbent Assay

In this test, IgM antibodies from patient serum is captured on wells coated with antihuman IgM. CHPV extracted from mouse brain by a sucrose-acetone method is the source of an antigen. Captured antigen can be detected with the IgG fraction of polyclonal anti-Chandipura virus mouse serum conjugated with biotin (Sigma, St. Louis, MO) and avidin-conjugated horseradish peroxidase; o-phenylenediamine and hydrogen peroxide are added for color development. Negative controls included age-matched serum from apparently healthy children from an area not affected by the outbreak and serum and CSF from children with flavivirus encephalitis. Similarly, IgG ELISA is also useful for serological examination.

Neutralization Tests

All serum samples can be tested with in vitro neutralization to detect antibodies to CHPV. In this test, the CHPV immune mouse serum is used as a positive control and normal mouse serum as a negative control. Virus controls and cell controls should include in each plate and carry out virus titration. The virus neutralizing antibody titer can be expressed the reciprocal of the highest antibody dilution capable of neutralizing 100 $TCID_{50}$s of virus. A titer of 1:10 is considered a positive result.

Complement Fixation Test

The complement fixation test (CFT) is a classical serological method designed for parallel antibody detection of multiple parameters in infection serology. The process depends on the fixation of complement during antigen antibody reaction.

The test consists of two experimental phases. In the first phase, CHPV antigen or antibodies and corresponding antibodies or antigen are mixed with a standard amount of

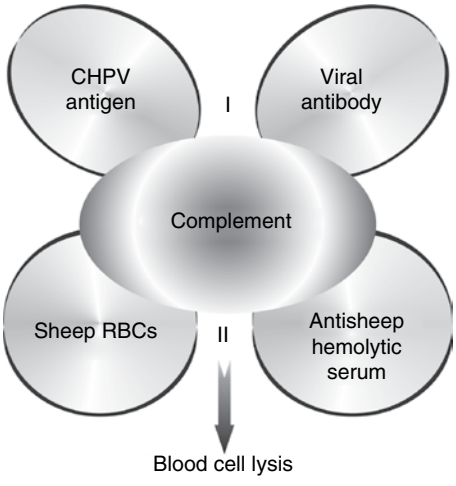

Figure 12.11. Complement fixation test. CHPV, Chandipura virus; RBCs, red blood cells.

complement and incubated for an hour at 98.6° F (37° C). The amount of residual or nonfixed complement is determined by a second test, which uses sheep blood cells that have been sensitized with anti-heep hemolytic serum. The sensitized sheep blood cells are added to the initial mixture, and they will be lysed in the presence of free complement. Whereas if all the complement components have been used up or fixed in the first antigen antibody reaction, there will be no lysis (Figure 12.11). By the spectrophotometric analysis, the amount of lysis can be determined, and the assay can be made highly quantitative. The results are generally expressed as units of complement fixed (CF units) and are, therefore, directly related to the amount of antigen present.

Virus Isolation

For the isolation of viral RNA, RNA-positive clinical samples are inoculated in cell culture (i.e., rhabdomyosarcoma [RD], porcine stable kidney [PS] cell lines, Vero, Madin-Darby canine kidney [MDCK, and Vero E6 cell lines) (Rao et al., 2004). Among these cell lines, the Vero E6 cells and RD cells are widely used for CHPV propagation. Apart from these cells, infant mice (i.e., Swiss albino mice) and embryonated eggs are also found to be useful for the isolation of CHPV. Cultures are observed for the development of cytopathic effects (CPEs), and the cultures not showing CPEs for three passages are considered negative for CHPV.

Differential Diagnosis

The characteristic presentation of CHP viral encephalitis usually consists of fever, headache, and clouding of consciousness together with seizures and focal neurology (in some cases). However, there should be a clear cut distinction between infective viral encephalitis and a metabolic encephalopathy or ADEM. There are numerous medical conditions that may produce an encephalopathic illness, which may mimic viral encephalitis (Table 12.3).

Table 12.3. Differences between encephalopathy and encephalitis.

Features	Encephalopathy	Encephalitis
Clinical features		
Fever	Uncommon	Common
Headache	Uncommon	Common
Depressed mental status	Steady deterioration	May fluctuate
Focal neurologic signs	Uncommon	Common
Types of seizures	Generalized	Generalized or focal
Laboratory findings		
Blood	Leucocytosis uncommon	Leucocytosis common
CSF	Pleocytosis uncommon	Pleocytosis common
EEG	Diffuse slowing	Diffuse slowing and focal abnormalities
MRI	Often normal	Focal abnormalities

CSF, cerebrospinal fluid; EEG; electroencephalogram; MRI, magnetic resonance imaging.

On differentiating the illness from encephalopathy, it should also be differentiated from encephalitis caused by other rhabdoviruses as rabies.

Clinical Management

Once Chandipura encephalitis is suspected, the patient should be managed with supportive care.

At Primary Health Centers

The basic supportive protocol includes:

1. Assessment of vital signs
   (a) Through airway maintenance.
   (b) Breathing measurements: It is necessary to check the respiratory rate whether it is abnormal or is irregular.
   (c) Circulation monitoring: Pulse, blood pressure, capillary refill time should be monitored after respiratory measurements.
2. Investigations: percentage of hemoglobin (HB%), total leucocyte count (TLC)/ differential leucocyte count (DLC), peripheral smear (PS) for malaria parasite (MP) should be investigated.
3. Basic support
   (a) By maintaining air way (suction, position [supine with head elevated by 300 degress]), oxygen therapy by nasal catheter, by face mask, or oxygen tent.
   (b) Through the use of Ambu bag and face mask for manual resuscitation.
   (c) By maintaining nutrition: Through intravenous fluid maintenance.
   (d) Through adequate nursing care: care of eyes, mouth, skin, bladder, bowel, and back.
4. Drug therapy
   (a) Giving paracetamol treatment: per rectal suppository, injectable 10 mg/kg per dose or oral dose at the interval of 8 hours.
   (b) Through intravenous fluid maintenance: 100 mL/kg in 24 hours
   (c) Providing anti malarial treatment
   (d) OR
   (e) Injectable intravenous Artisunate 3 mg/kg state followed by 1.5 mg/kg in the right eye for 3 days.
   (f) Intravenous mannitol: 5 mL/kg single dose bolus stat. in 20 minutes and can be repeated 6 hourly for 48 hours.
   (g) Intravenous Diazepam (only if seizure present): 0.3 mg/kg over a period of 5 to 10 minutes if seizure recurs.
5. Antibiotics therapy
   (a) Injectable ampicillin: 100 mg/kg per day in 8 hourly divided doses.
   (b) Injectable gentamycin: 5 to 7.5 mg/kg per day twice daily.
   (c) Injectable ciprofloxacin: 10 mg/kg per dose twice daily.

If necessary early refer to the nearest hospital after giving basic supportive care, and a referral slip with details of medication should be given with a patient transfer.

Rural Health/Subdistrict Hospital/District Hospital Level

Standard encephalitis management protocol includes:

1. Immediate hospitalization and examination by the available specialists within the shortest time.
2. Basic supportive care as per protocol for primary health center.
3. Investigations should be done urgently as: HB%, TLC/DLC, PS for MP, CSF study, blood sugar, serum electrolytes, bleeding time (BT)/clotting time (CT), platelet count, Widal test, liver function test (LFT). Serum and CSF sample should be preserved and sent for serological and virological study as per NIV in Pune guidelines.

Standard Encephalitis Management Protocol

1. If airway maintained and no shock: In this condition basic support care should continue with timely monitoring the patient and its medication.
2. If airway is not maintained :
   (a) Check head position, elevation of the head by 300 degrees.
   (b) Head tilt and chin lift.
   (c) Suction.
   (d) Tracheostomy if necessary.
   (e) Nothing by mouth, insert gastrointestinal tube and watch for bleeding.
3. Assesment of shock:
   (a) Pulse rate and volume (tachycardia, feeble, absent)
   (b) Systolic blood pressure (mm Hg): 1 to 10 years of age, <70 + (2 x age in years) mm Hg; for older than 10 years <90 mm Hg.
   (c) Delayed capillary refill time more than 3 seconds.
4. No shock:
   (a) Maintain airway.
   (b) Use Ambu bag and face mask for manual resuscitation.
   (c) Start intravenous fluid maintenance: Dextrose in normal saline (DNS)/Isolyte, P doses or intravenous fluid.
      (i) Age 1 to 3 years: 1,200 mL/24 hr
      (ii) Age 3 to 6 years: 1,500 mL/24 hr
      (iii) Aged 6 to 12 years: 1,800 mL/24 hr
      (iv) Age older than 12 years: 2,000 mL/24 hr
      (v) If intravenous access not available, intraosseous or intragastric can be used.
5. Continue drug management as previously described.

If signs of shock present suggested treatment of shock is:

1. Fluid resuscitation: 30 mL/kg over a period of 30 minutes.
2. If shock persists, start vasopresers.
   (a) Dopamine: 10/20 micrograms/kg per minute in intravenous infusion; maintenance drip (0.6 x body weight = dose in mg) dissolved in 100 mL of intravenous fluid, rate to be adjusted for appearance of pulse.

(b) If not responding to dopamine drip, start adrenaline drip: 0.06 x body weight in kg is the amount of milligrams of adrenaline to be dissolved in 100 mL of intravenous fluid, given slowly till pulse appears.

3. Drugs for seizures:
   (a) Intravenous diazepam: 0.3 mg/kg bolus slowly over a period of 3 to 5 minutes can be repeated after 10 minutes, up to maximum three times.
   (b) Intravenous Dilantin sodium: 10 to 15 mg/kg bolus dose followed by 5 mg/kg per day in two divided doses daily
4. Drugs for raised intracranial pressure:
   (a) Intravenous mannitol: 5 mL/kg (1.2 gm/kg) over a period of 20 minutes every six hourly for 48 hours.

OR

   (b) Intravenous Lasix: 2 mg/kg every 12 hours
5. Other drugs

   (a) Antimalarial, intravenous quinine; if not responding, injectable artether or injectable Artesunate: 1–5 mg/kg once a day intravenously or intramuscularly
   (b) Broad-spectrum antibiotics:
      (i) Injectable amoxicillin: 100 mg/kg per day.
      (ii) Injectable cefotaxime: 100 mg/kg per day divided in 8-hourly doses.

OR

      (iii) Injectable Ceftriaxone: 50 to 100 mg/kg per day divided in twice a day.

OR

      (iv) Injectable Ciprofloxacin – 10 mg/kg per dose after every 12 hours.
   (c) Management of gastrointestinal bleeding: injectable vitamin K 5 mg intramuscularly stat. Cold bowel wash, blood fresh-frozen plasma or platelet transfusion.

If the patient is not responding to preceding management, needs ventilator support, has profuse bleeding or refractory seizures, is not responding to treatment, then the patient must be referred to tertiary-care hospital, medical college hospital, or specialist hospital in an ambulance with basic life support and detailed referral slip.

Investigation of Outbreaks

Selection and timing of an investigation is crucial to making a diagnosis in mystery diseases. Laboratory contamination or errors must be considered whenever unexpected results are obtained. Fever, alteration of sensorium without rash or meningeal signs of irritation, normal CSF, epidemic within 2 days of heavy rain after a hot summer, and neuroimaging features of infarction should suggest the diagnosis of epidemic brain attack (EBA) in cases of patients with Chandipura encephalitis.

Case Definition

Like other diseases transmitted by flies, the case diagnosis of Chandipura encephalitis can only be made by laboratory means; it should be suspected when epidemic occurs in children younger than 15 years of age presenting with acute onset of fever and

central nervous system involvement in the form of one or more symptoms such as altered sensorium, unconsciousness, coma and convulsions; without signs of meningeal involvement; and negative for malaria, tuberculosis, and other common bacterial causes.

- Clinical criteria: High-grade fever of short duration, vomiting, altered sensorium, generalized convulsions, or decerebrate posture
- Epidemiological criteria: Recently visited epidemic areas, having reported transmission within 15 days prior to the onset of symptoms
- Laboratory criteria: At least one of the following tests in the acute phase:

1. Confirmation by virus isolation through cell culture, embryonated egg, or mice inoculation.
2. Presence of viral RNA by real time RT-PCR.
3. Detection of viral antigen by ELISA or IFA.
4. Serological examination by ELISA, IFA, CFT, or neutralization tests.

Surveillance and Outbreak Response

Detection of virus specific IgM antibody in patient's serum and an increase of IgG concentration in samples collected 3 to 4 weeks later. On the basis of sample investigation, Chandipura cases can be categorized as:

- **Possible case**: A patient meeting clinical criteria
- **Probable case**: A probable case was defined as a patient with a sudden onset of high-grade fever, followed by central nervous system involvement, altered senses, convulsions, or a comatose state, singly or in combination and negative test results for malaria and other common causes of illness
- **Confirmed case**: A confirmed case was defined as a patient with one or more of the following laboratory results: presence of viral RNA, IgM antibodies against CHPV in a clinical sample, seroconversion in a convalescent sample, and virus isolation.

Host Range

CHPV has a wide host range starting from mammalian (human) and other vertebrates, such as camels, sheep, goats, cows, buffaloes, horses rhesus, and other monkeys up to arthropods like mosquitoes and sand flies.

Notification and Reporting

Chandipura encephalitis is not a disease that requires notifying health authorities; however it also depends on the spread of virus in a particular region or area of a country. Country authorities may make it mandatory for the health-care centers and hospitals in the public and private sectors to notify suspected cases to authorities. Probable and confirmed cases may be associated with a laboratory investigation of possible cases and the laboratory should report it to the nearest district or civil authorities. Any case compatible

with the definition of a suspected case further indicates a possible outbreak, which should immediately be reported to the nearest health authority.

Prevention and Control

Because of the unavailability of any antiviral or therapeutics for CHPV infection, NIV in Pune has developed a recombinant glycoprotein (rGp)-based vaccine against CHPV, which achieved great success in mice (Venkateshwarlu and Arankalle, 2009). Assessment of this candidate vaccine in higher animals is further need to be investigated keeping recent outbreaks in mind.

Following activities should be monitored while suspecting CHPV epidemic:

1. Fever surveillance of affected children population.
2. Antilarval intra- and paradomestic activity.
3. Entomological observation
4. Morning collection of vectors, such as sand flies and indoor resting mosquito
5. Indoor residual spraying by Alpha cypermethrin 5%.
6. Using mosquito net treated with insecticide, Deltamethrin flow 2.5%.
7. Day to day (i.e., regular) monitoring of the affected population.
8. Pyrethrum fogging

In the forest areas, most of the houses are open (doorless or made with bamboos that leaves gaps) and made by mud plaster. Due to mix dwelling, sand flies reside in the houses and breed there. In the rural areas, there is a lack of knowledge and awareness among the people and an appropriate health specialist is also not available, hence in most of cases, the treatment may be delayed. Thus, there are several recommendations to prevent and control the Chandipura encephalitis, which can be further categorized into short-term and long-term category as:

- Short term:

1. Regular and timely surveillance of Chandipura encephalitis in high-risk villages, regular and timely intra- and paradomestic anti-Chandipura activity.
2. Timely surveillance of the fever cases in cluster associated with flulike symptoms, vomiting, and altered sensorium.
3. Review weekly and give feedback to block health officer to take actions.

- Long term:

1. Find out all water logging of mosquito breeding areas in the whole district, blocks, primary health centers, and village areas, and per requirement introduce abate and larvivorous fishes.
2. Pre-monsoon and post-monsoon activity should be done against sand flies with 100 percent coverage.
3. Focal point spraying with insecticide (Alphacypermethrine 5%)
4. Chandipura-positive cases should be given second-line treatment.
5. Use of insecticide-treated mosquito nets (ITMN).

References

Ahmed M, Lyles DS. 1998. Effect of vesicular stomatitis virus matrix protein on transcription directed by host RNA polymerases I, II, and III. *J Virol* 72:8413–8419.

Ball LA, White CN. 1976. Order of transcription of genes of vesicular stomatitis virus. *Proc Natl Acad Sci USA* 73(2):442–446.

Banerjee AK. 1987a. Transcription and replication of rhabdoviruses. *Microbiol Rev* 51:66–87.

Banerjee AK. 1987b. The transcription complex of vesicular stomatitis virus. *Cell* 48:363–364.

Barr JN, Whelan SP, Wertz GW. 2002. Transcriptional control of the RNA dependent RNA polymerase of vesicular stomatitis virus. *Biochim Biophys Acta* 1577:337–353.

Basak S, Mondal A, Polley S, Mukhopadhyay S, Chattopadhyay D. 2007. Reviewing Chandipura: a vesiculovirus in human epidemics. *Biosci Rep* 27(4–5):275–298.

Bhatt PN, Rodrigues FM. 1967. Chandipura: A new arbovirus isolated in India from patients with febrile illness. *Indian J Med Res* 55:1295–1305.

Bhattacharya R, Basak S, Chattopadhyay DJ. 2006. Initiation of encapsidation as evidenced by deoxycholate-treated Nucleocapsid protein in the Chandipura virus life cycle. *Virology* 349:197–211.

Chadha MS, Arankalle VA, Jadi RS, Joshi MV, Thakare JP, et al. 2005. An outbreak of Chandipura virus encephalitis in the eastern districts of Gujarat state, India. *Am J Trop Med Hyg* 73:566–570.

Cherian SS, Gunjikar RS, Banerjee A, Kumar S, Arankalle VA. 2012. Whole genomes of Chandipura virus isolates and comparative analysis with other rhabdoviruses. *PLoS ONE* 7(1):e30315.

Clague MJ, Schoch C, Zech L, Blumenthal R. 1990. Gating kinetics of pH-activated membrane fusion of vesicular stomatitis virus with cells: Stopped-flow measurements by dequenching of octadecylrhodamine fluorescence. *Biochemistry* 29:1303–1308.

Das T, Mathur M, Gupta AK, Janssen GM, Banerjee AK. 1998. RNA polymerase of vesicular stomatitis virus specifically associates with translation elongation factor1 alpha beta gamma for its activity. *Proc Natl Acad Sci USA* 95:1449–1454.

Davis NL, Wertz GW. 1982. Synthesis of vesicular stomatitis virus negative strand RNA in vitro: Dependence on viral protein synthesis. *J Virol* 41(3):821–832.

Dhanda V, Rodrigues FM, Ghosh SN. 1970. Isolation of Chandipura virus from Sand flies in Aurangabad. *Indian J Med Res* 58:179–180.

DNA Correspondent. 2010. Chandipura Virus Kills 17 in Gujrat. *DNA News & Analysis*. Accessed February 19, 2013, at http://www.dnaindia.com/india/report_chandipura-virus-kills-17-in-gujarat_1419111.

Durrer P, Gaudin Y, Ruigrok RW, Graf R, Brunner J. 1995. Photolabeling identifies a putative fusion domain in the envelope glycoprotein of rabies and vesicular stomatitis viruses. *J Biol Chem* 270:17575–17581.

Enninga J, Levy DE, Blobel G, Fontoura BM. 2002. Role of nucleoporin induction in releasing an mRNA nuclear export block. *Science* 295:1523–1525.

Fontenille D, Traore-Lamizana M, Trouillet J, Leclerc A, Mondo M, et al. 1994. First isolations of arboviruses from phlebotomine sand flies in West Africa. *Am J Trop Med Hyg* 50(5):570–574.

Fredericksen BL, Whitt MA. 1998. Attenuation of recombinant vesicular stomatitis viruses encoding mutant glycoproteins demonstrate a critical role for maintaining a high pH threshold for membrane fusion in viral fitness. *Virology* 240(2):349–358.

Gurav YK, Tandale BV, Jadi RS, Gunjikar RS, Tikute SKS, et al. 2010. Chandipura virus encephalitis outbreak among children in Nagpur division, Maharashtra, 2007. *Indian J Med* 132:395–399.

Jayakar HR, Jeetendra E, Whitt MA. 2004. Rhabdovirus assembly and budding. *Virus Research* 106:117–132.

Majumdar A, Bhattacharya R, Basak S, Shaila MS, Chattopadhyay D, et al. 2004. P-protein of

Chandipura virus is an N-protein-specific chaperone that acts at the nucleation stage. *Biochemistry* 43:2863–2870.

Marriott AC. 2005. Complete genome sequences of Chandipura and Isfahan vesiculoviruses. *Arch Virol* 150(4):671–680.

Matlin KS, Reggio H, Helenius A, Simons K. 1982. Pathway of vesicular stomatitis virus entry leading to infection. *J Mol Biol* 156(3):609–631.

Peiris JS, Dittus WP, Ratnayake CB. 1993. Seroepidemiology of dengue and other arboviruses in a natural population of toque macaques (Macaca sinica) at Polonnaruwa, Sri Lanka. *J Med Primatol* 22(4):245–245.

Rao BL, Basu A, Wairagkar NS, Gore MM, Arankalle VA, et al. 2004. A large outbreak of acute encephalitis with high case fatality rate in children in Andhra Pradesh, India in 2003 associated with Chandipura virus. *Lancet* 364:869–874.

Rodrigues JR, Singh PB, Dave DS, Prasan R, Ayachit VL, et al. 1983. Isolation of Chandipura virus from the blood in acute encephalopathy syndrome. *Indian J Med Res* 77:303–307.

Rose JK, Whitt MA. 2001. Rhabdoviridae: The viruses and their replication. In: Knipe DM, Howley PM, eds., *Fields Virology*, 4th ed., pp. 1221–1224. Philadelphia: Lippincott-Raven Publishers.

Thornton GB, Kopchick JJ, Stacey DW, Banerjee AK. 1983. Microinjection of vesicular stomatitis virus ribonucleoprotein into animal cells yields infectious virus. *Biochem Biophys Res Commun* 116:1160–1167.

Traore-Lamizana M, Fontenille D, Diallo M, Ba Y, Zeller HG, et al. 2001. Arbovirus surveillance from 1990 to 1995 in the Barkedji area (Ferlo) of Senegal, a possible natural focus of Rift Valley fever virus. *J Med Entomol* 38(4):480–492.

Venkateswarlu CH, Arankalle VA. 2009. Recombinant glycoprotein based vaccine for Chandipura virus infection. *Vaccine* 27:2845–2850.

Yao Y, Ghosh K, Epand RF, Epand RM, Ghosh HP. 2003. Membrane fusion activity of vesicular stomatitis virus glycoprotein G is induced by low pH but not by heat or denaturant. *Virology* 310:319–332.

Zagouras P, Rose JK. 1993 Dynamic equilibrium between vesicular stomatitis virus glycoprotein monomers and trimers in the Golgi and at the cell surface. *J Virol* 67:7533–7538.

Chapter 13
Kyasanur Forest Disease

Introduction

Kyasanur Forest disease (KFD) is an arbovirus infection found only in southwest peninsular India. It is an influenza-like illness and is marked with an onset of sudden chills, fever, frontal headaches, stiffness of the neck, and body pain. The disease was first reported from Kyasanur Forest of the Shimoga district, Karnataka state, in India in March 1957 and was first manifested as an epizootic outbreak among monkeys. Hence the disease is also locally known as monkey disease or monkey fever (Mark, 1987). The exact cause of its emergence in the mid-1950s is still unknown. A variant of Kyasanur Forest disease virus (KFDV) is characterized serologically and genetically as Alkhurma hemorrhagic fever virus (AHFV) and has recently been identified in Saudi Arabia. KFDV and AHFV share 89 percent sequence homology, suggesting a common ancestral origin. It was suggested that these viruses diverged 700 years ago (Dodd et al., 2011). A variety of animals is thought to be the reservoir hosts for the disease, including porcupines, rats, squirrels, mice, and shrews (Dobler, 2010). *Haemaphysalis spinigera*, a forest tick, acts as a vector for disease transmission. Humans contract infection from the bite of nymphs (a developmental stage) of the tick. KFDV is a member of the mammalian tick-borne virus group (previously referred to as the tick-borne encephalitis serogroup) of the family *Flaviviridae* and genus *Flavivirus* (Thiel et al., 2005). In addition to KFDV, this group contains Louping ill, tick-borne encephalitis, Omsk hemorrhagic fever, Langat, Powassan, Royal Farm, and Gadgets Gully viruses. Over the past five decades, KFD represented a newly emerged disease and is responsible for the continuing deaths in monkeys. An average of 400 to 500 human cases have been seen annually, usually occurring in evergreen, semi-evergreen, and neighboring, moist, deciduous forest areas. Human infections were also observed among persons who visited forests to collect firewood, grass, and other forest products and is characterized by an incubation period of 3 to

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

8 days, followed by chills, frontal headache, body ache, and high fever for 5 to 12 days (Banerjee, 1990).

Epidemiology

India

KFDV was first isolated from sick and dying monkeys in the Kyasanur Forest in 1957 (Work and Trapido, 1957; Pattnaik, 2006; Gould and Solomon, 2008) (Figure 13.1). Veterinary scientists involved in the investigation of the sick monkeys, as well as local people using the forest, were bitten by ticks infected with KFDV and developed a hemorrhagic disease. During the initial outbreak, there were 466 human cases and 181 more the following year (Acha and Szyfres, 2003). The disease is common in young adults that have been exposed during the dry season in the forest, and it has caused epidemic outbreaks of hemorrhagic fever affecting 100 to 500 people per year since then, with a case fatality rate between 2 and 10 percent (Acha and Szyfres, 2003; Heymann, 2004; Brown et al., 2005; Gould and Solomon, 2008). Table 13.1 shows the KFD epidemic cases in India during the period in between 1957 tand 2004.

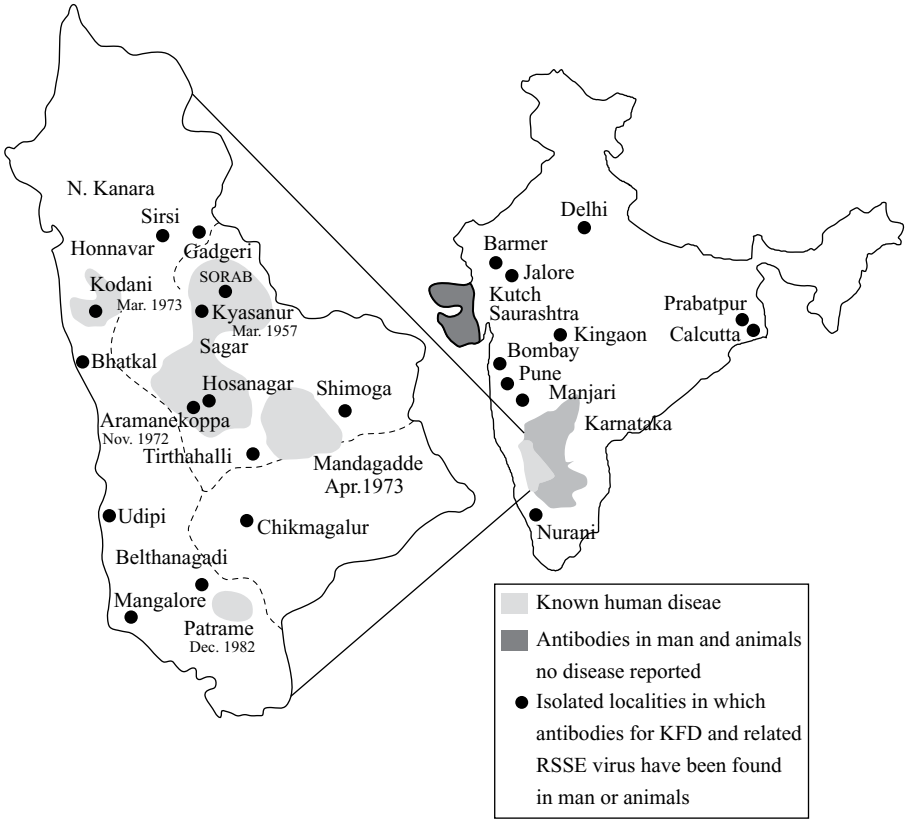

Figure 13.1. Distribution of Kyasanur Forest disease in the state of Karnataka in India. KFD, Kyasanur Forest disease; RSSE, Russian spring-summer encephalitis.

Table 13.1. Kyasanur Forest disease cases from various sources for certain segments of epidemic. Varying numbers are reported for 1999–2004.

KFD Epidemic	KFD Cases Reported
1957	466
1958	181
1981	>550
1982–1984	1117
1999	1,085
2000	130
2001	435
2002	98,625
2003	25,3920
2004	2,760

KFD, Kyasanur Forest disease.

Global

KFDV has also been isolated in Saudi Arabia and the People's Republic of China (Zaki, 1997; Wang et al., 2009). One of the variants of KFDV was isolated from the patients of hemorrhagic fever during 1994 and 1995 in the Makkah region of Saudi Arabia referred to as the Alkhurma variant or subgroup (Qattan et al., 1996; Charrel et al., 2001). The prototype strain of KFDV that had been isolated from Saudi Arabia (strain 1176, isolated in 1995) and the KFDV reference strain from India (P-9605, isolated in 1957) differ from each another by only 8 percent at the genome nucleotide level, despite their temporal (38 years) and geographic (~2,500 miles [~4,000 km]) separation. Apart from these, a related virus was isolated from a febrile patient in Nanjian County in the Hengduan Mountain region of Yunnan Province in southwestern China in 1989; initially it was referred to as Nanjianyin virus, but now it is considered as a strain of KFDV (Wang et al., 2009). However, it is still unclear whether this KFDV 1989 isolate from China is an authentic virus isolate because it is virtually identical at the nucleotide level with the 1957 reference strain from India (P-9605).

Vector Biology

Vector Species

H. spinigera is the most abundant tick species in the enzootic area and is also the major vector responsible for epizootics and epidemics of KFD. Cattle and birds act as hosts for the vector ticks. Man is parasitized almost exclusively by larvae and nymphs of *H. spinigera*, and in the absence of natural transovarial transmission of the virus in this species, transmission to man is by bite of infected nymphal ticks. Other *Haemaphysalis* spp., particularly *Haemaphysalis turturis* have been found to maintain the enzootic cycle. KFDV has also been isolated from 14 other species of ticks, including genus *Haemaphysalis*, *Dermacentor*, and *Ixodes*.

Breeding Places

H. spinigera is a forest-inhabiting species where its larvae and nymphs parasitize several species of small forest mammals and birds and monkeys. Cattle are also heavily parasitized by the adults of *H. spinigera* and help to amplify the tick population. *H. spinigera* is the most common of all ticks found in ground drags and on vegetation in the KFD area. The species is widely distributed in the tropical evergreen and deciduous forests of southern and central India and Sri Lanka.

Life Cycle of Haemaphysalis Spinigera

H. spinigera is an arachnid, which has only two body segments a fused head and thorax and an abdomen. An adult has four pairs of legs without any wings or antennae. The life cycle of arachnids involves incomplete metamorphosis during which the eggs hatch into nymphs that superficially resemble adults. In enzootic stage, KFDV circulates through small mammals such as rodents, shrews, porcupines, squirrels, rats, and an array of tick species including *H. spinigera*. These are the main reservoirs of the virus. Birds and bats are comparatively less important hosts. Man is an incidental host and does not play any part in virus transmission. The silent enzootic situation was perhaps dramatically altered by man's need for more land for construction, for grazing, and for other purposes as agricultural practices. Cattle were put to graze around the forest and thus provided ticks with a new and plentiful source of blood meals, which in turn resulted in a population explosion among the ticks. Cattle are very important in maintaining tick populations, but do not play any part in virus maintenance. When monkeys come in contact with the infected ticks, they get infected, amplify, and disseminate the infection creating hot spots of infection. The monkeys show marked viremia, which is an illness from which they may die, and are recognized as amplifying hosts for the virus. Seasonal epidemics of KFD have been associated with epizootics in monkeys; the most important is the black faced langur (*Presbytis entellus*) and the south Indian bonnet macaque (*Macaca radiata*).

Stages of the Life Cycle

Ticks begin their life cycle as eggs (I stage) that hatch into six-legged larvae (II stage). Larvae lives and feed on small mammals, monkeys, or birds for about a week before detaching and then molting (shedding) anywhere from 1 week to 8 months later (Figure 13.2). The larvae then become eight-legged nymphs (III stage). Nymphs feed on humans or animals, engorge for 3 to 11 days, detach, and molt about a month later (depending on the species and environmental conditions). Once the nymph molts, it becomes an adult tick (male or female). Ticks climb grass and plants and hold their legs up "sensing" and "looking" for their prey. Ticks are attracted to their hosts by detecting carbon dioxide and heat through special organs located on the first pair of the tick's legs (Haller's organs). When a warm-blooded animal walks past, the tick can crawl onto them and begin feeding by inserting their mouths, attaching to their prey, and engorging themselves with a blood meal (IV stage). During feeding, tick saliva can get into the host's body, especially in the blood stream, and infect the host with KFDV, thus transmitting this virus to the host.

Male and female ticks usually mate while attached to the host. A few weeks later, the engorged female detaches from the host and lays her eggs (1000–8000 eggs) on a leaf. A tick usually lives a year before dying.

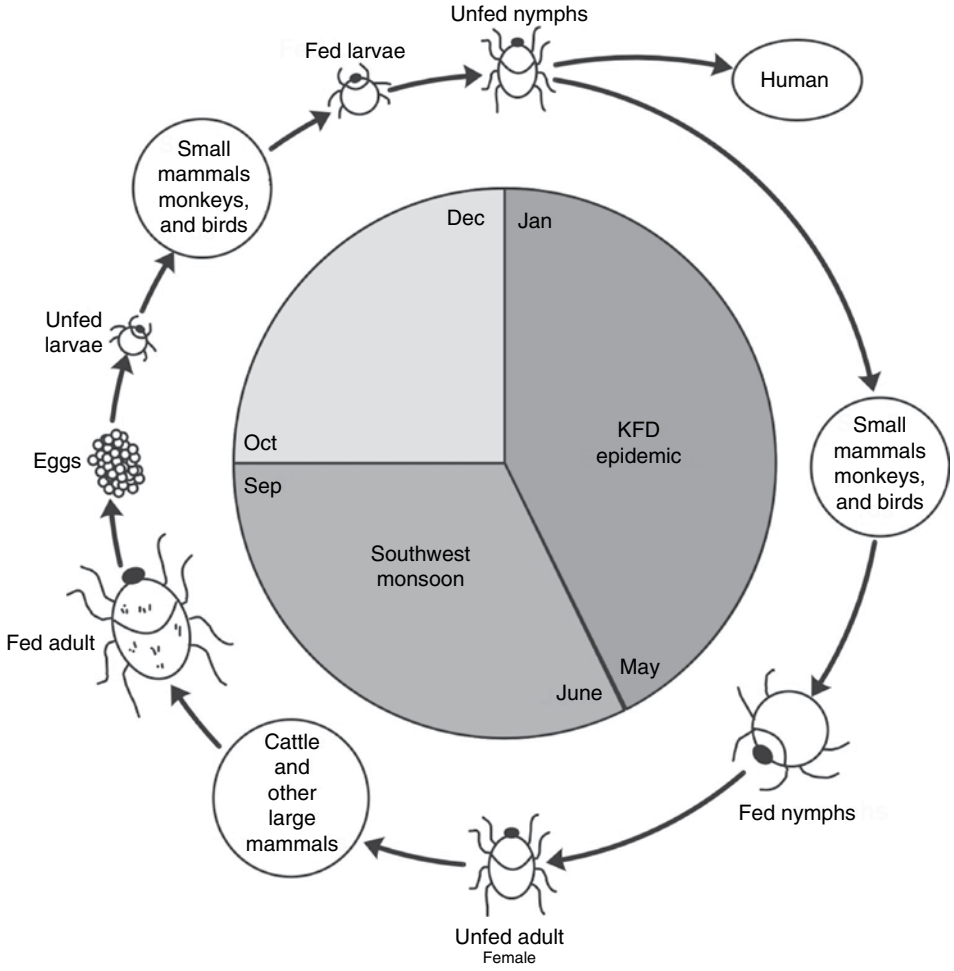

Figure 13.2. Life cycle of Kyasanur Forest disease virus vector tick, *Haemaphysalis spinigera*. KFD, Kyasanur Forest disease.

Ticks Seasonal Activity

Ticks show a definite stage wise seasonal activity. The adult becomes active after a few monsoon rains in the month of June and the adult population reaches its peak during July and August and gradually declines in September. Each fed female lays large number of eggs, which then convert into larvae. This larval population builds up in the monsoon months but remains dormant under the forest litter and becomes suddenly active when the litter dries up during the post-monsoon months of October to December. Epidemics actually coincide with nymphal activity, which is high from January to May; therefore, the nymph is considered the most important stage for human transmission. Adult ticks feed on large animals such as cattle and monkeys, which are good hosts for ticks proliferation,but are not significant for virus dissemination because of insignificant viremia in them.

Clinical Features

Classically KFD is a biphasic illness starts after an incubation period of 3 to 8 days. The onset is sudden with chills, high fever of around 104° F (40° C), frontal headache, sensitivity to light, followed by continuous fever of 12 days or longer, which is often associated with diarrhea, vomiting, cough, severe pain in the neck, lower back and extremities, accompanied by severe prostration. In some of the patients, papulo-vesicular eruption on the soft palate (blisters on the upper, inner mouth) is an important diagnostic sign. Bleeding in the gum, nose (epistasis), cough (hemoptysis), gastrointestinal bleeding resulting in dark feces (melena), and discharge of fresh blood in the stools are also common. The convalescent phase is generally prolonged, that is, maybe up to 4 weeks and constitutes the recovery after the onset of KFD. Relapse of the symptoms, often observed after 1 to 2 weeks of the first febrile period and lasts for 2 to12 days. The relapse phase showing the same symptoms as the first phase and, in addition symptoms, such as mental disturbance, giddiness, and reflex abnormality, are often seen. Leucopenia (reduction in the number of leucocytes) and accompanying thrombocytopenia (reduction in the number of thrombocytes) are constant hematological features in KFD. Intra-alveolar hemorrhage (oozing of blood into the lungs) and massive gastrointestinal hemorrhage are terminal complications resulting secondary infection that could lead to death.

This biphasic illness can further be subdivided into four stages, each lasting around a week in length:

- **I stage**: The initial prodromal stage starts with a sudden onset of fever and severe headache, hypotension and hepatomegaly, sore throat, diarrhea and vomiting, anorexia, insomnia, severe pain in the lower and upper extremities, and prostration (Acha and Szyfres, 2003; Heymann, 2004; Brown et al., 2005; Pattnaik, 2006). Bradycardia and inflammation of the conjunctiva are also commonly observed, along with acute lymphopenia and eosinopenia occurring within the first or second week of infection (Pattnaik, 2006).
- **II stage**: This is characterized by hemorrhagic complications such as intermittent epistaxis, hematemesis, melena, and blood in stool; neurological manifestations such as mental confusion, tremors, and abnormal reflexes; and bronchopneumonia or development of coma, which may occur in few cases prior to death (Acha and Szyfres, 2003; Heymann, 2004; Pattnaik, 2006).
- **III and IV stage**: A stage of recovery may be observed next, followed by a last stage of fever in certain cases (Pattnaik, 2006).

Some other pathologic manifestations have also been observed in human patients, including parenchymal degeneration of the liver and kidney, hemorrhagic pneumonitis, and a moderate to marked prominence of the reticuloendothelial elements in the liver and spleen with marked erythrophagocytosis (Iyer et al., 1959; Burke and Monath, 2001).

Differential Diagnosis

In the initial prodromal stage KFD should be differentiated from influenza-like illness. In the meningoencephalitic stage, the spectrum narrows toward causative agents of serious meningitis or encephalitis (e.g., mumps virus and many enteroviruses). Garin-Bujadoux-Bannwarth

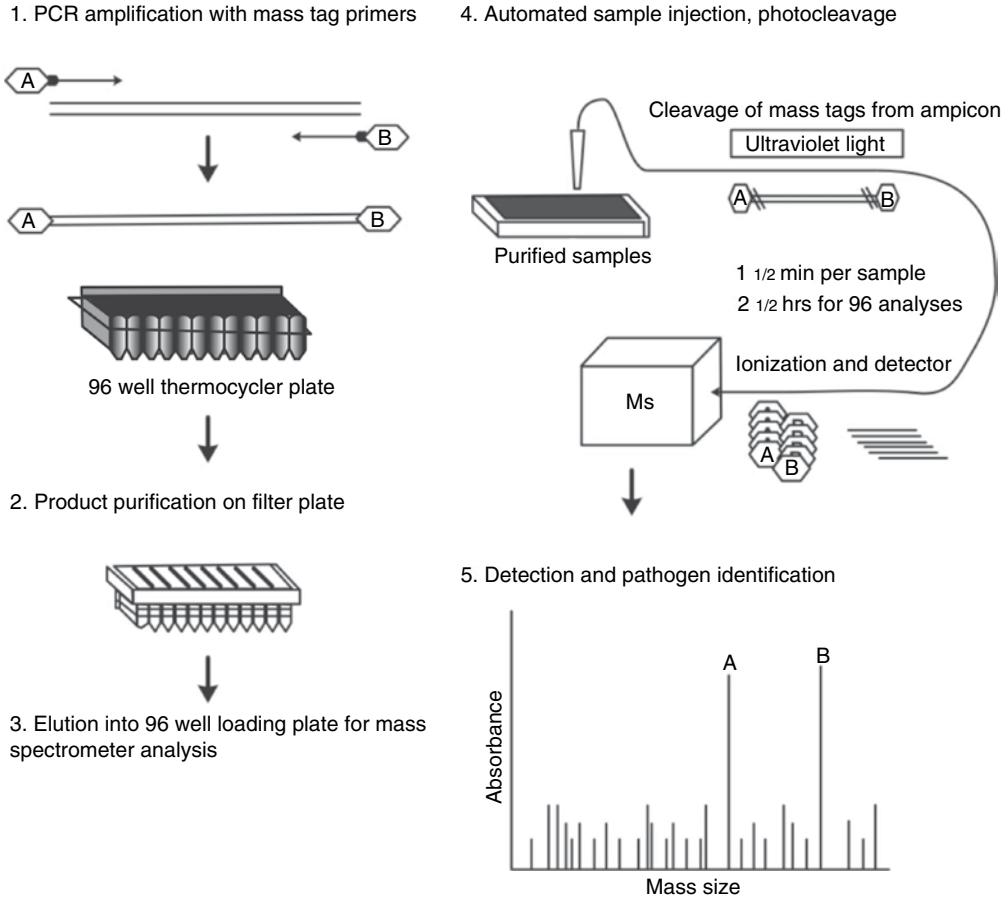

Figure 13.3. Mass Tag polymerase chain reaction method. A and B indicate mass tags; MS, mass spectrometer; PCR, polymerase chain reaction.

meningopolyneuritis caused by *Borrelia garinii* should be considered, especially after a tick bite. Apart from this other tick-borne encephalitis and hemorrhagic fever should also be included in the differential diagnosis.

Mass Tag polymerase chain reaction (PCR) could be applied for the differential diagnosis of respiratory disease (Briese et al., 2005). Mass Tag PCR is a multiplex assay in which microbial gene targets are coded by a library of 64 distinct mass tags (Figure 13.3). Nucleic acids (RNA or DNA) are amplified by multiplex reverse transcription-polymerase chain reaction (RT-PCR) using up to 64 primers, each labeled by a photo-cleavable link with a different molecular weight tag. On separating the amplification products from unincorporated primers and release of the mass tags from the amplicons by ultraviolet irradiation, tag identity is analyzed by mass spectrometry. The viral identity in the clinical sample is then determined by the presence of its two cognate tags, one from each primer.

Because only released mass tags are analyzed, the staggering the size of amplification products created in multiplex reactions is unnecessary; thus, primers are selected for efficient and consistent performance irrespective of their amplicon size (around 80–200 base

pair long). Before committing to the synthesis of tagged primers, the functionality of each candidate multiplex primer panels is examined through a series of amplification reactions that use prototype templates, which represents individual microbial targets. Primers that fail to yield a single, specific product band in agarose gel electrophoresis are replaced in the process.

Laboratory Diagnosis

The preliminary diagnosis of KFD is usually made by suspicion through clinical signs and symptoms as well as through the occupation of the patient whether related to forest traveling or not. Diagnosis can also be made by the detection of tick on the animal's body; however, for diagnostic confirmation it is important to detect the virus serologically by hemagglutination and immunofluorescence, which should be followed by the isolation of virus.

Serological Testing

The virus is stable in the blood; thereby isolation of KFDV from patient's serum can substantiate the clinical diagnosis of KFD. Any rapid diagnostic kit is not available and thus precludes early diagnosis. Enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) are commonly used for the demonstration of anti-KFD antibodies. Presence of immunoglobulin M (IgM) antibodies in serum and in cerebrospinal fluid (CS) are indicative of acute infection. The virus can also be diagnosed by complement fixation (CF) and hemagglutination inhibition (HI) tests.

Isolation of Kyasanur Forest Disease Virus

KFDV has been observed circulating in the blood from 2 days before until as long as 10 days after the onset of the disease. The virus can easily be isolated by intracerebral inoculation into infant or adult mice. Isolation of virus from the blood collected during the acute phase of illness has been the most frequent substantiation of a clinical diagnosis. The virus caused a typical cytopathic effect within 4 days after its injection in BHK-21 cells, killed 100 percent of 3-days-old mice within 2.5 days after their intracerebral inoculation with a 25 μL culture supernatant, and killed 100 percent of 50-day-old adult mice within 11–13 days of their intraperitoneal inoculation with a 30-μL culture supernatant.

Virus Selection and Reverse Transcription-Polymerase Chain Reaction

Samples of suspected KFDV can be grown in Vero E6 cell lines. Primers are designed to target regions of structural genes (premembrane/envelope) and the nonstructural protein 5 (NS5) gene (viral polymerase) for PCR and phylogenetic analysis. Total RNA can then extracted from infected Vero cell lysates by using Trizol reagent as per the manufacturer's protocol. RNA is then dissolved in nuclease-free water. cDNA is prepared separately for structural genes and NS5 by using avian Moloney virus reverse transcriptase (Mehla et al., 2009). The produced cDNA is amplified by using Taq DNA polymerase, PCR buffer, dNTPs, $MgCl_2$, and primer pair in a reaction volume of 25 μL. PCR conditions included are denaturation at 201.2° F (94° C) for 5 minutes; 35 cycles of 1-min steps at

201.2° F (94° C), 131° F (55° C), and 161.6° F (72° C); and a 5-minute extension at 161.6° F (72° C). Amplified products are then analyzed by agarose gel electrophoresis. Bands of interest are recovered by using a DNA gel extraction kit. Finally, direct sequencing of the gene amplified product is performed.

Case Management

Cases of KFD can be managed through the assessment of patients with suspected KFD. First they are isolated, and then specific laboratory diagnostic tests are performed. Finally, selective treatment methods are applied.

Assessment of Patients with Suspected Kyasanur Forest Disease

Most of patients with KFD do not show any specific distinguishing features in the early stages of the disease. It is, therefore, necessary to pay particular attention to epidemiological evidence while assessing a patient from an epidemic or endemic area with an unexplained fever.

Patients in an Endemic Area

In an endemic area assessment should be based on the analysis of the epidemiological and clinical features of the illness. The movement of the patient within an endemic area should be kept to a minimum, and whenever transport of the patient within or from an endemic area is essential, appropriate protective measures should be based on the epidemiology of the disease. In febrile patients from an endemic area those are admitted to hospital with possible KFD malaria should be excluded by examining a blood smear and giving antimalarial treatment. The blood film should be rendered safe by dipping it in a 10% solution of formol in buffered saline. After excluding malaria further, it requires clinical laboratory investigations to establish diagnosis.

Patients in a Nonendemic Area

Travelers from an endemic area that arrive with an unexplained fever or those develop a fever within the quarantine period after departure from an endemic area should also be assessed on epidemiological and clinical evidence. They should then further be classified into one of the following three categories according to the level of suspicion.

Minimal Suspicion

Patient visited cities where KFD has not been reported to be endemic should be admitted to standard isolation in hospital or may be confined at home in cases where there is no immediate threat to life. The patient may be transported in an ambulance taking the standard precautions for all the infectious diseases. Specimens may be sent to routine laboratories as early as possible. Close contacts should be identified but need not be placed under surveillance.

Moderate Suspicion

Patients from small towns or country districts in an endemic area should be regarded with more suspicion, especially if the course of the illness is consistent with a dangerous

hemorrhagic fever. They should be admitted to an isolation room with filtered negative-pressure ventilation and separate facilities for dealing with contaminated waste. In these cases, close contacts should be identified, but they need not be placed under immediate surveillance.

High Suspicion

Patients from rural areas and towns where a dangerous viral hemorrhagic fever is known to be endemic, and cases among medical and nursing staff from country hospitals, contacts of confirmed cases, and laboratory staff processing dangerous material should be admitted directly to a designated high-security unit. Specimens should be taken with the greatest care, securely packaged, and dispatched by special messenger to a designated maximum security laboratory. Close contacts should be placed under surveillance.

Management Policy in a General Hospital

Patients with an unexplained fever from an endemic area should be admitted in the emergency department. Patients fall into the categories of minimal or high risk should be dealt accordingly, and those in the moderate risk group require careful clinical and epidemiological assessment, and blood films should be examined to exclude malaria. These patients should be nursed in single rooms with strict isolation techniques.

Isolation Facilities in Designated Units

The designated unit should have some basic facilities and policies; these are:

1. The unit should be self-contained and separated from the rest of the hospital.
2. Access should be restricted to authorized personnel, and a record should be kept of anyone entering the unit.
3. There should be mechanical ventilation to maintain a negative pressure within the possibly contaminated areas, and exhaust air should be filtered.
4. All waste must be rendered safe before disposal.
5. Facilities should be provided within the unit for patient-management tests and for radiology.
6. Patients in the intermediate category should be nursed in single rooms by staff wearing protective clothing.
7. Patients in maximum-risk category should be nursed in flexible film, negative-pressure isolators, or by staff wearing full protective clothing.
8. Staff should be kept under surveillance for 21 days after their last exposure to a confirmed case. Their health status and their body temperature should be recorded each day.

Isolation of Patient

Once admitted into maximum security unit, the patient should be strictly isolated until a firm diagnosis has been made, and the fever has subsided completely and it is apparent that the course of illness has not been consistent with that of classical KFD or until laboratory tests have proved negative.

Laboratory Investigations

Diagnostic tests are required to establish the cause of illness in a patient with suspected KFD. These tests fall into three distinct categories:

1. Test for viral hemorrhagic fever: virus cultures and antibody studies should be considered.
2. Test may be required to exclude other causes of unexplained fever. This may include routine hematology, blood films for malaria, blood cultures, and serological examinations for KFD.
3. Hematological and biochemical investigations for the optimal management of the patient including hematology, clotting studies, urea estimation electrolytes, glucose, and liver function tests.

Treatment

Treatment might fall into two categories, general and specific depending upon the severity of illness.

General Treatment

It includes basic care by skilled nursing and careful attention to water and electrolyte balance. The health workers including nursing team and doctors looking after the patient should be well trained to ensure that there is no spread of infection. During the febrile phase of illness, symptomatic relief may be obtained by tepid sponging, but antipyretics such as salicylates are contraindicated. Analgesics can be prescribed for the relief of pain and mild sedation to control restlessness.

Specific Treatment

Although specific treatment is not available for KFD, it can be controlled to some extent by supportive therapy, including analgesics and antipyretics, intravenous fluids for those with hypotension, blood transfusion or fresh-frozen plasma and platelets for those with hemorrhagic symptoms, antibiotics for bronchopneumonia, and corticosteroids and anticonvulsants for neurological symptoms (Borio et al., 2002; Bronze and Greenfield, 2005).

Prevention and Control

KFDV is one of the highest-risk category pathogens designated as a Biosafety Level-4 pathogen. It is also considered as one of the potential bioterrorist weapons, which requires timely control to decrease the mortality and morbidity so observed. Prophylactic control can be achieved using inactivated chick embryo tissue culture vaccine developed by National Institute of Virology (NIV) in Pune, India. This can be used for vaccinating people those at high risk, such as villagers living near forest, forest workers, and other occupational personnel concerned with forest.

Control Measures for Self-Protection

Avoidance

Fields and forests infested with ticks should be avoided if possible. Beds, especially metal ones, may provide some protection because the ticks have difficulty in climbing the legs. However, they may still be able to reach hosts by climbing up the walls.

Repellents

Repellents can be used that prevent ticks from attaching to the body. Effective repellents include DEET, dimethyl phthalate, benzyl benzoate, dimethyl carbamate, and indalone (Schreck et al., 1980). These substances can be directly applied on to the skin or clothing. On the skin, repellents often do not last more than a few hours because of absorption and removal by abrasion whereas on clothing they last much longer, sometimes for several days (Schreck et al., 1982).

Clothing

Clothing can provide some protection, for example, if trousers are tucked into boots or socks and shirts are tucked into trousers. After visiting a tick-infested area, clothing should be removed and examined for the presence of ticks.

Impregnated Clothing

People who frequently enter tick-infested areas should impregnate their clothing by spraying or soaking with a pyrethroid insecticide such as permethrin or cyfluthrin. Ticks crawling up trousers or shirts are quickly knocked down, preventing biting and also killing the ticks.

Removal of Attached Ticks

During and after visits to tick-infested areas, the body should be examined frequently for ticks. They should be removed as soon as possible because the risk of disease transmission increases with the duration of attachment. A tick should be removed by pulling slowly but steadily, preferably with forceps to avoid contact between the fingers and the tick's infective body fluids. The tick should be grasped as close as possible to where the head enters the skin, so as not to crush it, and care should be taken not to break off the embedded mouthparts because they may cause irritation and secondary infection. In areas where ticks are only a nuisance, they can be coated with oil, paraffin, Vaseline, or nail varnish to prevent them from obtaining oxygen. Hard ticks then dissolve the cement so that they can withdraw their mouthparts, but this may take several hours. However, these methods are not recommended in areas where ticks are vectors of disease because they work too slowly and may cause ticks to regurgitate into wounds, injecting disease organisms. In such circumstances, it is recommended to pull the ticks out immediately, even if the head is left in the wound.

Application of Insecticides to Animals

Domestic animals are often hosts to ticks that can feed on humans and transmit disease to people and animals. Insecticides could be effective if directly applied to the bodies of

Table 13.2. Insecticidal formulations effective against ticks.

Application Method	Insecticide Formulation
Dipping, washing, or spray-on	Malathion (5%), dichlorvos (0.1%), carbaryl (1%), dioxathion (0.1%), naled (0.2%), coumaphos (1%)
Insecticidal powder (dust)	Carbaryl (5%), coumaphos (0.5%), malathion (3–5%), trichlorphon (1%)
Residual spray on floors, etc.	Oil solutions or emulsions of DDT (5%), lindane (0.5%), propoxur (1%), bendiocarb (0.25–0.48%), pirimiphos methyl (1%), diazinon (0.5%), malathion (2%), carbaryl (5%), chlorpyrifos (0.5%)
Ultra-low-volume fogging (area spraying)	Organophosphorus insecticides, carbamate compounds and pyrethroids
Flea and tick collars for dogs and cats	Dichlorvos (20%), propoxur (10%), propetamphos (10%), permethrin (11%)

these animals in the form of dusts, sprays, dips, or washes by means of a shaker, puff-duster, or plunger-type duster or with hand-compression sprayers. Pour-on formulations are applied over the back of animals.

Spraying Insecticides in Houses and Resting Places for Animals

Ticks can be killed by insecticides (Table 13.2) sprayed on floors in houses, porches, verandas, dog kennels and other places where domestic animals sleep.

Control Measures for Community Protection

Large-scale control activities are sometimes carried out in recreational areas or in areas where ticks transmit diseases. Several integrated methods can be used which provide economical and effective control strategy, including:

- **Surveillance**: Sampling to identify tick habitats wherever control is needed.
- **Vegetation management**: Applying physical or chemical measures to reduce and isolate tick habitats.
- **Host management**: Removal or exclusion of host animals.
- **Targeted chemical control**: Pesticide applications against ticks, targeted at the tick host or habitat.
- **Cultural practices**: Lifestyle changes to limit exposure to ticks.
- **Personal protection**: Protective clothing, repellents, and checking for and removing of ticks.

Area Spraying with Insecticides

Spraying insecticides directly in the natural habitats of ticks in forests and fields may be helpful in controlling KFD outbreaks. Large areas may be treated by ultra-low-volume spraying of liquid acaricide concentrates from fixed-wing aircraft or helicopters. Small areas may be sprayed by means of motorized knapsack sprayers or mist blowers, applying either ultra-low-volume formulations or formulations of water-based emulsions or wettable powders. Control lasts for a month or longer, depending on environmental conditions, ticks density, and the size of the treated area.

References

Acha PN, Szyfres B. 2003. *Zoonoses and Communicable Diseases Common to Man and Animals*, 3rd ed., p. 384. Washington, DC: Pan American Health Organization .

Banerjee K. 1990. Kyasanur Forest disease. In: Monath TP, ed., *Arboviruses: Epidemiology and Ecology*, pp. 93–116. Boca Raton: CRC Press.

Borio L, Inglesby T, Peters CJ, Schmaljohn AL, Hughes JM, et al. 2002. Hemorrhagic fever viruses as biological weapons: Medical and public health management. *JAMA* 287:2391–2405.

Briese T, Palacios G, Kokoris M, Jabado O, Liu Z, et al. 2005. Diagnostic system for rapid and sensitive differential detection of pathogens. *Emerg Infect Dis* 11:310–313.

Bronze MS, Greenfield RA. 2005. *Biodefense Principles and Pathogens*, pp. 377–389. Oklahoma City: Horizon Bioscience.

Brown RN, Lane RS, Dennis DT. 2005. Geographic distributions of tick-borne diseases and their vectors. In: Goodman JL, Dennis DT, Sonenshine DE, eds., *Tick-Borne Diseases of Humans*, pp. 363–391. Washington, DC: ASM Press.

Burke DS, Monath TP. 2001. Flaviviruses. In: Knipe DM, Howley PM, eds., *Fields Virology*, 4th ed., pp. 1046–1109. Philadelphia: Lippincott-Raven.

Charrel RN, Zaki AM, Attoui H, Fakeeh M, Billoir F, et al. 2001. Complete coding sequence of the Alkhurma virus, a tick-borne flavivirus causing severe hemorrhagic fever in humans in Saudi Arabia. *Biochem Biophys Res Commun* 287:455–461.

Dobler G. 2010. Zoonotic tick-borne flaviviruses. *Veterinary Microbiology* 140:221–228.

Dodd KA, Bird BH, Khristova ML, Albariño CG, Carroll SA, et al. 2011. Ancient ancestry of KFDV and AHFV revealed by complete genome analyses of viruses isolated from ticks and mammalian hosts. *PLoS Negl Trop Dis* 5:e1352.

Gould E, Solomon T. 2008. Pathogenic flaviviruses. *Lancet* 371:500–509.

Heymann DL. 2004. An official report of the American Public Health Association. In: Heymann DL, ed., *Control of Communicable Diseases Manual*, 18th ed., 35–37. Washington, DC: American Public Health Association.

Iyer CG, Laxmana Rao R, Work TH, Narasimha Murthy DP. 1959. Kyasanur Forest Disease VI. Pathological findings in three fatal human cases of Kyasanur Forest Disease. *Indian Journal of Medical Sciences* 13:1011–1022.

Mark N. 1987. Kyasanur Forest disease: An ethnography of a disease of development. *Medical Anthropology Quarterly, New Series* 1:406–423.

Mehla R, Kumar SRP, Yadav P, Barde PV, Yergolkar PN, et al. 2009. Recent ancestry of Kyasanur Forest disease virus. *Emerg Infect Dis* 15:1431–1437.

Pattnaik P. 2006. Kyasanur forest disease: An epidemiological view in India. *Reviews in Medical Virology* 16:151–165.

Qattan I, Akbar N, Afif H, Abu Azmah S, Al-Khateeb T, et al. 1996. A novel flavivirus: Makkah Region, 1994–1996. *Saudi Epidemiology Bulletin* 3:1–3.

Schreck CE, Mount GA, Carlson DA. 1982. Wear and wash persistence of permethrin used as a clothing treatment for personal protection against the lone star tick (Acari: Ixodidae). *Journal of Medical Entomology* 19:143–146.

Schreck CE, Snoddy EL, Mount GA. 1980. Permethrin and repellents as clothing impregnants for protection from the lone star tick. *Journal of Economic Entomology* 73:436–439.

Thiel HJ, Collett MS, Gould EA, Heinz FX, Houghton M, et al. 2005. Flaviviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desslberger U, Ball LA, eds., *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, 981–998. San Diego: Elsevier Academic Press.

Wang J, Zhang H, Fu S, Wang H, Ni D, et al. 2009. Isolation of Kyasanur Forest Disease virus from febrile patient, Yunnan, China. *Emerg Infect Dis* 15:326–328.

Work TH, Trapido H. 1957. Kyasanur Forest disease, a new virus disease in India. *Indian Journal of Medical Sciences* 11:341.

Zaki AM. 1997. Isolation of a flavivirus related to the tick-borne encephalitis complex from human cases in Saudi Arabia. *Trans R Soc Trop Med Hyg* 91:179–181.

Chapter 14

Hantavirus Disease

Introduction

Hantavirus disease is a life-threatening disease spread to humans by rodents, and it has symptoms similar to influenza. Hantaviruses are negative-sense RNA viruses in the *Bunyaviridae* family, which is a relatively newly discovered genus of viruses. Several thousand United Nations soldiers became ill with "Korean hemorrhagic fever" (now called HFRS) during the Korean War. The isolation of *Hantaan* virus (HTNV), the founding member of the group, was reported in the late 1970s by Ho-Wang Lee of South Korea and his colleagues (Lee et al., 1978), and the name *Hantavirus* is derived from the Hantan River area in South Korea. Humans might be infected with Hantaviruses through rodent bites, urine, saliva, or contact with rodent waste products. The virus may also enter the body with contaminated dirt or dust through a cut or wound, the eyes, by ingestion of contaminated water or food, or inhalation of contaminated dust. It neither seems to be transferred from one human to another nor to be transmitted by bites from fleas, ticks, mosquitos, or other biting insects. Although cats and dogs are not known carriers of the disease, they may bring infected rodents into contact with humans. Hantaviruses have also been found in multiple species of shrews and moles (Kang et al., 2011). Some Hantaviruses cause potentially fatal diseases in humans, hemorrhagic fever with renal syndrome (HFRS), and Hantavirus pulmonary syndrome (HPS), but others have not been associated with human disease. The pathogenesis of *Hantavirus* infections is not clear because of the lack of animal models to study (because rats and mice do not seem to acquire severe disease). Although the primary site of viral replication in the body is not known, in HFRS the main effect is in the blood vessels and in HPS, most symptoms are associated with the lungs. In HFRS, there is increased vascular permeability and decreased blood pressure resulting from the endothelial dysfunction and the most dramatic damage is seen in the kidneys, whereas in HPS, the lungs, spleen, and gall bladder are the most affected.

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Epidemiology and Global Scenario

The first outbreak of Hantavirus disease occurred during the Korean War (1950–1953), in which more than 3,000 United Nations troops fell ill with Korean hemorrhagic fever (HFRS). The second outbreak of the disease occurred in the Four Corners region of the United States in 1993,;hence initially it was referred to as "Four Corners disease," but is now called HPS or Hantavirus cardiopulmonary syndrome (HCPS). Table 14.1 shows the geographic distribution of and disease associated with Old World and New World strains of Hantavirus.

The epidemiology of hantavirus infections in human populations is based on incidences of peridomestic exposure of humans to rodents in endemic areas. In most of the cases, humans acquired infection on direct contact with infected rodents or their excreta, which occurs mostly by inhaling virus-contaminated aerosol. In the United States, person-to-person transmission has not been observed in the majority of hantavirus infections (Wells et al., 1997a); however, person-to-person transmission of Andes virus (ANDV) has been reported in Argentina and Chile (Padula et al., 1998; Wells et al., 1997b). Figure 14.1 shows the geographical representation of the relative global distributions of HFRS and HPS cases by country.

Epidemiology of Old World Hantaviruses

After the discovery of hantavirus, epidemiological studies were started in both human and rodent populations. Farmers, soldiers, and rural inhabitants were the most likely victim of HFRS. Initially, it was believed that HFRS occurred only in rural areas of Eurasia, specifically China, South Korea, eastern Russia, and northern Europe (Lee, 1982). HFRS caused by Seoul virus (SEOV) could also occur in urbanized cities and in many parts of the world (Lee and van der Groen, 1989).

In Europe most of the HFRS cases caused by Puumala virus (PUUV), of which 35,424 cases reported by the end of 2006 (Heyman et al., 2009), whereas 95 percent of the cases were reported after 1990. In general, PUUV infections occur throughout the continent within the range of the *Myodes glareolus* habitat. Dobrava-Belgrade virus (DOBV; derived from *Apodemus flavicollis*) infections have been identified only in the Balkan region (Antoniadis et al., 1996; Avsic-Zupanc et al., 1999), although the host species is distributed in a much larger area. Saaremaa virus (SAAV) carried by *Apodemus agrarius* has been detected in field mice in Central and Eastern Europe (Estonia, Russia, Denmark, Slovenia, and Slovakia), and it has also been associated with human disease in outbreaks in Russia (Klempa et al., 2008; Lundkvist et al., 1997; Plyusnin et al., 1999), individual cases in Germany (Klempa et al., 2003), and Slovakia (Sibold et al., 1999). In northern Europe, the epidemics typically occurs in November to December during high-rodent-density years, when the infected rodents come into contact with humans in the countryside, with another peak in August (typically when urban dwellers have been exposed during their summer holidays) (Brummer-Korvenkontio et al., 1999). The average sero prevalence in Finland and northern Sweden are about 5 percent and more than 50 percent, respectively; seroprevalence has been detected in rural communities especially for elderly males (Ahlm et al., 1994; Brummer-Korvenkontio et al., 1999). Up to 2,195 and 3,200 cases per calendar year have been detected in 2007 and 2008 in Sweden and Finland, respectively (Olsson et al.,

Table 14.1. Geographic distribution of and disease associated with Old World and New World strains of Hantavirus.

Group and subfamily	Virus Isolate or Strain	Abbreviation[a]	Geographic Distribution	Rodent Host	Associated Disease
Old World hanta viruses					
Murinae	Hantaan virus	HTNV	China, South Korea, Russia	*Apodemus agrarius*	HFRS
	Dobrava-Belgrade virus	DOBV	Balkans	*Apodemus flavicollis*	HFRS
	Seoul virus	SEOV	Worldwide	*Rattus*	HFRS
	Saaremaa virus	SAAV	Europe	*A. agrarius*	HFRS
	Amur virus	AMRV	Far East Russia	*Apodemus peninsulae*	HFRS
	Soochong virus	—	South Korea	*A. peninsulae*	Unknown
Arvicolinae	Puumala virus	PUUV	Europe, Asia, and Americas	*Clethrionomys glareolus*	HFRS/NE
	Khabarovsk virus	KHAV	Far East Russia	*Microtus fortis*	Unknown
	Muju virus	MUJV	South Korea	*Myodes regulus*	Unknown
	Prospect Hill virus	PHV	Maryland	*Microtus pennsylvanicus*	Unknown
	Tula virus	TULV	Russia/Europe	*Microtus arvalis*	Unknown
	Isla Vista virus	ISLAV	North America	*Microtus californicus*	Unknown
	Topografov virus	TOPV	Siberia	*Lemmus sibericus*	Unknown
New World hanta viruses					
Sigmodntinae	Sin Nombre virus	SNV	North America	*Peromyscus maniculatus*	HPS
	Monongahela virus	MGLV	North America	*Peromyscus leucopus*	HPS
	New York virus	NYV	North America	*P. leucopus*	HPS
	Black Creek Canal virus	BCCV	North America	*Sigmodon hispidus*	HPS
	Bayou virus	BAYV	North America	*Oryzomys palustris*	HPS
	Limestone Canyon virus	—	North America	*Peromyscus boylii*	Unknown
	Playa de Oro virus	—	Mexico	*Oryzomys couesi*	Unknown
	Catacamas virus	—	Honduras	*O. couesi*	Unknown

(*Continued*)

Table 14.1. (*Cont'd*)

Group and subfamily	Virus Isolate or Strain	Abbreviation[a]	Geographic Distribution	Rodent Host	Associated Disease
	Choclo virus	—	Panama	*Oligoryzomys fulvescens*	HPS
	Calabazo virus	—	Panama	*Zygodontomys brevicauda*	Unknown
	Rio Segundo virus	RIOSV	Cost Rica	*Reithrodontomys mexicanus*	Unknown
	Cano Delgadito virus	CADV	Venezuela	*Sigmodon alstoni*	Unknown
	Andes virus	ANDV	Argentina, Chile	*Oligoryzomys longicaudatus*	HPS
	Bermejo virus	BMJV	Argentina	*Oligoryzomys chocoensis*	HPS
	Pergamino virus	PRGV	Argentina	*Akodon azarae*	Unknown
	Lechiguanas virus	LECV	Argentina	*Oligoryzomys flavescens*	HPS
	Maciel virus	MCLV	Argentina	*Bolomys obscurus*	HPS
	Oran virus	ORNV	Argentina	*O. longicaudatus*	HPS
	Laguna Lechiguanas virus Negra virus	LANV	Paraguay, Bolivia, Argentina	*Calomys laucha*	HPS
	Alto Paraguay virus	—	Paraguayan Chaco	*Holochilus chacoensis*	Unknown
	Ape Aime virus	—	Eastern Paraguay	*Akodon montensis*	Unknown
	Itapúa virus	—	Eastern Paraguay	*Oligoryzomys nigripes*	Unknown
	Rio Mamore virus	—	Bolivia, Peru	*Oligoryzomys microtis*	Unknown
	Araraquara virus	—	Brazil	*Bolomys lasiurus*	HPS
	Juquitiba virus	—	Brazil	*Oligoryzomys nigripes*	HPS
	Jaborá virus	—	Brazil, Paraguay	*Akodon montensis*	

HFRS, Hemorrhagic fever with renal syndrome; HPS, Hantavirus pulmonary syndrome; NE, nephropathia epidemica (milder HFRS form).

Figure 14.1. 7 + Geographical representation of approximate annual hanta viral disease incidence by country. HFRS, hemorrhagic fever with renal syndrome; HPS, Hantavirus pulmonary syndrome; NE, nephropathia epidemica (milder HFRS form).
Source: Courtesy of Douglas Goodin, Kansas State University.

2009). On average, the disease predominates in individuals of 40 to 45 years age, with a male-to-female ratio of 2:1 (Brummer-Korvenkontio et al., 1999).

Since the disease was first reported in Germany, an average of 400 cases has been documented per year, with a record-high peak of 1,687 cases in 2007. Another region of endemicity is northeastern France and the Ardennes region in Belgium. In both France and Belgium, 50 to 150 cases were seen in the beginning of this millennium; however, 2005 was a record-high epidemic year (i.e., 253 and 372 cases in France and Belgium, respectively). As in Germany, the rodent and HFRS peaks were highest during the summer, followed by good mast years of trees the previous year, and, therefore, were a consequence of climate factors (Heyman et al., 2008; Tersago et al., 2009).

In Russia, 89,162 HFRS cases were detected from 1996 to 2006, and the mean incidence was 5.8 cases per 100,000 individuals (1997–2007), peaking in 1997, with 14.3 cases per 100,000 individuals. The incidence of HFRS in Russia varies geographically and the areas where the disease is the most endemic are the regions in the Volga federal district, especially Tatarstan, Udmurtia, Samara, Orenburg, and Bashkirostan, the latter with the highest incidence (68 cases per 100,000 individuals per year) in these regions where PUUV infection prevails (Garanina, et al., 2009).

In China during the last few years, 12,000 to 20,000 HFRS cases have been registered with the China Centre for Disease Control, with a mortality rate of approximately 1 percent (Lee, 1996; Song, 1999; Jonsson et al., 2010). These include cases caused by *Apodemus*-borne HTNV and HTNV-like viruses (Amur/Soochong virus) as well as rat-borne SEOV. Although cases occur throughout the year, the peak in incidence occurs in November and December. However, much higher case numbers (as well as mortality) were previously reported (Lee, 1996; Song, 1999), which suggested 1,256,431 cases from 1950 to 1997 and 44,304 deaths (3.53 percent), with the highest annual peak in 1986, with 115,985 cases. The HFRS incidence data for 1994 to 1998 showed that the highest incidence was in the middle and eastern part of China, with top incidences of 20.3, 18.9, 8.2, 7.7, 5.0, and 4.6 cases per 100,000 individuals in the Heilongjiang, Shandong, Zhejiang, Hunan, Hebei, and Hubei Provinces, respectively, covering 70 percent of all cases (Yan et al., 2007).

In South Korea, most cases are caused by HTNV (Amur virus [AMRV]/Soochong virus [SOOV]), and a small percentage of HFRS cases may be caused by Muju virus (MUJUV), harbored by *Myodes regulus* (Song et al., 2007). Hundreds of HFRS cases were registered in the 1970s and 1980s, with a sharp increase in the number of cases in the early 1990s, up to 1,200 cases per year. From 2001 to 2008, 323 to 450 HFRS cases were registered annually in the Republic of Korea.

HFRS probably occurs in other countries in Asia because antibodies to Thailand virus (THAIV) have been found in rodents and humans in Thailand (Pattamadilok et al., 2006; Suputthamongkol et al., 2005), Indonesia (Plyusnina et al., 2009), and India (Chandy et al., 2009).

Epidemiology of New World Hantaviruses

In 1993, a group of individuals living in rural areas in the Four Corners regions of the Southwestern United States died of acute unexplained respiratory distress (Chapman and Khabbaz, 1994). This was probably the initial outbreak of HPS, which was further identified as a newly recognized hantavirus, Sin Nombre virus (SNV). American Indian healers living in the affected areas were aware of similar deaths occurring over three cycles during the 20th century in association with identifiable ecological markers and, therefore,

had developed preventative measures. An emergency phone hotline was established by the Center for Disease Control and Prevention (CDC), which was successful in rapidly identifying the widespread sporadic geographic distribution of HPS cases throughout the United States (Tappero et al., 1996). Since the time of this outbreak though March 2007, 465 HPS cases in the United States have been reported to the CDC among which 35 percent resulted in death (64 percent male and 37 percent female), with a mean age of patients of 38 years (range, 10–83 years). HPS cases have been reported in 30 US states, with the majority in the western half of the country, especially in residents of rural areas.

The first outbreak of HPS cases outside the United States occurred in Paraguay from July 1995 through January 1996 (Williams et al., 1997) in which 17 cases of HPS caused by Laguna Negra virus (LANV) were confirmed for persons living in the western part of Paraguay. The first outbreak of HPS in Paraguay was reported in 1996 and 1997 with more than 125 cases. In Argentina, the first recorded outbreak of HPS cases was reported from September through December of 1996 in the southern Andean city of El Bolson, a ski resort nestled in the Andes Mountains, and it involved 18 cases, which also included three doctors who treated patients with the disease and became ill themselves. The physicians' illness strongly suggested person-to-person transmission of hantaviruses. This was also confirmed in case of a physician in Buenos Aires who became ill 27 days after taking care of a patient with HPS who had been transferred from the outbreak area to Buenos Aires (Wells et al., 1997a).

The first outbreak of HPS in Panama occurred in 1999 to 2000 in Los Santos, with reported 12 cases and a 25 percent case fatality rate (Vincent et al., 2000). However, the three fatal cases were not confirmed by serological tests. Sequence analysis of the virus genome showed that this virus belongs to the category of novel hantavirus, Choclo virus. Serological and virus genetic analyses of rodents trapped in the area showed *Oligoryzomys flavescens* to be the likely reservoir of Choclo virus. In addition, *Zygodontomys brevicauda* rodents were shown to harbor another genetically unique hantavirus, Calabazo virus (Vincent et al., 2000).

In Uruguay, the first three cases of HPS were reported in 2004 in the area bordering Brazil. Two closely related hantaviruses, Lechiguanas virus (LECV) and Andes Central Plata virus, are also found to be associated with HPS (Delfraro et al., 2008). Both of these viruses are harboured by *O. flavescens*. However, a Juquitiba virus (JUQV)-like hanta virus in Uruguay, carried by two rodent species, *Oligoryzomys nigripes* and *Oxymycterus nasutus*, was recently described (Delfraro et al., 2008). In Brazil, the first reported HPS cases occurred in 1993 in the southeast, caused by JUQV, and in this small outbreak, 66 percent of the patients reported fatality (Vasconcelos et al., 1997). The overall analysis reported (from 1993 through April 2009) 1,145 HPS cases in Brazil, with a 39.5 percent case fatality rate, which were caused mostly by five lineages of hantavirus: Araucária strain, Araraquara, LANV-like, Castelo dos Sonhos, and Anajatuba viruses (Johnson et al., 1999; Mendes et al., 2004).

The Etiological Agent

Classification of Hantaviruses

Hantaviruses belong to the category of negative-stranded RNA viruses. All the negative-stranded RNA viruses are broadly classified into two main groups, one group consists of nonsegmented RNA and another group consists of segmented genomic RNA (Figure 14.2). The nonsegmented group comprises four families *Paramyxoviridae*, *Filoviridae*,

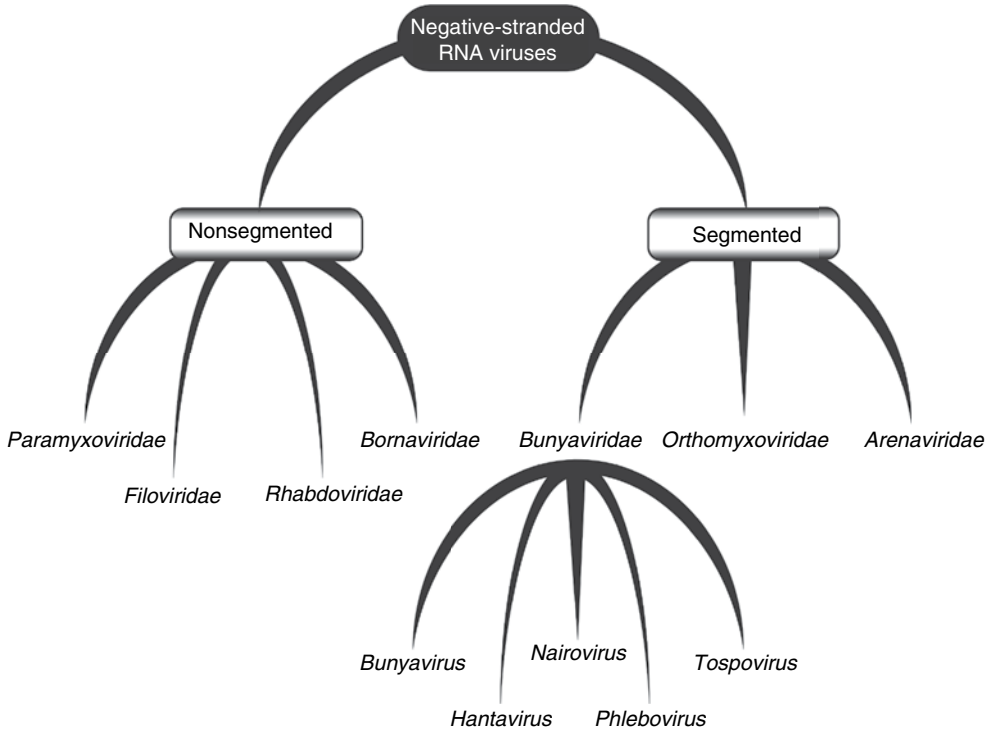

Figure 14.2. Classification of negative-stranded RNA viruses.

Rhabdoviridae, and *Bornaviridae*, whereas segmented group includes three families *Bunyaviridae*, *Orthomyxoviridae*, and *Arenaviridae*. The *Bunyaviridae* family is further divided into five genera: *Orthobunyavirus*, *Nairovirus*, *Phlebovirus*, *Tospovirus* and *Hantavirus*. Like all members of this family, hanta viral genome comprising three negative-sense, single-stranded RNA segments. Most of the generic members of *Bunyaviridae* family generally are arthropod-borne viruses, but hantaviruses are transmitted to humans mainly through the inhalation of aerosolized rodent excreta (carrying pathogenic virus) or rodent bites.

Newly emerged hantaviruses are being discovered all of the time, and many of them are not found to be associated with human disease. The ones that cause human illness are divided into two major groups.

1. The first group is found mostly in Asia and Europe. These hantaviruses include HTNV, SEOV, and PUUV, which are found to be associated with HFRS.
2. The second group is found in North, South, and Central America. These hantaviruses include SNV, New York virus (NYV), Bayou, and Black Creek Canal, which result in HPS, causing severe respiratory distress often leading to death

Structure of Hantaviruses

Like other members of the *Bunyaviridae* family, hantaviruses are enveloped RNA viruses having an average diameter of about 80 to 120 nm. Hanta viral particles appear enveloped,

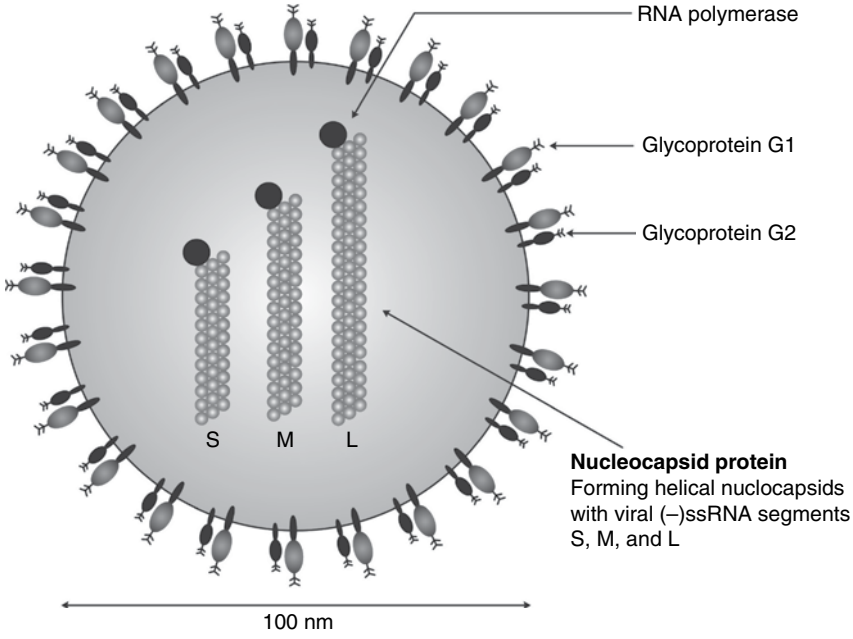

Figure 14.3. Morphology of Hantavirus. The negative ssRNA segments: S (small), M (medium), and L (large) are encoded for the nucleocapsid protein, the glycoproteins G1 and G2, and the RNA-dependent RNA polymerase, respectively.

spherical, or pleomorphic in electron micrographs with a lipid bilayer of about 5-nm thick, embedded with viral surface proteins to which sugar residues are attached. These glycoprotein, referred to as G1 and G2, are heterodimerize (i.e., tend to associate with each other and have an interior tail and an exterior domain that extends to about six nm beyond the envelope surface; Figure 14.3). Inside this envelope virally encoded RNA polymerase and nucleocapsid are present, composed of many copies of the nucleocapsid protein, N.

Hanta Viral Genome

Viral genome consists of three (tripartite) single-stranded, negative-sense RNA segments designated as L (large), M (medium), and S (small) (Figure 14.4). The largest segment L (6.5–6.6 kb) encoding L protein, which functions as the viral transcriptase/replicase, that converts the negative-sense genomic RNA into messenger RNA (mRNA), which can further be translated into proteins. The transcriptase protein is also found associated with each segment of the genome to quickly initiate this process on cellular entry. The M segment (3.7–3.8 kb) encodes a polyprotein, which is a glycoprotein precursor (GPC) and is cotranslationally cleaved to yield the envelope glycoproteins G1 and G2 that regulate the viral entry into the cell. The S segment (1.8–2.1 kb) codes for the N protein that make up the nucleocapsid.

The negative-sense RNA genomes serve as templates for producing positive-sense complementary RNA (cRNA) and m RNA. The total size of RNA genomes ranges from

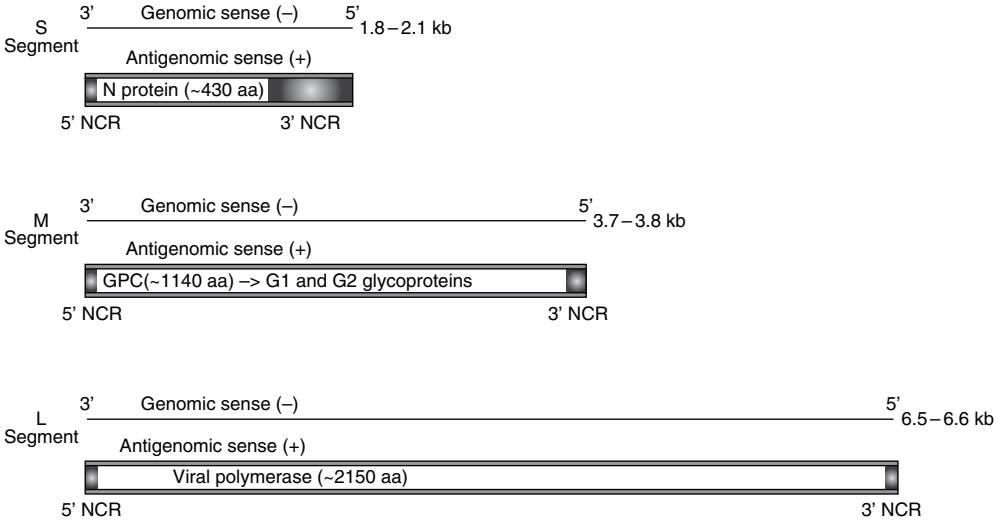

Figure 14.4. Genomic structure of Hantaviruses.

11,845 nucleotide for HTNV to 12,317 for SNV (Jonsson et al., 2010). Each of these viral RNA segments is covered with nucleocapsid proteins forming three helical ribonucleoprotein (RNP) complexes. The viral RdRp (RNA-dependent RNA polymerase) is associated with these nucleocapsid.

The 3' and 5' termini of hanta viral genome are highly conserved and complementary and thus capable of forming pan-handle-type of structures, a hallmark of *Bunyaviridae* (Elliott et al., 1991), which are at least 17-bp long, 14 of the 17 bases from each end are genus specific known as hantavirus sequences.

Life Cycle of Hantaviruses

Transmission of hantavirus in host populations occurs horizontally and more frequently among male than female rodents (Figure 14.5). Rodent-to-rodent transmission is believed to occur primarily after weaning and through physical contact, perhaps through aggressive behavior, such as fighting. Infection is also found occasionally in numerous other species of rodents and their predators (e.g., dogs, cats, and coyotes), indicating that many mammalian species coming into contact with an infected host might become infected. Infection cannot be transmitted to other animals or to humans from these "dead-end" hosts. However, domestic cats and dogs may bring infected rodents into contact with humans.

Entry of Virion

Each virus is highly specific because it may only infect a certain species or even a certain cell type within an organism; this property is known as *viral tropism*. Hantaviruses show tropism toward endothelial, epithelial, macrophage, follicular dendritic, and lymphocyte cells via the attachment of the viral glycoprotein to the host's cell surface receptor(s) (Mackow and Gavrilovskaya, 2001). In the initial step, of cellular attachment hantavirus binds to a family of proteins called as β-integrins, which are the large proteins present at

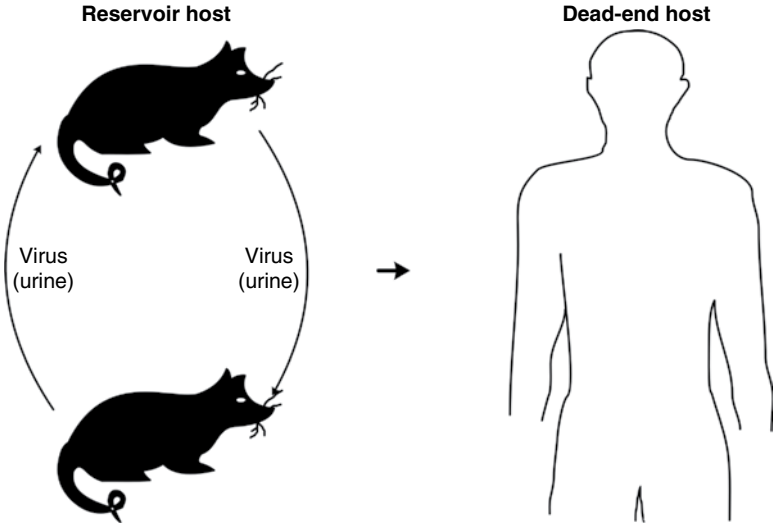

Figure 14.5. Transmission of Hantaviruses.

the cell membrane. There may be multiple β-integrin isoforms expressed on a cell. The pathogenic hantavirus strains (HTNV, SEOV, PUUV, SNV, NYV) use the β3-integrin as a receptor, whereas the nonpathogenic strains (Prospect Hill, Tula) recognize the β1-integrin receptor. On successful attachment to its cellular receptor, the penetration phase begins, which is carried out by the cell as the cell internalizes the integrin receptor and viral complex through the process of endocytosis. The endosomal membrane is then fused with a lysosome (highly acidic sac). Normally proteins are degraded by an acidic environment; however, the virus uses the acidic environment of the lysosome to its advantage by fusing with the lysosomal membrane. Hence the nucleocapsids are then released into the cytoplasm by pH-dependent fusion of the virion with the endosomal membrane. Subsequently with the release of the nucleocapsids into the cytoplasm, the complexes are targeted to the endoplasmic reticulum–Golgi intermediate compartments (ERGIC) through microtubular associated movement resulting in the formation of viral factories at ERGIC, which then facilitate transcription and subsequent translation of the viral proteins (Figure 14.6).

Transcription, Translation, and Replication of Virion

On uncoating the virion liberate all the three RNP molecules into the cell cytoplasm. Viral RdRp initiates primary transcription to give rise to S, M, and L mRNAs. The translation of the S and L mRNA transcripts occurs on free ribosomes, whereas the M-segment transcript occurs on membrane-bound ribosomes, which is cotranslated on rough endoplasmic reticulum (rER). The N protein is the most abundant viral protein for hantaviruses, which is synthesized early in infection (Schmaljohn and Hooper, 2001) and plays key roles in several important steps, in the virus life cycle, including translation, trafficking, and assembly (Jonsson and Schmaljohn, 2001). The viral L protein has a transcriptase, replicase and an endonuclease activity that cleaves cellular mRNA for the production of capped primers used to initiate transcription of viral mRNAs. As a result

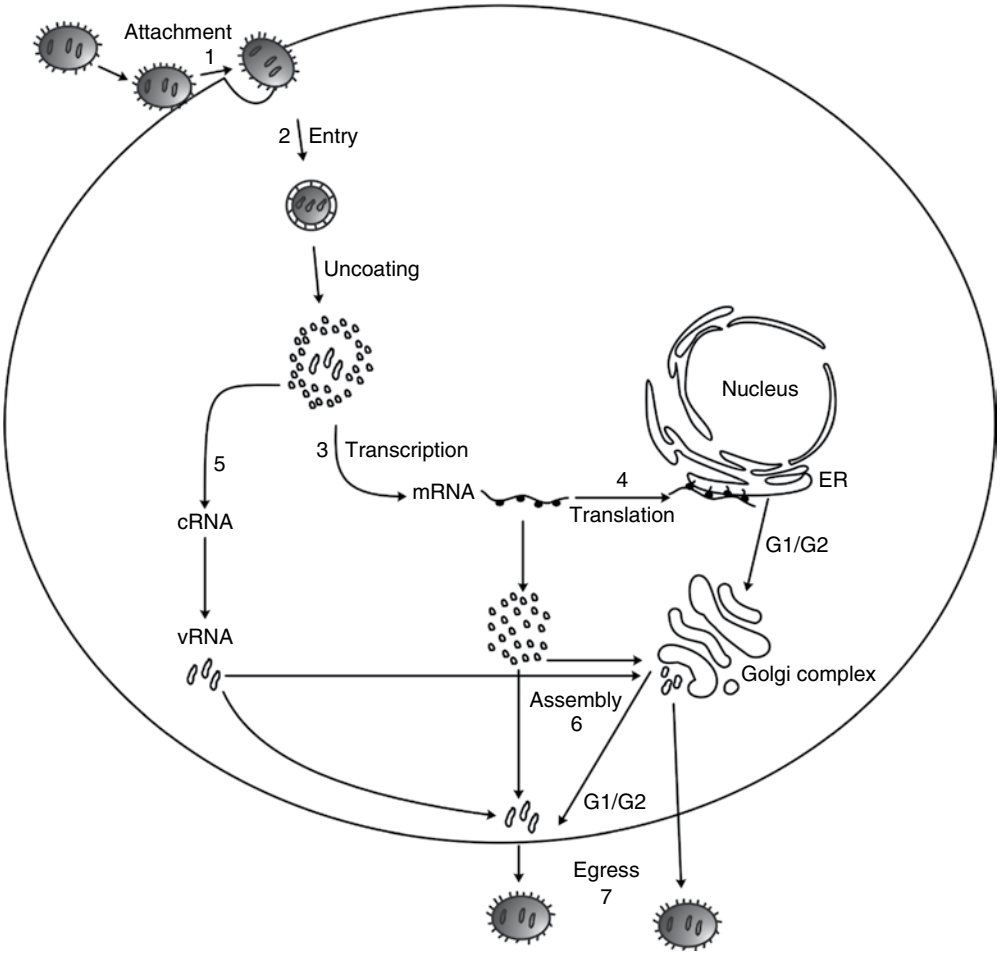

Figure 14.6. The Hantavirus life cycle. (1) The attachment of the infectious viral particle to the host cells' surface through interactions between the host's cell surface receptors and the viral glycoprotein. (2) Entry through receptor-mediated endocytosis and the uncoating and release of the viral genomes. (3) Immediately thereafter transcription of complementary RNA (cRNA) from the viral RNA (vRNA) genome using host-derived primers. (4) Translation of large (L), medium (M), and small (S) messenger RNA (mRNA) to form viral proteins using host machinery. (5) Replication and amplification of vRNA, assembly with the N protein, and finally transport to the Golgi apparatus. (6) Assembly of all viral components at the Golgi apparatus or at the plasma membrane (alternative assembly) possibly for New World viruses. (7) Viral egress through the fusion of the Golgi vesicle harboring the mature virion particles with the plasma membrane of the infected host cell.

of this "cap snatching," the mRNAs of hantaviruses are capped containing nontemplated 5' terminal extensions. The glycoprotein precursor is proteolytically processed into G1 and G2 during import into the ER (Ruusala et al., 1992; Spiropoulou, 2001), which further glycosylates in the ER and then is transported to the Golgi complex (Antic et al., 1992; Ruusala et al., 1992). When there is the production of sufficient amount of N

protein it signals a switch from transcription of the viral genome to replication, resulting the production of newly formed negative-sense viral RNA bounded by L protein polymerase and encapsidated by the N protein to form the RNPs (Schmaljohn and Hooper, 2001). The process occurs in perinuclear region (around the nucleus), but the final assembly with G1 and G2 glycoproteins occur at either the Golgi (Old World) or plasma membrane (New World viruses). The newly formed viral envelope is derived from the cellular lipid bilayer and simply buds off from the cell. These newly assembled progeny viruses are then free to target and infect other uninfected cells.

Clinical Features

Hantaviruses do not readily cause overt illness in their reservoir hosts. The infected rodents shed virus in saliva, urine, and feces for many weeks or months or for the whole life, and the quantity of virus shed can be at its greatest approximately 3 to 8 weeks after infection. Human infection occurs most commonly through the inhalation of infectious aerosolized saliva or excreta. Persons visiting laboratories housing infected rodents can easily be infected only after a few minutes of exposure to animal holding areas. Transmission can occur when dried materials contaminated by rodent excreta are inhaled or directly introduced into broken skin or conjunctiva, or possibly, when ingested with contaminated food or water. HPS can be acquired after being bitten by rodents. High risk of exposure has been found associated with entering or cleaning rodent-infested structures especially in viral laboratories. These viruses mainly cause two serious illnesses in humans, HFRS and HPS.

Hemorrhagic Fever with Renal Syndrome HFRS

As the name indicates, the three most common symptoms of HFRS are fever, malfunction of the kidneys, and the thrombocytopenia (low platelet count). Platelets are responsible for blood clotting, when the platelet count in blood gets reduced, blood clotting does not occur properly, as a result, the patient tends to hemorrhage or bleed easily.

Initial onset of HFRS is marked by nonspecific flulike symptoms of fever, myalgia, headache, abdominal pain, nausea, and vomiting sensation. There is a characteristic facial flushing and usually a petechial rash (limited to the axilla, or armpit). Sudden and extreme albuminuria occur about fourth day, which is a characteristic of severe HFRS followed commonly by ecchymosis, scleral injection, and bloodshot eyes. Additional symptoms include hypotension, shock, respiratory distress or failure, and renal impairment or failure especially the characteristic damage to the renal medulla, which is unique to hantaviruses.

Hantavirus has an incubation period of 2 to 4 weeks in humans before the occurrence of symptoms of infection. These symptoms of HFRS can be divided into five different phases (Figure 14.7):

1. **Febrile phase**: Symptoms of this phase include fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as those common in influenza infection, as well as gastrointestinal problems. These symptoms normally occur for 3 to 7 days and arise about 2 to 3 weeks after exposure (Canadian Lung Association, 2009).

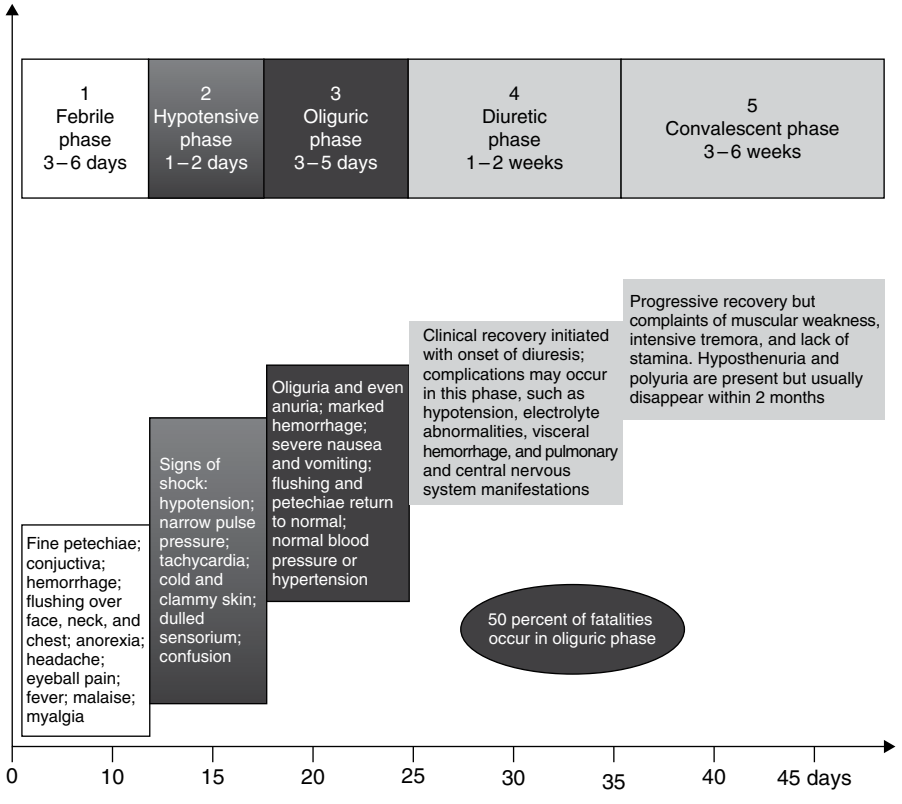

Figure 14.7. Clinical course and symptoms of hemorrhagic fever with renal syndrome.

2. **Hypotensive phase**: It starts with shock, during which nausea and vomiting are common. This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days, and approximately one-third of deaths occur at this stage.
3. **Oliguric phase**: This phase lasts for 3 to 5 days and sometimes extends up to 7 days, which is characterised by the onset of proteinuria and renal failure through renal impairment and relative hypervolemia resulting half of all deaths.
4. **Diuretic phase**: This is characterized by diuresis of 3 to 6 L/day, which can last for a couple of days up to weeks.
5. **Convalescent phase**: It is actually a recovery phase during which symptoms begin to improve and is characterized by fluid and electrolyte imbalance and can last 3 to 6 weeks or months.

Hantavirus Pulmonary Syndrome

HPS is often a fatal disease caused by hantaviruses. The symptoms are similar to those of HFRS including tachycardia and tachypnea; patients additionally develop difficulty in breathing, coughing, and shortness of breath (Canadian Lung Association, 2009). The first symptoms of HPS are fever and a sudden drop in blood pressure, which may be

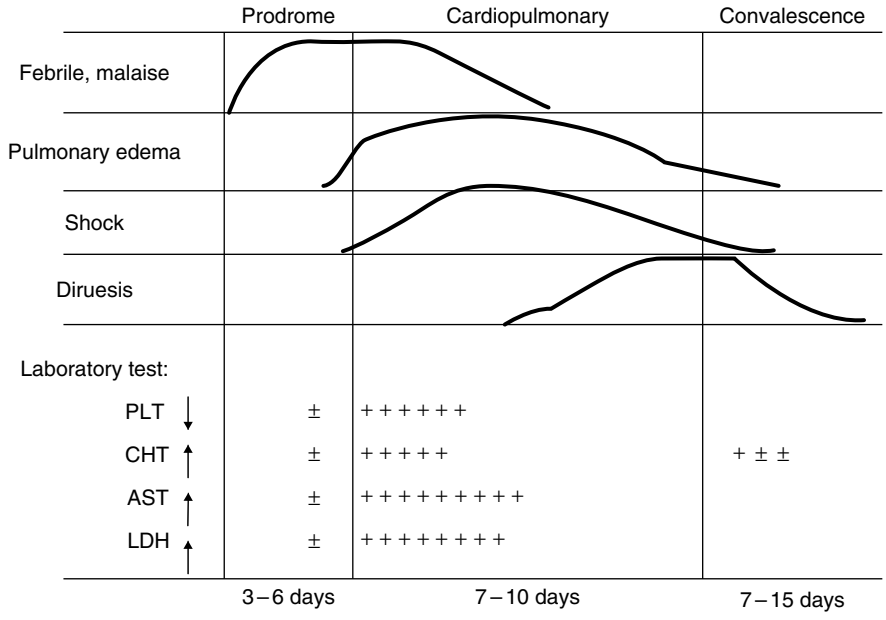

Figure 14.8. Clinical course of Hantavirus pulmonary syndrome. AST, aspartate aminotransferase; CHT, hematocrit; LDH, lactic dehydrogenase; PLT, platelets.

followed by shock and loss of blood in the lungs (Figure 14.8). During this phase, fluids may accumulate in the lungs, leading to shortness of breath. The symptoms occur so quickly that the patient goes into respiratory failure in a matter of hours. Such conditions lead to a cardiopulmonary phase characterized by a cardiovascular shock, which requires hospitalization of the patient. HPS was first recognized in 1993 in the southwest of the United States by Bruce Tempest, and it was originally called "Four Corners disease." It has since been identified throughout the United States. Although rare, HPS is fatal in up to 60 percent of recorded cases (Jonsson et al., 2010).

HPS develops in four stages. These are:

1. **Incubation period**: This lasts from 1 to 5 weeks from exposure. Here, the patient remains asymptomatic.
2. **Prodrome (warning signs) stage**: The symptoms begin with a fever, muscle aches, backache, and abdominal pain and upset stomach, sometimes followed by vomiting and diarrhea.
3. **Cardiopulmonary stage**: The patient rapidly slips into this stage, sometimes within 1 or 2 days of initial symptoms; sometimes as long as 10 days later. There is a drop in blood pressure, shock, and leaking of the blood vessels of the lungs, resulting in fluid accumulation in the lungs and subsequent shortness of breath. The fluid accumulation can be so rapid and so severe as to put the patient in respiratory failure condition within only a few hours. Some patients may experience severe abdominal tenderness.
4. **Convalescent stage**: If the patient survives the respiratory complications of the previous stage, there is a rapid recovery, usually within 1 or 2 days; otherwise it can take 7 to 15 days to recover partially as abnormal liver and lung functioning may persist up to 6 months.

Table 14.2. Comparison of hemorrhagic fever with renal syndrome and Hantavirus pulmonary syndrome.

Features	HFRS	HPS
Major target organ	Kidney	Lung
First phase	Febrile	Febrile prodrome
Second phase	Shock	Shock, pulmonary edema
Evolution	Oliguria, diureses, convalescence	Diureses, convalescence
Mortality	1–15%	60%

HPS, Hantavirus pulmonary syndrome; HFRS, hemorrhagic fever with renal syndrome.

Table 14.2 differentiates between the HFRS and HPS. It clearly shows that febrile phase and shock is common in both the illnesses, but the major target organ is different, which further leads to the difference in mortality rate.

Differential Diagnosis

The prodromal phase of HPS is clinically indistinguishable from numerous other related viral infections. The etiology of the patient's illness could be checked by the blood picture, which may show circulating immunoblasts appearing as large atypical lymphocytes and a decrease in platelets (thrombocytopenia). However, unlike other viral infections, patients with HPS usually have concurrent left-shifted neutrophilia (increase in neutrophils) with circulating myelocytes.

During the cardiopulmonary stage of the disease, the patients show a diffuse pulmonary edema. The most frequent cause for it is myocardial infarction, so it is important to obtain an electrocardiogram (ECG) and echocardiogram during the initial assessment, which can help to distinguish these patients from patients with acute respiratory distress syndrome (ARDS) because cardiac function is depressed to a much greater degree in the patients with HPS and cardiac output does not respond to fluid challenge as it tends to with ARDS.

Infections in the patient who is immunocompetent, leading to acute cardiopulmonary deterioration as in HPS, include leptospirosis, Legionnaire's disease, mycoplasma, Q fever, chlamydia, and septicemic plague, tularemia, coccidioidomycosis, and histoplasmosis (in the presence of receptive infectious organisms). Some noninfectious conditions such as Goodpasture's syndrome should also be considered. Lack of coryza clinically distinguishes between HPS and Influenza A infection.

HPS is relatively uncommon disease in patients who are immunocompromised, hence cytomegalovirus (CMV), *Cryptococcus, Aspergillus,* and graft-versus-host disease are more likely to be the cause of diffuse pulmonary infiltrates than a hanta viral infection.

Laboratory Diagnosis

Laboratory diagnosis of hanta viral diseases can only be confirmed by a positive serological test result. Detection of hanta viral antigen is in tissue by immunohistochem-

istry (IHC) or the presence of amplifiable viral RNA sequences in blood or tissue with a compatible history of HPS/HFRS.

A complete blood count (CBC) and blood chemistry should be repeated after every 8 to 12 hours in all the suspected hantavirus cases. A fall in the serum albumin and a rise in the hematocrit may indicate a fluid shift from the patient's circulation into the lungs. The white blood cell (WBC) count tends to be elevated with a marked left shift. The WBC precursors may be as high as 50 percent with the presence of atypical lymphocytes, usually at the onset of pulmonary edema. In about 80 percent of individuals with HPS, the platelet count is below 150,000 units. A dramatic fall in the platelet count may indicate a transition from the prodrome phase to the pulmonary edema phase of the illness. The most severe cases of HPS develop disseminated intravenous coagulation (DIC), but this is more common in hantavirus-related hemorrhagic fevers seen in Asia.

Serologic Assays

With the onset of symptoms, all patients with acute HFRS and HPS patients have immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to the N protein of hantavirus. Hence, serological tests detecting IgM or IgG antibodies to hanta viral antigens in serum are the most common tests for the laboratory diagnosis of suspected HPS and HFRS cases.

Immunofluorescence Assay

Indirect immunofluorescence assay (IFA) was one of the first serological tests used for laboratory diagnosis of HFRS in Europe and Asia using hantavirus-infected cells as a fixed antigen on microscopic slides. However, the virus-infected cells are not widely used for serological tests because it requires testing in a laboratory that is Biosafety Level-3. Thus, most hanta viral antigens currently used in serological tests are actually derived from recombinant DNA techniques. These antigens mostly are N proteins, but G1 and G2 glycoproteins have also been produced and used. These N proteins have been expressed and purified from a number of recombinant expression systems, including bacterial (Jonsson et al., 2001), baculovirus (Schmaljohn et al., 1988), insect (Vapalahti et al., 1996), *Saccharomyces* spp. (Schmidt et al., 2005), plant (Kehm et al., 2001), and mammalian cells. All the three structural proteins (Gn, Gc, and N) can induce a high level of IgM detectable at the onset of symptoms (Figueiredo et al., 2008), but the IgG response to the glycoproteins may be delayed, and in the acute phase, IgG IFA shows a granular pattern (Kallio-Kokko et al., 2001).

Enzyme Linked Immunosorbent Assay

The N protein is also used as an antigen in immuno-enzymatic assays (EIAs) for the diagnosis of Hantavirus infection (Figueiredo et al., 2008) as well as strip immunoblot tests (Hjelle et al., 1997). The most common serological tests for Hantaviruses are indirect IgG and IgM enzyme-linked immunosorbent assays (ELISAs) as well as IgM capture ELISAs. The rapid IgM capture ELISA, which was developed by the US Army Medical Research Institute of Infectious Diseases and the CDC is effective for the diagnosis of HFRS and HPS (Feldmann et al., 1993) and takes about 4 to 6 hours when performed by trained and skilled personnel. An IgG test is also used in conjunction with the IgM-capture test for best results. An acute and convalescent-phase serum showing a fourfold rise in IgG

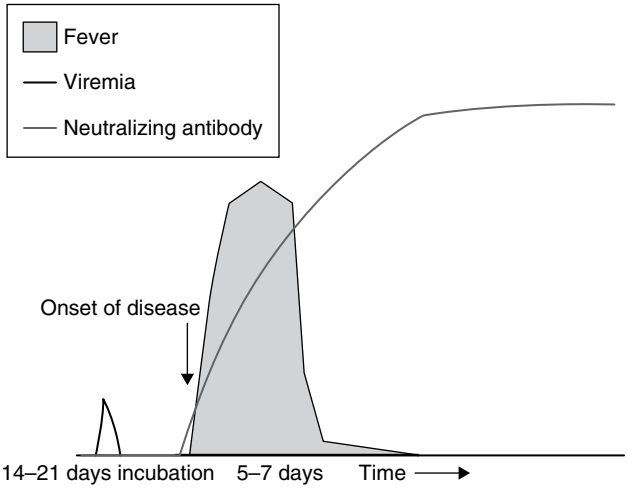

Figure 14.9. Viremia and antibody response in human Hantavirus infection.

antibody titer or the presence of IgM in acute-phase serum, considered being diagnostic indication for Hantavirus disease. The acute-phase serum of the patients sent as an initial diagnostic specimen does not have IgG because IgG is the antibody of secondary immune response, which is long lasting, and sera of patients retrospectively identified retained this antibody for many years. The SNV IgG ELISA has, therefore, been found appropriate for the serologic investigations during studying the epidemiology of the disease.

Blotting Techniques

A Western blot assay has also been developed using recombinant antigens and isotype-specific conjugates for IgM-IgG differentiation, and its results are quite confirmatory as with those of the IgM-capture format. Another blotting technique designated as a rapid immunoblot strip assay (RIBA) is an investigational prototype assay that identifies serum antibody to recombinant proteins and peptides specific for Hantaviruses.

Neutralization Tests

Neutralization tests have also been used as routine tests for the serological diagnosis and typing of hanta viral infections (Prince et al., 2007). The plaque reduction neutralization test (PRNT) is the most definitive method to facilitate the identification and differentiation of Hantaviruses (Chu et al., 1994). It is a specific test for the detection and measurement of viral neutralizing antibodies, produced after 14 to 21 days post-infection (Figure 14.9). Cross-PRNT permits the serotypic classification of Hantavirus infection in rodents and humans (Chu et al., 1995). This is a highly specific assay for distinguishing Hantaviruses with serum from experimentally infected animals but shown to be less specific for the diagnosis of human acute-phase sera from patients with HFRS and HPS. Neutralization tests are laborious and require laboratories that are Biosafety Level-3, however, remain the methods of choice for distinguishing between related Hantavirus infections serologically.

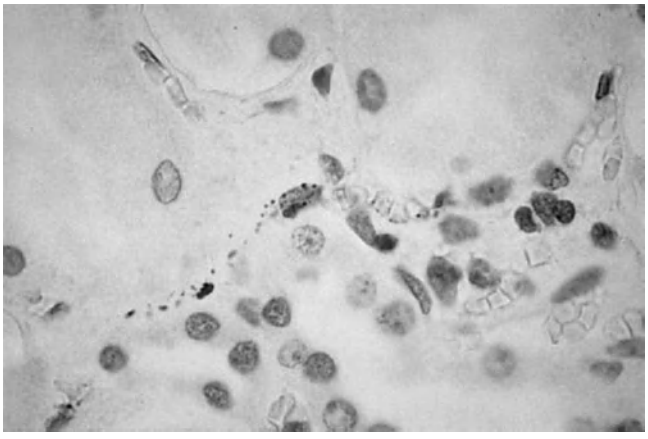

Figure 14.10. High power magnification showing immunostaining of renal interstitial capillaries using peromyscus serum.

Immunohistochemistry

IHC testing (using formalin-fixed tissues) with specific monoclonal and polyclonal antibodies can be used to detect Hantavirus antigens and has been proven to be a sensitive method for laboratory confirmation of hanta viral infections (Figure 14. 10). IHC plays an important role in the diagnosis of HPS in patients from whom serum samples and frozen tissues are unavailable for diagnostic testing and also in the retrospective assessment of disease prevalence in a particular geographic region.

Polymerase Chain Reaction

HPS is a fast-evolving disease with a high case fatality rate, and thus, there is a clear need for rapid diagnostic tests. A patient can evolve from acute febrile illness to severe pneumonia within 12 to 24 hours with respiratory failure and cardiac shock requiring rapid diagnosis. For the detection of the Hantavirus genome, a reverse transcriptase-PCR (RT-PCR) test is frequently used, involving fresh-frozen lung tissue, blood clots, or nucleated blood cells; however, it is prone to cross-contamination and should be considered an experimental technique only. Because the viral RNA is present at extremely low levels in human and rodent tissue samples, this requires nested-RT-PCR techniques, which uses primers selected for regions with high homology. Various differences in Hantaviruses complicate the use and sensitivity of RT-PCR for the routine diagnosis of these viral infections.

Case Management

Currently, there is no specific treatment available for both the clinical outcomes of Hantavirus (HFRS and HPS); therefore, the cornerstone of treatment remains supportive measures. In cases high degree of suspicion of HPS, the clinical management must include:

1. The patient should be transferred immediately to an emergency department or intensive care unit (ICU), where blood and tissue oxygenation, cardiac output, central blood pressure, and cerebral pressure can be frequently monitored (Lázaro et al., 2007). Although some of patients with HPS presenting with fulminant illness usually have a poor prognosis despite ICU care and require proper ICU management, including careful assessment, monitoring and adjustment of volume status and cardiac function, along with the inotropic and vasopressor support if needed.
2. Hypoxemic cases should be treated with oxygen. Shock and hypotension may be treated with drugs to increase blood flow to improve the supply of blood and oxygen to organs.
3. Fluids balance maintenance is also important while managing patients with HFRS and HPS and should be carefully monitored according to the patient's fluid status, amount of diuresis, and kidney function. Fluids should be administered carefully because there might be the potential for capillary leakage.
4. HFRS treatment requires one or two hemodialysis sessions; mechanical ventilation when indicated; and appropriate use of pressures is crucial to patients with HPS (Vapalahati et al., 2003).
5. Extracorporeal membrane oxygenation has been found useful as a rescue therapy in patients with severe HPS (Crowley et al., 1998).
6. Although corticosteroids are not advised for the treatment of hanta viral infection, steroids were used to treat severe HFRS and HPS cases. Two HPS cases caused by PUUV were reported to be successfully treated with corticosteroids combined with continuous veno-venous hemodiafiltration (Seitsonen et al., 2006). However, the utility of steroids in treating Hantavirus infections further needs systematic evaluation.
7. The drug Ribavirin shows anti-hanta viral effect both in vitro and in vivo by causing error catastrophe during Hantavirus replication (Huggins et al., 1991). Ribavirin has been used in the treatment of HFRS in China, and clinical trials suggested that Ribavirin therapy can significantly reduce the mortality and the risk of entering the oliguric phase and experiencing hemorrhage (Huggins et al., 1991). It was also included in the WHO Model List of Essential Medicines for HFRS treatment in 2007. However, Ribavirin was not that effective in the treatment of HPS in the cardiopulmonary stage (Mertz et al., 2004), which suggests that it should not be recommended for routine therapy of HPS.
8. Synergistic effect of the combined use of amixine, an interferon inducer, and Ribavirin was also demonstrated in experimental HFRS in suckling albino mice (Loginova et al., 2005).

Prevention and Control

The most efficient way of controlling Hantavirus diseases is to reduce human exposure to infected rodents and their excrement. Monitoring Hantavirus prevalence in rodent populations in the community may give some warning of an expected increase in the numbers of human cases (Mills, 2005). Rodent control in and around the residential area remains the primary strategy for preventing Hantavirus infection (CDC, 2012). The CDC recommends rodent-proofing of homes, reduction of rodent population around the houses, minimization of food availability for rodents, trapping in and around dwellings, and the careful and appropriate disposal of dead rodents. Removal of rodents from a ranch building that was not rodent-proof did not generally reduce rodent infestation, whereas

Table 14.3. Inactivated hanta viral vaccines used in China and Korea.

Virus Strains	Produced in	Vaccination Program	Protection (%)	Country
HTNV	MGKC+	3 basic doses + 1 booster	>90	China
SEOV	GHKC++	2 basic doses + 1 booster	>95	China
HTNV	SMB+++	3 basic doses + 1 booster	>90	China
HTNV/SEOV	MGKC	2 basic doses + 1 booster	100	China
HTNV	SMB	2 basic doses + 1 booster	75–100	Korea

++GHKC, Gold hamster kidney cell; HTNV, Hantaan virus; +MGKC, Mongolian gerbil kidney cell; SEOV, Seoul virus; +++SMB, Suckling mouse brain.

the application of simple rodent-proofing measures to dwellings can significantly decrease the frequency and intensity of rodent intrusion, which reduces the risk of HPS and HFRS among rural residents. In a similar manner, workplaces and conditions in agriculture, forestry, and military activities should be modified as possible to reduce human exposure to rodents. To prevent laboratory-acquired infections, all the laboratory work involving the propagation and cultivation of Hantaviruses in cell culture or animals should always be conducted in laboratory with Biosafety Level-4 conditions.

In China and the Republic of Korea, people have been using vaccines against hanta viral infections (Table 14.3). They are using four different kinds of Hantavirus vaccines based on inactivated HTNV and SEOV, which have been demonstrated safe and effective in China (Chen et al., 2002). In the Republic of Korea, an inactivated vaccine purified from suckling mouse brain (Hantavax) against HTNV was approved in 1990 and started to be used in 1991 (Cho et al., 2002).

To prevent hanta viral disease, the easiest way is to limit contact with deer mice and their droppings, urine, or saliva. In cases where there is a large population of mice, the following tips offer guidance.

Outdoors

1. Food, water, and garbage should be stored always in metal or heavy plastic containers with tight-fitting lids. Pet food or water should not left out overnight.
2. Seal holes around doors, windows and roofs with steel wool or cement, and place gravel around the base of the building to discourage digging and nesting.
3. Should not keep any abandoned vehicles, old tires, or cast-off furniture in home area; cut back thick bush and keep grass short in garden area.
4. While hiking or camping, a ground cover or a tent with a floor should be used; areas where there are rodent burrows or droppings should be avoided. Bottled or disinfected water should always be used for drinking purposes.

Indoors

1. Mice traps should set indoors. Traps and nests should be treated with household disinfectant or diluted bleach; for such purposes, household bleach can be diluted by mixing 1 tablespoon of the bleach in 1 L of water (or 4 tablespoons of bleach for each gallon of water).

2. Always use gloves (rubber or plastic) while handling dead rodents and other materials, put them inside a plastic bag and after sealing the bag, put it in a sealed garbage can or bury it.
3. Before removing the gloves rinse them in disinfectant or diluted bleach and then wash gloves and hands both using hot soapy water.
4. Floors should be wet and then washed with soap, water, and disinfectant to minimize dust.
5. A breathing mask should be used in poorly ventilated area. Countertops, drawers, and cupboards are washed with disinfectant. If the clothing or bedding gets contaminated with droppings, wash them properly and then dry in the sun or in a hot dryer.
6. After every clean-up, wash hands and face properly before eating, drinking or smoking.

References

Ahlm C, Linderholm M, Juto P, Stegmayr B, Settergren B. 1994. Prevalence of serum IgG antibodies to Puumala virus (haemorrhagic fever with renal syndrome) in northern Sweden. *Epidemiol Infect* 113:129–136.

Antic D, Wright KE, Kang CY. 1992. Maturation of Hantaan virus glycoproteins G1 and G2. *Virology* 189:324–328.

Antoniadis A, Stylianakis A, Papa A, Alexiou-Daniel S, Lampropoulos A, et al. 1996. Direct genetic detection of Dobrava virus in Greek and Albanian patients with hemorrhagic fever with renal syndrome. *J Infect Dis* 174:407–410.

Avsic-Zupanc T, Petrovec M, Furlan P, Kaps R, Elgh F et al. 1999. Hemorrhagic fever with renal syndrome in the Dolenjska region of Slovenia—a 10-year survey. *Clin Infect Dis* 28:860–865.

Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P, et al. 1999. Epidemiological study of nephropathia epidemica in Finland 1989–96. *Scand J Infect Dis* 31:427–435.

Canadian Lung Association. 2009. Hantavirus. Accessed March 17, 2011, at http://www.lung.ca/diseases-maladies/a-z/hantavirus-hantavirus/index_e.php.

Centers for Disease Control and Prevention (CDC). 2012. Hantavirus. Accessed March 25, 2013, at http://www.cdc.gov/hantavirus/.

Chandy S, Boorugu H, Chrispal A, Thomas K, Abraham P, et al. 2009. Hantavirus infection: A case report from India. *Indian J Med Microbiol* 27:267–270.

Chapman LE, Khabbaz RF. 1994. Etiology and epidemiology of the Four Corners hanta virus outbreak. Infect. *Agents Dis* 3:234–244.

Chen H X, Luo ZZ, Zhang JJ; Hantavirus Vaccine Efficacy Evaluation Working Group. 2002. Large scale field evaluation on vaccines of hemorrhagic fever with renal syndrome in China. *Chin J Epidemiol* 23:145–147.

Cho HW, Howard CR, Lee HW. 2002. Review of an inactivated vaccine against hantaviruses. *Intervirology* 45:328–333.

Chu YK, Jennings G, Schmaljohn A, Elgh F, Hjelle B, et al. 1995. Cross-neutralization of hantaviruses with immune sera from experimentally infected animals and from hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome patients. *J Infect Dis* 172:1581–1584.

Chu YK, Rossi C, Leduc JW, Lee HW, Schmaljohn CS, et al. 1994. Serological relationships among viruses in the Hantavirus genus, family Bunyaviridae. *Virology* 198:196–204.

Crowley MR, Katz RW, Kessler R, Simpson SQ, Levy H, et al. 1998. Successful treatment of adults with severe Hantavirus pulmonary syndrome with extracorporeal membrane oxygenation. *Crit Care Med* 26:409–414.

Delfraro A, Clara M, Tome L, Achaval F, Levis S, et al. 2003. Yellow pigmy rice rat (Oligoryzomys flavescens) and hantavirus pulmonary syndrome in Uruguay. *Emerg Infect Dis* 9:846–852.

Delfraro A, Tome L, D'Elia G, Clara M, Achaval F, et al. 2008. Juquitiba-like hantavirus from 2 nonrelated rodent species, Uruguay. *Emerg Infect Dis* 14:1447–1451.
Elliott RM, Schmalijohn CS, Collett MS. 1991. Bunyaviridae genome structure and gene expression. *Curr Top Microbiol Immunol* 169:91–141.
Feldmann H, Sanchez A, Morzunov S, Spiropoulou CF, Rollin PE, et al. 1993. Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. *Virus Res* 30:351–367.
Figueiredo LT, Moreli ML, Borges AA, Figueiredo GG, Souza RL, et al. 2008. Expression of a hantavirus N protein and its efficacy as antigen in immune assays. *Braz J Med Biol Res* 41:596–599.
Garanina SB, Platonov AE, Zhuravlev VI, Murashkina AN, Yakimenko VV, et al. 2009. Genetic diversity and geographic distribution of hantaviruses in Russia. *Zoonoses Public Health* 56:297–309.
Heyman P, Vaheri A, ENIVD Members. 2008. Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006. *Euro Surveill* 13(28):18925.
Heyman P, Vaheri A, Lundkvist A, Avsic-Zupanc T. 2009. Hantavirus infections in Europe: from virus carriers to a major public-health problem. *Expert Rev Anti Infect Ther* 7:205–217.
Hjelle B, Jenison S, Torrez-Martinez N, Herring B, Quan S, et al. 1997. Rapid and specific detection of Sin Nombre virus antibodies in patients with hantavirus pulmonary syndrome by a strip immunoblot assay suitable for field diagnosis. *J Clin Microbiol* 35:600–608.
Huggins JW, Hsiang CM, Cosgriff TM, Guang MY, Smith JI, et al. 1991. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous Ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis* 164:1119–1127.
Johnson AM, de Souza LT, Ferreira IB, Pereira LE, Ksiazek TG, et al. 1999. Genetic investigation of novel hanta viruses causing fatal HPS in Brazil. *J Med Virol* 59:527–535.
Jonsson CB, Figueiredo LTM, Vapalahti O. 2010. A global perspective on Hantavirus ecology, epidemiology, and disease. *Clin Microbiol Rev* 23(2):412–414.
Jonsson CB, Gallegos J, Ferro P, Severson W, Xu X, et al. 2001. Purification and characterization of the Sin Nombre virus nucleocapsid protein expressed in Escherichia coli. *Protein Expr Purif* 23:134–141.
Jonsson CB, Schmaljohn CS. 2001. Replication of hanta viruses. *Curr Top Microbiol Immunol* 256:15–32.
Kallio-Kokko H, Leveelahti R, Brummer-Korvenkontio M, Lundkvist A, Vaheri A, et al. 2001. Human immune response to Puumala virus glycoproteins and nucleocapsid protein expressed in mammalian cells. *J Med Virol* 65:605–613.
Kang HJ, Bennett SN, Hope AG, Cook JA, Yanagihara R. 2011. Shared ancestry between a newfound mole-borne Hantaviruses and Hantaviruses harbored by cricetid rodents. *J Virol* 85(15):7496–7503.
Kehm R, Jakob NJ, Welzel TM, Tobiasch E, Viczian O, et al. 2001. Expression of immunogenic Puumala virus nucleocapsid protein in transgenic tobacco and potato plants. *Virus Genes* 22:73–83.
Klempa B, Meisel H, Rath S, Bartel J, Ulrich R, et al. 2003. Occurrence of renal and pulmonary syndrome in a region of northeast Germany where Tula hantavirus circulates. *J Clin Microbiol* 41:4894–4897.
Klempa B, Tkachenko EA, Dzagurova TK, Yunicheva YV, Morozov VG, et al. 2008. Hemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. *Emerg Infect Dis* 214:617–625.
Lázaro ME, Cantoni GE, Calanni LM, Resa AJ, Herrero ER, et al. 2007. Clusters of hantavirus infection, southern Argentina. *Emerg Infect Dis* 13:104–110.
Lee H. 1996. Epidemiology and pathogenesis of haemorrhagic fever with renal syndrome. In: Elliot RM, eds., *The Bunyaviridae*, pp. 253–267. New York: Plenum Press.
Lee HW, Lee PW, Johnson KM. 1978. Isolation of the etiologic agent of Korean Hemorrhagic fever. *The Journal of Infectious Diseases* 137(3):298–308.

Lee HW, Vander Groen G. 1989. Hemorrhagic fever with renal syndrome. *Prog Med Virol* 36:62–102.

Lee HW. 1982. Korean hemorrhagic fever. *Prog Med Virol* 28:96–113.

Loginova SI, Koval'chuk AV, Borisevich SV, Kopylova NK, Pashchenko IUI, et al. 2005. Antiviral effectiveness of the combined use of amixine and virasole in experimental hemorrhagic fever with renal syndrome in sucking albino mice. *Vopr Virusol* 50:30–32.

Lundkvist A, Apekina N, Myasnikov Y, Vapalahti O, Vaheri A, et al. 1997. Dobrava hantavirus outbreak in Russia. *Lancet* 350:781–782.

Mackow ER, Gavrilovskaya IN. 2001. Cellular receptors and hantavirus pathogenesis. *Curr Top Microbiol Immunol* 256:91–115.

Mendes WS, Silva AA, Aragao LF, Aragao NJ, Raposo MDL, et al. 2004. Hantavirus infection in Anajatuba, Maranhao, Brazil. *Emerg Infect Dis* 10:1496–1498.

Mertz GJ, Miedzinski L, Goade D, Pavia AT, Hjelle B, et al. 2004. Collaborative Antiviral Study Group. Placebo-controlled, double-blind trial of intravenous ribavirin for the treatment of hanta virus cardiopulmonary syndrome in North America. *Clin Infect Dis* 39:1307–1313.

Mills JN. 2005. Regulation of rodent-borne viruses in the natural host: implications for human disease. *Arch Virol Suppl* 19:45–57.

Olsson GE, Hjertqvist M, Lundkvist A, Hornfeldt B. 2009. Predicting high risk for human hantavirus infections, Sweden. *Emerg Infect Dis* 15:104–106.

Padula PJ, Edelstein A, Miguel SD, Lopez NM, Rossi CM, et al. 1998. Hantavirus pulmonary syndrome outbreak in Argentina: Molecular evidence for person-to-person transmission of Andes virus. *Virology* 241:323–330.

Pattamadilok S, Lee BH, Kumperasart S, Yoshimatsu K, Okumura M, et al. 2006. Geographical distribution of hantaviruses in Thailand and potential human health significance of Thailand virus. *Am J Trop Med Hyg* 75:994–1002.

Plyusnin A, Nemirov K, Apekina N, Plyusnina A, Lundkvist A, et al. 1999. Dobrava hantavirus in Russia. *Lancet* 353:207.

Plyusnina A, Ibrahim IN, Plyusnin A. 2009. A newly recognized hanta virus in the Asian house rat (*Rattus tanezumi*) in Indonesia. *J Gen Virol* 90:205–209.

Prince HE, Su X, Hogrefe WR. 2007. Utilization of hantavirus antibody results generated over a five-year period to develop an improved serologic algorithm for detecting acute Sin Nombre hantavirus infection. *J. Clin Lab Anal* 21:7–13.

Ruusala A, Persson R, Schmaljohn CS, Pettersson RF. 1992. Coexpression of the membrane glycoproteins G1 and G2 of Hantaan virus is required for targeting to the Golgi complex. *Virology* 186, 53–64.

Schmaljohn CS, Hooper JW. 2001. *Bunyaviridae*: The viruses and their replication. In Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, et al., eds., *Fields Virology*, 4th ed., 2:1581–1602. Philadelphia: Lippincott Williams & Wilkins.

Schmaljohn CS, Sugiyama K, Schmaljohn AL, Bishop DH. 1988. Baculovirus expression of the small genome segment of Hantaan virus and potential use of the expressed nucleocapsid protein as a diagnostic antigen. *J Gen Virol* 69"777–786.

Schmidt J, Jandrig B, Klempa B, Yoshimatsu K, Arikawa J, et al. 2005. Nucleocapsid protein of cell culture-adapted Seoul virus strain 80–39: Analysis of its encoding sequence, expression in yeast and immuno-reactivity. *Virus Genes* 30:37–48.

Seitsonen E, Hynninen M, Kolho E, Kallio-Kokko H, Pettilä V. 2006. Corticosteroids combined with continuous veno-venous hemodiafiltration for treatment of hantavirus pulmonary syndrome caused by Puumala virus infection. *Eur J Clin Microbiol Infect Dis* 25:261–266.

Sibold C, Meisel H, Lundkvist A, Schulz A, Cifire F, et al. 1999. Simultaneous occurrence of Dobrava, Puumala, and Tula hantaviruses in Slovakia. *Am J Trop Med Hyg* 61:409–411.

Song G. 1999. Epidemiological progresses of hemorrhagic fever with renal syndrome in China. *Chin Med J (Engl)* 112:472–477.

Song KJ, Baek LJ, Moon S, Ha SJ, Kim SH, et al. 2007. Muju virus, a novel hantavirus harboured by the arvicolid rodent Myodes regulus in Korea. *J Gen Virol* 88:3121–3129.

Spiropoulou CF. 2001. Hantavirus maturation. Curr. Top. *Microbiol Immunol* 256:33–46.

Suputthamongkol Y, Nitatpattana N, Chayakulkeeree M, Palabodeewat S, Yoksan S, et al. 2005. Hantavirus infection in Thailand: first clinical case report. *Southeast Asian J Trop Med* 36: 700–703.

Tappero JW, Khan AS, Pinner RW, Wenger JD, Graber JM, et al. 1996. Utility of emergency, telephone-based national surveillance for hantavirus pulmonary syndrome. Hantavirus Task Force. *JAMA* 275:398–400.

Tersago K, Verhagen R, Servais A, Heyman P, Ducoffre G, et al. 2009. Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. *Epidemiol Infect* 137:250–256.

Vapalahti O, Lundkvist A, Kallio-Kokko H, Paukku K, Julkunen I, et al. 1996. Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. *J Clin Microbiol* 34:119–125.

Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, et al. 2003. Hantavirus infections in Europe. *The Lancet Infect Dis* 3:653–752.

Vasconcelos MI, Lima VP, Iversson LB, Rosa MD, Da Rosa AP, et al. 1997. Hantavirus pulmonary syndrome in the rural area of Juquitiba, Sao Paulo metropolitan area, Brazil. *Rev Inst Med Trop Sao Paulo* 39:237–238.

Vincent MJ, Quiroz E, Gracia F, Sanchez AJ, Ksiazek TG, et al. 2000. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. *Virology* 277:14–19.

Wells RM, Sosa Estani S, Yadon ZE, Enria D, Padula P, et al. 1997a. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia. *Emerg Infect Dis* 3:171–174.

Wells RM, Young J, Williams RJ, Armstrong LR, Busico K, et al. 1997b. Hantavirus transmission in the United States. *Emerg Infect Dis* 3:361–365.

Williams RJ, Bryan RT, Mills JN, Palma RE, Vera I, et al. 1997. An outbreak of hantavirus pulmonary syndrome in western Paraguay. *Am J Trop Med Hyg* 57:274–282.

Yan L, Fang LQ, Huang HG, Zhang LQ, Feng D, et al. 2007. Landscape elements and Hantaan virus-related hemorrhagic fever with renal syndrome, People's Republic of China. *Emerg Infect Dis* 13:1301–1306.

Chapter 15
Influenza

Historical Aspects

Early Descriptions

It is believed that influenza disease was first appeared in the first half of the 16th century. Caius, an English physician described a "sweating disease" in 1551, characterized by headache, fever, and myalgias that killed some patients in hours, but lasted only a few days in survivors. Caius's descriptions are not entirely convincing that he was dealing with viral influenza. This sweating sickness did not resemble the influenza epidemic that occurred in 1173 in England, Germany, and Italy. Subsequent influenza epidemics occurred in Italy and France in 1323. The Italians used the expression *ex influentia colesti*, believing that probably some celestial influence was responsible for the epidemic. Villaini and Segui modified the term and designated it as *una influenza* (Aufderheide and Rodriguez-Martin, 1998; Crosby, 1993). The French referred to influenza as *the grippe*, suggesting an acute onset in which the patient suddenly was seized or gripped by the disease. Further influenza epidemics followed in Paris in 1411, 1414, and 1427 and again returned to Italy in 1414. Although thousands of individuals were affected by these early epidemics, there was a little or almost negligible influenza activity until the 1510 pandemic, which spread rapidly and began in Sicily, spread to Italy, and then to the rest of Europe (Debre and Couvreur, 1970; Nicholson et al., 1998; Sydenham, 1978). In 1679, Sydenham gave the first accurate description of influenza (Sydenham, 1978), which greatly resembles the convulsive whooping cough of children, except it was not so severe.

Historical Influenza Pandemics

The first influenza pandemic followed the 1580 epidemic and spread from Europe to Asia and Africa.

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Influenza Pandemics of 18th Century

During the 17th century, influenza epidemics occurred throughout Europe, but three major influenza pandemics occurred in the 18th century during the years of 1729 to 1730, 1732 to 1733, and 1781 to 1782. The most severe pandemic occurred in 1781 to 1782 and affected North America, South America, and most of the Europe (Crosby, 1993; Knipe and Howley, 2001).

Influenza Pandemics of 19th Century

During the 19th century, three influenza pandemics occurred in 1830 to 1831, 1833 to 1834, and 1889 to 1890, and some major epidemics also occurred in between these pandemics. Worldwide, the influenza activity was light between the 1847 to 1848 pandemic and the 1889 to 1890 pandemic. The 1889 to 1890 pandemic basically originated in Russia and devastated Europe before reaching North America. The exact cause of those pandemics was unknown at that time; hence the influenza epidemics were named according to their country of origin (e.g., the 1889 to 1890 pandemic was called the Russian flu). From North America, the Russian flu spread to Latin America, New Zealand, Australia, and finally to Africa and Asia. The 1889 to 1890 pandemic was the first pandemic for which detailed records are available. Approximately 1 million people died worldwide; of the low mortality rate (1 percent) in the 1889 to 1890 pandemic, the deaths primarily occurred among the elderly. Influenza activity was relatively insignificant for the next two decades, and many people considered influenza as an episodic mild respiratory infection until 1918 (Christie, 1974; Nicholson et al., 1998).

Influenza Pandemic of 20th Century

Sporadic influenza activity was noted in the United States in the spring of 1918. The Spanish influenza (1918 to 1919 pandemic) originated in the United States and was transported by US troops to Europe before spreading worldwide, and the pandemic killed hundreds of thousands and infected millions. Unlike previous influenza epidemics, the 1918 to 1919 pandemic affected healthy young adults and had an unusually high mortality rate. Another unusual feature of this pandemic was that death in young adults was caused primary by influenzal pneumonia and not other complications, as had occurred in previous pandemics. The pandemic occurred in three simultaneous waves, with each wave bringing increased death rates. The initial first wave occurred in the spring followed by the second wave in August, which tripled the death rates and subsided in the fall. In the winter and spring of 1919, a third wave occurred before the pandemic subsided resulting more than a half a million deaths in the United States alone. In terms of fatalities, this pandemic was the single most lethal infectious outbreak occurred worldwide over a 1-year period.

Influenza Pandemic of 21st Century

With the global spread of a new strain of influenza, the World Health Organization (WHO) on June 11, 2009, declared the outbreak to be influenza pandemic. It began in Mexico in March 2009 then the "H1N1 swine flu" was first identified in California in late April 2009, whereas in Alberta, the pandemic occurred in two waves; the first wave peaked in June 2009 followed by the second in October 2009. During this pandemic, it was observed that the younger people were at more risk to acquire the virus with a particularly high illness reported among school-aged children (aged 5–18 years) but with less severe

symptoms compared to the seasonal influenza virus strains seen each year. Finally, WHO declared the H1N1 pandemic over on August 10, 2010.

Global Scenario

In tropical countries, sporadic influenza cases occur throughout the year but are more common in the rainy season (LaForce et al., 1994; Cox and Subbarao, 2000; Monto, 2008). Some localized outbreaks of seasonal influenza also occur in interpandemic years, especially when viral strains infect communities with little or no pre-existing immunity to the circulating virus. The exact reason for seasonal infection remains unclear, but because the primary mode of transmission is by large air droplets, increased crowding in the colder months, return to schools and university dormitories, and the start of military recruiting courses have been suggested as contributing factors (LaForce et al., 1994; Cox and Subbarao, 2000; Monto, 2008). The secondary mode of transmission is the fomites. The intensity of seasonal influenza actually varies from year to year and particularly depends on the size of the susceptible population, which in turn depends on the degree of antigenic drift in the viral strains that have occurred in the previous seasons (Monto et al., 2006; Garten et al., 2009). Other factors, including overcrowding, crowded sleeping arrangements, and unhygienic living conditions, can contribute to increased localized incidence (LaForce et al., 1994; Monto, 2008) and explains the higher incidence of seasonal influenza observed in colleges, individuals living in low socioeconomic conditions, daycare centers, and military settings. In seasonal outbreaks, age-specific incidence generally follows a predictable pattern in which children are affected early in epidemics, followed by their adult caregivers, and, last, the elderly (Cox and Subbarao, 2000).

Three major pandemics were recorded in the 20th century: the 1918 to 1919 pandemic (H1N1), the 1957 to 1958 pandemic (H2N2), and the 1968 pandemic (H3N2) (Cox and Subbarao, 2000), each with a unique epidemiologic pattern and origin. Table 15.1 shows the known influenza pandemics of 19th, 20th and 21st centuries.

1918 to 1919 Pandemic: H1N1 "Spanish" Influenza

The exact geographic origin of the 1918 pandemic virus remains unclear; the most probable considering origin is either China or in military camps in the United States soon

Table 15.1. Known flu pandemics.

Name of pandemic	Date	Deaths	Case Fatality Rate (%)	Subtype Involved	Pandemic Severity Index
Asiatic (Russian Flu), Valleron et al., 2010	1889–1890	1 million	0.15	Possibly H3N8	NA
1918 flu pandemic (Spanish flu), Mills et al., 2004	1918–1920	20 to100 million	2	H1N1	5
Asian Flu	1957–1958	1 to 1.5 million	0.13	H2N2	2
Hong Kong Flu	1968–1969	0.75 to 1 million	<0.1	H3N2	2
2009 flu pandemic, Donaldson et al., 2009	2009–2010	18,000	0.03	H1N1	NA

after the return of soldiers from the European front (Reid and Taubenberger, 2003; Olson et al., 2005). The first wave showed a relatively low incidence of nonsevere clinical disease but with a limited spread. In August 1918, there was a sudden change in disease pattern, with widespread reports of a severe influenza compatible clinical disease that emerged simultaneously in North America, Africa, and Europe (Cox and Subbarao, 2000). The second wave peaked in October 1918 globally, when school absenteeism was approximately 40 percent, which was followed by a third small wave in February 1919 (Frost, 2006). Although the rate of infection and age-specific incidence of this pandemic did not differ significantly from subsequent pandemics, this pandemic was characterized by its particularly high rates of morbidity and mortality, especially observed among the young adults (Cox and Subbarao, 2000; Frost, 2006).

1957 to 1958 Pandemic: H2N2 Asian Influenza

This pandemic was much better characterized than the 1918 pandemic because of the availability of more reliable intercontinental communication and the advanced laboratory facilities of viral isolation in cell cultures. The virus originated in Guizhou, China, in February 1957, and then spread rapidly throughout the whole country (Cox and Subbarao, 2000). In April 1957, the epidemic reached Hong Kong and the causative virus was isolated in Japan in May 1957. The virus was found to be unique in the human population because of the presence of different HA and NA antigens. By November 1957, the outbreak reached the pandemic proportions (Cox and Subbarao, 2000), and in the Northern Hemisphere, peak incidence occurred in October followed by a second wave observed in January 1958. Similar to other influenza epidemics, this influenza also showed the highest age-specific attack rates in children (aged 5–19 years), although excess mortality was noted in both the waves.

1968 Pandemic: H3N2 Hong Kong

This pandemic was first notified in Hong Kong in July 1968 (Cox and Subbarao, 2000) and appeared to spread globally but more slowly than the previous pandemics, reaching the United States in December 1968/January 1969 and 1 year later in Europe. This pandemic had the lowest excess mortality of the 20th century influenza pandemics, which is probably because of the presence of partial immunity to the N2 component of the virus from previous circulating H2N2 in most of the individuals (Cox and Subbarao, 2000). As the H3N2 emerged in the human population, the H2N2 virus became extinct, supporting cross-immunity.

Epidemiology of the 2009 H1N1 Pandemic

In April 2009, an epidemic of severe influenza was reported in various parts of Mexico caused by a H1N1 strain of influenza and capable of human-to-human transmission. Although initially the misnomer "swine flu" was used for the virus, it was later given the more appropriately named swine-origin H1N1 influenza virus, human-adapted swine H1N1 influenza virus, novel H1N1, or 2009 H1N1. This pandemic reported cumulative deaths ranges from four categories as 1–10, 11–50, 51–100, and 100 or more (Figure 15.1).

Influenza can be seasonal, which further leads to the outbreak of an epidemic, and it may also become pandemic. Table 15.2 shows the difference between seasonal and pandemic influenza.

Figure 15.1. Countries, territories, and areas with laboratory confirmed cases and number of deaths reported to the World Health Organization during the 2009 H1N1 influenza pandemic.

Table 15.2. Comparison of seasonal and pandemic flu.

Seasonal Influenza	Pandemic Influenza
In seasonal influenza, outbreaks follow predictable seasonal patterns: occurs annually, usually in winter, in temperate climates	Pandemic influenza occurs rarely (a few times a century)
Immunity built up from previous exposure	No previous exposure thereby little or no pre-existing immunity
Healthy adults usually not at risk for serious complications; the very young, elderly, and those with certain underlying health conditions at increased risk for serious complications	Healthy people may be at increased risk for serious complications
Health systems can usually meet public and patient needs	Health systems may be overwhelmed
Vaccine developed based on known influenza strains and available for annual influenza season	Vaccine probably would not be available in the early stages of a pandemic
Adequate supplies of antivirals are usually available	Effective antivirals may be in limited supply
Average US deaths approximately 36,000 a year	Number of deaths could be quite high (e.g., US 1918 death toll approximately 675,000)
Symptoms are general: fever, cough, runny nose, and muscle pain. Deaths often caused by complications such as pneumonia.	Symptoms may be more severe and involving more frequent complications
Generally causes modest impact on society (e.g., closing of school, sick people confined at home)	May cause major impact on society (e.g., widespread restriction on travel, closing of school and businesses, cancellation of large public gatherings)
Manageable impact on domestic and world economy	Potential for severe impact on domestic and world economy

Agent Factors

Types of Influenza Virus

Influenza viruses are RNA viruses that constitute three of the five genera of the family *Orthomyxoviridae* (Kawaoka, 2006); these are as Influenza A virus, Influenza B virus, and Influenza C virus. These viruses are distantly related to the human parainfluenza viruses, the RNA viruses of *Paramyxoviridae* family, which are a common cause of respiratory infections in children and cause a disease similar to influenza in adults (Hall, 2001).

Influenza Virus A

This genus constitutes single species, Influenza A virus. Wild aquatic birds are the natural hosts for this virion, but occasionally, these viruses are transmitted to other species and might cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics (Klenk et al., 2008). The type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease.

This virus can further be subdivided into different serotypes (designated as H1N1, H2N2, H3N2, H5N1, H7N7, H1N2, H9N2, H7N2, H7N3, and H10N7) based on the

Table 15.3. Comparison of Influenzas A, B and C.

Characters	Influenza A	Influenza B	Influenza C
Severity of illness	++++	++	+
Animal reservoir	Yes	No	No
Human pandemics	Yes	No	No
Human epidemics	yes	Yes	No (sporadic)
Antigenic changes	Shift and drift both	Drift	Drift
Segmented genome	Yes	Yes	Yes
Amantadine, rimantadine	Sensitive	No effect	No effect
Zanamivir (Relenza)	Sensitive	Sensitive	Sensitive
Surface glycoproteins	2	2	1

antibody response against them (Hay et al., 2001). These serotypes have already been confirmed in humans and ordered by the number of known human pandemic deaths.

Influenza Virus B

Influenza B exclusively infects humans (Fouchier et al., 2004) and is less common than influenza A. Apart from humans, the other susceptible animals to influenza B infection are the seal and the ferret. This virus mutates at a rate two to three times slower than type A (Nobusawa and Sato, 2006) and consequently is less genetically diverse as having only one influenza B serotype (Hay et al., 2001). Because of this lack of antigenic diversity, the immunity to influenza B is usually acquired at an early age. The reduced rate of antigenic variation along with its limited host range (inhibiting cross-species antigenic shift) ensures that pandemics of influenza B do not occur (Zambon, 1999).

Influenza Virus C

It infects humans, dogs, and pigs, sometimes causing both severe illness and local epidemics (Taubenberger and Morens, 2008). However, influenza C is less common than the other types and causes mild disease in children only.

Structure of Influenza Virus

Influenza viruses are enveloped single-stranded RNA viruses having a diameter ranging from 80 to 120 nm. They tend to be pleomorphic and may appear roughly spherical, although filamentous forms also occur (Lamb and Choppin, 1983), which are more common in influenza C and form cordlike structures up to 500 micrometers long on the surfaces of infected cells. However, despite these varied shapes, the viral particles of all influenza viruses are similar in composition (Bouvier and Palese, 2008).

The Central Core

The central core contains the viral RNA and viral proteins that package and protects this RNA. RNA tends to be single stranded, but in special cases, it is double-stranded (Lamb and Choppin, 1983). The genome of influenza virus contains seven or eight

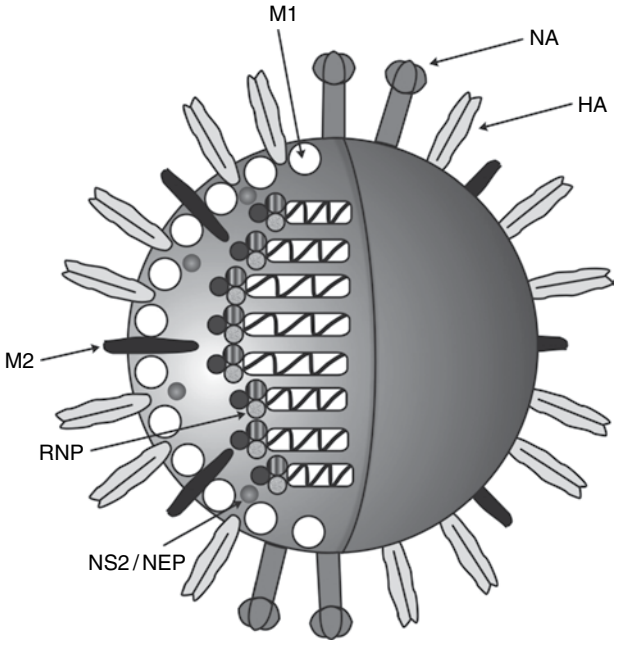

Figure 15.2. Structure of the influenza virus. HA, hemagglutinin; M1, matrix protein; M2, matrix protein; NA, neuraminidase; NEP, nuclear export protein; NS1, nonstructural protein 1; NS2, nonstructural protein 2; RNP, ribonucleoprotein.

pieces of segmented negative-sense RNA, each piece containing either one or two genes, which code for a gene product (Bouvier and Palese, 2008). For example, the influenza A genome contains 11 genes on eight separate segments of RNA (13.5 kb total), covered by the nucleocapsid protein, which together build the ribonucleoprotein (RNP), each encoding for 11 different proteins as hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M1, M2, NS1(nonstructural protein), NS2 (nuclear export protein [NEP]), PA (Polymerase A protein), PB1 (polymerase basic 1), PB1-F2, and PB2 (Ghedin et al., 2005). M1 constructs the matrix, and in influenza A viruses only, M2 acts as an ion channel pump, which lowers or maintains the pH inside the endosome, whereas the function of NS2 is hypothetical. PB2, PB1, and PA form an active RNA-RNA polymerase, which is responsible for replication and transcription. It has an endonuclease activity and is linked to the RNP. The NS1 and NS2 proteins have a regulatory function promoting the synthesis of viral components in the infected cell.

Viral Envelope

The viral envelope is a lipid bilayer containing two main types of glycoproteins, HA and NA wrapped around a central core (*see* Figure 15.2). The HA and NA proteins are the major targets for antiviral drugs (Wilson and von Itzstein, 2003) and are recognized by antibodies (i.e., they are antigens) (Hilleman, 2002). The responses of antibodies to these proteins are used to classify the different serotypes of influenza A viruses.

Hemagglutinin

HA is a glycoprotein containing either two or three glycosylation sites having the molecular weight of approximately 76,000. It spans the lipid membrane so that the major part containing at least five antigenic domains is presented at the outer surface. HA is a lectin that mediates binding of the virus and entry of the viral genome into the target cell (Suzuki, 2005) by serving as a receptor by binding to sialic acid residues (N-acetylneuraminic acid) and induces the penetration of the interior of the virus particle by membrane fusion. HA is the main influenza virus antigen; the antigenic sites being A, B (carrying the receptor binding site), C, D, and E, presented at the head of the molecule and the feet are embedded in the lipid bilayer. The HA molecule composed of a stalk region and the fusogenic domain responsible for membrane fusion when the virus infects a new cell. At low pH, the fusion peptide is turned to an interior position, the HA forms trimers and several trimers form a fusion pore. There are 16 HA antigenic subtypes, which are labeled H1 through H16. Each HA molecule is actually a combination of three identical proteins bound together to form an elongated cylindrical shape. A mutation that changes just one amino acid in any of the protein structure can alter the antigenic properties significantly.

Neuraminidase

NA is a glycoprotein, which is also found as a projection on the surface of the virus. It forms a tetrameric structure with an average molecular weight of 220,000. The NA molecule presents its main part at the outer surface of the cell, spans the lipid layer, and has a small cytoplasmic tail forming mushroom-like projections on to the surface of the virion. The top is roughly spherical in shape consisting of four identical proteins, which acts as an enzyme (sialidase) cleaving sialic acid from the HA molecule, from other NA molecules, and from glycoproteins and glycolipids at the cell surface. It is also responsible for the penetration of the virus through the mucin layer of the respiratory epithelium. There are nine neuraminidase subtypes labeled N1 through N9.

The matrix and nucleoprotein antigens are type specific and are, therefore, used to differentiate types A, B, and C, whereas subtyping is based on HA and NA antigens. The nomenclature of influenza virus is performed by soluble nucleoprotein group antigen (A, B, C), geographic location, original isolation date, and the type of HA and NA antigens. For example, a strain of influenza virus might be designated A/Bangkok/1/79(H3N2). This designates an influenza A virus first isolated in Bangkok in January 1979, containing HA (H3) and NA (N2) antigens (Figure 15.3). Strains of influenza B are designated by type, geography, and date of isolation as B/Singapore/3/64, without specific mention of HA or NA antigens.

Antigenic Variation in Influenza Virus

The immune system of the body recognizes viruses when viral surface antigens bind to specific immune receptors. Following infection, the body starts producing many more of these virus-specific receptors, which prevent reinfection by this particular strain of the virus and generate adaptive immunity. Similarly, a vaccine works against a virus by guiding the immune system to recognize the antigens exhibited by this virus on future exposure; however, influenza viral genomes are constantly mutating, producing new forms of these antigens. In case one of these new forms of antigen is sufficiently different from the old antigen, it will no longer bind to the immune receptors and viruses with

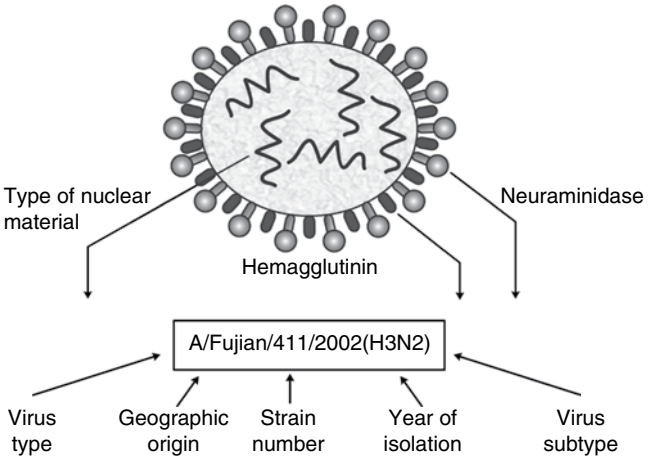

Figure 15.3. Nomenclature of the influenza virus.

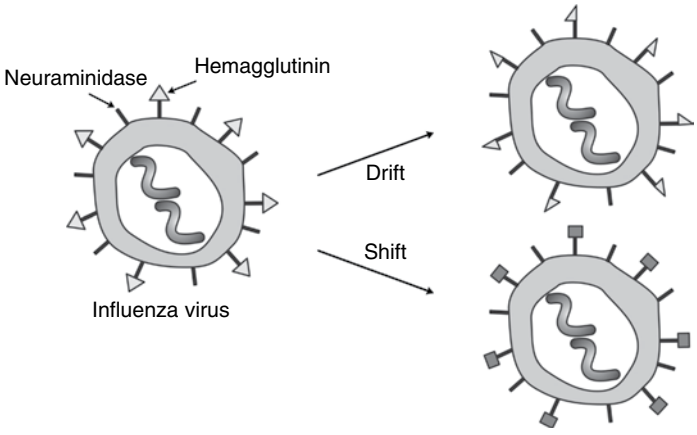

Figure 15.4. Antigenic variations in the influenza virus.

these newly evolved antigens can evade immunity to the original strain of the virus. When such a change occurs, people who have had the illness in the past and have immunity against the previous original strain will lose their immunity and become infected by a new strain and resulting vaccines against the original virus will also no longer be effective. Theses antigenic variations occur by two different processes, antigenic drift and antigenic shift (Figure 15.4).

Antigenic Drift

Prominent mutations in the antigenic sites inhibit the binding of neutralizing antibodies, thereby allowing a new subtype to spread within a nonimmune population. This phenomenon is called *antigenic drift*. The mutations causing the antigenic drift are the molecular explanation for the seasonal influenza epidemics during winter time in temperate climatic zones. The rate of antigenic drift is dependent on two characteristic

features: the duration of the epidemic and the level of host immunity. A longer epidemic session allows for selection pressure to continue over an extended period of time and stronger host immune responses increase selection pressure for the development of novel antigens (Boni et al., 2006).

The immune response to the HA antigen, results in the production of neutralizing antibody, which is the basis for resolving infection in an individual, and is sometimes part of the cross-immunity found in elderly individuals in the emergence of a new pandemic virus strain. Antigenic drift can also occur in the NA spike. The NA carries several important amino acid residues, which, on mutation, can lead to resistance against various NA inhibitors. Antigenic drift generates an increasing variety of strains until one evolves that can infect people who are immune to the pre-existing strains. This newly generated variant then replaces the older strains as it rapidly sweeps through the human population often causing an epidemic (Wolf et al., 2006). However, because the strains produced by antigenic drift will still show similarity to the older strains, some people will still possess immunity against them. Antigenic drift occurs in all types of influenza viruses, including influenza virus A, influenza virus B, and influenza virus C, after every 2 to 3 years, causing local outbreaks of influenza infection.

The influenza vaccine preparation contains three influenza strains, generally two influenza A strains and one influenza B strain, which can change from year to year. On vaccination, the body produces neutralizing antibodies against the three strains present in the vaccine. If people are exposed to any of the three strains during the influenza season, the antibodies will neutralize virus HA antigens, preventing the virus from attaching to healthy body cells and infecting them; but if the HA gene changes, it will then encode a different-shaped HA antigen on to the newly evolved virion's surface, which results in the neutralizing antibodies not being capable of neutralizing newly mutated virus. Hence, the person gets a strain of that influenza. This type of genetic mutation is called *antigenic drift* (Figure 15.5).

Antigenic shift

Antigenic shift is also termed as a gene reassortment or reassortment. It occurs only in influenza A virus and arises when the HA is exchanged in a virus, for example, H1 replaced by H5, resulting in the formation of a mosaic virus. This may happen when a host cell is infected simultaneously by two different influenza viruses, and their genome segments are exchanged during replication. The genome reassortment is frequently observed in water birds, especially ducks. Although the birds are seldom symptomatic after infection, they shed virus in their feces for several months. During reassortment, the virus completely acquires new surface antigens (e.g., reassortment between avian strains and human strains [Figure 15.6]). As a result, the newly produced human influenza virus has entirely new antigens, thereby making everybody susceptible, and the novel influenza will spread uncontrollably, causing a pandemic (Parrish and Kawaoka, 2005). Antigenic shifts occur infrequently, approximately after every 10 years. For example, in 1947, the prevalent influenza A virus was the H1N1 subtype, and in 1957 there was a shift in both the antigens, resulting in H2N2 subtype, H3N2 appeared in 1968, and in1978 H1N1 reappeared. The reappearance of H1N1 reflected a large susceptible population, all those younger than 30 years of age lacked protective antibodies. Those older than 30 years of age were protected despite the fact that H1N1 had not been present for 20 years. This is attributable to an anamnestic antibody response

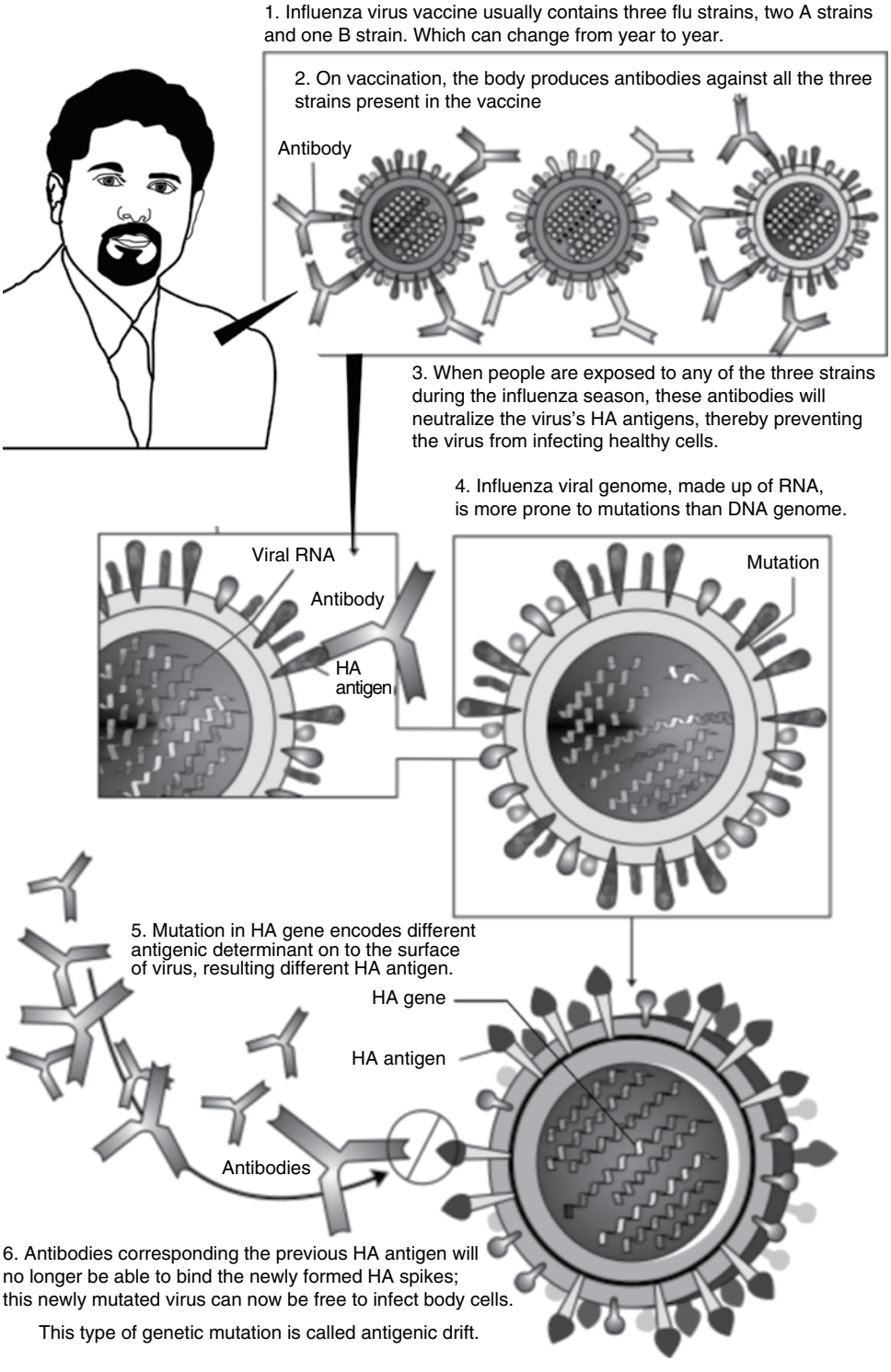

Figure 15.5. Antigenic drift in influenza virus. HA, hemagglutinin.
Source: Modified from image illustrated by the National Institute of Allergy and Infectious Diseases.

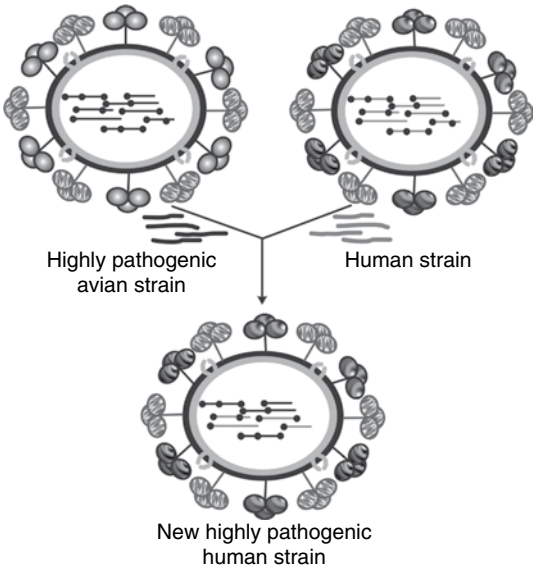

Figure 15.6. Antigenic shift or reassortment in the influenza A virus.

because during the first exposure of any influenza subtype, individuals develop antibodies and anamnestic response (memory response) to that virus.

The antigenic shift can occur by three ways (Figure 15.7)

1. When an intermediate host, such as pig or chicken, is simultaneously infected with human and avian strain of influenza A virus. During infection in the same cell of the host, both the viral strains exchange their genes resulting in the formation of new viral strain, which can further spread from the intermediate host to humans.
2. When a bird strain of influenza A directly infects humans.
3. When a bird strain of influenza A infects an intermediate animal host without any genetic change acquired by human population.

Pathogenesis and Spread of Influenza

The pathogenicity and virulence of the influenza virus can be determined by several interacting factors those present in the host and the virion:

1. Host factors:
   (a) Presence of target receptors on host cells
   (b) Availability of enzymes in host cells, which are essential for viral entry and replication
   (c) State of immunocompetence of the individual host
   (d) Specific immunity against certain viral epitopes in the individual host and target population
   (e) Ability of the immune system to control the viral replication effectively without causing serious collateral damage for the host by its inflammatory response

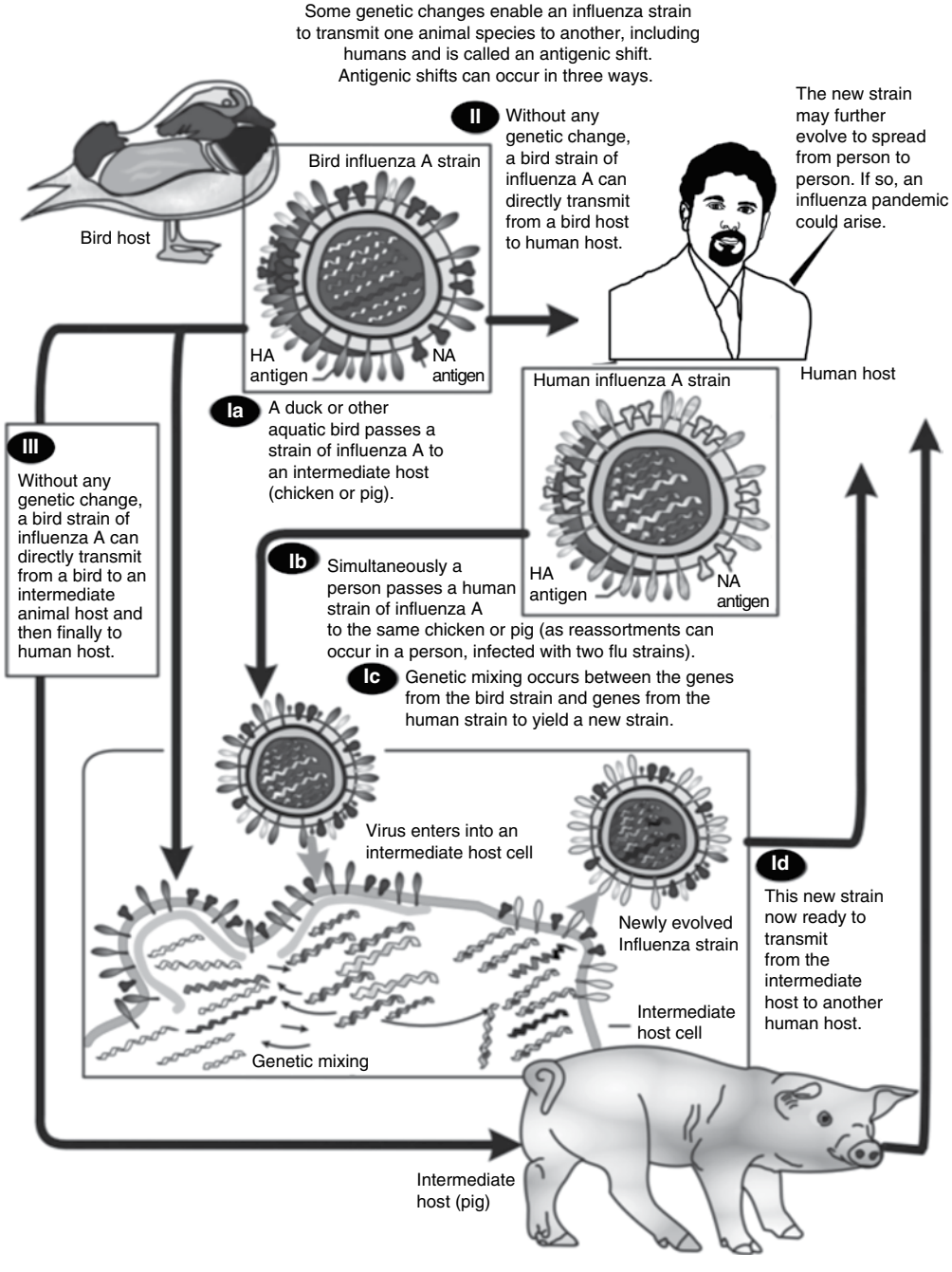

Figure 15.7. Ways that a virus undergoes an antigenic shift or reassortment. HA, hemagglutinin; NA, neuraminidase.

2. Viral factors:
   a. Ability to bind to host cells
   b. Ability of virus shedding
   c. Restriction of cytopathogenic effects to allow for an appropriate balance between viral replication and control by the host
   d. Escape from immunosurveillance by evolution of antigenic variation driven by selective pressure of the immune response
   e. Escape from immunosurveillance by recombination with different virus strains from zoonotic disease
   f. Modulation of the immune response to attenuate effective host defense mechanisms

The pathogenesis of influenza starts with the entry of virus through the respiratory tract. In a human lung, there are about 300 million terminal sacs or alveoli, which are involved in gaseous exchange between inspired air and the blood. The resting ventilation rate in humans is about 6 L/min of air, which introduces a large numbers of foreign particles and aerosolized droplets potentially containing virus into the lungs. Foreign particles get deposited on the basis of their size because small particles do not absorb through the alveoli or the bronchial system. Small droplets with a diameter of approximately 1 to 4 μm precipitate in the small airways whereas larger particles are either not able to enter the respiratory system or are deposited in the upper respiratory tract (Figure 15.8A).

Numerous host defense mechanisms, including mechanical barriers, protect the respiratory tract from infection. The respiratory tract is covered with a mucociliary layer consisting of ciliated cells, mucus-secreting cells, and glands (Figure 15.8 B); foreign particles coming through the nasal cavity or upper respiratory tract get trapped in mucus, are carried back to the throat, and then are swallowed, whereas foreign particles of the lower respiratory tract are brought up by the ciliary action of epithelial cells. In the alveoli that lack cilia or mucus, macrophages are principally responsible for destroying invading particles (*see* Figure 15.8 A).

In the respiratory tract, the main targets of the influenza virus are the columnar epithelial cells. These cells may be susceptible to infection in the presence of a functional viral receptor, which is the determinant of viral tropism. In an influenza infection, the receptor binding site of viral HA is required for binding to galactose-bound sialic acid on the surface of host cells. Hosts may prevent the attachment by one of the following mechanisms:

1. Viral specific immune response and secretion of specific immunoglobulin A (IgA) antibodies.
2. Nonspecific mechanisms, such as mucociliary clearance through the production of mucoproteins capable of binding to viral HA.
3. Genetic diversification of the host receptor (sialic acid residue), which is highly conserved in the same species but differs between avian and human receptors (Matrosovich et al., 2000).

Once influenza has efficiently infected respiratory epithelial cells, it replicates within hours, producing numerous virions. Infectious particles are preferentially released from the apical plasma membrane of epithelial cells into the airways by budding process favoring the spread of virus within the lungs because of the rapid infection of neighboring cells.

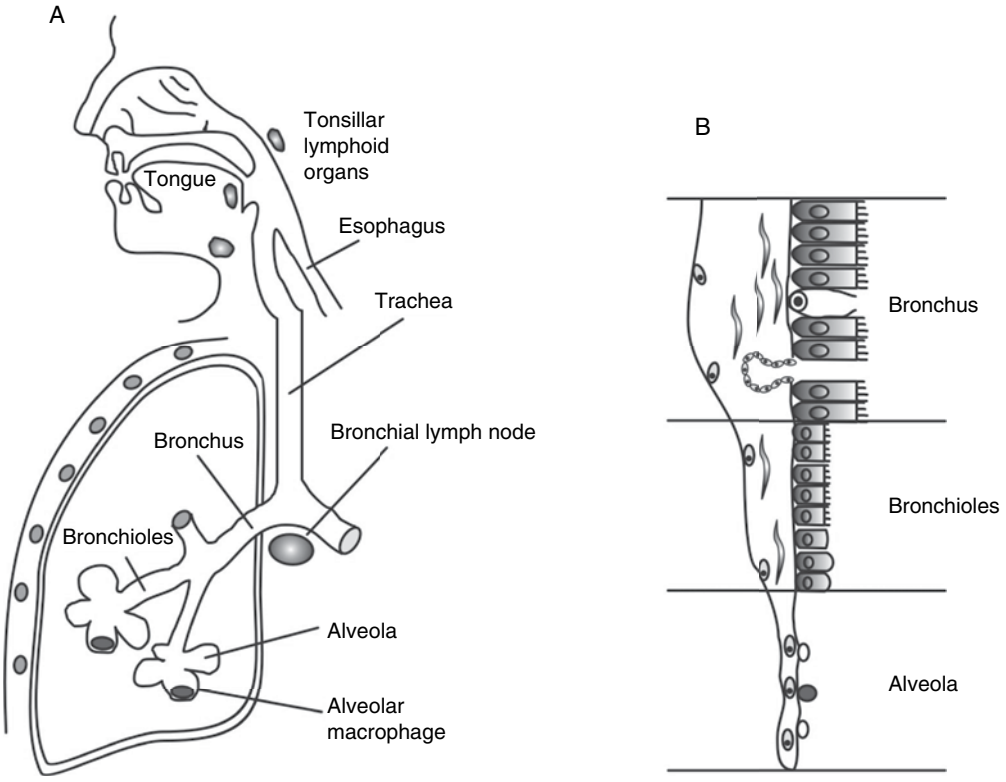

Figure 15.8. Sites of influenza entry in the respiratory tract. A, Influenza first infects the upper airway and the ciliated cells in the bronchus and bronchioli. Resulting clinical syndromes include tracheitis, bronchitis, bronchiolitis, and bronchopneumonia. B, The respiratory epithelia defends from incoming pathogens by mucus layer (bronchus), ciliated cells (bronchus and bronchioli), and alveolar macrophages (alveoli).

Life Cycle

Adsorption of the Virus

As described previously, the influenza virus binds to the host cell receptor proteins that contain polysaccharides terminating with sialic acid, which provides a recognition site for the virus's HA protein. This sialic acid linkage to the penultimate galactose, and either alpha 2, 3 (in birds) or alpha 2, 6 (in humans) determines host specificity. In humans the alpha 2, 3-linked carbohydrates are found only deep within the lungs; thereby the avian strain (H5N1), which requires alpha 2, 3 linkage, is rarely transmitted between humans. In contrast, the upper respiratory tract of humans contains mainly alpha 2, 6-linked receptors and thus provides a binding site for human influenza A virus. Sialic acid-presenting carbohydrates are present on several cells of the organism, indicating the influenza infection of multiple cell types.

For influenza virus to become infective, an HA spike must be cleaved, which is mediated by enzyme released from the epithelial lining of the human respiratory tract. This cleavage frees one end of a segment, called as *fusion peptide*, which is hydrophobic and buries within the core of the HA trimeric complex.

Entry of the Virus

After adsorption, the host cell takes up the virus via a clathrin-coated receptor-mediated endocytosis process. When internalized, the clathrin molecules are liberated and the vesicle harboring the whole virus fuses with endosomes. The endocytic vesicle then fuses with the lysosome and its interior acidifies as a result of the increased activity of the M2 ion channel. Thus, ions flood into the particle, inducing a lowering of pH. The lowered pH induces a conformational change, shifting the receptor binding region back and triggering the translocation of the fusion peptide forward to penetrate the endosomal membrane.

Uncoating of the Virus

The ion influx from the endosome to the virus particle leads to the disconnection of the different viral proteins; M1-protein aggregation is disrupted and RNPs are then detached to the M1-protein complex. After a certain level, the lowering of the pH is stopped by the action of the M2 protein, which induces the partial liberation of the fusion peptide of the HA and allows the fusion of the HA with the membrane of the vesicle and liberation of the RNPs into the host cytoplasm (Bouvier and Palese, 2008). Within 20 to 30 minutes of attachment, the virion becomes uncoated, transporting the RNPs to the cell nucleus.

Synthesis of Viral RNA and Viral Proteins

Once the viral RNA releases inside the nucleus, the RNA-dependent RNA polymerase binds to it, cleaves viral RNA by its endonuclease activity, and begins transcribing complementary positive-sense vRNA (Cros and Palese, 2003). Human messenger RNA carries a poly-A tail at the 5' end; NS1 (MW 26000) forms a dimer that inhibits the export of poly-A containing mRNA molecules from the nucleus, thus giving preference to viral RNA, which is transported to the ribosome to undergo translation. NS1 might also inhibit splicing of pre-mRNA, and part of the viral mRNA is spliced by cellular enzymes resulting viral proteins, such as M1 and NS2, and can be synthesized without any further cleavage. Newly synthesized viral proteins (PB1, PB2, PA, NS1, NS2, NP, M1) are then finally transported to the nucleus (*see* Figure 15.9) where they bind to newly synthesized viral RNA to form RNPs.

Assembly and Release

After translation of viral proteins and replication of the viral genome, NS2 facilitate the transport of RNPs from the nucleus to the cytoplasm to accelerate the production of new viral progeny. Other newly synthesized viral proteins are processed in the endoplasmic reticulum and the Golgi apparatus where glycosylation occurs. These modified proteins are transported to the cell membrane and stick in the lipid bilayer, when their concentration is high enough at the plasma membrane; RNPs and M1 proteins aggregate and condense to produce new viral particle. Finally, the particle is extruded from the membrane and will then be liberated by the NA activity. After releasing new influenza viruses, the host cell dies and newly produced viral progeny are now ready to infect another nearby healthy cell. The time from entry to the production of progeny viruses is around 6 hours.

There is the absence of RNA proofreading enzymes, thus the RNA-dependent RNA polymerase that copies the viral genome makes an error roughly after every ten thousand nucleotides, which is the approximate length of the influenza vRNA, and as a result, the

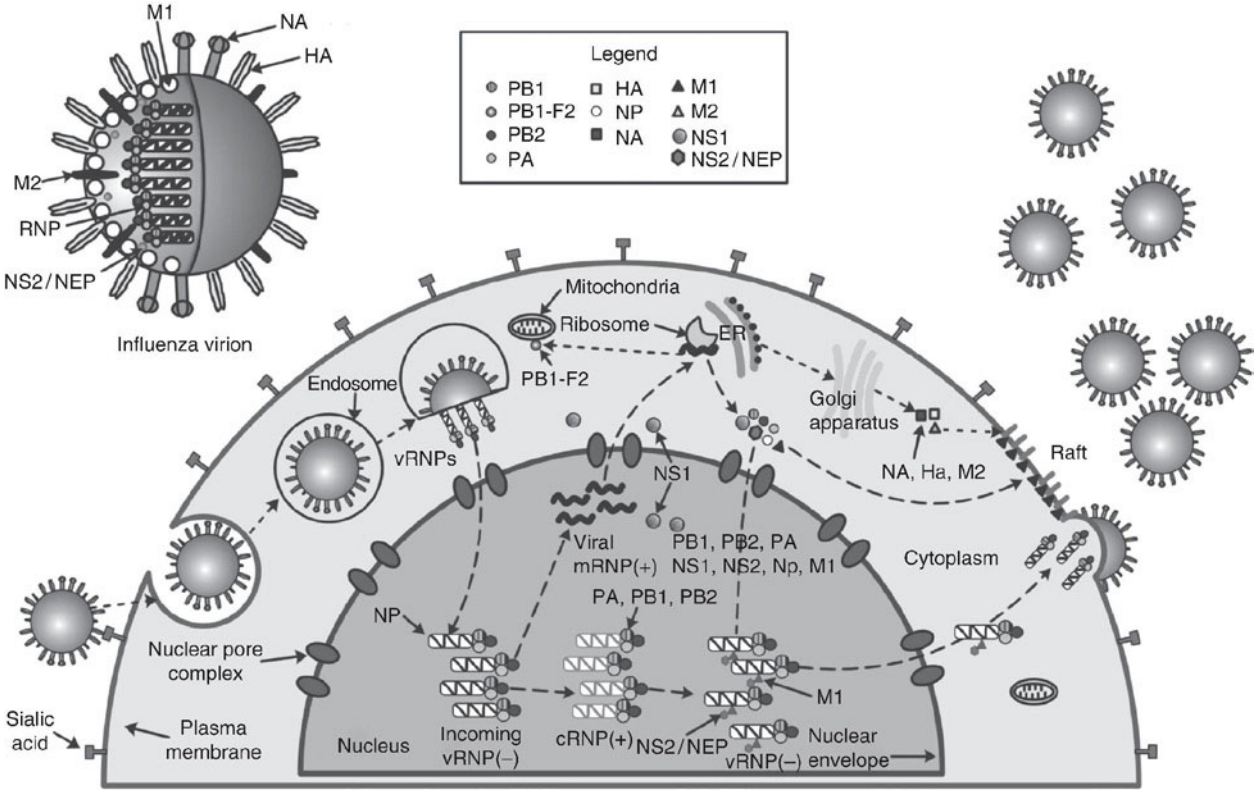

Figure 15.9. Life cycle of the influenza virus. HA, hemagglutinin; M1, matrix protein; M2, matrix protein; NA, neuraminidase; NEP, nuclear export protein; NS1, nonstructural protein 1; NS2, nonstructural protein 2; RNP, ribonucleoprotein. *Source*: Image modified from Lamb RA, Krug RM. 2011. Orthomyxoviridae: The viruses and their replication. In: DM, Howley PM, eds. *Fields Virology*, 4th ed., pp. 1487–1532. Philadelphia: Lippincott Williams & Wilkins.

majority of newly formed influenza viruses are mutants causing antigenic drift (Drake, 1993). The separation of vRNA into eight separate genomic segments allows mixing or reassortment of vRNAs, if more than one type of influenza virus infects a single cell. The resulting rapid change in viral genetics produces second antigenic variation, which is the antigenic shift responsible for the emergence of pandemics.

Host Factors

Influenza is more severe and more complex in the very young, the elderly, and individuals who are immunocompromised. Several factors may increase the risk of developing influenza or its complications.

Age

Seasonal influenza generally targets young children and people older than 65 years of age. The very young and the very old people are at higher risk for upper respiratory tract infections and related complications. All infants are highly susceptible to upper respiratory tract infections because of their immature immune system, with a possible frequency of a cold every 1 to 2 months. Smaller nasal and sinus passages of younger children also make them more vulnerable to colds than older children and adults. These upper respiratory infections gradually decrease with the growth of children, and at school age, the rate of such infections is about the same as in adults. Elderly patients have diminished cough and gag reflexes, and their immune system is also weaker; they are, therefore, at greater risk for serious respiratory infections than young and middle-aged adults. However, the recent 2009 H1N1 pandemic appeared to be the most common in teenagers and young adults.

Occupation

Health-care workers and child-care personnel are more likely to have close contact with people infected with influenza; thus, they can easily develop influenza.

Living Conditions

People who live in areas along with many other residents, such as nursing homes or military barracks, are more likely to develop influenza.

Medical Conditions

Patients undergoing cancer therapy, transplantation surgery, receiving corticosteroids or other medications that suppress the immunity, and have viral infections, such as HIV or AIDS, or have weakened immune systems are more susceptible to influenza and may increase the risk of developing complications. Cancers, especially leukemia and Hodgkin's disease (blood malignancies), chronic conditions, such as asthma, diabetes, or heart problems, put patients at risk of developing influenza. Apart from these disorders, certain genetic diseases predispose people to respiratory infections, including sickle-cell disease, cystic fibrosis, and Kartagener syndrome (cilia malfunctioning).

Pregnancy

Pregnant women especially in the second and third trimesters of pregnancy are more likely to develop influenza complications.

People under Stress

Stress appears to increase the susceptibility to cold regardless of lifestyle or health habits, and as a person acquires a cold or influenza, stress can make symptoms worse. Stress might alter specific immune factors causing inflammation in the airways.

Excessive Exercise

In people suffering from cold, exercise has no obvious effect on the severity of the illness or duration of the infection, but some highly trained athletes, for instance, report being susceptible to colds after strenuous events, which suggests that people should avoid strenuous physical activity when they have high fevers or there is widespread viral illnesses.

Immunomodulating Factors

High levels of vitamin D levels and other immunomodulating factors associated with ultraviolet light levels have also been suggested as factors influencing influenza attack rates (Cannell et al., 2008).

Overcrowding

Because influenza is principally an airborne disease, the person-to-person spread of virus-laden aerosol particles is greatly enhanced by having a dense population of susceptible individuals surrounding each infective subject, thereby maximizing the potential for the spread of infection. Overcrowding has, therefore, been considered as a risk factor for a wide range of viral and bacterial diseases, including influenza (Souza et al., 2003). For an example, the origin and spread of the 1918 influenza pandemic has been attributed to the overcrowded populations on military bases during the First World War.

Traveling in Trains, Buses, and Planes

Traveling brings close contact with people, whether on trains, planes, or buses. If any of these people are infected, this can increase the risk for respiratory infections, For example, during the Hong Kong pandemic of 1968, air trafficking was considered to be responsible for rapid and wide dissemination of the virus across both the hemispheres (Grais et al., 2003).

Environmental Factors

Ambient Temperature

Decrease in temperature is an environmental variable that is frequently found to be associated with high levels of seasonal influenza virus infection (Cox and Subbarao, 1999). Low temperature induces behavioral changes, such as increased crowding and increase

physiological stress and energy costs for thermoregulation, and these could, in turn, weaken the immune system, which increases the susceptibility to infection from an unaltered rate of exposure.

Indoor Heating

Along with the direct effects of altering weather conditions on the biology of either the host or the pathogen, human defenses against declining temperature may also contribute to the seasonality of influenza. Indoor heating levels should increase as the temperature drops, resulting in a continuously recirculation of air with a low humidity. This makes conditions ideal for the persistence of viral particles in the environment. In contrast, there are a few cases of influenza in the summer season, when the use of air conditioning systems is high, which lowers the absolute humidity of the air via condensation (potentially trapping virus-bearing aerosols within the unit itself), whereas heating lowers only the relative humidity and never exposes the air to a wet-condensing surface. Large-scale heating systems, especially in apartments, offices, and university dormitories, can create a viral dispersion mechanism resembling the unintentional one potentially responsible for the Amoy Gardens severe acute respiratory syndrome outbreak (Li, Duan, et al., 2005).

Exposure to Smoke and Environmental Pollutants

Exposure to smoke and environmental pollutants can injure airways and damage the cilia (tiny hair-like structures that help keep the airways clear) and thereby increase the risk of respiratory infections. Other risk factors include air pollutants, such as toxic fumes and industrial smoke. Parental smoking also increases the risk of respiratory infections in children.

Seasonal Incidence

Influenza predominantly occurs in the winter season; the season typically starts in October and lasts into mid March. The reasons for this seasonal bias are not only as a result of the cold itself, but other associated factors. For example, in winter people spend more time indoors and, are therefore, exposed to higher concentrations of air-borne viruses. Winter weather also dries up nasal passages, making them more susceptible to air-borne viruses.

Bulk Aerosol Transport

The aerosolization of influenza virus particles discharged from infectious individuals might be directly responsible for the disease's seasonality. Coughing and sneezing produce massive amounts of small-sized aerosol droplets containing high viral titers, which travel through the air at speeds of nearly 100 ft/s (Tyler and Nathanson, 2001). Modeling of the 2003 severe acute respiratory syndrome outbreak at the Amoy Gardens housing complex in Hong Kong (329 people infected and 42 killed) has thoroughly examined the role of so-called "bioaerosols" in the transmission of disease (Li, Duan, et al., 2005).

Mode of Transmission

Influenza is highly contagious disease, particularly in persons without pre-existing antibodies against influenza, such as young children during the interpandemic phase and anyone during a pandemic phase; however, 50 percent of all infections are asymptomatic. Influenza virus may be transmitted among humans by one of the three ways:

1. Through direct contact with infected individuals.
2. Through contact with contaminated objects (fomites, such as toys, doorknobs).
3. Through the inhalation of virus-laden aerosols.

Direct-Contact Transmission

It involves person-to-person direct contact and physical transfer of the virus to a susceptible host from an infected or colonized person, which occurs when personnel turn patients, bathe patients, or perform other patient-care activities that require physical contact. Direct-contact transmission also becomes possible between two patients, with one serving as the source of infectious microorganisms and the other as a susceptible host.

Indirect-Contact Transmission

It involves the transfer of virus particle from a patient to a susceptible host by means of a contaminated intermediate object, which is usually inanimate, such as needles or countertops.

Droplet Transmission

In droplet transmission, contagious droplets are produced by the infected host, which then are propelled a short distance through coughing or sneezing and then come into contact with another person's conjunctiva, mouth, or nasal mucosa. Influenza can be transmitted by large droplets (greater than 10 micrometers), which generally travel 3 to 6 feet, and it is not affected by special air handling or control of room pressures. These large droplets are the primary route of nosocomial infection in hospital premises.

Another droplet transmission is through infectious droplet nuclei, which are generally 5 or less micrometers in diameter. In contrast to the larger droplets, these droplet nuclei can remain suspended in the air and be disseminated by air currents in a room or through a facility to be inhaled by a susceptible host; hence, these small particles appear to be more infectious, with both the degree of infectivity and the severity of illness. Among droplet nuclei, aerosol smaller than 10 microns has been shown to cause more severe disease and require a smaller inoculum as compared to the large intranasal droplets. To prevent the spread of these droplet nuclei, the use of special air handling and ventilation procedures are then required.

The exact contribution of each mode of transmission of influenza is not known, although droplet transmission is thought to be the predominant form. When there is the absence of appropriate ventilation and air exchange, air-borne transmission may play a greater role, such as in a crowded space.

Clinical Manifestations

Classical Symptoms

The typical symptoms of influenza start appearing at least after an incubation period of 48 hours. The first symptoms are chills or a chilly sensation along with the fever with body temperatures ranging from 100.4 to 102.2° F (38–39° C). Further the onset is marked with headache, photophobia, shivering, a dry cough, nasal congestion, malaise, myalgia, irritated watering eyes, reddened eyes, skin especially of face, mouth, throat, and nose, petechial rash (Silva et al., 1999), and a dry tickling throat (Figure 15.10). In children, gastrointestinal symptoms, such as diarrhea and abdominal pain, may also be present (Heikkinen, 2006), which may be more severe in children with influenza B (Kerr et al., 1975). The fever is continuous and takes around 3 days to subside completely; many people are so ill that they are confined to bed for several days, with aches and pains throughout their bodies, especially in their backs and legs. Influenza B infection is more or less similar to influenza A, but infection with influenza C is usually subclinical or very mild.

Diarrhea is not normally a symptom of influenza, although it has been seen in some human cases with H5N1 (bird flu) infection in adults (Hui, 2008) and can be a symptom in children. The symptoms most commonly seen in influenza are fever, cough, and nasal congestion (Table 15.4) (Call et al., 2005). All the three findings, especially fever, are less sensitive in patients older than 60 years of age.

Influenza is sometimes confused with the common cold, and it can be difficult to distinguish between the common cold and influenza in the early stages of these infections (Eccles, 2005), but influenza can be identified by a high fever with a sudden onset and extreme fatigue (Table 15.5).

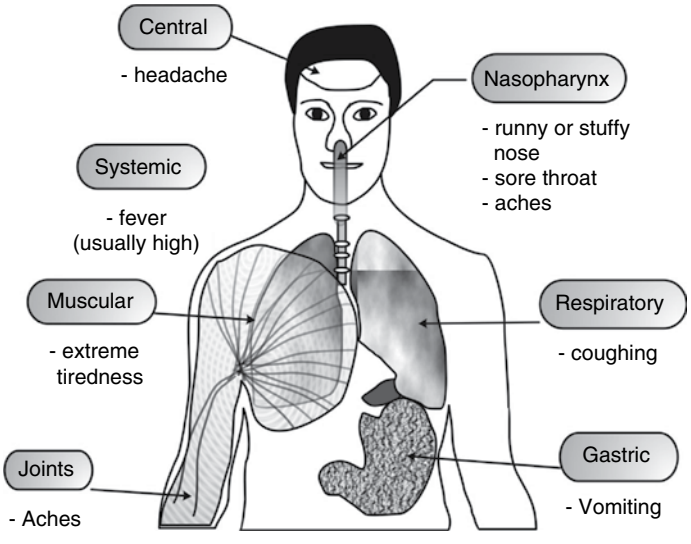

Figure 15.10. Symptoms of influenza.

Table 15.4. Most sensitive symptoms to diagnosing influenza.

Symptom	Sensitivity (%)	Specificity (%)
Fever	68–86	25–73
Cough	84–98	7–29
Nasal congestion	68–91	19–41

Table 15.5. Difference between influenza and common cold.

Symptoms	Influenza	Common Cold
Fever	Usually high, lasts 3–4 days	Unusual
Headache	Yes	Unusual
Fatigue or weakness	Can last up to 2–3 weeks	Mild
Pains and aches	Unusual and often sever	Slight
Exhaustion	Early and sometimes severe	Slight
Stuffy nose	Sometimes	common
Sore throat	Sometimes	common
Cough	Yes	Unusual
Chest discomfort	Common and sometimes severe	Mild to moderate
Complications	Bronchitis, pneumonia; in severe cases life-threatening	Sinus congestion

Complications

1. **Tracheobronchitis and bronchiolitis**: Some patients with influenza develop more severe respiratory symptoms in which rales and rhonchi are heard but with a radiologically clear chest.
2. **Pneumonia**: Primary viral pneumonia may develop, which is relatively uncommon, but many cases have been demonstrated in various influenza epidemics. It may be observed in previously young and healthy persons, but usually are associated with patients with pre-existing cardiovascular disease, such as Rheumatic fever.
3. **Secondary bacterial pneumonia**: Secondary bacterial pneumonia is more commonly observed than primary viral pneumonia. It was speculated that the high incidence of deaths in young people during the Spanish influenza pandemics of 1917 to 1918 may have been the result of secondary bacterial pneumonia. It usually occurs late during the acute disease course, after a period of improvement. The symptoms and signs are that of a typical bacterial pneumonia involving mainly *Staphylococcus aureus*, although *Streptococcus pneumoniae* and *Haemophilus influenzae* may also be found. Influenza A infection requires cleavage of the virus HA by proteases, which are produced by strains of *S. aureus* thereby promoting the influenza infection.
4. **Myositis and myoglobinuria**: In addition to myalgia, a characteristic of acute influenza infection, clinical myositis and myoglobinuria may also occur.
5. **Reye's syndrome**: This is characterized by encephalopathy and fatty liver degeneration. The disease has 50 percent mortality among hospitalized patients, which had been

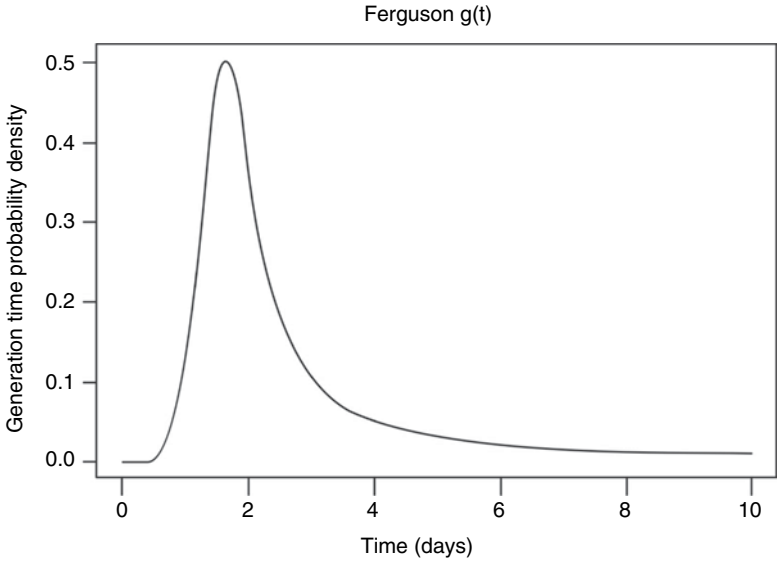

Figure 15.11. Generation time probability density of the influenza virus.

associated with several viruses including influenzas A and B, Coxsackie B5, echovirus, herpes simplex virus (HSV), varicella zoster virus (VZV,) cytomegalovirus (CMV) and adenovirus.

6. **Other complications**: Influenza infection have also been implicated in acute viral encephalitis and Guillain-Barré syndrome. Influenza A was also found to be associated with the Cot death syndrome.

Shedding of the Virus and Infectivity

The generation time (the time from one infection to the next) for influenza is very short (only 2 days). Hence, influenza epidemics start and finish in a time of only a few months (Ferguson et al., 2005) (Figure 15.11). Adults start shedding influenza virus from the day before symptoms begin through days 5 to 10 after illness onset. However, the amount of virus shed and infectivity decreases rapidly by days 3 to 5 after onset in an experimental human infection model. Young children also might shed virus several days before illness onset and can be infectious for 10 or more days after the onset of symptoms, whereas persons who are severely immunocompromised can shed virus for weeks or months.

Immune Response to Influenza

Influenza virus causes acute infection and thereby induces a cascade of immune reactions activating almost all parts of the immune system. Innate immunity is an essential prerequisite for the adaptive immune response because it limits the initial viral replication and antigen load and also activates the antigen-specific lymphocytes of the adaptive immune response through the costimulatory molecules, which are stimulated on cells of the innate

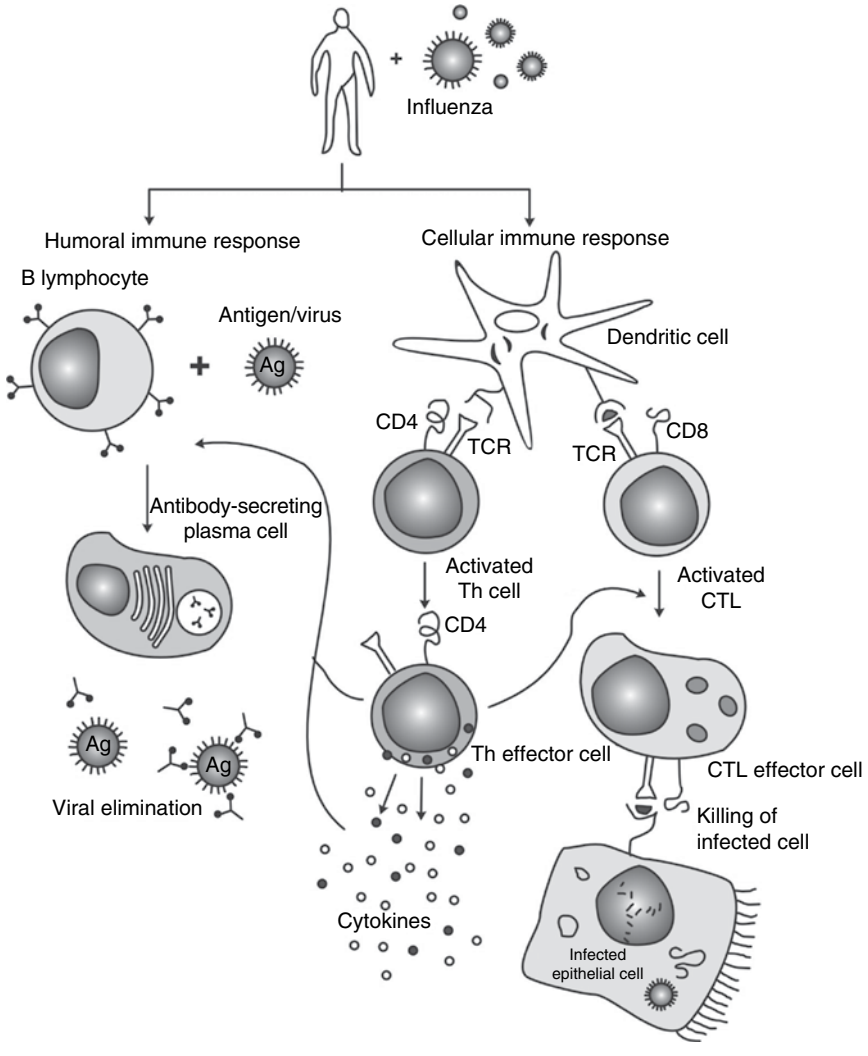

Figure 15.12. Immune response to influenza virus infection. The humoral immune system comprises B-lymphocytes, which on antigenic stimulation by influenza virus differentiate into antibody-secreting plasma cells and memory cells. The cell-mediated immunity starts with antigen presentation via MHC I and II molecules by antigen presenting cells, which then leads to activation, proliferation, and differentiation of antigen-specific T cells (CD4 or CD8). These cells perform effector cell function by releasing cytokines or mediate cytotoxicity following recognition of an antigen. CTL, cytotoxic T cell; TH, T helper.

immune system during their primary interaction with infected virion particles (Figure 15.12). The adaptive immune response does, however, require some days to become effective but once it develops, it prevents the further spread of virus, tries to eradicate it, and finally establishes an anamnestic response resulting in a long-lived immunity to reinfection with homologous virus. Influenza infection induces both systemic and local antibody (humoral

immunity), as well as cytotoxic T cell responses (cellular immunity), each of which is important in recovery from acute infection and gives resistance to reinfection.

The Humoral Immune Response

Humoral immune response is mediated by B lymphocytes. B lymphocytes recognize viral antigen through their surface-bound antibody receptors, become activated, switch from immunoglobulin M (IgM) to immunoglobulin G (IgG) production (class-switch if required), increase their immunoglobulin specificity and affinity, and differentiate into plasma cells or memory B cells as the cell continues to divide in the presence of cytokines. The other immunoglobulin secretory IgA is transported across the mucosal epithelium of the upper airway and serves to neutralize and clear viral infection, whereas IgG is principally responsible for the protection of the lower respiratory tract infection (Palladino et al., 1995; Renegar et al., 2004). Influenza infection results in the systemic production of antibody against both HA and NA (influenza glycoproteins), as well as M and NP proteins. The HA-specific immunoglobulins, including IgM, IgA, and IgG, appear within 2 weeks of virus inoculation and the development of anti-NA antibodies parallels that of HA-inhibiting antibodies. The peak in antibody titers is seen between 4 and 7 weeks after infection followed by a steady decline, whereas antiviral antibodies remain detectable for years after infection even without re-exposure. Serum HA-inhibiting titers of 1:40 or greater, or serum neutralizing titers of 1:8 or greater, are supposed to protect against influenza infection, but higher levels of antibody are required for completely protect older individuals (Treanor, 2004).

In contrast to anti-HA antibody, anti-NA antibody does not neutralize virus infectivity, instead it reduces the efficient release of virus from infected cells (Johansson et al., 1989) by cleaving the cellular-receptor sialic acid residues to which the newly formed particles are attached. Thus, anti-NA antibody can also protect against the disease and result in decreased virus shedding and severity of symptoms.

The nasal secretions exhibit immune response against influenza, which is probably because of the presence of IgA and IgG_1 antibody against viral HA protein. The mucosal anti-HA IgG levels correlate with the respective serum levels, indicating passive diffusion of IgG from the systemic compartment, whereas IgA is produced locally in the mucosal surface. The immunity to reinfection is predominantly mediated by locally produced anti-HA IgA, although IgG might also be relevant as well (Renegar et al., 2004). Either mucosal or systemic antibody alone can be protective if present in sufficient concentrations, but for optimal protection both the serum and nasal antibodies should be present (Treanor, 2004). Antibodies exhibit immunity against influenza either by neutralizing the virus particles or by lysing virally infected cells via complement or antibody-dependent cellular toxicity (ADCC).

The Cellular Immune Response

The cellular immune response involves an antigen presenting cell and an effector cell. During infection with influenza virus, the dendritic cells serve as antigen presenting cells playing a central role in initiating and driving T lymphocyte (effector cell) responses against influenza virus. These cells are sparsely distributed and are specialized for the uptake, transport, processing, and presentation of antigens to T cells (*see* Figure 15.12). The dendritic cells of the lungs acquire antigen from the infecting influenza virion, become activated, and subsequently travel to the local draining lymph nodes (Legge and Braciale, 2003). The viral antigen is processed and then presented along with the major

histocompatibility complex (MHC) molecules on the surface of dendritic cell. Endogenous antigens are presented with MHC I molecule to CD8 T cell, whereas exogenous antigens are presented with MHC II molecules to CD4 T lymphocytes. Alternatively, dendritic cells may also present antigens acquired from infected cells or transfer them to neighboring dendritic cells in the lymph node which then initiate a cytotoxic T cell response by a process called cross-presentation (Heath et al., 2004). The newly activated T cells acquire effector cell functions and migrate to the site of infection in the lung and mediate antiviral activities. In human beings, the T lymphocyte responses at about 14th day post-infection and influenza-specific cytotoxic T cell levels correlate with a reduction in the level and duration of viral replication in adults.

After the patient get recovers from influenza infection, the antigen-specific T cells maintain the immunological memory of influenza virus, which without costimulatory signal, responds quickly to antigenic restimulation.

Laboratory Diagnosis

During epidemics, a presumptive diagnosis can always be made on the basis of the clinical symptoms. However, influenza A and B can co-circulate, and mixed infections of influenza and other viruses have also been reported.

Specimen

Respiratory Specimens

The time scheduling for collecting specimen is important because the highest yield can be obtained within 4 days of onset of symptoms especially for respiratory specimens. Different types of respiratory specimens can be collected, but nasal washes and nasopharyngeal aspirates tend to be more sensitive than pharyngeal swabs. Tracheal aspirates and bronchial lavages can also be collected (WHO, 2005) provided washes and aspirates contain sufficient respiratory epithelium for immunofluorescence tests. However, specimens without sufficient cells could be collected and diagnosed by other methods such as rapid antigen detection, virus isolation, and reverse transcriptase-polymerase chain reaction (RT-PCR). All the swabs should be transported in virus transport medium to prevent desiccation and arrive at the laboratory as soon as possible to avoid any degradation because it may lead to misdiagnosis. If there is any delay expected in transportation, transportation in virus transport medium on ice or with refrigeration at 35.6° to 46.4° F (2–8° C) should be recommended.

Blood Specimens

Blood (whole blood or serum) specimens are collected to detect the presence of antibodies against influenza through various serological techniques. Acute and convalescent serum samples 14 to 21 days apart should also be collected to demonstrate a significant (at least fourfold) rise in strain-specific antibody titer.

Laboratory Tests

The various factors should be considered before deciding the type of test, including sensitivity, specificity, turn-around-time, repeatability, ease of performance, and costs.

RT-PCR is generally found to be more sensitive than serological and culture techniques, and the combination of RT-PCR with serology make it more sensitive than the combination of any other two methods (Zambon et al., 2001). Serology is less expensive than RT-PCR, but because it requires acute and convalescent blood specimens, diagnosis is only retrospective. Traditional culture is time consuming, but shell vial culture techniques can diagnose within 48 to 72 hours.

Direct Methods

Some methods can directly detect influenza viruses in patient's sample, such as direct immunofluorescence, enzyme immunoassays (EIAs), and RT-PCR. These methods can either detect both influenza A and B and can also differentiate between types (influenza A or B). RT-PCR is the only direct technique having the potential to differentiate between subtypes (on the basis of HA and NA spikes).

1. Immunofluorescence: This test employs the fixation of infected respiratory epithelial cells on to a slide and viral antigens contained in the cells are detected by specific antibodies, which are either directly conjugated to a fluorescent dye (in direct immunofluorescence assay) or detected by anti-antibodies linked to a fluorescent dye (in indirect immunofluorescence assay). In both cases, reactions are then visualized under the fluorescent microscope, and viral positive cells are distinguished on the basis of color intensity and morphology of fluorescent areas. Direct immunofluorescence tends to allow faster results but probably is less sensitive than indirect immunofluorescence. Another advantage of an indirect immunofluorescence test is that pooled antisera can be used to screen for viral infection using a single anti-antibody conjugated to a fluorescent dye (e.g., fluorescein isothiocyanate), which generally allows for the rapid diagnosis of respiratory specimens.
2. EIAs or immunochromatographic assays: EIAs employ antibodies directed against viral antigen that are conjugated to enzyme. On incubation with a chromogenic substrate, a color change appears indicating the presence of viral antigen in the sample. Certain EIAs as well as similar assays using immunochromatography allow for bedside testing (Allwinn et al., 2002) and takes about 10 to 30 minutes. These assays are rapid and generally more expensive than direct immunofluorescence or virus culture showing sensitivity between 64 and 78 percent. Different rapid tests can easily detect the type of influenza viruses, but they cannot detect subtypes (H1N1 and H3N2).
3. RT-PCR: This test can be performed in well-equipped laboratories by trained personnel. The process employs the conversion of viral RNA to complementary DNA followed by the amplification of genomic section through the use of primers, which specifically bind to this target area. Thus, a small amount of nucleic acid is exponentially amplified through the action of a thermostable DNA polymerase enzyme, which enables highly sensitive detection of minute amounts of viral genome. RT-PCR is highly sensitive diagnostic method (Steininger et al., 2002) because it can differentiate between subtypes and conduct phylogenetic analysis (Allwinn, 2002); however, RNA degradation of archival samples can sometimes decrease the sensitivity of RT-PCR (Frisbie et al., 2004). Therefore, specimens should always be processed as fast as possible after collection.

Isolation Methods

Virus isolation or culture techniques employ the inoculation of specimen in a live culture system and then detect the presence of virus in this culture system. Because culture naturally amplifies the amount of virus, this process is more sensitive than direct methods with the exception of RT-PCR (that also employs amplification). Isolation requires the rapid and immediate transport of specimens to the laboratory because delay may lead to inactivation of the virus (Allwinn, 2002). Throat swabs and nasal washings of patients can be used for virus isolation; however, the nasal washings are considered the best specimens for virus isolation. The specimen may then be inoculated in embryonated chick eggs, cell/tissue culture, or in susceptible experimental animals.

1. Embryonated egg culture: The specimen is inoculated directly into the amniotic cavity of 10- to 12-days embryonated chicken eggs. The virus replicates in the cells of the amniotic membrane and large quantities of them is then released back into the amniotic fluid. High viral yield could be harvested after 3 days of incubation (WHO, 2005) by adding aliquots of harvested amniotic fluid to chick, guinea pig, or human erythrocytes. This technique requires fertilized chicken eggs and special incubators and time; hence, it is no longer used for the routine diagnosis of influenza infection. However, egg inoculation provides high yield of virus and is a sensitive culture system. Reference laboratories, therefore, use this to ensure high sensitivity, especially to enable the production of virus stocks for the epidemiological monitoring.
2. Cell Culture:
   - **Conventional culture**: Pathological specimens can be inoculated on various cell lines to isolate influenza viruses, as primary monkey kidney cells and Madin-Darby canine kidney (MDCK) cells. Although conventional cell culture takes around 2weeks, it has a high sensitivity. Influenza virus (mainly influenza B and occasionally influenza A) produces cytopathic effects as syncytia and intracytoplasmic basophilic inclusion bodies following incubation. The presence of influenza virus can also be ascertained by hemadsorption test using guinea pig red blood cells (Weinberg et al., 2005) and by HA test using the culture medium containing free virus particles, although immunofluorescence has a higher sensitivity in detection of positive cultures than hemadsorption. Further influenza viruses, which have been isolated from embryonated eggs or tissue culture, can be identified by serological or molecular methods. They can be diagnosed as A, B, or C types by the complement fixation tests against the soluble antigen (A soluble antigen is found for all influenza A, B, or C type virus and antibody against one type does not cross-react with the soluble antigen of the other). The further classification of influenza isolates into subtypes and strains is a highly specialized responsibility that must be carried out in WHO reference laboratories.
   - **Shell vial culture**: Shell vial culture allows for diagnosis within 48 hours (Allwinn, 2002) and is brought about by centrifugation of the inoculum onto the cell culture monolayer and the performance of immunofluorescence before the observance of a cytopathic effect. Shell vial culture can, however, be less sensitive than conventional culture (Weinberg et al., 2005).
3. **Animal inoculation**: Ferrets are often used in research facilities as an experimental model of human influenza infection, but they do not have any role in routine diagnosis.

Serology

Serology refers to the detection of influenza virus-specific antibodies in patient serum or other body fluids. This can either detect total antibodies or be class-specific as IgG, IgA, or IgM alone. The diagnosis is made retrospectively by the demonstration of a rise in serum antibody titer to the infecting virus. Various serological techniques are available for the diagnosis of influenza including hemagglutination inhibition (HI), compliment fixation (CF), EIA, and indirect immunofluorescence. Serological diagnosis has a little role in diagnosing acute influenza because to diagnose an acute infection, at least a fourfold rise in titer needs to be demonstrated, which necessitates both an acute and a convalescent specimen. However, it may have value in diagnosing influenza in recently infected patients. Serology is also used check the response to influenza vaccination (Prince and Leber, 2003).

1. HI: HI assays are labor intensive and time consuming and require several controls for standardization. However, the assay reagents are cheap and easily available. These tests employ various red blood cells such as a guinea pig, fowl, and human blood group "O" erythrocytes and the serum is pretreated to remove nonspecific HA and inhibitors. A viral HA preparation showing visible HA (usually 4 HA units) reaction is then preincubated with two-fold dilutions of the serum specimen. The lowest serum dilution that inhibits HA reaction is considered as the HI titer. This test is more sensitive than CF and it is more specific in differentiating between HA subtypes (Julkunen et al., 1985).
2. CF: CF tests are based on the ability of antigen-antibody complexes to fix complement resulting unavailability of complement to lyse sensitized sheep red blood cells. It is the most common test, which uses type-specific soluble antigen. Although this assay is labor intensive and necessitates complete control of each step, but it is economic and easy because its reagents are cheap and widely available. The only drawback is CF assays are less sensitive than HI both in the diagnosis of acute infection and the determination of immunity after vaccination (Prince and Leber, 2003).
3. Indirect immunofluorescence: Cells from pathological specimens could also be examined for the presence of influenza A and B antigens by indirect immunofluorescence test. In this test, the specificity of the antisera and the level of background fluorescence make it difficult to interpret properly. Thus, indirect immunofluorescence is not commonly used as a method to detect influenza virus specific antibodies.

Rapid Tests

The clinical value of an individual diagnostic test for influenza is dependent on the particular test's turnaround time. The first tests that were developed for the diagnosis of influenza were virus isolation and serological assays, which took more than 2 weeks to exclude influenza infection. Although shell vial tests have reduced the turn-around time of isolation, they are not generally regarded as rapid tests for diagnosing influenza.

The direct tests, such as immunofluorescence, although enabled the diagnosis within a few hours (1 to 2 incubation and wash steps), require skilled laboratory workers and immunofluorescence microscopes. The revolution in rapid diagnosis of influenza was actually brought about by the development of rapid antigen assay kits (most of which work on an EIA or immunochromatographic principle). These assays enable the diagnosis of influenza within 10 to 30 minutes and are so easy to perform that even nonlaboratory

Table 15.6. Characteristic comparison of influenza test methods.

Test	Sensitivity	Turnaround Time	Ease of Performance	Affordability
Direct Detection				
Rapid tests (EIA/chromatography)	–2	+2	+2	0
Immunofluorescence	0	+1	+1	+1
Gel electrophoresis RT-PCR	+2	0	–1	–2
Real-time RT-PCR	+2	+1	–1	–2
Viral Culture				
Routine viral culture	+2	–2	–1	+2
Shell vial culture	+1	0	–1	+1
Serology				
EIA	+2	–2	+1	+1
Hemagglutination inhibition	+1	–2	–1	+2
Complement fixation	0	–2	–2	+2

(–2) very unfavorable characteristic; (–1) unfavorable characteristic; (0) average characteristic; (+1) favorable characteristic; (+2) very favorable characteristic.
EIA, enzyme immunoassay; RT-PCR, reverse transcription-polymerase chain reaction.

trained people can perform these tests. RT-PCR reactions requiring a gel electrophoresis step were initially time consuming, but the relatively recent development of real-time technology made RT-PCR diagnosis possible within about 2 hours. Although antigen assays are generally user friendly, they are not as sensitive as direct immunofluorescence, isolation, or RT-PCR. Table 15.6 compares the characteristics of different available test methods for influenza diagnosis in which minus (–) sign indicates the unfavorable characteristic and a plus (+) sign indicates favorable characteristic.

Clinical Management

Before starting the treatment, clinical data and samples should be collected for virological monitoring, which allow assessment of the effects of treatment regimens. In this regard, the retrospective reporting of clinical and laboratory data from patients who had recently been infected may also be helpful. When a patient is suspected with influenza, the specimen should be sent to a WHO H5 reference laboratory for further testing and confirmation of the infection.

Infection Control

To limit the infection, the patient should first be isolated and infection control precautions should be implemented.

1. All body fluids, secretions, and clinical specimens should be considered potentially infectious and handled accordingly.

2. Proper personal protective equipment (PPE) should be provided to the caregivers. For a health-care provider, PPE is considered the first line of defense against influenza infection.

Supportive Care

Most of the human cases with new influenza infection have had uncomplicated illness of limited duration, requiring supportive care, which includes antipyretics, such as paracetamol or acetaminophen for fever or pain, and fluid rehydration rather than hospitalization or antiviral therapy. Salicylates (such as aspirin and aspirin-containing products) are not recommended to children and young adults (aged <18 years) because of the risk of Reye's syndrome. Clinicians and caregivers should watch for signs of possible clinical deterioration (e.g., difficulty in breathing, chest pain, coughing up colored sputum, altered level of consciousness, and confusion) and should refer such patients immediately to a hospital. Clinicians should also observe and record any underlying comorbidities (as immunocompromising conditions, pre-existing chronic lung or cardiovascular disease, or diabetes). Pregnant women are known to be at increased risk of influenza infection; consequently, pregnant women with suspected or confirmed influenza infections warrant closer observation.

Oxygen Therapy

During managing patients with influenza, oxygen saturation should be monitored by pulse oximetry and followed by supplemental oxygen to correct hypoxemia. WHO recommendations for influenza advise oxygen therapy to maintain oxygen saturations above 90 percent; however, this threshold may be increased to 92 to 95 percent in some special situations, for example, during pregnancy. Patients with severe hypoxemia need high flow oxygen (e.g., 10 L/min), which should be delivered by face mask. Some patients experiencing difficulties with compliance (such as children) may require the close involvement of nursing staff or their family members.

Antibiotic Treatment

Antibiotic chemotherapy should be avoided; however, seasonal influenza and past influenza pandemics have been found associated with an increased risk of secondary *S. aureus* infections, which may be severe, rapidly progressive, necrotizing, and in some areas, caused by methicillin-resistant strains. Although in cases where viral influenza is present, proper antibiotic should be administered, probably based on the results of microbiological studies.

Antiviral Therapy

The newly evolved influenza viruses are currently susceptible to the neuraminidase inhibitors (NAIs), oseltamivir and zanamivir, but resistant to amantadine or rimantadine (adamantine or M2 inhibitor drugs). Early administration of NAIs (Table 15.7) might reduce the severity and duration of influenza illness caused by the new influenza virus infection and may also contribute to prevent progression to severe influenza disease resulting death. Antiviral therapy may be beneficial for the following groups of persons:

Table 15.7. Recommended antiviral treatment regimens.

Modalities	Strategies
Antibiotics	In case of pneumonia, empiric treatment for community-acquired pneumonia, thereafter tailor therapy on identification of pathogen
Antiviral therapy	Neuraminidase inhibitors as oseltamivir or zanamivir as some strains (e.g., H1N1) are resistant to amantadine and rimantadine.
Corticosteroids	Moderate to high dose of steroids are not recommended because they are not beneficial and potentially harmful.
Infection control	Standard as well as droplet precautions. In case of aerosol generating procedures, particular respirator (N95, FFP2 etc.), eye protection devices, gowns, gloves, and an air-borne precaution room should be recommended, which can be naturally or mechanically ventilated.
Nonsteroidal anti-inflammatory drugs or antipyretics	Paracetamol or acetaminophen should be given orally or by suppository. But administration of salicylates (as aspirin and aspirin-containing products) should be avoided in children and young adults (<18 years old) because of risk of Reye's syndrome.
Oxygen therapy	Monitor oxygen saturation and maintain SaO_2 over 90% (95% for pregnant women) with nasal cannulae or face mask.

SaO_2, oxygen saturation.

1. Pregnant women.
2. Patients with progressing lower respiratory disease or pneumonia.
3. Patients with underlying medical conditions.

The antiviral treatment should be started ideally as early as possible, but it may also be administered at any stage of the disease when ongoing viral replication is anticipated or documented. There are also the chances of viral replication for a prolonged period of time in some patients because of the lack of pre-existing protective immunity. Some important pharmacological differences should be considered when choosing NAIs for influenza treatment. Oseltamivir can be administered orally, which provides a higher systemic level, and thereby is recommended treatment for lower respiratory tract complications (Table 15.8). Zanamivir is delivered by oral inhalation with low systemic absorption. At the beginning of treatment for seasonal influenza with oseltamivir, some rare neuropsychiatric symptoms such as confusion or abnormal behavior have occurred, particularly in children and adolescents, but the contribution of oseltamivir to these events is unknown. Similarly inhaled zanamivir has been temporally found associated with bronchospasm and patients with pre-existing airway disease appear to be at increased risk for this severe adverse reaction.

Corticosteroids

Corticosteroids are not recommended for the routine treatment of patients with the new influenza viruses, although low doses of corticosteroids may be prescribed for patients in septic shock who require vasopressors and have suspected adrenal insufficiency. Prolonged use of or high dose corticosteroids can result in serious adverse events in influenza virus-infected patients, such as a secondary infection with opportunistic pathogens and possibly prolonged viral replication.

Table 15.8. Recommended antiviral treatment regimens by age and weight.

Drug	Recommended Dose
Oseltamivir	Oseltamivir is prescribed for treating patients 1 year of age and older. For adolescents (13 to 17 years of age) and adult dose: 75 mg orally twice a day for 5 days. For infants older than 1 year of age and for children 2 to 12 years of age recommended doses are depending on the body weight: 15 kg or less: 30 mg orally twice a day for 5 days 15–23 kg: 45 mg orally twice a day for 5 days 24–40 kg: 60 mg orally twice a day for 5 days >40 kg: 75 mg orally twice a day for 5 days
Zanamivir	Zanamivir is indicated for treatment of influenza in adults and children (>5 years). The recommended dose for treatment of adults and children from age of 5 years is two inhalations (5 mg) twice a day for up to 5 days.

Advance Respiratory Support

Treatment of acute respiratory distress syndrome (ARDS) associated with the new influenza virus infection should be based on published evidence-based guidelines for sepsis-associated ARDS (*see* Table 15.8). Mechanical ventilation strategies should be used to protect lungs.

Surveillance

High-quality influenza surveillance systems are needed that enable countries to better understand influenza epidemiology, including disease incidence and severity, and help health authorities in implementing appropriate prevention strategies.

Global Influenza Surveillance

The WHO Global Influenza Surveillance Network (GISN) has provided all the virologic information required in the biannual process of selecting strains for the formulation of Northern and Southern Hemisphere influenza vaccine. However, the provided epidemiologic data or an alert of an emerging pandemic is limited. GISN currently comprises 122 National Influenza Centers in 87 countries and four WHO Collaborating Centers for Reference and Research on Influenza.

Influenza in Developing Countries

The transmission of influenza virus or its clinical presentation may be altered depending on the differences in cultural practices, the environment, geography, human genetics, and social structures. Advanced influenza surveillance can permit assessment of a number of factors that may affect disease severity, population density, differences in prevalence and spectrum of chronic illness, proximity of the young and elderly affected, low proportion of elderly in the population, and in case of child, patients' low school attendance and school schedules that may not correspond with peak transmissibility season. The effectiveness of control measures adopted, such as social distancing and vaccination schedule, may differ between developed and developing structure due to all these factors.

According to the available epidemiologic evidence, influenza is common in tropical regions; some tropical countries report year-round human influenza activity (Nguyen et al., 2007), unlike temperate regions where transmission occurs with marked seasonality.

Objectives

The most efficient process for generating high-quality epidemiologic data for influenza-associated illness is hospital-based sentinel surveillance. It is the most efficient way to collect clinical data and laboratory specimens from patients with prevalent and severe infectious disease.

A sentinel surveillance system can be used to monitor a specific disease, can be sustainable, and can integrate with and build on existing systems. The system objectives include:

1. Describing the disease impact and epidemiology of severe, acute, and febrile respiratory illness and also defining the proportion that is associated with influenza.
2. Providing influenza virus isolates for monitoring changes in viral antigens and development of new vaccines accordingly.
3. Contributing data for local pandemic planning and making decisions regarding vaccination policy.
4. Providing infrastructure for an early warning system for the upcoming outbreaks of new subtypes of influenza A viruses and new strains of existing subtypes.
5. Serving as a monitoring tool for influenza pandemic.

Case Definitions

These surveillance guidelines use the existing WHO case definition and incorporate WHO guidance to define severe acute respiratory infection (SARI) in adults and children (Table 15.9).

Sentinel Site Selection

The choice of sentinel hospitals will entirely based on practical issues, including human resources, communication infrastructure, and availability of specimen transport, its testing, and diagnosis of the disease. There is no ideal number of surveillance sites; therefore, the number chosen by a particular country will totally depend in part on sustainability and resources available.

Data Collection

The collected data should be adequate for routine public health surveillance and to descript the key epidemiologic features of the disease. Data should include clinical signs and symptoms, potential exposures, laboratory data, and drug therapies. According to Ortiz et al. (2009), data should include:

1. General information regarding the patient, such as unique identification number, medical record number, name of patient and parent's name in case of minor, date of birth, sex, address, date of onset of symptoms, date of collection of epidemiological data, and suspected novel influenza case.
2. All the observed clinical signs and symptoms, such as fever, cough, sore throat, and shortness of breath or difficulty in breathing.

Table 15.9. Influenza sentinel surveillance case definitions.

Case	Definition Criteria
Influenza-like illness	1. Sudden onset of fever >100.4° F (38° C) 2. Cough or sore throat 3. Absence of other diagnoses
Severe acute respiratory infection in persons >5 years of age	4. Sudden onset of fever >100.4° F (38° C) 5. Cough or sore throat 6. Shortness of breath or difficulty in breathing 7. Requires hospitalization
Severe acute respiratory infection in persons <5 years of age	IMCI criteria for pneumonia Any child 2 months to 5 years of age with cough or difficult breathing and 1. breathing faster than 60 breaths/min (infants <2 months) 2. breathing faster than 50 breaths/min (2–12 months) 3. breathing faster than 40 breaths/min (1–5 years) OR IMCI criteria for severe pneumonia Any child 2 months to 5 years of age with cough or difficult breathing and any of the following general danger signs: 1. unable to drink or breastfeed 2. vomits everything 3. convulsions 4. lethargic or unconscious 5. chest indrawing or stridor in a calm child 6. Requires hospital admission

3. Type of specimen collected as throat swab, nasal swab, or other specimens along with their date of collection
4. Pre-existing medical condition of the patient as liver disease, kidney disease, AIDS, cancer or other immunocompromised state, neuromuscular dysfunction, diabetes, cardiac disease, or lung disease.
5. Other information, such as patient's occupation, part of an outbreak investigation, contact with sick or dead poultry or wild birds, contact with a person having an infection, or traveling in an infected area.
6. Previous history of therapy or immunization (drug treatment/vaccine).

Specimen Collection

Respiratory specimens should be collected as early as possible from all patients with SARI. Their number must be determined by the primary surveillance objective (e.g., understanding of seasonality, risk factor analysis, or determination of clinical outcomes) and must represent climatic and geographic regions.

Outpatient Surveillance

The highest priority of outpatient surveillance is to collect data on SARI cases because they are suffering from the most influenza-associated disability and premature death. Weekly cases

should be categorized by age group or categories (e.g., 6–23 months, 2–4 years, 5–17 years, 18–49 years, 50–64 years, and >65 years) (Fiore et al., 2008). Ideally, the total number of patients weekly seen by clinics would also be collected by age group.

Laboratory Testing

Clinical specimens are generally processed in sentinel site laboratories, but these further require analysis in additional specialized laboratories. Ideally, specimens should be tested for the presence of influenza viruses by RT-PCR reaction, although a subset of specimens should also undergo viral culture and antigenic characterization of infecting virion. All the data related with the surveillance should then be submitted to WHO FluNet, and, if possible, national laboratories should work with a WHO collaborating center laboratory to submit sample virus isolates for vaccine strain selection. The sensitivity and specificity of influenza testing will depend on the laboratory in which the test is performing, the quality of the clinical specimen, the manner in which the specimen is processed, and the type of specimen collected.

Timely Data Analysis and Reporting

Timely analysis and reporting of surveillance data is important to facilitate the treatment decisions by clinicians and control measures by public health officials. It will also encourage continuous reporting of cases by clinicians in the influenza surveillance system. Weekly reports of clinical and laboratory confirmed case counts should be disseminated throughout the surveillance system to encourage the participation of health-care providers and all stakeholders during influenza peak seasons.

Investigation of an Outbreak

Objectives for Investigations of Human Cases Influenza

The objectives for the investigation of human cases of influenza include:

1. Confirmation of the diagnosis of recent infection with influenza virus.
2. To reduce morbidity and mortality through rapid identification, isolation, treatment and clinical management of cases, and follow-up of contacts.
3. To reduce further spread of the virus through identification of potential human, animal, or environmental sources of exposure, risk factors, and implementation of appropriate prevention and control measures, including stamping out of vulnerable flocks, environmental decontamination, communication, and social mobilization activities.
4. To determine the risk for pandemic influenza as evidenced by increased efficiency of human-to-human transmission.
5. To determine the key epidemiological, clinical, and virological characteristics for cases including the mode(s) of transmission and disease diagnosis, manifestations, and responses to treatment.
6. Finally ensure timely exchange of information among clinicians, investigators of public and animal health, and government officials to facilitate critical and informed decision making at subnational, national, and international levels during the investigation.

Key Steps for Investigation of Human Cases of Influenza

A series of critical activities must be undertaken as a part of every influenza investigation. The order of these activities may vary depending on local circumstances and often require multiple activities in parallel.

Preparation for the Investigation

A multidisciplinary team should be constructed to investigate seasonal influenza cases. The team should consist of the members having experience in the field of epidemiology, clinical assessment, laboratory specimen collection, infection control, and social mobilization. Some additional team members include logisticians, public health veterinarians, and environmental health specialists. The size and structure of the initial investigation team may vary depending in part on the size and complexity of the anticipated investigation. Before deploying, the team has to gather preliminary background information (a description of the area of investigation, including a description of the health infrastructure and the agricultural sector), assemble the required materials and supplies (e.g., PPE, antiviral drugs, collection of specimens and transport materials), and finally inform relevant local public health and animal health authorities.

Confirming the Diagnosis, Interviewing the Case Patient, and Visiting the Patient's Home

The patient or its family members (in case the patient is too ill to be interviewed or has died), should be interviewed within the first 24 to 48 hours of the investigation to collect basic demographic, clinical, and epidemiological information. It starts with the date of the case patient's illness because the onset needs to be ascertained. Exposures to all the potential influenza sources in the 7 days before illness onset (i.e., 7 days is considered to be the usual upper limit of the incubation period for human cases of influenza) should be sought. If the diagnosis has not yet been confirmed, collection and testing of appropriate clinical samples from the case patients(s) is an immediate priority.

Define a Case and Actively Search for Cases and Their Contacts

Information obtained from the case patient's interview and home visit can be used to develop a working case definition. WHO has already developed surveillance case definitions (*see* Table 15.9) for the classification of human cases of influenza, which can be adapted for this purpose; however, it will be necessary further to develop a locally implemented case definition for influenza that incorporates time periods, localities, illness characteristics, exposure, and other information, which is specific to the investigation. Table 15.10 defines all cases of influenza virus in human beings.

Contact Tracing

Tracing efforts focus on close unprotected persons (i.e., were not wearing PPE) contact with the case patient in the 1 day before through 14 days after the case patient's illness onset. Contact tracing activities should be prioritized in case a large number of contacts are eligible for tracing or personnel resources are limited. Factors that can be used to prioritize among contacts should include:

Table 15.10. Cases of influenza virus in human beings.

Case Status	Definition
Confirmed	A person with acute respiratory illness and 1. an epidemiological link with the area as school (e.g., student, staff, close contact of ill student or staff) and 2. laboratory confirmed swine-origin influenza A (H1N1) confirmed by one or more of: (a) reverse transcriptase-polymerase chain reaction, with genotyping or sequence confirmation of H1N1 swine-origin influenza virus (b) viral culture with strain typing (c) serological testing, showing fourfold rise in antibody titer specific to swine-origin influenza A (H1N1) virus
Probable	A person with an acute respiratory illness and 1. an epidemiologic link with the area as school (e.g., student, staff, close contact of ill student or staff) and 2. laboratory confirmed influenza A, untypeable
Suspect	A person with an acute respiratory illness and an epidemiological link with the area as school (e.g., student, staff, close contact of ill student or staff).
Acute respiratory illness	Cough or fever and one or more of sore throat, headache, eye pain or myalgia.

1. Probability of influenza A (H5N1) infection in the case patient (e.g., contacts of confirmed or probable cases).
2. Duration, spatial proximity, and intensity of exposure to the case patient.
3. Likelihood that human-to-human transmission has resulted from contact with the influenza patient.

Symptomatic patients should be referred for collection and laboratory testing of specimens and advised appropriate medical care including antiviral therapy (WHO, 2006). Depending on the severity of illness, acceptability, and the availability of hospital beds, ill contacts should be isolated at a health-care facility or at home while awaiting test results. However, asymptomatic contacts require active monitoring for the development of fever or respiratory symptoms for up to 7 days after the last exposure to the case patient.

Collection of Specimens

Early and rapid collection and testing of appropriate specimens from case patients, as well as symptomatic contacts, are the highest priority. Under some specific circumstances and if resources permit, specimens can be collected from asymptomatic contacts or asymptomatic persons exposed to another source of influenza A (H5N1) for complimentary study purposes.

Visit Hospitals and Other Sites of Health Care

All locations where the patient is receiving or received traditional or nontraditional care should be visited, regardless whether the patient has died or been discharged, which is followed by the identification of any health-care workers, patients, or others who were close contacts, especially those require chemoprophylaxis.

Initiation of Enhanced Surveillance

During the investigation, in addition to active case searches and contact tracing, efforts should be undertaken to enhance routine surveillance systems in the area(s) where case patient(s) reside or where animal outbreaks are occurring. The targeted geographical area for enhanced surveillance will need to be assessed on a case-by-case basis. Enhanced surveillance should be based on the health-care–seeking behavior of the population and can include various active and passive approaches that are health-care and community-based. For example, surveillance can be further enhanced by:

1. Active surveillance in hospitals particularly targeting in-patient and emergency departments.
2. Inclusion of other sources, such as traditional healers, private practitioners, and private laboratories.
3. Active surveillance of groups that may be at higher occupational risk of exposure (e.g., health- care workers, persons exposed to live or dead birds or animals).

Analysis of Data

The data should be analyzed in terms of a person, place, and time. During analysis of multiple cases, graphical or tabular descriptions of cases by date of onset (an epidemic curve), geographical location (maps of the locale, case patients' homes), relationships (transmission or family trees), and demographic characteristics (distribution by age) should be checked and interpreted.

Conduct Complementary Studies

Based on the results of the field investigation, additional studies may be undertaken to advance the understanding of the epidemiological, virological, and clinical aspects of infection with the influenza virus. This includes:

1. Sero-incidence studies of close contacts of case patients to document new infection incidences using paired sera.
2. Seroprevalence surveys of persons living in the affected area with possible occupational risk (e.g., health-care workers, veterinarians, cullers, farmers, zoo personal, live poultry market workers), residents of an area experiencing bird or animal or human outbreaks, blood donors, or persons registered in ongoing sentinel surveillance for influenza or encephalitis. Such surveys include collection of appropriate epidemiological data to assess various risk factors for infection.
3. Case-control or cohort studies to evaluate risk factors for infection.
4. Systematic evaluation of the safety and efficacy of antiviral drugs or other treatment regimens.

Implementing Prevention and Control Measures

There are some standard prevention and control measures which reduce the opportunity for further transmission of influenza virus as:

1. Proper infection control, the use of PPE, and isolation of cases.
2. Use of antiviral drugs for the treatment of influenza cases and targeted prophylaxis of close contacts.
3. Initiation of active case-finding and notification and enhanced surveillance.

4. Active monitoring of contacts for the development of viral fever and respiratory symptoms.
5. In case of human-to-human transmission, voluntary home quarantine of asymptomatic case contacts.

Reporting and Notification

Detection of a possible human case(s) of influenza should immediately be made known to the local, subnational, and national public health and agricultural authorities so that they can make immediate decisions about the indication of an investigation, which in turn results in the notification of health-care providers (traditional as well as non-traditional), hospitals and outpatient facilities, community leaders in the area where the case patient resided or traveled as part of active case-finding efforts. Further the national health authority must notify WHO of any human cases of influenza, the new human influenza virus subtype, and should disseminate relevant information and biological materials in a timely and consistent manner to WHO collaborating center.

Prevention and Control

Vaccination

As with other infectious diseases, the most effective strategy for preventing influenza infection is immunoprophylaxis through annual vaccination. Routine vaccination of certain persons, such as children, contacts of persons at risk for influenza complications, and health-care personnel serving as a source of influenza virus transmission, might provide additional protection to persons at risk for influenza complications and help in reducing the overall influenza burden. The other strategy to prevent influenza illness involves chemoprophylaxis with antiviral drugs. Antiviral medications have been demonstrated to reduce the severity and duration of illness, particularly if administered within the first 48 hours after illness onset. However, antiviral medications are adjuncts to vaccine in the prevention and control of influenza, and primary prevention through annual vaccination is the most effective and efficient influenza prevention strategy.

Influenza Vaccine Efficacy, Effectiveness, and Safety

The efficacy (prevention of illness among vaccinated persons in controlled trials) and effectiveness (prevention of illness in vaccinated populations) of influenza vaccines depend on the age and immunocompetency of the vaccine recipient, the degree of similarity between the viruses present in the vaccine preparation and those in circulation, and the outcome being measured. Influenza vaccine efficacy and effectiveness studies have used multiple possible outcome measures, including the prevention of medically attended acute respiratory illness (MAARI), laboratory-confirmed influenza virus illness, influenza or pneumonia-associated hospitalizations or deaths, or seroconversion illnesses.

Influenza Vaccine Composition

Two vaccine preparations have been recommended for preventing influenza infection: the live attenuated vaccine (LAIV) and the inactivated influenza vaccine (TIV). Both LAIV and TIV consists of strains of influenza viruses that are antigenically equivalent to the annually recommended strains: one influenza A virus of H3N2 type, one influenza A virus

of H1N1 type, and one influenza B virus. Based on the global surveillance for influenza viruses and the emergence and spread of new strains, each year one or more virus strains in the vaccine preparation might be changed. For example, the 2010–2011 trivalent vaccines contained A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like, and B/Brisbane/60/2008-like antigens, whereas the A/California/7/2009 (H1N1)-like antigen are derived from a pandemic 2009 influenza A (H1N1) virus. The A/Perth/16/2009 (H3N2)-like antigen is different from the H3N2-like antigen recommended for the 2009–2010 northern hemisphere seasonal influenza vaccine. The influenza B vaccine strain will remain as B/Brisbane/16/2008 and did not change compared with the 2009–2010 northern hemisphere seasonal influenza vaccine (Food and Drug Administration, 2010). Viruses for currently licensed TIV and LAIV preparations are prepared by growing virus in chicken eggs.

Both of these vaccines are effective and do not contain any adjuvant, but Table 15.11 shows the difference in several respects. TIV contains inactivated viruses and thus cannot cause influenza disease. LAIV contains live influenza viruses (although attenuated) and therefore have the potential to cause mild signs or symptoms related to vaccine virus infection (e.g., rhinorrhea, nasal congestion, fever, or sore throat). LAIV is administered intranasally by sprayer, whereas TIV is administered intramuscularly by an injection.

Some adverse effects have been observed in children after TIV vaccination, such as fever, malaise, myalgia and in adults, the most frequent side effect is soreness at the vaccination site that lasted <2 days (Govaert et al., 1993); however, no adverse fetal effect have been observed in pregnant women (Munoz et al., 2005). LAIV also contains the same three vaccine antigens used in TIV; however, the antigens are constituted as live, attenuated, cold-adapted and temperature-sensitive vaccine viruses. LAIV does not typically cause more prominent systemic symptoms of influenza, such as high fever, myalgia, and severe fatigue, and generates prolonged immunity against influenza. A universal influenza vaccine has researched which produces antibodies against viral coat proteins that mutate less rapidly, and thus a single shot could potentially provide long-lasting protection (Ekiert et al., 2011). Figure15.13 shows the number of seasonal influenza vaccine doses was recommended for children in 2010–2011.

Influenza vaccination is particularly important for persons who are at increased risk of developing severe complications from influenza or at higher risk for influenza-related outpatient or hospital visits. If vaccine supply is limited, vaccination efforts should focus on administering vaccination to the following persons:

1. All children 6 months to 4 years of age (59 months).
2. All persons aged ≥50 years.
3. Adults and children suffering from chronic pulmonary (including asthma) or cardiovascular (except isolated hypertension), renal, hepatic, neurological, hematologic, or metabolic disorders (including diabetes mellitus).
4. For patients who are immunosuppressed (including immunosuppression caused by medications or by HIV).
5. Pregnant women.
6. Children and adolescents (aged 6 months–18 years) those receiving long-term aspirin therapy and might be at risk for experiencing Reye's syndrome after acquiring influenza virus infection.
7. Residents of nursing homes and other long-term care facilities.
8. Persons who are morbidly obese (BMI ≥40).
9. Household contacts and caregivers of children <5 years of age and adults ≥50 years of age, with particular emphasis on vaccinating contacts of children <6 months of age.
10. Household contacts and caregivers of persons with medical conditions that put them at higher risk of developing severe complications from influenza.

Table 15.11. Comparison of live, attenuated influenza vaccine with inactivated influenza vaccine for seasonal influenza*.

Factor	LAIV	TIV
Route of administration	Intranasal spray	Intramuscular injection
Type of vaccine	Live virus	Killed virus
Number of included virus strains	Three (two influenza A, one influenza B)	Three (two influenza A, one influenza B)
Vaccine virus strains updated	Annually	Annually
Frequency of administration	Annually	Annually
Approved age	Persons aged 2 to 49 years	Persons aged ≥6 months (varies by formulation)
Interval between two doses recommended for children aged ≥6 months to 8 years who are receiving influenza vaccine for the first time	≥4 weeks	≥4 weeks
Can be given to persons with medical risk factors (as asthma, diabetes, hepatic or neurological complications, pregnancy) for influenza-related complications	No	Yes
Can be given to children with asthma or children aged 2 to 4 years with wheezing in the past year	No	Yes
Can be administered to family members or close contacts of persons who are immunosuppressed not requiring a protected environment	Yes	Yes
Can be administered to family members or close contacts of persons who are immunosuppressed requiring a protected environment (e.g., hematopoietic stem cell transplant recipient)	No	Yes
Can be administered to family members or close contacts of persons at higher risk including pregnant women, but not severely immunosuppressed	Yes	Yes
Can be administered simultaneously with other vaccines	Yes (in children aged 12 to 15 months who received measles, mumps, and rubella vaccine)	Yes (coadministration only in adults who received pneumococcal polysaccharide or zoster vaccine
If not administered simultaneously, can be administered within 4 weeks of another live vaccine	Prudent to space ≥4 weeks apart	Yes
If not administered simultaneously, can be administered within 4 weeks of an inactivated vaccine	Yes	Yes

LAIV, live, attenuated influenza vaccine; TIV, inactivated influenza vaccine.
*US formulations.

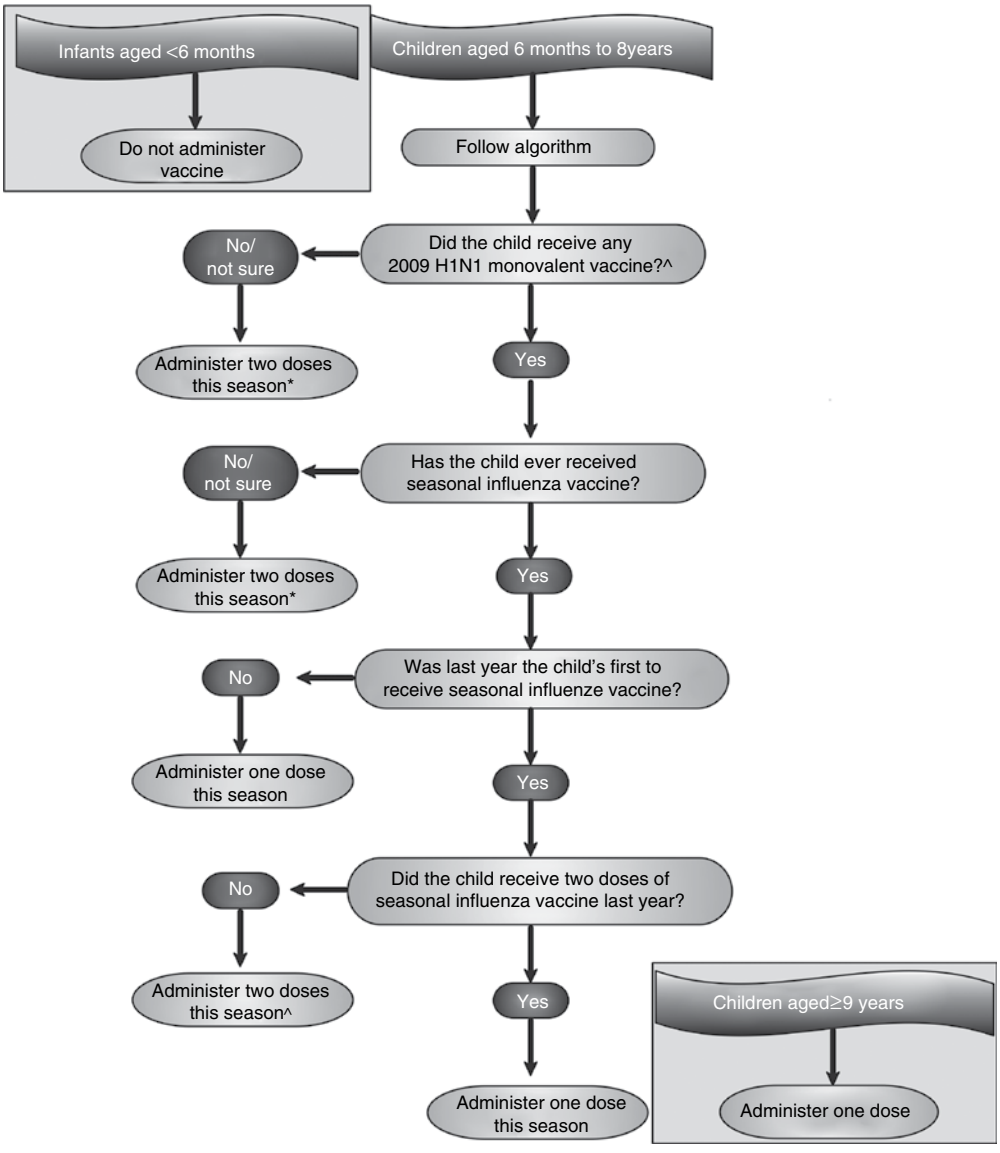

Figure 15.13. Number of 2010–2011 seasonal influenza vaccine doses recommended for children as developed by the Centers for Disease Control and Prevention with the American Academy of Pediatrics, Committee on Infectious Diseases. *Children who had a laboratory-confirmed 2009 pandemic H1N1 virus infection (e.g., reverse transcription-polymerase chain reaction or virus culture specific for 2009 pandemic influenza A[H1N1] virus) appear to be immune to this virus. ^Interval between two doses is ≥4 weeks.

Control

Good personal health and hygiene habits are effective in reducing the transmission and spread of influenza such as not touching the eyes, nose, or mouth, frequent hand washing with soap and water, or with alcohol-based hand rubs (Grayson et al., 2009; Jefferson et al., 2011),

covering coughs and sneezes; avoiding close contact with sick people and staying home if sick. Face masks also proved to be helpful in preventing transmission while caring for the sick (MacIntyre et al., 2009). Because influenza spreads through both aerosols and contact with contaminated surfaces, surface sanitizing with appropriate sanitizer may help prevent some infections (Hota, 2004). Alcohol is considered an effective sanitizer against influenza viruses, and quaternary ammonium compounds can also be used with alcohol to prolong the sanitizing effect (McDonnell and Russell, 1999). In hospitals, quaternary ammonium compounds and bleach are used to sanitize rooms or equipment that was occupied by patients with influenza symptoms. At home, this can effectively be done by using diluted chlorine bleach.

During past pandemics, closing of schools, churches, and theaters slowed the spread of the virus, but did not have a large effect on the overall death rate (Hatchett et al., 2007). It is uncertain if reducing public gatherings, by, for example, closing schools and work places, will reduce transmission because people with influenza may just be moved from one area to another; such measures would also be difficult to enforce and might be unpopular (Aledort et al., 2007). In cases of small numbers of people infected, isolating the sick might reduce the risk of transmission.

Avian Influenza

Avian influenza, commonly called as bird flu ,refers to an infectious disease of birds, ranging from mild to severe form of illness caused by viruses. All known viruses that cause influenza in birds belong to the species influenza A virus and all subtypes (but not all strains) of influenza A virus are adapted to birds. Less pathogenic viruses can, after circulation for some time in a poultry population, mutate into highly pathogenic viruses. To date, all outbreaks of the highly pathogenic form have been caused by influenza A/H5N1 virus, the only subtype causing outbreaks of severe disease in humans. The ability of this avian strain H5N1 to evade the body's defense mechanism by evading cytokines (the first line of defense against 'flu') may be responsible for the high pathogenicity of this particular strain. (Whitfield, 2003). H5N1 is an emerging avian influenza virus having global concern as a potential pandemic threat. It has killed millions of poultry population in a growing number of countries throughout Asia, Europe, and Africa. Health experts are concerned that the coexistence of human influenza viruses and avian influenza viruses (especially H5N1) will provide an opportunity for genetic material to be exchanged between species-specific viruses, possibly creating a new virulent, influenza strain, which is easily transmissible and proves lethal to humans.

Avian influenza was first transmitted from birds to humans in 1997 and caused an outbreak in Hong Kong. Hong Kong conducted a mass slaughter of chickens following the death of people by H5N1 avian influenza. To protect its poultry, Chinese poultry producers use an inactivated H5N1 virus for vaccinating birds. However, the vaccination does not confer complete immunity, which is evident from the fact that the vaccinated birds may develop the disease possibly due to infection by new strains resulting out of genetic reassortments, as is the case with H5N1 strain sweeping Asia. The avian strains differ from human strains in that they have all the 16 subtypes of HA in contrast to only the 3 present in case of humans. The virulent avian influenza H5N1 strains differ from other avirulent avian strains and there lies a link between HA cleavage and degree of virulence. In virulent strains, the HA spikes contain multiple basic amino acids/amino acids chain at the cleavage site, which are cleaved intracellularly by endogenous proteases. In contrast, in cases of

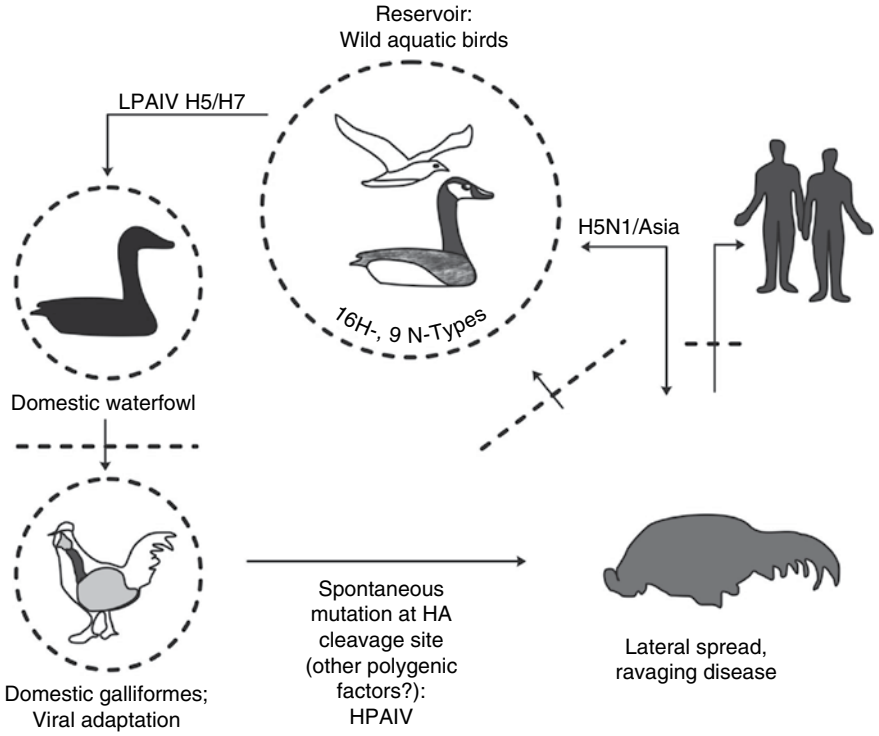

Figure 15.14. Scheme of avian influenza pathogenesis and epidemiology. HA, hemagglutinin protein; HPAIV, highly pathogenic avian influenza virus; LPAIV, low pathogenic avian influenza virus; dotted lines with arrows represent species barriers.

avirulent avian strains and nonavian influenza A viruses, the HAs lack these basic amino acid residues and thereby are not subjected to cleavage by endogenous proteases. Moreover, all types of influenza A viruses are antigenically labile, well adapted to evade various host defenses, and lack proofreading mechanisms. The disease is transmitted to humans by direct or indirect contact with infected wild ducks and chickens through infected aerosols, discharges, and surfaces because large amounts of the virus are excreted in bird droppings, which can survive for some time in the environment, but there is no evidence of human-to-human transmission of avian influenza to this date.

The highly pathogenic form of avian influenza disease has been caused by influenza A viruses of the H5 and H7 subtypes. However, only a few H5 and H7 subtypes display a highly pathogenic biotype (Swayne and Suarez, 2000). Usually, H5 and H7 viruses are stably maintained in their natural hosts in a low pathogenic form and from this reservoir, the viruses can easily be introduced by various pathways (Figure 15.14) into poultry flocks. Following a variable and indecisive period of circulation (and presumably because of adaptation) in susceptible poultry populations, these viruses can saltatorily mutate into the highly virulent pathogenic form, HPAIV (Rohm et al., 1995), capable of infecting mammals, especially humans, and has been observed for the Asian lineage H5N1(WHO, 2006). Host-dependent pathogenicity of HPAIV H5N1 for mammals has also been studied in several animal model species, such as in

mice (Li, Chen, et al., 2005), ferrets (Govorkova et al., 2005), cynomolgus monkeys (Rimmelzwaan et al., 2001), and pigs (Choi et al., 2005).

The infection outcome was entirely dependent on the viral strain and species of host. Ferrets appeared to mirror pathogenicity in humans better than mice (Maines et al., 2005).

The classical clinical symptoms of avian influenza are quite similar to that of the disease caused by other influenza viruses. Fever, malaise, myalgia, sore throat, and cough are found in most of the patients, and conjunctivitis is seen rarely in some of the patients. Persistent high fever is a useful sign and various life-threatening complications such as viral pneumonia, respiratory distress syndrome, and multiorgan failure may result in the death of the patient. A patient is suspected to be suffering from avian influenza if he or she has any respiratory illness and has had recent direct or indirect contact by handling, or by having taken care or by exposure to sick chickens or other birds. Besides the classical clinical presentations chest X-rays proved to be useful in detecting early viral pneumonia. Specimens such as nasopharyngeal aspirate, endotracheal aspirate, sputum, and serum from clinically suspected cases should be subjected for laboratory investigations for further confirmation. If avian influenza is suspected in a person, treatment should immediately be started without waiting for laboratory confirmation, which is essentially similar to that employed for infections resulting from the other influenza viruses. Unfortunately, the current strain of H5N1 has already shown resistance to amantadine and rimantadine, two of the antiviral drugs commonly used for influenza; however, other antivirals including oseltamivir and zanamivir are still effective against this strain of H5N1 (CDC, 2004).

Swine Influenza

Swine influenza is also related to human influenza and was first proposed during the 1918 influenza pandemic, when pigs became sick at the same time as humans (Knobler et al., 2005). However, the influenza virus was first identified as a causative agent of disease in pigs 12years later in 1930 (Olsen, 2002). Further, the H1N1 was the strain frequently found to be associated with the infections in pigs in the next 60 years. Then in 1997 and 1998, H3N2 strains emerged, including genes derived by reassortment from human, swine, and avian viruses and have become a major cause of swine influenza in North America. Reassortment between H1N1 and H3N2 produced H1N2. Between 1990 and 1993, a triple re-assortment occurred in a pig host involving North American H1N1 swine virus, the human H3N2 virus, and avian H1N1. This generated the swine H1N2 strain. Finally, in 2009, H1N2 and the euro-asiatic H1N1 swine strain coinfected a human host, leading to the emergence of a new human H1N1 strain, which caused the 2009 pandemic. Recently three people are believed to have developed a strain of influenza virus known as variant H1N2 (H1N2v) after exhibiting pigs or spending time in the swine barn at the Minnesota State Fair (Steller, 2012).

Swine influenza is transmitted through direct contact between infected and uninfected animals (Kothalawala et al., 2006), particularly during the transportation of these animals. The transmission may also occur during intensive farming as the pigs are raised in proximity to each other (Gilchrist et al., 2007), and the virus is directly transferred either by pigs touching noses or through their dried mucus. Another important mode of transmission is air-borne transmission through the aerosols produced by pigs during coughing or sneezing (Kothalawala et al., 2006), resulting in quick spread of a virion through a herd, infecting all

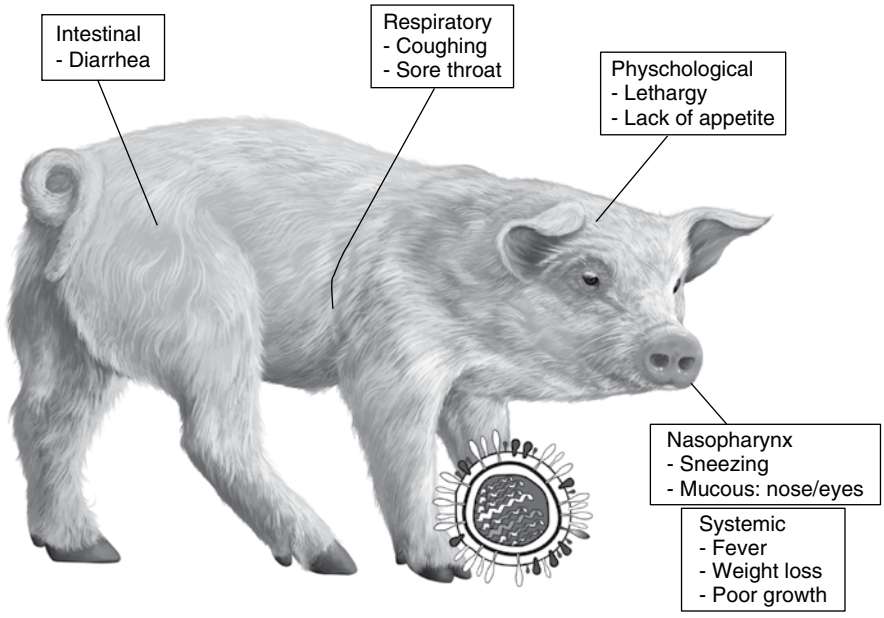

Figure 15.15. Main symptoms of swine influenza in swine.

the pigs within just a few days. People who work with poultry and swine and those with intense exposures are at increased risk of this type of zoonotic infection with influenza virus endemic in these animals and constitute a population of human hosts in which zoonosis and reassortment can co-occur (Gray and Kayali, 2009).

In pigs, the illness is characterized by fever, lethargy, sneezing, coughing, difficulty breathing, and decreased appetite (Kothalawala et al., 2006) (Figure 15.15). In some cases, the infection can also cause abortion, although with low mortality (1–4 percent). The virus may result in weight loss and poor growth because infected pigs can lose up to 12 pounds of body weight over a 3- to 4-week period of time, causing economic loss to farmers (Kothalawala et al., 2006).

In cases of human, swine influenza produces severe influenza-like illness (CDC, 2009). Symptoms include fever, cough, sore throat, body aches, headache, chills, and fatigue. The 2009 outbreak of swine influenza had an increased percentage of patients reporting diarrhea and vomiting (Figure 15.16). The 2009 H1N1 virus was principally transmitted from person to the person not from pigs to humans.

Because the swine influenza can be transmitted from pigs to humans, as well as from humans to humans, its proper control and management requires three components: prevention of transmission in swine, prevention of transmission to humans, and prevention of its spread among humans. Among swine, spread can be prevented by facility management, herd management, and proper vaccination. The transmission from swine to human is mainly occurring in swine farms, where farmers are in close contact with live pigs. Although strains of swine influenza are usually not potent enough to infect humans, this may occasionally happen; hence, farmers and veterinarians are encouraged to use a face mask while dealing with infected animals. The use of vaccines on swine to prevent their infection is a major method of limiting swine-to-human transmission. Risk factors

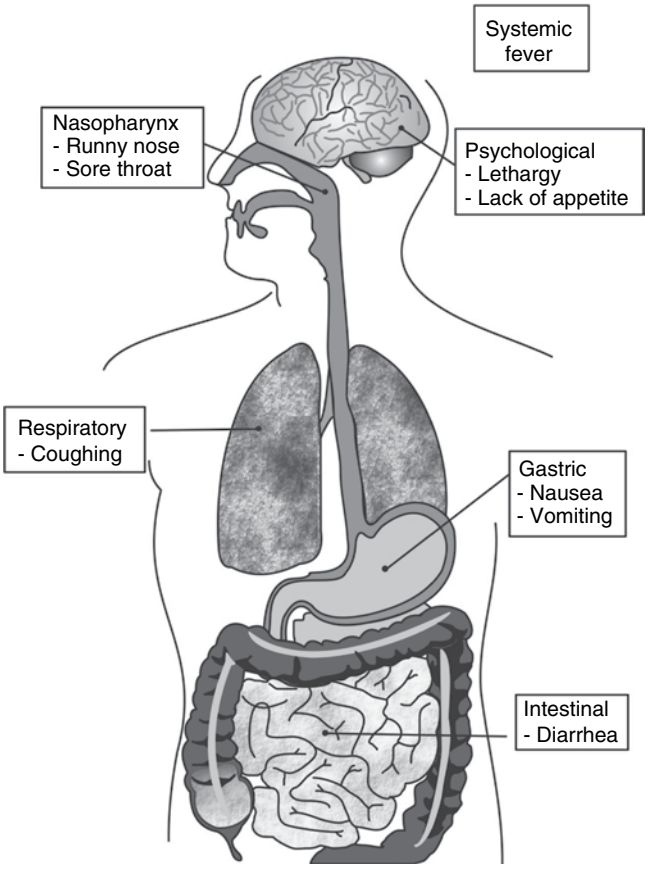

Figure 15.16. Main symptoms of swine influenza in humans.
Source: Centers for Disease Control and Prevention (CDC). 2009. Key facts about human infections with variant viruses (Swine origin influenza viruses in humans). Accessed February 19, 2013, at http://www.cdc.gov/flu/swineflu/keyfacts-variant.htm.

that may contribute to swine-to-human transmission include smoking and, especially, not wearing gloves when working with sick animals, and thereby increasing the risk of subsequent hand-to-eye, hand-to-nose, or hand-to-mouth transmission (Ramirez et al., 2006). When these infectious persons cough or sneeze and other noninfectious persons breathe or touch something with the virus on it and then touch their own face, the influenza spreads. It cannot be spread by pork products because the virus is not transmitted through food. It is the most contagious in humans during the first 5 days of the illness; although some people, most commonly children, can remain contagious for up to 10 days. Viral spread among humans can be prevented using standard infection control against influenza, which includes frequent hand washing with soap and water or with alcohol-based hand sanitizers, especially after being out in public (CDC, 2009). Transmission can also be reduced by disinfecting household surfaces, using a diluted chlorine bleach solution. Maintaining social distancing is another means of preventing

influenza, which involves staying away from other people who might be infected and can include avoiding large gatherings, spreading out a little at work, or perhaps staying home and lying low when an infection is spreading in a community.

References

Aledort JE, Lurie N, Wasserman J, Bozzette SA. 2007. Non-pharmaceutical public health interventions for pandemic influenza: an evaluation of the evidence base. *BMC Public Health* 7:208.

Allwinn R, Preiser W, Rabenau H, Buxbaum S, Sturmer M, et al. 2002. Laboratory diagnosis of influenza—virology or serology? *Med Microbiol Immunol* (Berl) 191:157–160.

Aufderheide AC, Rodriguez-Martin C. 1998. *The Cambridge Encyclopedia of Human Paleopathology*, pp. 210–212. New York: Cambridge University Press.

Boni MF, Gog JR, Andreasen V, Feldman MW. 2006. Epidemic dynamics and antigenic evolution in a single season of influenza A. *Proc R Soc* 273:1307–1316.

Bouvier NM, Palese P. 2008. The biology of influenza viruses. *Vaccine* 26:49–53.

Call S, Vollenweider M, Hornung C, Simel D, McKinney W. 2005. Does this patient have influenza? *JAMA* 293:987–997.

Cannell JJ, Zasloff M, Garland CF, Scragg R, Giovannucci E, et al. 2008. On the epidemiology of influenza. *Virol J* 5, 29.

Centers for Disease Control and Prevention (CDC). 2004. Past avian influenza outbreaks. Accessed February 19, 2013, at http://www.cdc.gov/flu/avian/outbreaks/past.htm.

Centers for Disease Control and Prevention (CDC). 2009. Key facts about human infections with variant viruses (Swine origin influenza viruses in humans). Accessed February 19, 2013, at http://www.cdc.gov/flu/swineflu/keyfacts-variant.htm.

Choi YK, Nguyen TD, Ozaki H, Webby RJ, Puthavathana P, et al. 2005. Studies of H5N1 influenza virus infection of pigs by using viruses isolated in Viet Nam and Thailand in 2004. *J Virol* 79:10821–10825.

Christie AB. 1974. Influenza. In: *Infectious Diseases Epidemiology and Clinical Practice*, pp. 222–222. New York: Churchill Livingston.

Cox NJ, Subbarao K. 1999. Influenza. *Lancet* 354:1277–1282.

Cox NJ, Subbarao K. 2000. Global epidemiology of influenza: Past and present. *Annu Rev Med* 51:407–421.

Cros J, Palese P. 2003. Trafficking of viral genomic RNA into and out of the nucleus: Influenza, Thogoto and Borna disease viruses. *Virus Res* 95:3–12.

Crosby AW. 1993. Influenza. In: Kiple KF, ed., *The Cambridge World History of Human Disease*, pp. 807–811. New York: Cambridge University Press.

Debre R, Couvreur J. 1970. Influenza: Clinical features. In: Debre R, Celers J, eds., *Clinical Virology: The Evaluation and Management of Human Viral Infections*, pp. 507–515. Philadelphia: WB Saunders.

Donaldson LJ, Rutter PD, Ellis BM, Greaves FEC, Mytton OT, et al. 2009. Mortality from pandemic A/H1N1 2009 influenza in England: Public health surveillance study. *BMJ* 339:b5213.

Drake J. 1993. Rates of spontaneous mutation among RNA viruses. *Proc Natl Acad Sci USA* 90:4171–4175.

Eccles R. 2005. Understanding the symptoms of the common cold and influenza. *Lancet Infect Dis* 5:718–725.

Ekiert DC, Friesen RHE, Bhabha G, Kwaks T, Jongeneelen M, et al. 2011. A highly conserved neutralizing epitope on Group 2 influenza a viruses. *Science* 333:843–850.

Ferguson NM, Cummings DAT, Cauchemez S, Fraser C, Riley S, et al. 2005. Strategies for containing an emerging influenza pandemic in Southeast Asia. *Nature* 437:209–214.

Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, et al. 2008. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. *MMWR Recomm Rep* 57:1–60.

Food and Drug Administration. 2010. Influenza virus vaccine for the 2010–2011 season. Accessed February 19, 2013, athttp://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Post-MarketActivities/LotReleases/ucm202750.htm.

Fouchier R, Schneeberger P, Rozendaal F, Broekman J, Kemink S, et al. 2004. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome". *Proc Natl Acad Sci USA* 101:1356–1361.

Frisbie B, Tang YW, Griffin M, Poehling K, Wright PF, et al. 2004. Surveillance of childhood influenza virus infection: What is the best diagnostic method to use for archival samples? *J Clin Microbiol* 42:1181–1184.

Frost WH. 2006. The epidemiology of influenza. 1919. *Public Health Rep* 121:149–159.

Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, et al. 2009. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* 325:197–201.

Ghedin E, Sengamalay NA, Shumway M, Zaborsky J, Feldblyum T, et al. 2005. Large-scale sequencing of human influenza reveals the dynamic nature of viral genome evolution. *Nature* 437:1162–1166.

Gilchrist MJ, Greko C, Wallinga DB, Beran GW, Riley DG, et al. 2007. The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environ Health Perspect* 115:313–316.

Govaert TM, Dinant GJ, Aretz K, Masurel N, Sprenger MJ, et al. 1993. Adverse reactions to influenza vaccine in elderly people: Randomised double blind placebo controlled trial. *BMJ* 307:988–990.

Govorkova EA, Rehg JE, Krauss S, Yen HL, Guan Y, et al. 2005. Lethality to ferrets of H5N1 influenza viruses isolated from humans and poultry in 2004. *J Virol* 79:2191–2198.

Grais RF, Ellis JH, Glass GE. 2003. Assessing the impact of airline travel on the geographic spread of pandemic influenza. *Eur J Epidemiol* 18:1065–1072.

Gray GC, Kayali G. 2009. Facing pandemic influenza threats: The importance of including poultry and swine workers in preparedness plans. *Poult Sci* 88:880–884.

Grayson ML, Melvani S, Druce J, Barr IG, Ballard SA, et al. 2009. Efficacy of soap and water and alcohol-based hand-rub preparations against live H1N1 influenza virus on the hands of human volunteers. *Clin Infect Dis* 48:285–291.

Hall CB. 2001. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 344:1917–1928.

Hatchett RJ, Mecher CE, Lipsitch M. 2007. Public health interventions and epidemic intensity during the 1918 influenza pandemic. *Proc Natl Acad Sci USA* 104:7582–7587.

Hay A, Gregory V, Douglas A, Lin Y. 2001. The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci* =356:1861–1870.

Heath WR, Belz GT, Behrens GM, Smith CM, Forehan SP, et al. 2004. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol Rev* 199:9–26.

Heikkinen T. 2006. Influenza in children. *Acta Paediatr* 95:778–784.

Hilleman M. 2002. Realities and enigmas of human viral influenza: Pathogenesis, epidemiology and control. *Vaccine* 20:3068–3087.

Hota B. 2004. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? *Clin Infect Dis* 39:1182–1189.

Hui DS. 2008. Review of clinical symptoms and spectrum in humans with influenza A/H5N1 infection. *Respirology* 13 Suppl 1:S10–S13.

Jefferson T, Del Mar CB, Dooley L, Ferroni E, Al-Ansary LA, et al. 2011. Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane Database Syst Rev* 6 July 7: CD006207.

Johansson BE, Bucher DJ, Kilbourne ED.1989. Purified influenza virus hemagglutinin and neuraminidase are equivalent in stimulation of antibody response but induce contrasting types of immunity to infection. *J Virol* 63:1239–1246.

Julkunen I, Pyhala R, Hovi T. 1985. Enzyme immunoassay, complement fixation and hemagglutination inhibition tests in the diagnosis of influenza A and B virus infections. Purified hemagglutinin in subtype-specific diagnosis. *J Virol Methods* 10:75–84.

Kawaoka Y, ed. 2006. *Influenza Virology. Current Topics*. Norfolk, England: Caister Academic Press.

Kerr AA, McQuillin J, Downham MA, Gardner PS. 1975. Gastric flu influenza B causing abdominal symptoms in children. *Lancet* 1:291–295.

Klenk H, Matrosovich M, Stech J. 2008. Avian influenza: Molecular mechanisms of pathogenesis and host range. In: Mettenleiter TC, ed., *Animal Viruses Molecular Biology*, pp. 253–303. Norfolk, England: Caister Academic Press.

Knipe DM, Howley PM. 2001. *Influenza: Fields Virology*, 4th ed., pp. 1533–1579. Philadelphia: Lippincott Williams & Wilkins.

Knobler S, Mack A, Mahmoud A, Lemon S. 2005. *The story of Influenza. The Threat of Pandemic Influenza: Are We Ready? Workshop Summary*. Washington, DC: The National Academies Press.

Kothalawala H, Toussaint MJ, Gruys E. 2006. An overview of swine influenza. *Vet Q* 28,46–53.

LaForce FM, Nichol KL, Cox NJ. 1994. Influenza: Virology, epidemiology, disease, and prevention. *Am J Prev Med* 10:31–44.

Lamb RA, Choppin PW. 1983. The gene structure and replication of influenza virus. *Annu Rev Biochem* 52:467–506.

Lamb RA, Krug RM. 2011. Orthomyxoviridae: The viruses and their replication. In: DM, Howley PM, eds. *Fields Virology*, 4th ed., pp. 1487–1532. Philadelphia: Lippincott Williams & Wilkins.

Legge KL, Braciale TJ. 2003. Accelerated migration of respiratory dendritic cells to the regional lymph nodes is limited to the early phase of pulmonary infection. *Immunity* 18:265–277.

Li Y, Duan S, Yu IT, Wong TW. 2005. Multi-zone modeling of probable SARS virus transmission by airflow between flats in Block E, Amoy Gardens. *Indoor Air* 15:96–111.

Li Z, Chen H, Jiao P, Deng G, Tian G, et al. 2005. Molecular basis of replication of duck H5N1 influenza viruses in a mammalian mouse model. *J Virol* 79:12058–12064.

MacIntyre CR, Cauchemez S, Dwyer DE, Seale H, Cheung P, et al.2009. Face mask use and control of respiratory virus transmission in households. *Emerging Infect Dis* 15:233–241.

Maines TR, Lu XH, Erb SM, Edwards L, Guarner J, et al. 2005. Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. *J Virol* 79:11788–11800.

Matrosovich M, Tuzikov A, Bovin N, Gambaryan A, Klimov A, et al. 2000. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J Virol* 74:8502–8512.

McDonnell G, Russell A. 1999. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 12:147–179.

Mills CE, Robins JM, Lipsitch M. 2004. Transmissibility of 1918 pandemic influenza. *Nature* 432(7019):904–906.

Monto AS, Comanor L, Shay DK, Thompson WW. 2006. Epidemiology of pandemic influenza: Use of surveillance and modeling for pandemic preparedness. *J Infect Dis* 194:92–97.

Monto AS. Epidemiology of influenza. *Vaccine* 2008;26:45–48.

Munoz FM, Greisinger AJ, Wehmanen OA, Mouzoon ME, Hoyle JC, et al. 2005. Safety of influenza vaccination during pregnancy. *Am J Obstet Gynecol* 192:1098–1106.

Nguyen HL, Saito R, Ngiem HK, Nishikawa M, Shobugawa Y, et al. 2007. Epidemiology of influenza in Hanoi, Vietnam, from 2001 to 2003. *J Infect* 55:58–63.

Nicholson KG, Webster RG, Hay AJ. 1998. *Textbook of Influenza*. Oxford: Blackwell Science.

Nobusawa E, Sato K. 2006. Comparison of the mutation rates of human influenza A and B viruses. *J Virol* 80:3675–3678.

Olsen CW. 2002. The emergence of novel swine influenza viruses in North America. *Virus Research* 85:199–210.

Olson DR, Simonsen L, Edelson PJ, Morse SS. 2005. Epidemiological evidence of an early wave of the 1918 influenza pandemic in New York City. *Proc Natl Acad Sci USA* 102:11059–11063.

Ortiz JR, Sotomayor V, Uez OC, Oliva O, Bettels D, et al. 2009. Strategy to enhance influenza surveillance worldwide *Emerging Infect Dis* 15:1271.
Palladino G, Mozdzanowska K, Washko G, Gerhard W. 1995. Virus-neutralizing antibodies of immunoglobulin G (IgG) but not of IgM or IgA isotypes can cure influenza virus pneumonia in SCID mice. *J Virol* 69:2075–2081.
Parrish C, Kawaoka Y. 2005. The origins of new pandemic viruses: the acquisition of new host ranges by canine parvovirus and influenza A viruses. *Annual Rev Microbiol* 59:553–586.
Prince HE, Leber AL. 2003. Comparison of complement fixation and hemagglutination inhibition assays for detecting antibody responses following influenza virus vaccination. *Clin Diagn Lab Immunol* 10:481–482.
Ramirez A, Capuano AW, Wellman DA, Lesher KA, Setterquist SF, et al. 2006. Preventing zoonotic influenza virus infection. *Emerging Infect Dis* 12:996–1000.
Reid AH, Taubenberger JK. 2003. The origin of the 1918 pandemic influenza virus: A continuing enigma. *J Gen Virol* 84:2285–2292.
Renegar KB, Small PA Jr, Boykins LG, Wright PF. 2004. Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. *J Immunol* 173:1978–1986.
Rimmelzwaan GF, Kuiken T, Van Amerongen G, Bestebroer TM, Fouchier RA, et al. 2001. Pathogenesis of influenza A (H5N1) virus infection in a primate model. *J Virol* 77:3148–3156.
Rohm C, Horimoto T, Kawaoka Y, Suss J, Webster RG. 1995. Do hemagglutinin genes of highly pathogenic avian influenza viruses constitute unique phylogenetic lineages? *Virology* 209:664–670.
Silva ME, Cherry J D, Wilton RJ, Ghafouri NM, Bruckner DA, et al. 1999. Acute fever and petechial rash associated with influenza A virus infection. *Clin Infect Dis* 29:453–454.
Souza LS, Ramos EA, Carvalho FM, Guedes VM, Souza LS, et al. 2003. Viral respiratory infections in young children attending day care in urban Northeast Brazil. *Pediatr Pulmonol* 35:184–191.
Steller C. 2012. Minnesota State Fair on Saturday, Sept. 1, 2012. Accessed March 25, 2013, at http://fridley.patch.com/articles/minnesota-state-fair-on-saturday-sept-1-2012.
Steininger C, Kundi M, Aberle SW, Aberle JH, Popow-Kraupp T. 2002. Effectiveness of reverse transcription-PCR, virus isolation, and enzyme-linked immunosorbent assay for diagnosis of influenza A virus infection in different age groups. *J Clin Microbiol* 40:2051–2056.
Suzuki Y. 2005. Sialobiology of influenza: molecular mechanism of host range variation of influenza viruses. *Biol Pharm Bull* 28:399–408.
Swayne DE, Suarez DL. 2000. Highly pathogenic avian influenza. *Rev Sci Tech* 19:463–468.
Sydenham T. 1978. Influenza. In: Major RH, eds., *Classic Descriptions of Disease*, pp. 201–202. Springfield, IL: Charles C. Thomas.
Taubenberger JK, Morens DM. 2008. The pathology of influenza virus infections. *Annu Rev Pathol* 3:499–522.
Treanor JJ. 2004. Influenza virus. In: Mandell GL, Bennett JE, Dolin R, eds., *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 6th ed., pp. 2060–2085. Philadelphia: Elsevier Churchill Livingstone.
Tyler K, Nathanson N. 2001. Pathogenesis of viral infections. In: Knipe D, Howley P, Griffin D, Martin M, Lamb R, Roizman B, et al., eds., *Fields Virology*, vol. 1:199–241. Philadelphia: Lippincott Williams & Wilkins.
Valleron AJ, Cori A, Valtat S, Meurisse S, Carrat F, Boëlle PY. 2010. Transmissibility and geographic spread of the 1889 influenza pandemic. *Proc Natl Acad Sci USA* 107(19):8778–8781.
Weinberg A, Mettenbrink CJ, Ye D, Yang CF. 2005. Sensitivity of diagnostic tests for influenza varies with the circulating strains. *J Clin Virol* 33:172–175.
Whitfield J. 2003. Deadly flu evades body's defenses. Nature news Service. Accessed February 19, 2013, at http://www.nature.com/news/2002/020826/full/news020819-14.html.
World Health Organization (WHO). 2005. *Manual on Animal Influenza Diagnosis and Surveillance*. Geneva: WHO.

World Health Organization (WHO). 2006. Cumulative number of confirmed human cases of Avian Influenza A/(H5N1) Reported to WHO. Accessed February 19, 2013, at http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/.

Wilson J, Von Itzstein M. 2003. Recent strategies in the search for new anti-influenza therapies. *Curr Drug Targets* 4:389–408.

Wolf Yuri I, Viboud C, Holmes EC, Koonin EV, Lipman DJ. 2006. Long intervals of stasis punctuated by bursts of positive selection in the seasonal evolution of influenza A virus. *Biol Direct* 1:34.

Zambon M, Hays J, Webster A, Newman R, Keene O. 2001. Diagnosis of influenza in the community: Relationship of clinical diagnosis to confirmed virological, serologic, or molecular detection of influenza. *Arch Intern Med* 161:2116–2122.

Zambon M. 1999. Epidemiology and pathogenesis of influenza. *J Antimicrob Chemother* 44 Suppl B:3–9.

Chapter 16
Severe Acute Respiratory Syndrome

Introduction

Severe acute respiratory syndrome (SARS) is a serious form of pneumonia caused by a corona virus, which was first identified in 2003. The virus was discovered by a World Health Organization (WHO) physician, Carlo Urbani, in a 48-year-old businessman who had traveled from the Guangdong province of China, through Hong Kong, to Hanoi, Vietnam. The businessman and the doctor both died from the illness. Meanwhile, SARS was spreading rapidly and infected thousands of people around the world, including people in Asia, Australia, Europe, Africa, and North and South America. WHO further identified SARS as a global health threat and issued a travel advisory. The spread of SARS has been considered fully contained with the last infected human case as observed in June 2003 (except a laboratory induced infection case in 2004). However, it is not yet claimed to have been eradicated because it may still be present in its natural reservoirs (animal hosts) and may have the potential to return to the human population in the future. According to WHO, SARS showed fatality of less than 1 percent for people aged 24 years or younger, 6 percent for those aged 25 to 44, 15 percent for those aged 45 to 64, and more than 50 percent for those aged older than 65 (WHO, 2003), whereas the fatality in case of influenza disease are usually around 0.6 percent (primarily among the elderly) and can rise as high as 33 percent in severe epidemics of new strains.

Epidemiology

SARS was first recognized as a clinical entity in late February 2003, when atypical pneumonia cases of unknown origin began appearing among staff at a hospital in Hanoi. Within 2 weeks, similar outbreaks had been reported from various hospitals in Hong Kong, Singapore, and Toronto. In March 2003, WHO issued emergency

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

travel recommendations and alerted health authorities, physicians, and the traveling public against SARS and declared it a worldwide threat to health; this marked a turning point in the early course of the SARS outbreak. Some areas, including Vietnam, Hong Kong, Singapore, and Toronto, experienced the largest and most severe outbreaks, characterized by chains of secondary transmission outside the health-care setting. The disease moved out of southern China, but before that, Hanoi, Hong Kong, Singapore, and Toronto became the initial hot zones of SARS disease, which were characterized by rapid increases in the number of cases, especially in health-care workers and their close contacts. In Singapore and Taiwan, the epidemic involved cases that either had atypical clinical presentations or were otherwise not identified probably because of the lack of an initial history of direct contact with a known SARS case. These patients were the hidden reservoirs and resulted in continuous transmission of SARS cases, leading to the substantial morbidity and mortality. The disease transmitted around the world along international air travel routes.

Causative Agent

SARS is caused by a corona virus designated as SARS coronavirus (SARS-CoV), which is a positive-stranded enveloped RNA virus. It causes enteric or respiratory tract infections in a variety of animals including humans, livestock, and pets (Thiel, 2007). The genome of SARS-CoV contains five major open reading frames (ORFs) that encode: the replicase polyprotein, the spike (S), envelope (E), and membrane (M) glycoproteins, and the nucleocapsid protein (N). The S protein binds to species-specific host cell receptors and triggers fusion between the viral envelope and host cell membrane. It is the principal viral antigen eliciting neutralizing antibody production in the host. The M protein is the determinant of virion morphogenesis and is a major component of the virion envelope. Coronavirus RNA has a high rate of RNA-RNA recombination.

Transmission of Severe Acute Respiratory Syndrome Virus

SARS is predominantly spread through droplets shedding from the respiratory secretions of persons infected with it. Most of the new infections occurred through close contacts of patients, including household members, health-care workers, or other patients who were not protected with contact or respiratory precautions, which indicates that the virus is predominantly spread by droplets or by direct and indirect contact. The virus can be less frequently transmitted through oral-fecal or air-borne transmission because it has been found in the stool of patients (Drosten et al., 2003). Some patients can transmit the SARS virus to a large number of individuals. These patients and nosocomial infections were the main driving factors behind the early 2003 outbreaks. During transmission, the inoculums size (the number of infectious particles transmitted from one person to another) was of major importance and was determined, by:

1. The viral load in the secretion of the index patient.
2. The distance to the index patient (face to face contact, crowded locations etc.).

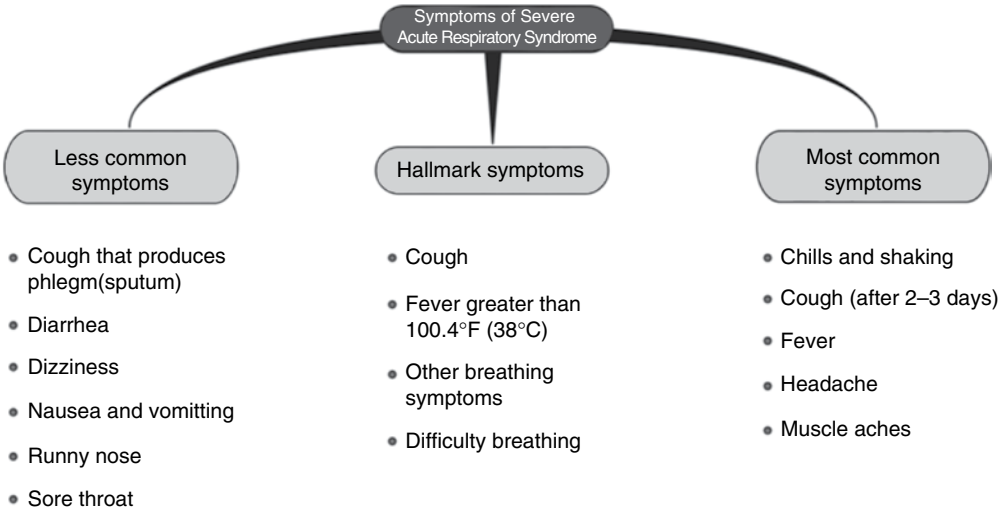

Figure 16.1. Symptoms of severe acute respiratory syndrome.

Clinical Features

SARS presents a variety of symptoms, which initially resemble a common cold, and these include fever, myalgia, lethargy, gastrointestinal pain, cough, sore throat, and some other nonspecific symptoms; later it resembles influenza. The common symptom appears in almost all the patients is a fever above 100.4° F (38° C) followed by shortness of breath. Figure 16.1 details all the symptoms of SARS

Laboratory Diagnosis

SARS viral RNA can be detected in various clinical specimens including blood, stool, respiratory secretions, or body tissues by various methods as virus isolation, serological tests (enzyme-linked immunosorbent assay [ELISA], immunofluorescence assay [IFA]) and through molecular methods, such as polymerase chain reaction (PCR; Figure 16.2).

Polymerase Chain Reaction Assay

A positive PCR test indicates the presence of SARS RNA in the patient's sample. However, it does not mean that the present viral particle is infectious and is present in a large enough quantity capable enough to infect another person. Similarly, negative PCR results do not exclude the possibility of SARS infection.

In Vitro Virus Isolation

The virion can be detected by inoculating the patient's samples, such as respiratory secretions, blood, or stool, in suitable cell cultures (e.g., Vero cell lines). This allows the

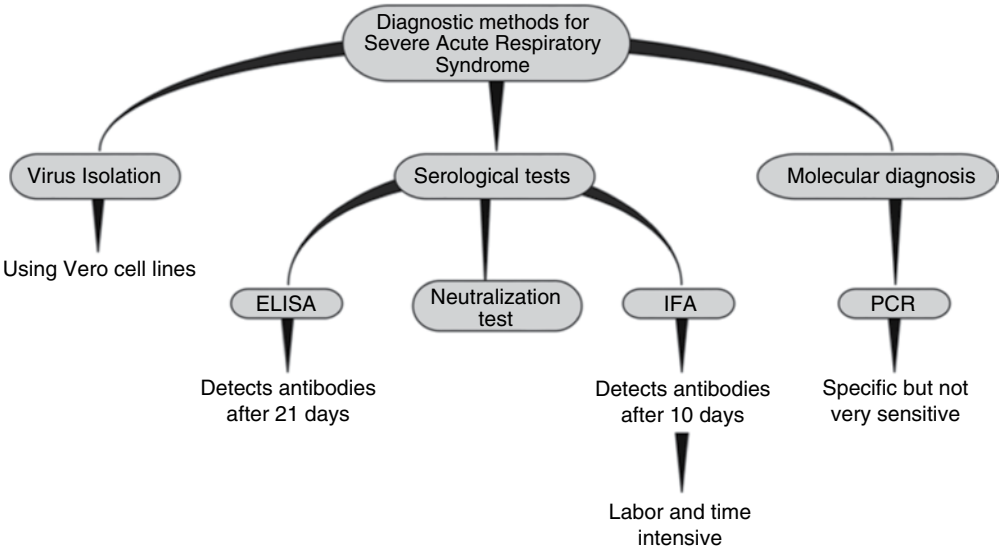

Figure 16.2. Diagnostic methods for severe acute respiratory syndrome virus. ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence assay; PCR, polymerase chain reaction.

propagation of virus in vitro. On isolation, the virus can further be identified using further tests. The virus isolation steps must be performed under at least Biosafety Level-3 conditions. Positive cell culture results indicate the presence of live SARS virus in the sample, whereas negative cell culture results do not exclude the possibility of SARS

Serological Methods

Various serological methods provide a means for the detection of antibodies produced in response to SARS-CoV. During the course of SARS infection, various types of antibodies (especially immunoglobulin M [IgM] and immunoglobulin G [IgG]) appear, and change is observed in their level in circulation. These antibodies are usually undetectable during the early stages of infection. ELISA and IFA have been developed to detect the mixture of IgM and IgG antibodies against SARS-CoV. ELISA shows positive results 21 day after the onset of illness, whereas IFA typically yields results 10 days after the onset of illness. Results can further be quantified using serial titrations of patient sera. Another test that assesses and quantifies the ability of patient's sera to neutralize the infectivity of SARS-CoV by is a neutralization test (NT). The positive serological tests also indicate previous infection with the SARS virus. A recent infection can be indicated by the seroconversion from negative to positive or a fourfold rise in the antibody titer from acute to convalescent serum.

Treatment

The therapeutic approach for SARS involves the administration of broad-spectrum antibiotics and supportive care with antipyretics, in association with antiviral agents and

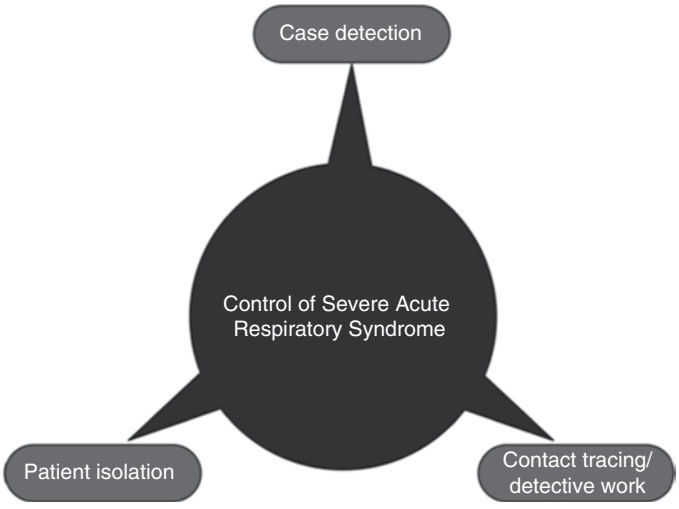

Figure 16.3. Control of severe acute respiratory syndrome.

immunomodulatory therapy. In patients with respiratory failure, ventilation should be provided. Patients suspected of having SARS must first be isolated without any delay, preferably in negative-pressure rooms.

Antibiotic Therapy

Antibiotic agents are routinely prescribed for the treatment of SARS because of its nonspecific features and unavailability of rapid laboratory tests that can reliably diagnose the SARS-CoV in the early stages of infection. Appropriate empirical antibiotics are therefore necessary to control common respiratory pathogens as per national or local treatment guidelines for community-acquired or nosocomial pneumonia (Niederman et al., 2001). Some antibiotics additionally have immunomodulatory properties, especially the quinolones (Dalhoff and Shalit, 2003) and macrolides (Labro and Abdelghaffar, 2001).

Antiviral Therapy

Because SARS is a viral disease, antibiotics are ineffective. Various antiviral agents have been prescribed during an epidemic, including ribavirin, protease inhibitor, and human interferons. Ribavirin is a nucleoside analog that was widely suggested as an empirical therapeutic agent for SARS probably because of its broad-spectrum antiviral activity against many DNA and RNA viruses. It was administered along with corticosteroids and has since become the most frequently administered antiviral agent for SARS (Peiris et al., 2003). A combination of lopinavir-ritonavir (protease inhibitor preparation)-ribavirin has also been prescribed for the treatment of SARS because it blocks the processing of the viral replicase polyprotein thereby preventing the replication of viral RNA.

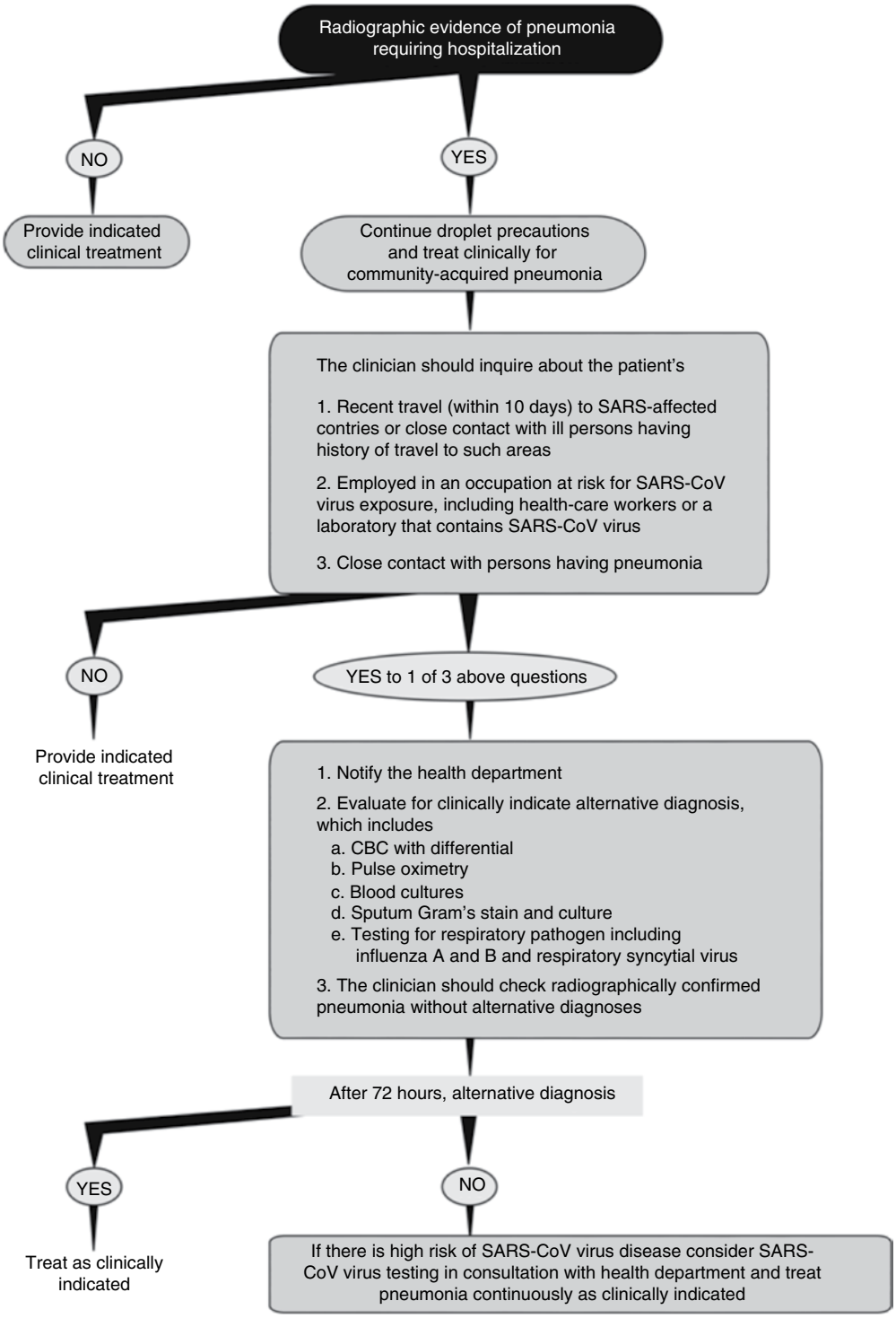

Figure 16.4. Evaluation and management of patients with radiologically confirmed pneumonia. CBC, complete blood count; SARS-CoV, SARS coronavirus.

Prevention and Control

SARS is only moderately transmissible disease because the number of SARS cases per index case, in one epidemiological study ranged from 2.2 to 3.6, which are considerably lower than those estimated for most other respiratory diseases. This feature indicates that a combination of various control measures, including shortening the time from symptom onset to isolation of patients, effective contact tracing, and quarantine of exposed persons, can be effective in containing SARS. Such measures have been successful and contributed to the prevention of major outbreaks in other countries, whereas in the absence of such measures, SARS has the potential to spread widely. Because an appropriate vaccine is not available, the most effective way to control SARS is by breaking the chain of transmission from infected to healthy persons. In most of the documented cases, SARS was spread through close face-to-face contact with infected droplets during sneezing or coughing. Three activities can reduce the number of people affected by SARS-CoV infection (Figure 16.3) and further eventually break the chain of transmission.

Case detection identifies SARS cases as soon after the onset of illness as possible. Once the cases have been identified, the next step is to ensure their prompt isolation using properly equipped facility and management according to strict infection control procedures. The third activity identifies all close contacts of each case and assures their careful follow-up, including daily health checks and possible voluntary home isolation. All of these activities work to shorten the time period between the onset of illness and isolation of the patient, thus reducing the opportunities for the virus to spread further. When the patient has pneumonia that has been radiologically confirmed, it can be managed as suggested in Figure 16.4.

References

Dalhoff A, Shalit I. 2003. Immunomodulatory effects of quinolones. *Lancet Infect Dis* 3:359–371.

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, et al. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 348:1967–1976.

Labro MT, Abdelghaffar H. 2001. Immunomodulation by macrolide antibiotics. *J Chemother* 13:3–8.

Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, et al. 2001. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. *Am J Respir Crit Care Med* 163:1730–1754.

Peiris JSM, Lai ST, Poon LLM, Guan Y, Yam LYC, et al. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 361:1319–1325.

Thiel V, ed. 2007. *Coronaviruses: Molecular and Cellular Biology*. Norfolk, England: Caister Academic Press.

World Health Organization (WHO). 2003. Update 49—SARS case fatality ratio, incubation period. Accessed February 20, 2013, at http://www.who.int/csr/sarsarchive/2003_05_07a/en/.

Chapter 17
Nipah Virus

Introduction

Nipah virus (NiV) was first identified in April 1999 in peninsular Malaysia when it caused an outbreak of neurological and respiratory disease on pig farms, resulting in 257 human cases, including 105 human deaths and the culling of one million pigs (Field et al., 2001; Centers for Disease Control and Prevention [CDC], 1999). This was followed by 11 cases reported in abattoir workers in Singapore who were exposed to pigs imported from the affected Malaysian farms. The name *Nipah* is originated from name of the place Kampung Nipah in Negeri sembilan, Malaysia, from where the virus was first isolated. The outbreak was actually mistaken for Japanese encephalitis (JE); however, the patients who had already been vaccinated against JE were not protected. During the Malaysian outbreak, humans primarily reported encephalitic symptoms, whereas pigs suffered respiratory symptoms. Later outbreaks have shown respiratory illness in humans, increasing the likelihood of human-to-human transmission. Pteropid fruit bats, including *Pteropus vampyrus* (large flying fox) and *Pteropus hypomelanus* (small flying fox), were the primary reservoir of NiV based on seroprevalence data and viral isolation. The CDC classified NiV as Category C agent, which makes it a potential bioweapon targeted to animals, humans, or both because:

- Even a single small outbreak in pigs could affect herds, causing substantial economic loss to the industry or to the national economy of the affected country.
- NiV can infect humans with a case fatality rate of approximately 50 percent because a Category C agent has a high morbidity and mortality rates and a major health impact.
- Currently there is no effective treatment or vaccine against NiV in either pigs or humans (although ribavirin may be effective in human encephalitic cases).
- An outbreak of NiV in animals or humans could cause substantial fear and social disruption probably as a result of the little known information regarding the causal agent.
- Ease of production and dissemination of the virus.

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Epidemiology

NiV outbreaks in south Asia showed a strong seasonal pattern and a limited geographical range. The emergence of bat-related NiV infections has been suggested to be communicable to humans as a result of the loss of natural habitats of bats. The habitat of flying fox is destroyed by human activity and the bats get stressed and hungry. This weakens their immune system, their virus load goes up, and high amount of virus spills out in their urine and saliva. This type of virus shedding may also found to be associated with stressful physiological conditions or seasons. Seasonal transmission in *Pteropus lylei* was demonstrated in a study in Thailand. The viral RNA could be mainly detected in urine between April to June (with highest in May). In 2001, some focal outbreaks of NiV were reported in Bangladesh and India during the winter. This was associated with the drinking of fresh date palm sap contaminated by fruit bats (*Pteropus giganteus*) during the winter season, which transmitted NiV indirectly to humans (Luby et al., 2006). Figure 17.1 shows the distribution of NiV outbreaks in Bangladesh and India from 2001 to 2008.

The human-to-human transmission of NiV was reported in India in 2001 when an outbreak in Siliguri reported nosocomial infection in 33 health-care workers and hospital visitors after having exposed to patients hospitalized with NiV illness (Chadha et al., 2006). Whereas, during an outbreak in Bangladesh, NiV was transmitted either directly or indirectly from infected bats to humans. Human- to-human transmission of NiV was strongly evidenced in Bangladesh in 2004 (Gurley et al., 2007). Table 17.1 presents the morbidity and mortality data of human NiV infection.

Etiological Agent

NiV belongs to the genus Henipa virus, a new class of virus in the *Paramyxoviridae* family. The genus also includes a closely related Hendra virus. The virus is spherical or filamentous but sometimes pleomorphic in nature with size ranging from 40 to 600 nm in diameter (Hyatt et al., 2001). It is an enveloped virus with a lipid bilayer overlying a shell of the viral matrix protein. The genome of the virion is a single strand of helical RNA, 15,200 to 15,900 nucleotides long and is tightly bound to a nucleocapsid (N) protein. N protein is further associated with the large (L) and phosphoprotein (P) proteins providing RNA polymerase activity during replication. Within the lipid bilayer, spikes of fusion (F) protein trimers and attachment (G) protein trimers are embedded (Figure 17.2). The G protein attaches the virus on to the surface of host cell via a highly conserved protein present in many mammals, which is designated as EFNB2 (Bonaparte et al., 2005; Negrete et al., 2005), whereas, the F protein mediates the fusion of the viral membrane with the host cell membrane, releasing the virion contents into the cell. The F protein also promotes the infected cells to fuse with neighboring cells and forms a large, multinucleated syncytium.

Transmission

Infected bats are the symptomless carriers of NiV, which shed virus in their excretions and secretions, including saliva, urine, semen, and excreta, but the shedding of NiV in urine only occurs sporadically.

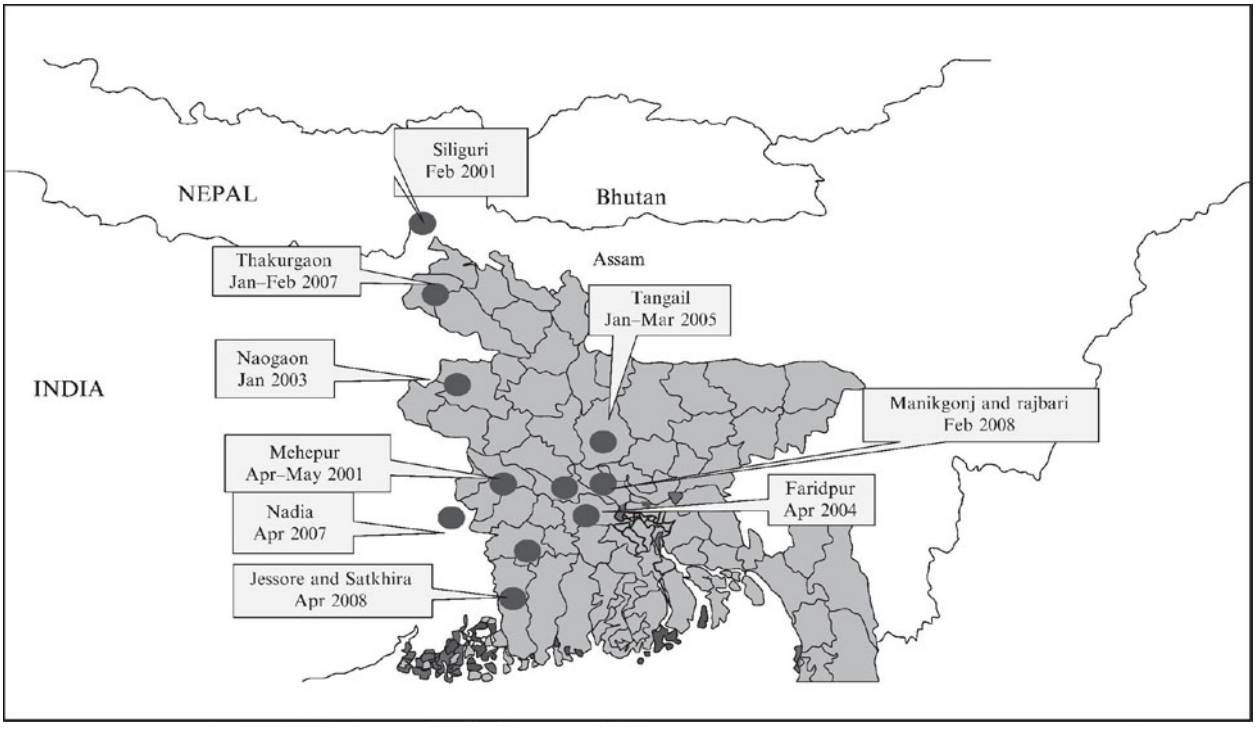

Figure 17.1. Chronological distribution of outbreak of Nipah virus infection in South Asia, 2001–2008.

Table 17.1. Morbidity and mortality associated with Nipah virus, Asia-Pacific Region, 1998–2008.

Year/Month	Location	Number of Cases	Number of Deaths	Case Fatality (%)
Sep 1998–Apr 1999	Malaysia (Perak, Selangor and Negeri Sembilan states)	265	105	40
Mar 1999	Singapore	11	1	9
Feb 2001	Siliguri (India)	66	45	68
Apr–May 2001	Meherpur, Bangladesh	13	9	69
Jan 2003	Naogaon, Bangladesh	12	8	67
Jan 2004	Goalando, Bangladesh	29	22	76
Apr 2004	Faridpur, Bangladesh	36	27	75
Jan–Mar 2005	Tangil, Bangladesh	12	11	92
Jan–Feb 2007	Thakurgaon, Bangladesh	7	3	43
Mar–Apr 2007	Kushtia, Bangladesh	8	5	63
April 2007	Nadia, India	5	5	100
Feb 2008	Manikgonj and Rajbari	11	6	55
Apr 2008	Shatkira and Jessore	2	1	50
Total		**477**	**248**	**52**

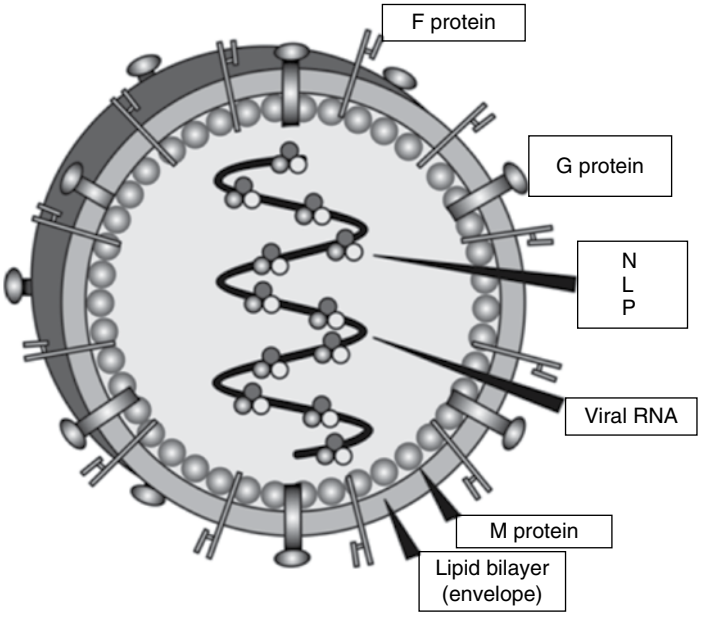

Figure 17.2. Structure of Nipah virus. F, fusion protein; G, attachment protein; L, large protein; M, matrix protein, N, nucleocapsid protein; P, phosphoprotein.

Pigs are highly contagious carrier for NiV infection, and the direct contact with the infected pigs is considered as the predominant mode of transmission in humans, which was first recognized in a large outbreak in Malaysia in 1999 (Goh et al., 2000). Approximately 90 percent of the infected people in the 1998–1999 outbreaks were pig farmers or had contact with pigs.

Horizontal transmission may also be possible in cats because the virus is found in respiratory secretions, urine, the placenta, and embryonic fluid in experimentally infected cats, although has not yet been demonstrated.

Clinical Features

In Humans

NiV causes asymptomatic or mild infections in some of the patients, but most of the clinical cases present with acute neurological signs and symptoms. The initial symptoms appear are influenzalike, with high fever, headache, and myalgia. In patients with encephalitis, the symptoms may include drowsiness, disorientation, convulsions, and coma (Figure 17.3). Nausea and vomiting can also be observed, and the patients sometimes develop respiratory signs, including acute respiratory distress syndrome. The patients who are severely ill show septicemia, bleeding from the gastrointestinal tract, renal impairment, and other associated complications. Cases will become fatal when they progress to encephalitis. Patients who survive the disease may still have mild-to-severe residual neurological deficits. The patients, once recovered from neurologic disease, may relapse with encephalitis several months to several years later. In humans, the case fatality rate ranges from 9 to 75 percent.

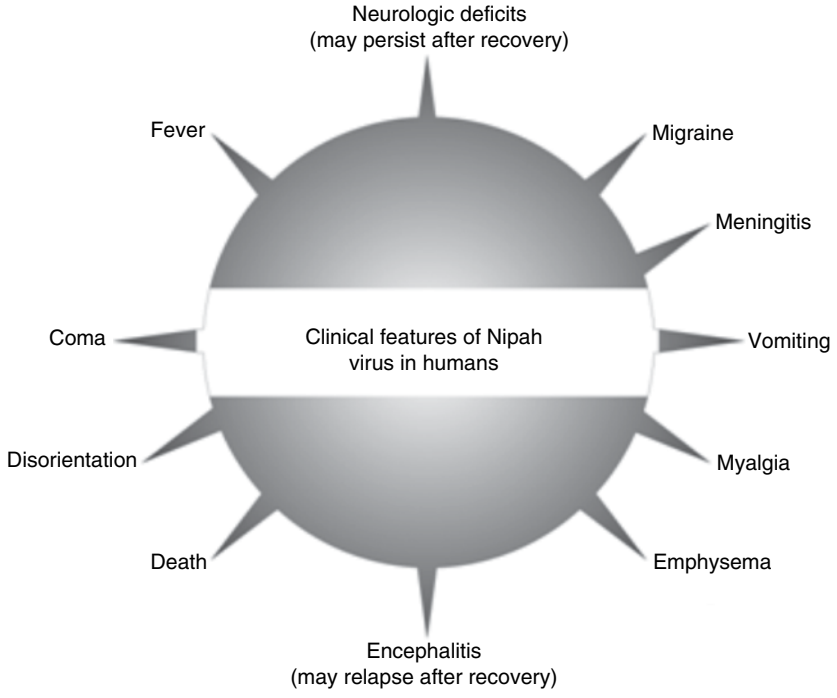

Figure 17.3. Clinical features of Nipah virus in humans.

In Animals

Among animals, the typical clinical symptoms observed in pigs are respiratory symptoms. The incubation period in pigs is estimated to be around 7 to 14 days, but it may be as short as 4 days. In experimentally infected cats, the reported incubation period was 6 to 8 days. Although in pigs asymptomatic infections are more common, symptomatic infections usually are acute febrile illnesses, which sometimes fulminate into infections. Sudden death has also been reported with low mortality except in young piglets. In pigs, between 1 and 6 months old, respiratory symptoms are more common than neurologic symptoms. The clinical signs are fever, nasal discharge, open mouth breathing, rapid and labored respiration, and a loud barking cough with hemoptysis in severe cases. Table 17.2 shows all the possible clinical signs and symptoms of NiV infection in swine.

Laboratory Diagnosis

Quite similar to other viral agents, NiV infections can also be diagnosed by virus isolation, polymerase chain reaction (PCR), and serological tests (Figure 17.4). Histopathology also helps in diagnosis. For the isolation of NiV, many cell lines can be used, including Vero, RK-13, BHK, and porcine spleen cells. Apart from these, the virus can also be cultured in embryonated chicken eggs, but because of the ease of culture in cells, it is predominantly isolated by cell culture. It should be cultured in high-security conditions because it is a Biosafety Level-4 pathogen. Immunostaining and viral neutralization techniques also

Table 17.2. Clinical features of Nipah virus in swine.

Developmental stage of life	Characteristics
All age groups	Clinical signs that vary with stage of development: Nervous signs, twitching/trembling, muscle fasciculation, tetanic spasm, hind limb weakness, death 1–2 days after onset of respiratory distress, coagulopathy leading to petechial and ecchymotic hemorrhage, some affected pigs may be asymptomatic and virus tends to be vasotropic and neurotropic
Suckling pigs	These are not affected by Nipah virus
Young pigs	Coughing, open-mouth breathing, abnormal posturing and convulsions
Weaners and growers	General flulike ailments, such as fever, anorexia, dyspnea, and coughing
Adults	Neuropathy, head bobbing/banging, spasms, agitation/biting, flatulence, early abortion, stillbirths, sudden death, fever, anorexia, dyspnea, pneumonia, coughing, mucoid nasal secretions, may be yellow-green in color or blood-tinged, and sudden death
Necropsy	Lung consolidation, especially diaphragmatic lobes, thickened interlobular septae, bronchi saturated with frothy exudates, may be bloody, renal congestion and hemorrhage, visceral organs appear normal

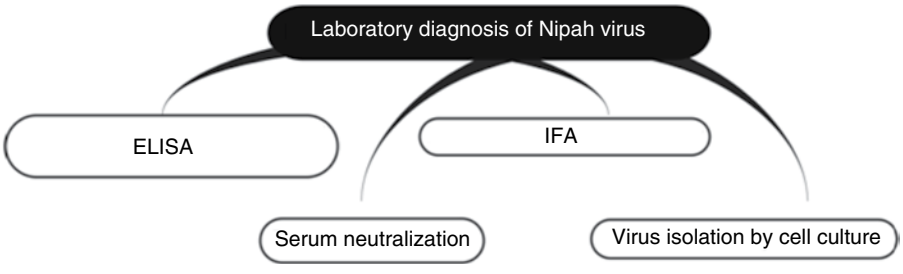

Figure 17.4. Laboratory diagnosis of Nipah virus. ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence assay.

found to be useful for the identification of NiV. NiV can be isolated under Biosafety Level-3 conditions, but with stringent precautions to protect laboratory personnel. Viral antigens can also be detected by serological methods, including immunofluorescence assays (IFAs) on formalin–fixed tissues, viral neutralization, and enzyme-linked immunosorbent assay (ELISA) tests.

Prevention and Control

To date, there is no effective treatment for NiV disease, but ribavirin may alleviate the symptoms of nausea, vomiting, and convulsions (Chong et al., 2001). Treatment is usually symptomatic by managing fever and the neurological symptoms. Patients who are severely ill should be hospitalized and may require the use of a ventilator. Health-care workers involved in the caring for patients with suspected or confirmed NiV should

follow standard precautions when caring for patients and handling specimens from them.

A recombinant subunit vaccine formulation has been developed for cats to protect against lethal NiV challenge (McEachern et al., 2008). ALVAC Canarypox vectored NiV F and G vaccine is a promising vaccine for swine and a potential vaccine for humans (Weingartl et al., 2006). For protecting humans, appropriate surveillance systems are necessary to detect NiV outbreaks quickly followed by suitable control measures.

By preventing pig infections, the risk of infection for humans can be decreased. Pigs and fruit bats should be avoided, especially in endemic areas. Good biosecurity practices should be employed in preventing infections on pig farms. Unpasteurized juices should be avoided, and fruit should be washed thoroughly and peeled or cooked properly. Good personal hygiene and sanitation also reduces the risk of infection. Because NiV is a Biosafety Level-4 pathogen, infected animals, their body fluids, and tissue samples must be handled with appropriate biosecurity precautions. Persons who generally come in contact with potentially infected animals, including laboratory personnel and health-care workers, should wear protective clothing, impermeable gloves, masks, goggles, and boots. Patients infected with NiV should be isolated and barrier nursing should be used while caring for infected patients because the virus can be transmitted from person to person.

References

Bonaparte M, Dimitrov AS, Bossart KN, Crameri G, Mungall BA, et al. 2005. Ephrin-B2 ligand is a functional for Hendra virus and Nipah virus. *Proc Natl Acad Sci USA* 102:10652–10657.

Centers for Disease Control and Prevention (CDC). 1999. Update: Outbreak of Nipah virus—Malaysia and Singapore, 1999. *Morb Mortal Wkly Rep* 48:335–337.

Chadha MS, Comer JA, Lowe L, Rota PA, Rollin PE, et al. 2006. Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis* 12:235–240.

Chong HT, Kamarulzaman A, Tan CT, Goh KJ, Thayaparan T, et al. 2001. Treatment of acute Nipah encephalitis with ribavirin. *Ann Neurol* 49:810–813.

Field H, Young P, Yob JM, Mills J, Hall L, et al. 2001. The natural history of Hendra and Nipah viruses. *Microbes Infect* 3:307–314.

Goh KJ, Tan CT, Chew NK, Tan PSK, Kamarulzaman A, et al. 2000. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 342:1229–1235.

Gurley E, Montgomery JM, Hossain MJ, Bell M, Azad AK, et al. 2007. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerg Infect Dis* 13:1031–1037.

Hyatt AD, Zaki SR, Goldsmith CS, Wise TG, Hengstberger SG. 2001. Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. *Microbes Infect* 3:297–306.

Luby SP, Rahman M, Hossain MJ, Blum LS, Mushtaq Husain M, et al. 2006. Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis* 12:1888–1894.

McEachern JA, Bingham J, Crameri G, Green DJ, Hancock TJ, et al. 2008. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* 26:3842–3852.

Negrete OA, Levroney EL, Aguilar HC, Bertolotti-Ciarlet A, Nazarian R, et al. 2005. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* 436:401–405.

Weingartl HM, Berhane Y, Caswell JL, Loosmore S, Audonnet JC, et al. 2006. Recombinant Nipah virus vaccines protect pigs against challenge. *J Virol* 80:7929–7938.

Chapter 18
Paragonimiasis

Magnitude of the Problem

Global

Paragonimiasis is a zoonotic disease in which humans are the definitive hosts. Many *Paragonimus* species reproduce and produce eggs in the human body, but humans are not essential for the survival of this parasite. It requires numerous definitive animal hosts to support infection (Blair et al., 2008). It is difficult to ascertain the prevalence of paragonimiasis throughout the world. It was estimated in 1995 that approximately 20.7 million people may be infected with the parasite *Paragonimus* and that another 195 million were at risk for disease (World Health Organization [WHO], 1995; Marty and Neafie, 2000). Recently, it has been estimated that 293 million people are at risk of the disease, and several million have actually been infected (Keiser and Utzinger, 2005; Blair et al., 2008). *Paragonimus* species are found in tropical, subtropical, and temperate climates. They occur throughout east and south Asia, throughout sub-Saharan Africa, and in the Americas from Peru to Canada (Blair et al., 2008). Geographic regions that have or have had a high prevalence of the disease include Cameroon, China, the Philippines, parts of Ecuador, parts of India, Japan, and Thailand (Chang et al., 1958; Shih et al., 1958; Yokogawa, 1965; Malek, 1980; Amunarriz, 1991; Ripert et al., 1992; Singh et al., 1993; Xu, 1991; Chen et al., 2001).

Some environmental factors, such as pollution, adversely affect intermediate host population, thereby affecting the prevalence of disease. However the prevalence of human paragonimiasis disease has declined substantially in many endemic areas over the past century. Many factors have contributed to this, but the most important is education concerning the risk of disease associated with the consumption of raw crustaceans and the use of raw animal products in folk medicine practices. For example, in Korea, the prevalence of disease was estimated to exceed 1.5 million people prior to educational

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

efforts focusing on healthier eating habits, cessation of the use of crayfish juice in folk medicine, and more widespread pollution that adversely affected intermediate hosts (Kim, 1984; Nana and Bovornkitti, 1991; Kagawa, 1997). Following the impact of these factors, the prevalence of paragonimiasis disease in Korea was substantially reduced (Kagawa, 1997). Although the prevalence of paragonimiasis has been reported to have declined in many regions, areas of high endemicity still exist.

Paragonimiasis uniformly occurs in men and women and in both adults and children, and there was no substantial difference in the male-to-female ratio (Uchiyama et al., 1999), although it was suggested that in some areas children may be infected at a higher rate than adults. Furthermore, it has been reported that children are more likely to develop ectopic paragonimiasis, with cerebral paragonimiasis the most severe form of ectopic disease.

India

Paragonimus westermani are the most widely distributed *Paragonimus* species in Asia and was first described by Kerbert from the lungs of a Bengal tiger captured in India and died at a zoo in Amsterdam more than a century ago. However, little attention was paid to the genus *Paragonimus* because paragonimiasis disease was never considered a public health problem in India and remained a neglected disease until the first case was reported from Manipur in 1982 (Singh et al., 1982). Afterward, many cases of paragonimiasis were reported from several parts of Manipur (Singh et al., 1986, 1993). In contrast to almost equal male-to-female ratio, Singh et al. (1986) reported that approximately 90 percent of the patients in Manipur, India, were male. Subsequently, endemic foci of paragonimiasis were also discovered in Arunachal Pradesh (Narain et al., 2003). Surprisingly *Paragonimus heterotremus* has been identified as the causative agent of human paragonimiasis in this part of India in comparison to the widely reported *Paragonimus westermani*. In 2008, seven paragonimiasis cases were reported in the Community Health Centre at Pfutsero town, Phek district, Nagaland, with a male-to-female ratio of 5:2. Additionally, a higher prevalence of paragonimiasis was detected among children and young adults in the age group ranging in between 7 and 32 years and was rare after 40 years of age (Singh et al., 2009).

The Parasite: Paragonimus

The lung flukes are the trematode (flat, leaf like) parasites of the genus *Paragonimus* most commonly *P. westermani*.

Morphology

Adult

The adult parasite is 4- to 6-mm wide, 3- to 5-mm thick, and 7- to 12-mm long of red brown color looking almost like a coffee bean. It is hermaphroditic, with two testes and one ovary, which is located above the testes and on the right side of the body. The trematode has two suckers both for attachment and locomotion, an oral cavity, and a genital pore. The oral sucker is in the front and just before the center of its lower body is the

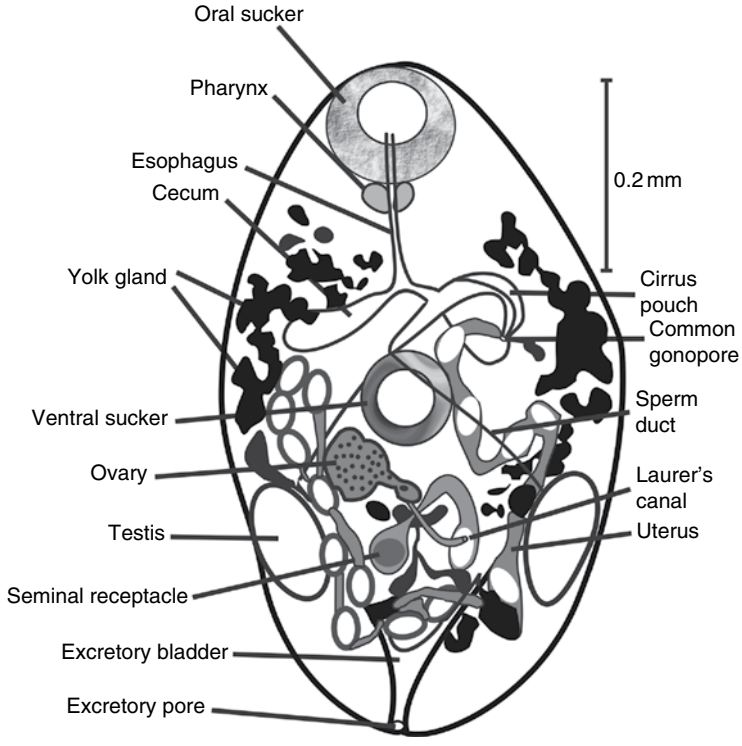

Figure 18.1. *Paragonimus westermani* (an adult of the hermaphroditic generation).

ventral sucker. Both the oral and the ventral suckers are similar in size, with the later placed slightly pre-equatorially. The excretory bladder extends from the posterior end to the pharynx (Figure 18.1). Most of the body is occupied by the various reproductive organs; the largest being the uterus, which is located in a tight coil to the right of the acetabulum and is connected to the vas deferens. The vitelline glands producing the yolk for the eggs are widespread in the lateral field from the pharynx to the posterior end, and the tegument/outer skin is covered with scalelike spine.

Egg

The eggs of *P. westermani* are 80 to 120 μm in length and 45 to 70 μm wide, having their greatest width near the equator.They are yellowish brown in color, ovoid or elongated, with a thick shell, and often asymmetrical with one end slightly flattened. At the large end, the operculum is clearly visible. The opposite (abopercular) end is thickened. The eggs are unembryonated when passed in sputum or feces.

Life Cycle

Paragonimus species present a complex life cycle, including a mammal as the definitive host (the host in which the adult is found, and sexual reproduction occurs), and snails and crustaceans serve as intermediate hosts (Sugiyama et al., 2004). *P. westermani* are

transmitted to humans and mammals through the consumption of raw or undercooked seafood. In Asia, an estimated 80 percent of freshwater crabs carry *P. westermani* (Pachucki et al., 1984). During seafood preparation, live crabs are crushed, resulting metacercariae (the infective stage) of the parasite, which may contaminate the fingers of the person preparing the meal. Infective cysts accidentally transfer via food preparers who handle raw seafood and subsequently contaminate cooking utensils and other foods (Yokogawa, 1965). Animals that consume crustaceans can also transmit the parasite as happened in Japan where raw boar meat was the source of human infection. Some other animals such as pigs, dogs, and a variety of feline species can also harbor *P. westermani* (Centers for Disease Control and Prevention [CDC], 2012).

The life cycle of *P. westermani* can be broadly divided into four steps.

Excretion of Eggs from the Human Host

The life cycle begins with the production and then passage of fertilized, operculate eggs from sexually competent adult trematode parasites residing within the lungs of their definitive mammalian host. These eggs are found in stool or sputum as golden eggs and are coughed up along with blood or they are swallowed and passed along with the stool. These eggs are excreted unembryonated and get embryonated in the external environment. As soon as they find the fresh water source, they will hatch and turn into ciliated larvae called *miracidia*. It takes around 2 weeks for an egg to hatch completely into a miracidium (Figure 18.2).

Primary Host: Snail

Miracidia hatch and seek the first intermediate host snail and penetrate its soft tissues. They asexually reproduce inside the snail and then go through several developmental stages, such as sporocysts, rediae, and finally produce thousands of another larval form called as *cercaria*. It takes approximately 3 to 5 months for asexual reproduction inside the snail. In the end, the cercaria crawls out of the snail and swims around until finding the secondary host, a fresh water crayfish (a lobster like creature) or crabs.

Secondary Host: Fresh Water Crayfish or Crab

Cercaria finds its way to the fresh water crayfish or crab, penetrates the gills, muscles and starts forming a cyst in crustacean tissues. It undergoes a secondary transformation within 2 months into the resting form of cercaria called *metacercaria*, the infective stage for mammalian host.

Human Infection

Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish harboring the infective stage (metacercaria) of the parasite. These metacercaria excyst in the duodenum and penetrate through the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs. In lungs, they become encapsulated and develop into an adult in 5 to 6 weeks and usually tend to reside in pairs Male and female lung worms start reproducing, and the female lays eggs, which are then carried out from the human lungs in the sputum by the motion of microvilli. Finally, these eggs are taken through the gastrointestinal tract and discharged from the body, and the cycle starts again.

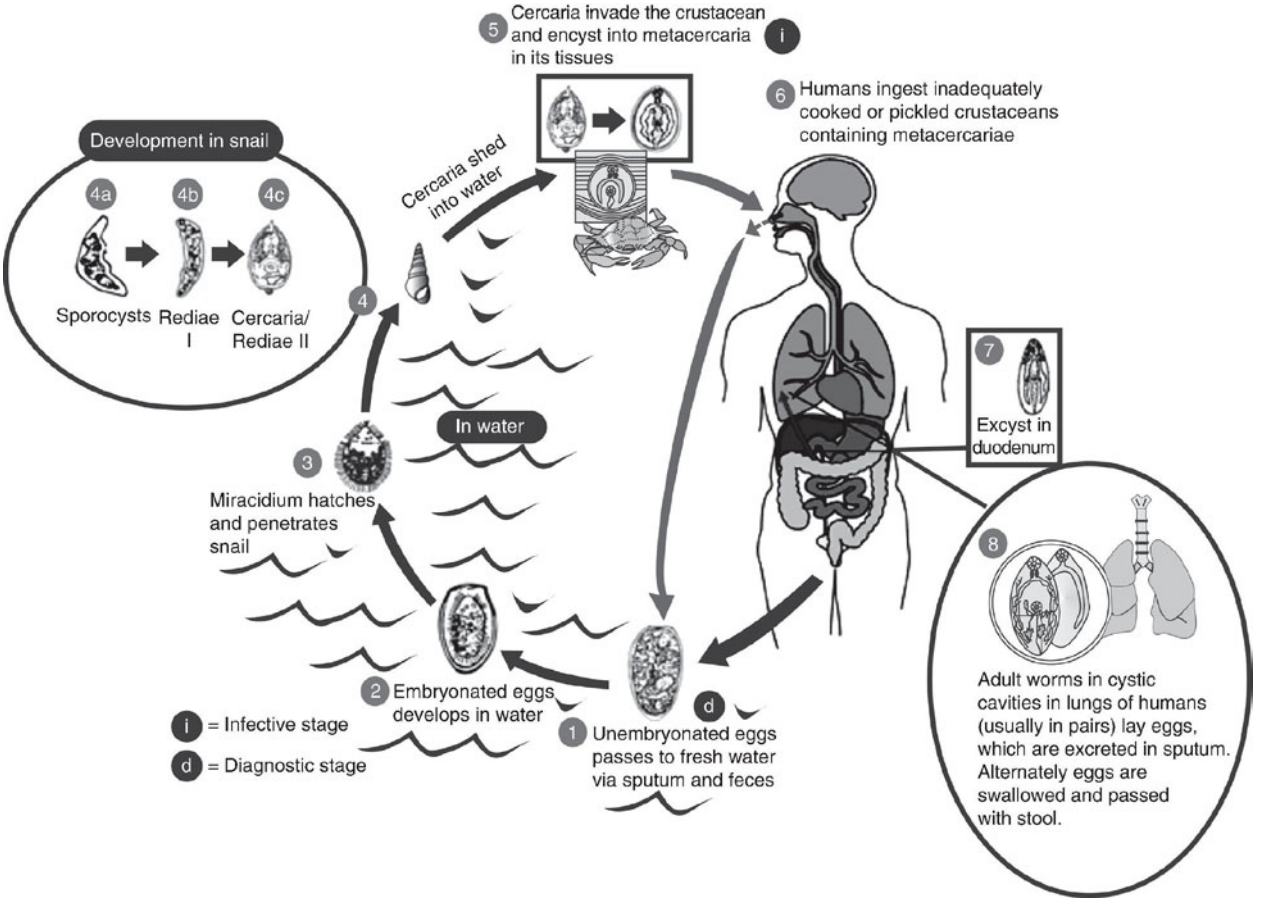

Figure 18.2. Life cycle of *Paragonimus westermani*.

These lung fluke larvae sometimes accidentally travel to other organs and tissues, such as the brain and striated muscles, respectively, and get reproduced there. But because these eggs are not further secreted from the brain, the life cycle will not be completed. In case the worm goes to the spinal cord instead of the lungs, it paralyzes the host, and if it infects the heart, the host could die. Time from infection until oviposition is 65 to 90 days, and the infection may persist for up to 20 years in humans. Apart from the human beings, animals such as pigs, dogs, and a variety of feline species can also harbor the parasite *P. westermani*.

Epidemiology

Paragonimus species are extremely successful parasites and are widely geographically distributed as being endemic in Asia, the Americas, and Africa (Table 18.1). Endemic *Paragonimus* species have yet to be reported from Europe, Australia, and Antarctica; imported paragonimiasis, however, may occur in any region (Mukerjee et al., 1992). Certain species are limited to a particular geographical area, whereas others are more widely distributed, for example, *Paragonimus miyazakii* is endemic in Japan and *P. heterotremus* is endemic in Thailand, but *P. westermani* occurs in both of these locations (Maleewong, 1997; Nawa, 2000). The distribution of *P. westermani* ranges from Japan throughout Southeast Asia to India, whereas *Paragonimus kellicotti* is the only *Paragonimus* species that is endemic in North America and *Paragonimus mexicanus* occurs in Central and South America.

The distribution of *Paragonimus* species is dependent on the distribution of permissive animals that support its infection (i.e., the natural distribution of the definitive and intermediate hosts) (Davis et al., 1994). The major faunas of North America that support the life cycle of *Paragonimus kellicotti* including various domestic animals such as dogs and cats, as well as many wild animals (Pechman, 1980). Although this parasite is still

Table 18.1. The predominant *Paragonimus* species that cause human infections and their geographic distribution.

Paragonimus Species	Area(s) of Endemicity
P. westermani	Asia, India, Philippines, and New Guinea
P. miyazakii	Japan
P. skrjabini	China, Southeast Asia
P. heterotremus	Thailand, China, and Southeast Asia
P. hueitungensis	China
P. uterobilateralis	West Africa
P. africanus	West Africa
P. kellicotti	North America
P. mexicanus	Central America and South America

Adapted from Marty AM, Neafie RC. 2000. Paragonimiasis. In: Myers WM, Neafie RC, Marty AM, Wear DJ, eds., *Pathology of Infectious Diseases*, vol. I, pp. 49–67. *Helminthiases*. Washington, DC: Armed Forces Institute of Pathology.

unknown to many physicians and medical microbiologists, it is well known to veterinarians and animal biologists in North America.

Countries Where Paragonimus Species Are Endemic

The distribution of human paragonimiasis is highly focal in endemic countries (Figure 18.3), which can be identified as those areas in which inhabitants consume raw crabs.

Cameroon

It is only one area of Africa where paragonimiasis is reported through eating raw crabs. The species of the parasite involved are *Paragonimus. africanus* and *Paragonimus uterobilateralis*. The most frequently affected people are in the 10- to 19-year-old age group and women. In southwest Cameroon, four foci exist of human paragonimiasis cases: where primates are naturally infected are the mount kupe area and foci of Mbam, Nyong, and Ntem, whereas in west Cameroon naturally infected drills (*Mandrillus leucophaeus*) are the reservoirs for lung flukes, but human infection has not yet been reported in this area.

China

In China, the first endemic focus was found in Zhejiang province in 1930. Human paragonimiasis cases have been reported from a total of 21 provinces in China. Approximately 20 *Paragonimus* species are reported in China, but only four are medically important: *P. westermani, Paragonimus skrjabini, P. heterotremus*, and *Paragonimus hueitungensis. P. westermani* are the most abundant as has been reported from 17 provinces, *P. skrjabini* is the second species that is endemic in a mountainous area of 14 provinces, which suffers from a cutaneous type of paragonimiasis. It estimated that about 185 million people are at risk of paragonimiasis.

Ecuador

Since 1980, infected freshwater crabs have been found in 15 of the 22 provinces of Ecuador particularly in the Amazonian region, which represents more than 70 percent of the total national territory. Approximately 20 percent of the total population of the country or about 50 percent of those living in rural areas, are at risk. Of the population at risk 24.3 percent are seroreactive, or egg positive and 12.3 percent are egg positive; thus at least 494,000 people are estimated to be infected.

Japan

Paragonimiasis has been reported in almost all parts of Japan with an average positivity of 3.5 percent between 1954 and 1968 as observed in skin-test surveys. The egg positivity was shown by approximately 10.4 percent of people who were both serologically and skin-test positive. The sporadic cases of *P. westermani* and *P. miyazakii* have also been diagnosed in various parts of Japan.

Peru

Crabs infected with *Paragonimus* are found in 5 out of 23 departments of Peru, including Cajamarca, Huanuco, Amazonas, Junin, and Ucayali. Human paragonimiasis is endemically

Figure 18.3. Geographic distribution of Paragonimiasis. Map from www.cdfound.to.it/html/par1.htm.

found mainly in the rural areas of the Condebamba Valley of the department of Cajamarca. Serological surveys indicate that 9.6 percent or approximately 27,000 people are infected in rural areas where people eat raw crabs.

Republic of Korea

About 6 million people in four provinces (Chejudo, Chollanamdo, Chollabukdo, and Kangwondo) reported being at risk of paragonimiasis because the presence of *P. westermani* or rarely, *Paragonimus Iloktsuenensis*.

Clinical Manifestations

Paragonimiasis was previously known by many names, including Oriental lung fluke, pulmonary distomatosis, and benign endemic hemoptysis (coughing up blood). In most cases, *Paragonimus* causes limited morbidity, and it rarely causes death and had been given the name benign endemic hemoptysis. The clinical features of paragonimiasis start with some nonspecific symptoms appearing during the migratory stage, such as diarrhea, abdominal and chest pain, allergic reaction, fever, and chills. Once the parasite establishes an infection in the lungs, it elicits an eosinophilic inflammatory reaction during which one or more parasites become encapsulated in 1- to 2-cm lesions and causes secondary infection on capsule burst that may clinically appear as chronic bronchitis with cough, night sweats, hemoptysis, and feeling of general malaise. This further leads to severe infection, resulting in pleurisy, clubbed fingers, and pneumothorax. Sometimes ectopic infection occurs in the brain, which will exhibit neurological symptoms, such as paralysis, epilepsy, homonymous hemianopsia, optic atrophy, and papilledema, which occurs more commonly in children.

Many patients are asymptomatic (Table 18.2), having subclinical disease and are unaware of the infection, whereas others have mild to moderate symptoms for many years before getting medical attention. Regardless of the mild nature of paragonimiasis in many patients, *Paragonimus* remains an important cause of morbidity and mortality throughout many parts of the world.

Three categories of paragonimiasis are acute paragonimiasis (relatively soon after infection), chronic pleuropulmonary paragonimiasis (the manifestations of established pulmonary disease), and ectopic paragonimiasis (the manifestations resulting from the presence of the parasite in a location other than the lungs). The clinical findings observed in an individual patient actually reflect the stage and type of disease. The acute phase is produced to some degree in all the patients because it is associated with the gastrointestinal penetration and initial migration of the parasite. However, many patients that seek medical attention at the chronic pleuropulmonary stage of disease may not recall or were actually unaware of the transient symptoms present during the early stage of infection (because they were either asymptomatic or suffering from subclinical acute paragonimiasis disease).

Acute Paragonimiasis (Early-Stage Disease)

The clinical manifestations of acute paragonimiasis appear following the ingestion of the infective metacercaria and their migration to the pleural space, which occurs between

Table 18.2. Clinical manifestations of paragonimiasis.

Clinical Feature	Frequency (%)		Comments
	Average	Range	
Cough	83	62–100	Cough may be exacerbated by physical strain.
Hemoptysis	70	61–95	The rusty discoloration of the sputum is caused not only by hemosiderin but also because of the presence of the pigmented (tan to brown) *Paragonimus* eggs; the sputum of these patients have been classically described as resembling iron filings (Malek, 1980).
Chest pain or discomfort	65	38–94	A predominance of pleuritic pain suggests a prominent pleural component. In cases where only pleural disease is present, eggs will not be detected in the sputum or stool
Dyspnea	42	5–53	
Fever or chills	37	11–67	These may occur as occasional febrile episodes, with what appears to be spontaneous resolution (Kagawa, 1997).
Asymptomatic	2	0–8	Approximately 20 percent of patients with paragonimiasis may be asymptomatic (Uchiyama et al., 1999)

2 and 15 days following the ingestion of the parasite. The possible early manifestations include abdominal pain, fever, and diarrhea commonly observed in patients with heavy worm burdens (Zhong et al., 1981). In contrast, patients are often asymptomatic or may have subclinical disease (Kagawa, 1997) in association with chest pain, fatigue, and urticaria caused by the presence of the immature, migrating forms in the pleural cavity, finally resulting pleuritic chest pain and pleural effusions.

Chronic Pleuropulmonary Paragonimiasis (Late-Stage Disease)

The chronic paragonimiasis occurs when the worms migrate to their final destination in the pulmonary parenchyma, adult worms are paired in a cyst and produce fertilized eggs. An exception to this is the infection caused by a triploid variant of *P. westermani*, wherein a single worm occupies a cyst and parthenogenically produces viable eggs (Blair et al., 2008). The observed clinical manifestations are directly related to the pathological process associated with this stage of infection that further depend on the location of the parasitic cyst, the number of cysts produced, and any associated sequelae. The chronic paragonimiasis begins with the migration of the parasites from the pleura to the location where the cyst will be formed, which is just under the pleural surface in many instances and these migrating worms may cause bronchiectasis, interstitial pneumonitis, transient hemorrhage, or bronchopneumonia to varying degrees (Kagawa, 1997; Nana and Bovornkitti, 1991). Cough and recurrent hemoptysis are predominantly observed clinical findings for patients with chronic paragonimiasis (*see* Table 18.2). Other important symptoms in patients with paragonimiasis include weakness, hoarseness, and breathlessness. Although some physical signs such as crepitation (a crackling sound heard on auscultation of the lungs) and rhonchi (a coarse rattling sound heard on auscultation of the

lungs) may also be present. The diagnosis will be suspected if the patient is from an endemic area, particularly if he or she has a history of eating crab or crayfish.

With the production of parasitic cyst near the pleura, the eggs exit into the pleural space, which is a dead end for the parasite (i.e., it is nonproductive for the completion of the life cycle); then it produces pleura-based disease. Eggs thereby released into the pleural space, become entrapped in the parietal and visceral pleurae, and finally elicit a substantial inflammatory response. These inflammatory mediators and the presence of foreign material (eggs) cause edema and effusions that then resolves into fibrosis that entraps and restricts the lungs. In patients having both parenchyma- and pleura-based disease, the infection is generally referred as pleuropulmonary or chronic pleuropulmonary paragonimiasis. Communications between the pleural space and patent airways, generated through cyst maturation or rupture, cause a pneumothorax. Hemorrhage that occurs into the airspace, causes hemoptysis, whereas hemorrhage into the pleural space results in hemothorax. Pleural effusions, fibrosis, and pneumo- or hemothorax are generally common in patients with a pleural component of disease. The common symptom, such as shortness of breath because of lung compression, is associated with a hemo- or pneumothorax. Another chronic form an extrapulmonary paragonimiasis can further be divided into cerebral, abdominal, subcutaneous, and miscellaneous forms of the disease (Cho et al., 2011). This extrapulmonary paragonimiasis can occur either from the migration of young or mature flukes to various organs or from eggs that enter the circulation and are carried to the various sites, such as the liver, spleen, kidney, brain, intestinal wall, peritoneum, mesenteric lymph nodes, muscle, testis/ovary, subcutaneous tissues, and spinal cord. Although cerebral paragonimiasis usually occurs in less than 1 percent of symptomatic patients, it is the most common extrapulmonary site of infection and is responsible for 50 percent of all extrapulmonary disease (Liu et al., 2008).

Ectopic Paragonimiasis

Quite similar to many other helminths, *Paragonimus* aberrantly migrates to other organs, especially in heavy infections (Nakamura-Uchiyama et al., 2002). The migrating immature parasites may rest in a variety of organs, but the brain is the primary site of ectopic paragonimiasis. Ectopic paragonimiasis has also been reported involving the breast (Fogel and Chandrasoma, 1994), adrenal gland (Hahn et al., 1996), heart and mediastinum (Saborio et al., 1995), and genital organs (Yokogawa, 1965). Ectopic paragonimiasis is an uncommon cause of infertility, probably secondary to fallopian tube obstruction in women and of marked swelling of the scrotum in men (Sadun and Buck, 1960; Harinasuta, 1993).

Cerebral paragonimiasis has two classical manifestations because a few patients present with the signs and symptoms of a meningitis or meningoencephalitis probably as a result of the migration of the worm (Marty and Neafie, 2000) or alternatively most patients present with an expansive, space-occupying lesion in the brain (Kusner and King, 1993). Patients with cerebral paragonimiasis present with a variety of signs and symptoms including headache, vomiting, and seizures, depending on the involved areas of the brain and also have a much poorer prognosis than patients with pleuropulmonary disease. Approximately 70 percent of patients will exhibit personality changes and a decline of cognitive function whereas 15 percent of patients with cerebral paragonimiasis will go into a coma (Marty and Neafie, 2000). Death may occur through herniation, which is caused

by the increase in intracranial pressure as occurs with other space-occupying lesions. Cerebral paragonimiasis is more commonly reported in children than in adults for unknown reasons. Most patients (90 percent) with cerebral paragonimiasis are younger than 30 years old, with 75 percent presenting before the age of 20, and the reported mean age is 15 years (Kusner and King, 1993). The patient's age seems to contribute to the aberrancy of worm migration in some way; in addition to cerebral paragonimiasis, increased hepatic involvement has also been observed in children (Blair et al., 2008). In addition to the brain, the skin is a common site for ectopic lesions of paragonimiasis with patients showing multifocal skin lesions, as well as lung lesions (Ashitani et al., 2000).

Radiological Features of Paragonimiasis

The radiologic features of paragonimiasis reflect the stage of disease and the anatomic location of the parasite. There are two types of pulmonary lesions, pleural and parenchymal; sometimes both may be present. Because of the migration of the parasite to ectopic sites (i.e., the brain or the skins), radiologic changes may be present in these locations, depending on the sensitivity of the imaging technique.

Radiological Features of Pleuropulmonary Paragonimiasis

When the worm starts penetrating the pleural cavity and subsequently gets into the lung early in the disease process, it produces a small and limited pleural , which can be seen in humans with imaging usually done at this stage of disease. Occasionally, however, a massive exudative effusion might occur at this stage (Kagawa, 1997). The penetration of the lung parenchyma and the subsequent migration result in a worm tract, which appears as a linear streak or streaks and localized air space consolidation of around 0.5 to 1.0 cm in diameter (Kagawa, 1997) and are observed in the chest radiograph and are more clearly demonstrated using computed tomography (CT) or magnetic resonance imaging (MRI) scans. Although the burrow tracts are not the most common sign, if found they strongly suggest the diagnosis of paragonimiasis (Singh et al., 1986; Kagawa, 1997). These radiologic changes correlate with the focal hemorrhage and local tissue damage especially caused by parasitic migration. When this migration ceases, a nodule or cystic lesion is formed in the pleura or in the distal aspects of the lungs near the pleura, which is responsible for pleural manifestations of chronic disease, such as pneumothorax and effusions. These pleura-based lesions, whether the result of the presence of early migratory forms or to later chronic disease, have already been described in up to 70 percent of patients with paragonimiasis (Shim et al., 1991; Im et al., 1993; Kagawa, 1997). The most common radiologic findings for patients with pleuropulmonary paragonimiasis are summarized in Table 18.3.

Radiological Features of Cerebral Paragonimiasis

The brain, particularly the cerebral cortex, is the second most common site of *Paragonimus* localization after the lung (Kadota et al., 1989). The changes observed in acute cases of cerebral paragonimiasis are less specific than those found in chronic cases of the disease, but are important to recognize because the disease is still progressing and further brain injury may be curtailed with appropriate therapy.

Table 18.3. Common radiologic features of pleuropulmonary paragonimiasis.

	Frequency (%)	
Radiographic Feature	Average (Number with Finding/ Number Examined for Finding)	Range
Consolidation	58 (123/211)	52–68
Pleural effusions	51 (108/211)	10–66
Cystic lesions	32 (67/211)	13–46
Linear streaks	25 (53/211)	3–41
Nodules	20 (43/211)	8–25
Pleural thickening	16 (33/211)	7–28
Ring shadow	14 (19/135)	3–23
Calcified lesions	6 (4/64)	4–8
Adenopathy	3 (1/39)	Not available; only a single study reported on adenopathy
Normal	8 (11/140)	5–13

Adapted from Kagawa FT. 1997. Pulmonary paragonimiasis. *Semin Respir Infect* 12:149–158.

Table 18.4. The radiologic features of cerebral paragonimiasis.

Disease Stage	Results with the Imaging Technique	
	Skull Film	CT/MRI
Early	Not required	Conglomerated, multiple ring-shaped enhancements with a variable degree of surrounding edema, which appear as a cluster resembling grapes; a minority of patients may have a solitary ring-shaped lesion; the nodules of early cerebral paragonimiasis have iso- or hypointense centers with a hyperintense periphery (T1-weighted image) or with an iso- to hypointense periphery compared with the center (unenhanced T2-weighted image); localized areas of hemorrhage may also be seen with both CT and MRI
Late	Four types of intracranial calcifications: type I, punctuate, amorphous calcified deposits that occasionally contain calcified trabeculae; type II, a spotty arrangement of round nodular calcifications (5–7 mm in diameter) with poor demarcation; type III, a solitary, round, well-defined cystic calcification (10–20 mm in diameter); type IV, congregated, multiple, round-to-oval, cystic calcifications that have a hypodense center in comparison with the periphery (7–30 mm in diameter) (these clustered, calcified cysts resembling "soap bubbles")	CT scans demonstrate multiple round or nodular densely calcified areas correlating the "soap bubble" or type IV calcifications on skull plain films; T1-weighted images are nodules with peripheral low density and central hyperintensity compared with the intensity of the gray matter; T2-weighted images have peripheral regions of low intensity and areas of central high intensity; CT scan may also show large low-density areas, surrounding or connected with the calcified area, and may also demonstrate ventricular dilatation and widening of the cortical sulci; MRI clearly demonstrates these lesions described and may also show areas of surrounding gliosis along with the changes in the cortical sulci more clearly than with the CT scans.

CT, computed tomography; MRI, magnetic resonance imaging.

Early Cerebral Paragonimiasis

Patients with early cerebral paragonimiasis are difficult to recognize because of the absence of calcifications that are readily detected by plain skull films (Li et al., 1992). To detect the early lesions of cerebral paragonimiasis, advanced imaging such as CT or MRI scanning is necessary (*see* Table 18.4) because plain skull films are not found to be useful.

Chronic Cerebral Paragonimiasis

Plain skull films, CT scans, and MRI studies should have been performed for patients with chronic cerebral paragonimiasis (Figures 18.4 and 18.5); the plain skull film is the most likely to be available in resource-limited areas and adequately demonstrates the characteristic cerebral calcifications (Udaka et al., 1988), which consists of multiple, round to oval areas of intracranial calcifications. Four different types of intracranial calcifications (Figure 18.4) in cerebral paragonimiasis have been reported in 1978.

The presence of a Type IV lesion is considered to be pathognomonic of chronic cerebral paragonimiasis (Figures 18.5 and 18.6).

A Type IV lesion, resembling "soap bubbles," is demonstrated by an arrow in Figure 18.6.

The CT scan findings of patients with chronic cerebral paragonimiasis may show multiple round or nodular densely calcified areas, correlating with the Type IV calcifications on plain skull films. Additionally, large low-density areas, surrounding or connected with the calcified area, may also be observed.

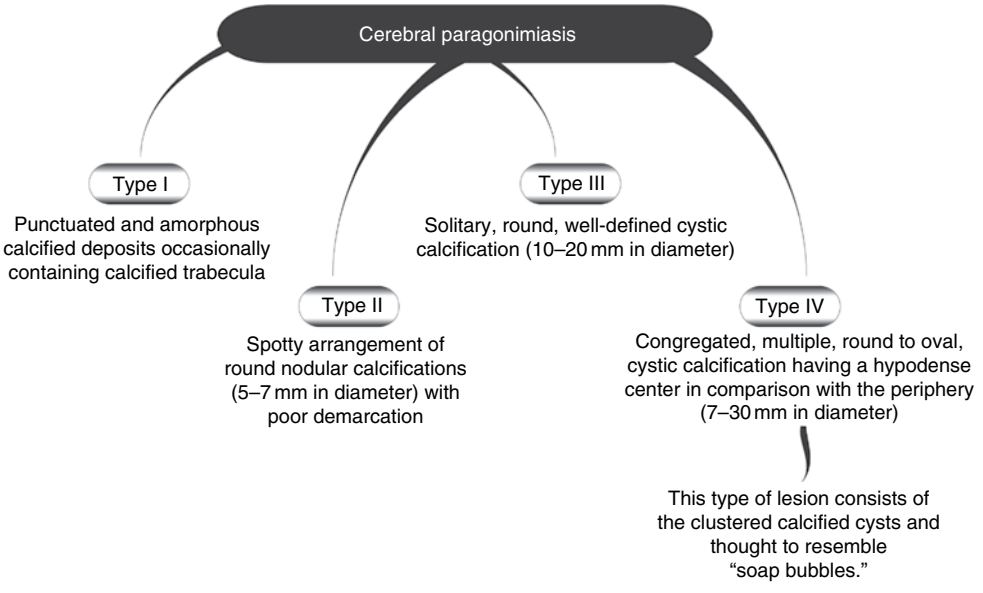

Figure 18.4. Types of intracranial calcifications in cerebral paragonimiasis..

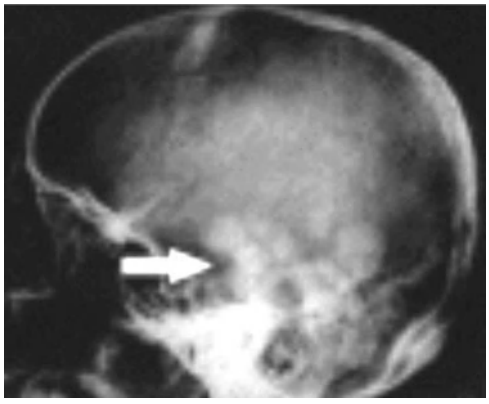

Figure 18.5. Plain skull film of a patient with chronic cerebral paragonimiasis. Arrow, Type IV lesion, which resembles "soap bubbles".

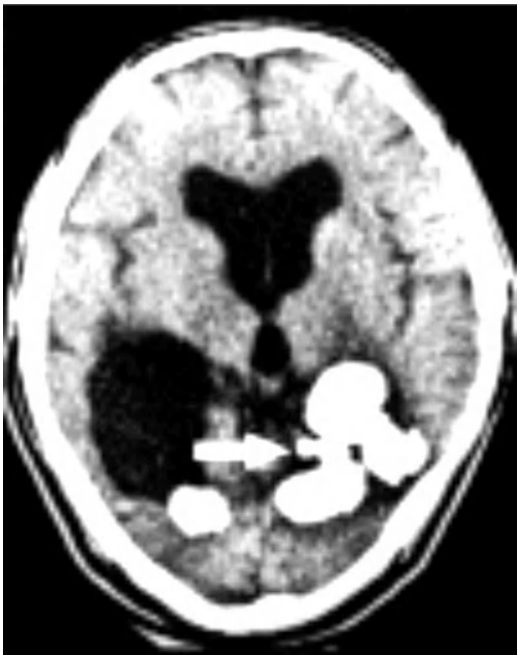

Figure 18.6. Clustered parasitic cysts/elements (*arrow*) are well-delineated in this computed tomography scan of a patient with cerebral paragonimiasis.

Laboratory Diagnosis

General Laboratory Findings

The general laboratory findings are nonspecific but could be useful for the presumptive diagnosis of paragonimiasis made on the basis of clinical and radiologic findings.

A marked increase in eosinophils (eosinophilia) is commonly observed in patients with paragonimiasis, with approximately two-thirds having more than 500 x 109 eosinophils/mm^3 (Nawa, 2000). Similarly, immunoglobulin E (IgE) levels are also frequently elevated in patients with paragonimiasis. Eosinophilia or an increased IgE were present in 80 percent of the 104 patients studied by Uchiyama et al. (1999).

Further, the definitive diagnosis of paragonimiasis is classically made by the detection and demonstration of eggs of *Paragonimus* in sputum, feces, pleural fluid, cerebrospinal fluid (CSF), or pus (Doanh et al., 2011) by microscopy. Unfortunately, the microscopic examination of respiratory and stool specimens is not sensitive. Therefore, in suspected cases of paragonimiasis based on clinical or radiologic diagnosis, a stool examination for parasite eggs should be performed in conjunction with an examination of respiratory secretions, even though the diagnostic yield of a stool examination is low.

For some cases, the species identification for *Paragonimus* can also be made with a high degree of certainty based predominantly on egg morphology. The characteristic features of eggs include egg size, the location of greatest width with respect to the diameter of the egg, the presence of abopercular thickening, and the type of the eggshell (i.e., smooth versus pitted or dimpled). For example, the eggs of *P. kellicotti* can be definitively differentiated by size from those of *P. mexicanus* as the eggs of *P. kellicotti* are average $91.22 \pm 3.60\,\mu m$ in length (range, 82.3–99.8 μm) with a mean width of $56.70 \pm 1.78\,\mu m$ (range, 54.3–61.3 μm), whereas the eggs of *P. mexicanus* are average $74.11 \pm 3.28\,\mu m$ in length (range, 64.8–78.8 μm) with a mean width of $44.45 \pm 1.97\,\mu m$ (range, 38.5–45.5 μm) (Miyazaki and Ishii, 1968a). The thickness of the egg shell of these species also varies as the eggshells of *P. kellicotti* having an average thickness of $2.27 \pm 0.26\,\mu m$ (range, 1.68–2.68 μm), whereas in *P. mexicanus* it averages $1.17 \pm 0.19\,\mu m$ (range, 0.67–1.34 μm) (Miyazaki and Ishii, 1968b). These features are important in differentiating *P. mexicanus* from *P. kellicotti*, which have a similar egg shape (broadest centrally), but it is distinctly different from that of *P. westermani*, which is broadest near the operculum and has more distinct abopercular thickening.

Immunodiagnostics

Antibody Detection

Antibody detection is particularly useful when the clinical suspicion is high, but the eggs cannot be demonstrated in a stool sample. Immunodiagnostic techniques are particularly helpful to assist in the diagnosis of patients with cerebral paragonimiasis, particularly early in the course of disease because antibodies wane with chronicity (Nakamura-Uchiyama et al., 2002). A series of serologic tests have been developed, which slightly vary from one another with respect to sensitivity and specificity, such as immunodiffusion assays, immunoelectrophoretic assays, and monoclonal antibody-based enzyme-linked immunosorbent assays (ELISAs), and have been reported to have 100 percent sensitivity (Nawa, 2000).

For the confirmation of diagnosis, adult parasites must be harvested from experimentally infected animals; hence crude parasite extracts as a source of antigen is difficult to obtain. A monoclonal antibody library has been constructed and *Paragonimus*-specific antibody-producing clones derived from this library are used to capture antigens from the crude extract preparation. These specific antigenic preparations were then used into an ELISA having a sensitivity, specificity, positive predictive value, and negative predictive

value of 100 percent each. ELISAs for the detection of immunoglobulin G (IgG) antibodies or total antibodies against *Paragonimus* are generally preferred by researchers because they are easier to perform, may take longer to become positive following infection (4 to 24 months), and longer to normalize after a cure.

The most accessible immunodiagnostics assay in the United States is an immunoblot assay, which has been offered by the Centers for Disease Control and Prevention (CDC) since 1988 (Slemenda et al., 1988); it targets antibodies directed against 8-kDa antigen of *P. westermani* and has a sensitivity of 96 percent. The antibodies detected in this immunoblot assay, decline more slowly than those detected by the complement fixation test. This assay is highly useful for patients with imported paragonimiasis, which in many instances may be the result of the causative agent, *P. westermani*.

Intradermal testing is another immunodiagnostics method that may be used to identify patients who have or have had paragonimiasis. Although this is a highly sensitive (80 to 90 percent) method, its utility as a diagnostic tool is limited because patients remain positive for years to decades following a cure (Blair et al., 1999), which results in a false-positive reaction. Therefore, it has been successfully used as an epidemiologic survey tool. It can also be used in conjunction with a serologic assay to diagnose active infection (Singh et al., 2004). In this type of application, the intradermal skin test is applied to the population under consideration and then the more specific serologic assay is performed to those individuals showing positive skin test.

Antigen Detection

The antigen detection assay is essentially the reversal of a serologic assay. In this assay rather than using a specific antigen to detect the presence of a specific antibody, an antibody is used for the detection of a particular antigen. This test employs the use of monoclonal antibodies directed against *Paragonimus*-specific epitopes alone or in conjunction with polyclonal antibodies to capture the *Paragonimus*-specific antigens. This application is particularly useful to detect the presence of antigens from a *Paragonimus* species that are shed into the blood, respiratory secretions, or stool.

Molecular Testing

Species-specific polymerase chain reaction (PCR) assays are used for the detection of *Paragonimus* species (Sugiyama et al., 2002) in various specimens. For this purpose, a variety of postamplification methods has been used to characterize the products of broad-range *Paragonimus* genus-generic assays. Traditional DNA sequencing methods has been used to determine the identity of the *Paragonimus* species under investigation (in patient's sample) through comparison with a genetic library of homologous DNA sequences from well-characterized species (Ryu et al., 2000; Iwagami et al., 2003). For such type of analyses, commonly used genetic targets are the internal transcribed spacer (ITS) regions of ribosomal genetic complexes, particularly ITS2, and the mitochondrial cytochrome c oxidase gene. Previously a DNA probe was used for the detection of *Paragonimus* in molecular methods, which could detect as few as five eggs or two metacercaria and was reported to be both highly sensitive and specific (Maleewong, 1997). Although its feasibility has been described, it has not yet been developed into a commonly used or commercially available assay.

Differential Diagnosis

The presence of fever, abdominal pain, and diarrhea observed in the early phase of infection in acute paragonimiasis raise the possibility of the sundry causes of gastroenteritis. These include many viral and bacterial diseases such as acute gastroenteritis caused by Caliciviruses and also by the common bacterial pathogens including *Salmonella*, *Shigella*, *Vibrio*, and *Campylobacter* species. Some gastrointestinal parasites, such as *Giardia*, should also be considered.

The latter aspects of the early stage of infection (i.e., the pleural manifestations with eosinophilia) raise the possibility of other migrating parasites as *Ascaris* and other infectious diseases (e.g., a parapneumonic effusion) must also be differentiated.

In cases with chronic paragonimiasis, cough and hemoptysis, particularly in conjunction with cavitary changes (i.e., cyst production), are common symptoms, which raise the possibility of tuberculosis. Furthermore, many geographic regions where *Paragonimus* is endemic also have high rates of tuberculosis; thus the disease is often misdiagnosed as tuberculosis (Lane et al., 2009) and paragonimiasis is also referred as "nonresponsive tuberculosis" (Blair et al., 2008). To distinguish these, a variety of simple laboratory techniques, such as acid-fast staining for respiratory specimens and *Paragonimus* serologic studies, are an effective means for achieving the correct diagnosis. Although North American paragonimiasis cases were not misdiagnosed as having tuberculosis because of the relatively low incidence of tuberculosis in the United States, the challenge of differentiating tuberculosis from paragonimiasis has occurred in the United States when imported paragonimiasis has been encountered in immigrants (Yee et al., 1992).

Some other causes of lung disease, such as bacterial pneumonia, lung abscess, and echinococcosis, must also be considered in the differential diagnosis of pleuropulmonary paragonimiasis. The elevated levels of eosinophils and IgE raise the possibility of a number of other parasitic diseases that are more common than paragonimiasis in North America, including strongyloidiasis, ascariasis, toxocariasis, and ancylostomiasis (Boe and Schwarz, 2007). Some fungal lung infections, particularly coccidioidomycosis, may also found to be associated with an eosinophilic infiltrate. Elevated IgE levels and eosinophilia may also be observed in patients with bronchopulmonary aspergillosis (Boe and Schwarz, 2007). Finally some noninfectious causes including Churg-Strauss syndrome (i.e., an autoimmune vasculitis that predominantly involves lungs blood vessels), must also be considered in patients with pulmonary disease eosinophilia and elevated IgE levels (Boe and Schwarz, 2007).

Clinical Management

Interventions

In patients with paragonimiasis, supportive treatment is advised depending on the patient's condition. Conservative medical therapy proved to be useful in conditions, such as bronchiectasis, coagulopathies, Goodpasture's syndrome, and acute bronchopulmonary infections. Preparation for other interventions (e.g., endobronchial tamponade, bronchial artery embolization [BAE], or surgery in eligible candidates) should also be undertaken in cases where bleeding fails to respond to conservative measures. Supportive therapy should be applied immediately to all patients with massive hemoptysis. In patients

with massive hemoptysis, surgery was regarded as the treatment of choice in operable patients. An excellent nonsurgical alternative is BAE, which is effective and lacks the mortality and morbidity encountered in surgical interventions. Nevertheless, surgery is recommended in patients with massive hemoptysis caused by thoracic vascular injury, arteriovenous malformation, leaking thoracic aneurysm with bronchial communication, hydatid cyst, and other in conditions during which BAE would be inadequate.

Medications

Three major antihelminthic drugs including praziquantel, bithionol, and triclabendazole are currently available for the treatment of paragonimiasis; among these praziquantel (e.g., Biltricide) is the drug of choice for the treatment of paragonimiasis with a recommended dose of 25 mg per kg body weight three times a day after meals for up to 3 days. There may be a relapse in 2 percent of the cases with the 3-day regimen and thereby a 5-day regimen for almost a 100 percent cure is recommended.

Public Health Importance

Prevention and Control of Paragonimiasis

The control of paragonimiasis in animals is impractical because of the widespread distribution of *Paragonimus* genus in a variety of carnivorous and omnivorous animal hosts. The control of the intermediate hosts as snail and crustacean is also impractical and may have untoward ecological consequences. However, the only thing that can be controlled is human paragonimiasis and to a large extent, through education and changes in customs and food preparation practices. Prevention programs for paragonimiasis should promote more hygienic food preparation by encouraging safer cooking techniques and more sanitary handling of potentially safe, as well as contaminated, seafood. The success to date in substantially reducing paragonimiasis cases in countries that traditionally have had high incidences of infection has been directly related to educational efforts. Interestingly, some areas that are experiencing emergence of disease have been linked with newly defined eating behaviors, such as the consumption of raw meat of wild boar (Blair et al., 2008). The thorough cooking of the crustacean such as crab or crayfish (intermediate host) effectively kills the parasite *Paragonimus*. The emergence of paragonimiasis in Japan because of the consumption of raw boar also indicated the importance of thorough cooking of any potential paratenic host (Blair et al., 2008). Infections with *P. kellicotti* may be avoided by not consuming crayfish or by thorough cooking the crayfish prior to consumption. Most of patients with North American paragonimiasis had a history of eating crayfish, and among them, some reportedly ate them raw, whereas a few reported eating undercooked crayfish. Raw crawfish is also popular in the Mississippi Basin (Diaz, 2011), which was probably the cause of paragonimiasis. Similarly, imported crabs or their meat should also be thoroughly cooked prior to consumption and should never be eaten raw. Alcohol preservation is insufficient to kill the parasite *Paragonimus*; hence crabs pickled in alcohol (i.e., drunken crabs) also pose a danger. Poor food preparatory practices may also lead to paragonimiasis disease, as well as other food-borne illnesses associated with the seafood. Whenever fresh crustaceans are processed with a food processor, the utensils and cutlery boards that have been used should be thoroughly cleaned prior to using these to prepare

any other foods, particularly for those foods that will be consumed without proper cooking (e.g., salads) (Singh et al., 1993; Kagawa, 1997).

In the future, the prevention and control of paragonimiasis will employ previously used highly effective educational methods, and additionally, information should be provided to target populations who are contracting paragonimiasis in unconventional ways (e.g., Japanese hunters, which can contract paragonimiasis through the consumption of raw or undercooked meat from wild boars). Although vaccination is conceivably possible, it will not likely occur in the near future, suggesting more pressing need for vaccinations for more prevalent, medically severe, and economically impactful infections with *Paragonimus* genus (Blair et al., 2008).

References

Amunarriz M. 1991. Intermediate hosts of *Paragonimus* in the eastern Amazonic region of Ecuador. *Trop Med Parasitol* 42:164–166.

Ashitani J, Kumamoto K, Matsukura S. 2000. *Paragonimiasis westermani* with multifocal lesions in lungs and skin. *Intern Med* 39:433–436.

Blair D, Agatsuma T, Wang W. 2008. Paragonimiasis. In: Murrell KD, Fried B, eds., *Food-Borne Parasitic Zoonoses*, vol. 11, pp. 117–150. New York: Springer.

Blair D, Xu ZB, Agatsuma T. 1999. Paragonimiasis and the genus *Paragonimus*. *Adv Parasitol* 42:113–222.

Boe DM, Schwarz MI. 2007. A 31-year-old man with chronic cough and hemoptysis. *Chest* 132:721–726.

Centers for Disease Control and Prevention (CDC). 2012. Paragonimiasis. Accessed September 6, 2012, at http://www.cdc.gov/parasites/paragonimus/.

Chang HT, Wang CW, Yu CF. 1958. Paragonimiasis: A clinical study of 200 adult cases. *Chin Med J* 77:3–9.

Chen MG, Chang ZS, Shao XY, Liu MD, Blair D, et al. 2001. Paragonimiasis in Yongjia County, Zhejiang Province, China: Clinical, parasitological and karyotypic studies on *Paragonimus westermani*. *Southeast Asian J Trop Med Public Health* 32:760–769.

Cho AR, Lee HR, Lee KS, Lee SE, Lee SY. 2011. A case of pulmonary paragonimiasis with involvement of the abdominal muscle in a 9-year-old girl. *Korean J Parasitol* 49:409–412.

Davis GM, Chen CE, Kang ZB, Liu YY. 1994. Snail hosts of *Paragonimus* in Asia and the Americas. *Biomed Environ Sci* 7:369–382.

Diaz JH. 2011. Boil before eating: Paragonimiasis after eating raw crayfish in the Mississippi River Basin. *J La State Med Soc* 163:261–266.

Doanh PN, Dung doT, Thach DT, Horii Y, Shinohara A, Nawa Y. 2011. Human paragonimiasis in Viet Nam: Epidemiological survey and identification of the responsible species by DNA sequencing of eggs in patients' sputum. *Parasitol Int* 60:534–537.

Fogel SP, Chandrasoma PT. 1994. Paragonimiasis in a cystic breast mass: Case report and implications for examination of aspirated cyst fluids. *Diagn Cytopathol* 10:229–231.

Hahn ST, Park SH, Kim CY, Shinn KS. 1996. Adrenal paragonimiasis simulating adrenal tumor—a case report. *J Korean Med Sci* 11:275–277.

Harinasuta T, Pungpak S, Keystone JS. 1993. Trematode infections. Opisthorchiasis, clonorchiasis, fascioliasis, and paragonimiasis. *Infect Dis Clin N Am* 7:699–716.

Im JG, Kong Y, Shin YM, Yang SO, Song JG, et al. 1993. Pulmonary paragonimiasis: Clinical and experimental studies. *Radiographics* 13:575–586.

Iwagami M, Monroy C, Rosas MA, Pinto MR, Guevara AG, et al. 2003. A molecular phylogeographic study based on DNA sequences from individual metacercariae of *Paragonimus mexicanus* from Guatemala and Ecuador. *J Helminthol* 77:33–38.

Kadota T, Ishikura R, Tabuchi Y, Nakao N, Fujikawa H, et al. 1989. MR imaging of chronic cerebral paragonimiasis. *Am J Neuroradiol* 10:S21–S22.
Kagawa FT. 1997. Pulmonary paragonimiasis. *Semin Respir Infect* 12:149–158.
Keiser J, Utzinger J. 2005. Emerging foodborne trematodiasis. *Emerg Infect Dis* 11:1507–1514.
Kim DC. 1984. *Paragonimus westermani*: Life cycle, intermediate hosts, transmission to man and geographical distribution in Korea. *Arzneimittelforschung* 34:1180–1183.
Kusner DJ, King CH. 1993. Cerebral paragonimiasis. *Semin Neurol* 13:201–208.
Lane MA, Barsanty MC, Santos CA, Yeung M, Lubner SJ, et al. 2009. Human Paragonimiasis in North America following Ingestion of Raw Crayfish. *Clin Infect Dis* 49:e55–e61
Li HZ, Xie FW, Sun SC. 1992. CT findings in "fresh" cerebral paragonimiasis. *No Shinkei Geka* 20:91–97.
Liu Q, Wei F, Liu W, Yang S, Zhang X. 2008. Paragonimiasis: An important food-borne zoonosis in China. *Trends Parasitol* 24:318–323.
Maleewong W. 1997. Recent advances in diagnosis of paragonimiasis. *Southeast Asian J Trop Med Public Health* 28(Sup. 1):134–138.
Malek EA. 1980. *Paragonimiasis*, Vol. II. Boca Raton: CRC Press.
Marty AM, Neafie RC. 2000. Paragonimiasis. In: Myers WM, Neafie RC, Marty AM, Wear DJ, eds., *Pathology of Infectious Diseases*, vol. I, pp. 49–67. *Helminthiases*. Washington, DC: Armed Forces Institute of Pathology.
Miyazaki I, Ishii Y. 1968a. Comparative study of the Mexican lung flukes with *Paragonimus kellicotti* Ward, 1908. *J Parasitol* 54:845–846.
Miyazaki I, Ishii Y. 1968b. Studies on the Mexican lung flukes, with special reference to a description of *Paragonimus mexicanus* sp.n. *Jpn J Parasitol* 17:445–453.
Mukerjee CM, Simpson SE, Bell RJ, Walker JC. 1992. Pleuropulmonary paragonimiasis in a Laotian immigrant to Australia. *Chest* 101:849–851.
Nakamura-Uchiyama F, Mukae H, Nawa Y. 2002. Paragonimiasis: a Japanese perspective. *Clin Chest Med* 23:409–420.
Nana A, Bovornkitti S. 1991. Pleuropulmonary paragonimiasis. *Semin Respir Med* 12:46–54.
Narain K, Devi RK, Mahanta J. 2003. Paragonimus and paragonimiasis: A new focus in Arunachal Pradesh, India. *Curr Sci* 84:985–987.
Nawa Y. 2000. Re-emergence of paragonimiasis. *Intern Med* 39:353–354.
Pachucki CT, Levandowski RA, Brown VA, Sonnenkalb BH, Vruno MJ. 1984. American Paragonimiasis treated with praziquantel. *New Eng J Med* 311:582–583.
Pechman RD Jr. Pulmonary paragonimiasis in dogs and cats: A review. 1980. *J Small Anim Pract* 21:87–95.
Ripert C, Couprie B, Moyou R, Gaillard F, Appriou M, et al. 1992. Therapeutic effect of triclabendazole in patients with paragonimiasis in Cameroon: A pilot study. *Trans R Soc Trop Med Hyg* 86:417.
Ryu JS, Hwang UW, Min DY, Shin KS, Nam SJ, et al. 2000. Molecular identification of *Paragonimus ohirai* and *P. westermani* from Anhui Province, China. *Parasite* 7:305–309.
Saborio P, Lanzas R, Arrieta G, Arguedas A. 1995. *Paragonimus mexicanus pericarditis*: Report of two cases and review of the literature. *J Trop Med Hyg* 98:316–318.
Sadun EH, Buck AA. 1960. Paragonimiasis in South Korea-immunodiagnostic, epidemiologic, clinical, roentgenologic and therapeutic studies. *Am J Trop Med Hyg* 9:562–599.
Shih YC, Ch'En YH, Chang YC. 1958. Paragonimiasis of central nervous system. Observations on 76 cases. *Chin Med J* 77:10–19.
Shim YS, Cho SY, Han YC. 1991. *Pulmonary paragonimiasis*: a Korean perspective. *Semin Respir Med* 12:35–45.
Singh TN, Singh HR, Devi KS, Singh NB, Singh YI. 2004. Pulmonary paragonimiasis. *Indian J Chest Dis Allied Sci* 46:225–227.
Singh TS, Mutum S, Razaque MA, Singh YI, Singh EY. 1993. Paragonimiasis in Manipur. *Indian J Med Res* 97:247–252.

Singh TS, Mutum SS, Razaque MA. 1986. Pulmonary paragonimiasis: clinical features, diagnosis and treatment of 39 cases in Manipur. *Trans R Soc Trop Med Hyg* 80:967–971.
Singh TS, Sugiyama H, Umehara A, Hiese S, Khalo K. 2009. *Paragonimus heterotremus* infection in Nagaland: A new focus of paragonimiasis in India. *Indian J Med Microbiol* 27:123–127.
Singh YI, Singh NB, Devi SS, Singh YM, Razaque M. 1982. Pulmonary paragonimiasis in Manipur. *Indian J Chest Dis Allied Sci* 24:304–306.
Slemenda SB, Maddison SE, Jong EC, Moore DD. 1988. Diagnosis of paragonimiasis by immunoblot. *Am J Trop Med Hyg* 39:469–471.
Sugiyama H, Morishima Y, Kameoka Y, Arakawa K, Kawanaka M. 2004. *Paragonimus ohirai metacercariae* in crabs collected along the Arakawa River in Tokyo, Japan. *J Vet Med Sci* =66:927–931.
Sugiyama H, Morishima Y, Kameoka Y, Kawanaka M. 2002. Polymerase chain reaction (PCR)-based molecular discrimination between *Paragonimus westermani* and *P. miyazakii* at the metacercarial stage. *Mol Cell Probes* 16:231–236.
Uchiyama F, Morimoto Y, Nawa Y. 1999. Re-emergence of paragonimiasis in Kyushu, Japan. *Southeast Asian J Trop Med Public Health* 30:686–691.
Udaka F, Okuda B, Okada M, Tsuji T, Kameyama M. 1988. CT findings of cerebral paragonimiasis in the chronic state. *Neuroradiology* 30:31–34.
World Health Organization (WHO). 1995. Control of foodborne trematode infections. Report of a WHO. Study Group. *WHO Tech Rep Ser* 849:1–157.
Xu ZB. 1991. Studies on clinical manifestations, diagnosis and control of paragonimiasis in China. *Southeast Asian J Trop Med Public Health* 22:345–348.
Yee B, Hsu JI, Favour CB, Lohne E. 1992. Pulmonary paragonimiasis in Southeast Asians living in the central San Joaquin Valley. *West J Med* 156:M423–M425.
Yokogawa M. 1965. Paragonimus and paragonimiasis. *Adv Parasitol* 3:99–158.
Zhong HL, He LY, Xu ZB, Cao WJ. 1981. Recent progress in studies of Paragonimus and paragonimiasis control in China. *Chin Med J* 94:483–494.

Chapter 19
Melioidosis

Introduction

Melioidosis is the etiological designation of a spectrum of clinical manifestations caused by the gram-negative bacillus *Burkholderia pseudomallei*. This organism was previously classified under the genus *Pseudomonas* because of similarities in culture, morphological characteristics, and biochemical properties with the *Pseudomonas* species. It is now assigned to a new genus, *Burkholderia* along with other members of ribosomal RNA homology group II (Yabuuchi et al., 1992). The bacterium *B. Pseudomallei* was first isolated in 1911 by Captain Alfred Whitmore (1913), a British pathologist at Rangoon General Hospital in Burma, and his assistant C. S. Krishnaswami, when they were treating a young boy dying with pneumonia. Hence, the disease is also called as Whitmore's disease. In 1921, Stanton and Fletcher (1921) coined the term *melioidosis*, which is originally derived from the Greek *melis* (μηλις) meaning "a distemper of asses" with the suffixes *-oid* meaning "similar to" and *-osis* meaning "a condition," that is, a condition similar to glanders disease. It is also referred to as Nightcliff gardener's disease because Nightcliff, a suburb of Darwin, Australia, is an endemic home of melioidosis (Barker, 2005). The organism *B. pseudomallei* lives below the soil's surface during the dry season but after heavy rainfall resides in surface water and mud and may become airborne further. It usually gains entry in the host body via cuts and sores in the skin or via inhalation of dust or droplets; rarely it enters the host body by ingestion of contaminated water. The disease has also been found among some domestic and farm animals and usually does not spread from one person to another or from animals to humans. It is found mainly in the tropical areas throughout the world, particularly in Southeast Asia and northern Australia. In Australia, cases typically occur in the Top End of the Northern Territory (NT), in far north Queensland, and in the Kimberley region of Western Australia. The disease has also been found associated with natural disasters, such as floods (Apisarnthanarak et al., 2012). Clinical symptoms of melioidosis depend on the

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

site of the infection, which often starts as a chest infection with a productive cough and difficult breathing. Other possible presentations including fever with headache and confusion, or pain or difficulty passing urine. Following infection, people can become ill from 1 to 21 days after being infected, and the onset of symptoms may be sudden or gradual. The infection can be fatal, and the patient requires urgent medical attention and treatment with specific antibiotics.

Epidemiology

Melioidosis is endemic to Southeast Asia and northern Australia; sporadic cases are observed in Central and South America (Brilhante et al., 2012). The worldwide epidemiology of melioidosis has been comprehensively studied by Dance (1991, 2000). Dance noted that because of the unavailability of appropriate culture facilities in most of the rural tropics where the infection is likely to be prevalent, the published case reports and series represented only the "tip of the iceberg" (Dance, 1991).

Krishnaswami documented melioidosis in 5 percent of all autopsy deaths in 1917, the only reported cases from Burma since 1945 have been in travelers (Hsueh et al., 2001). Similarly, Thailand and Australia, from where the highest rates of disease are noted, did not record any cases until 1947 and 1950, respectively (Dance, 1991). Other anomalies that may be associated to incomplete ascertainment include a high serological prevalence (7 percent) of melioidosis in returning US troops stationed in Vietnam (Clayton et al., 1973), but a low disease rate in the indigenous population (Parry et al., 1999).

Melioidosis in the Australia-Pacific Region

In Australia, the disease was first recognized from an outbreak in sheep in 1949 in Winton, northern Queensland (Cottew, 1950). The first human case described was a patient with diabetes who died from septicemic melioidosis in Townsville in 1950 (Rimington, 1962), and in Northern Territory the first case was reported in 1960 (Crotty et al., 1963). This late emergence of disease in northern Australia suggests that *B. pseudomallei* may have colonized Australia from Southeast Asia (Fournier, 1965). Although, the area where melioidosis is declared endemic has generally been regarded as restricted to the latitudes 20° S and 20° N in Southeast Asia and northern Asia (Leelarasamee and Bovornkitti, 1989), large outbreaks have occurred outside this area in Australia, including the first case in Winton (22° S) and 159 cases of melioidosis in pigs older than 3 years in the Burnett River region (25.5° S), which was attributed to a contaminated water supply (Ketterer et al., 1986). In contrast to many other countries of the world where the disease is declared endemic, in Australia most patients are from remote locations but are then transported to referral hospitals in the Top End region of the Northern Territory, the Kimberley region of Western Australia, and far north Queensland and the Torres Strait for management.

Melioidosis in Thailand

The annual incidence of 4.4 melioidosis cases per 100,000 persons studied was reported in Ubon Ratchathani province in Northeast Thailand (Suputtamongkol et al., 1994). Other centers in northeast Thailand, such as Khon Kaen, Nakhon Ratchasima, Buri

Ram, and Udon Thani, also reported large numbers of patients. In a national survey conducted in Thailand, 30 of the 125 hospitals did not have microbiological facilities, and from1994 to 1995, the annual number of isolates was more than 1,100, which probably represents a conservative estimate of the number of cases of melioidosis in Thailand (Leelarasamee et al., 1997). *B. pseudomallei* is widely distributed in soil, particularly in pooled surface water, such as in rice paddies in Thailand (Finkelstein et al., 2000); however, the rate of the closely related but less virulent *Burkholderia thailandensis*, which had previously been recognized as *B. pseudomallei*, may account for the variation in disease throughout the country (Trakulsomboon et al., 1999). The ratio of *B. pseudomallei* to *B. thailandensis* found in soil, which is highest in the northeast, matches rates of clinical *B. pseudomallei* isolation throughout the country (Leelarasamee et al., 1997). These findings along with the possibility of the existence of other, less virulent strains of *Burkholderia* spp. may also account for the much higher rates of seropositivity as seen in Thailand (Kanaphun et al., 1993), compared to the areas of endemicity of northern Australia.

Melioidosis in Vietnam

Melioidosis was first observed in southern Vietnam by Pons and Advier (1927). Large numbers of French, and later US, troops based in Vietnam, were exposed to environmental *B. pseudomallei* and had access to modern clinical and laboratory services; therefore, many cases were described from the 1940s to the 1970s (Dance, 1991). These cases continued to be observed in returning servicemen for up to 29 years following exposure (Chodimella et al., 1997); further, sporadic cases in Vietnamese emigrants and returned travelers to other countries also occurred (Heyse et al., 2003). However, recent attempts at systematic surveillance have not observed significant proportions of *B. pseudomallei* in blood culture isolates or soil around Ho Chi Minh City (Parry et al., 1999), although it is likely to be found elsewhere in the country.

Melioidosis in Malaysia and Indonesia

Stanton and Fletcher (1932) observed animal cases at the Institute of Medical Research of the Federated Malay States in 1913 and then published reports of subsequent human and animal cases. Puthucheary et al. (1992) reviewed 50 septicemic melioidosis cases in 1992 at a single referral center in Kuala Lumpur and reported a total of 85 cases from 1976 to 1991. Similarly some sporadic cases of melioidosis have been reported in the Dutch literature for many years from Indonesia (Beeker et al., 1999), in addition to cases exported to Australia (Grosskopf, 2000).

Melioidosis in Singapore

Since 1989 in Singapore, melioidosis has been a disease that should be reported and has an annual incidence rate of 1.7. The majority of melioidosis cases were documented in between 1989 and 1996; most of them (89 percent, or 337 cases) were culture-confirmed cases (Heng et al., 1998). In 2004, approximately 57 cases were reported, with an unusually higher case fatality rate of 40 percent, which was attributed to abnormally heavy rains and flooding. The fatality rate for patients with severe melioidosis otherwise appears in line with those in more developed countries (Chan et al., 2003). Serological surveys conducted in Singapore have consistently demonstrated a low rate of seropositivity

(0.2 percent in the general population and 1.6 percent in construction workers), except in immigrants from Thailand or Malaysia (Heng et al., 1998).

Melioidosis in China, Hong Kong Special Administrative Region, and Taiwan

A few cases of locally acquired melioidosis have been observed in Hong Kong (So et al., 1984; Tsang and Lai, 2001), and a seroprevalence of 14 percent was demonstrated by indirect hemagglutination assay (IHA) in a tuberculosis sanatorium (So et al., 1987). In Taiwan, some sporadic cases have been described; most of them were autochthonous (Hsueh et al., 2001; Luo et al., 2003; Ben et al., 2004). On mainland China, *B. pseudomallei* has been isolated from 4.2 percent of soil as well as water specimens in Hainan Island and adjoining coastal provinces (Yang et al., 1995).

Melioidosis in Other Parts of Asia

Until 1990, only two or three foreigners developed melioidosis subsequent to travel to India as reported in the medical literature, indicating the presence of *Burkholderia* on the Indian soil (Thurnheer et al., 1988). In 1991, a child case of melioidosis was reported in Maharashtra district of India (Raghavan et al., 1991), and then in 1993, a confirmed case of melioidosis was reported from Kerala district (John et al., 1996). Melioidosis is suspected to be widespread in India as human cases of melioidosis have been reported from several Indian states including Maharashtra, Kerala, Orissa, Tripura, Tamilnadu, West Bengal, and Assam.

Few melioidosis cases have been described in Sri Lanka (Van Peenen et al., 1976). Some sporadic cases of melioidosis have been reported in travelers returning from Bangladesh (Kibler et al., 1991; Dance et al., 1999). Although the disease was reported in up to 10 percent of autopsy death cases in Rangoon, Burma, since 1945, the only cases reported were one in a Dutch traveler (Leeuwenburgh et al., 2002) and a second possible exported case of *Burkholderia* in a Taiwanese traveler (Hsueh et al., 2001).

Melioidosis in Areas Outside Asia

Most cases of melioidosis reported outside Southeast Asia are from travelers to areas of endemicity; this requires clinical awareness regarding the disease worldwide. However, sporadic cases of melioidosis have been reported throughout the world, including west and east Africa, Caribbean, Central and South America, and Middle East (Dance, 1991, 2000).

The Etiological Agent: *Burkholderia Pseudomallei*

Morphological and Cultural Characteristics

B. pseudomallei are aerobic, motile rod-shaped bacteria appearing as gram-negative bacilli with bipolar staining and are vacuolated and slender having rounded ends showing a "safety pin" appearance. It infects humans and animals and causes the disease melioidosis and is also capable of infecting plants (Lee et al., 2010). The bacterium is 2 to 5 μm in length and 0.4 to 0.8 μm in diameter and has flagella; thereby it is capable of

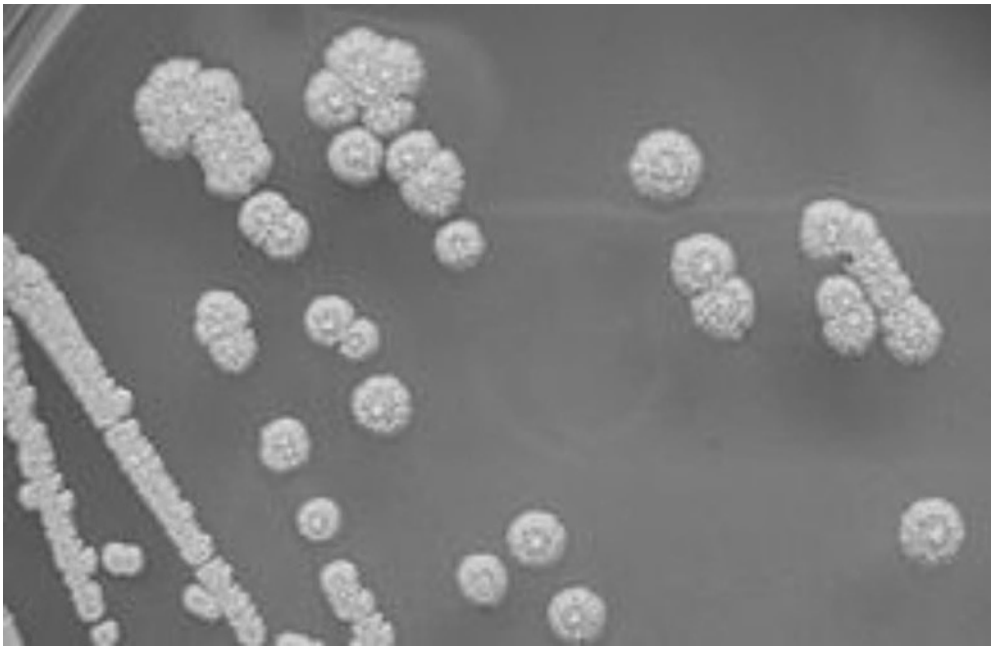

Figure 19.1. *Burkholderia pseudomallei* colonies on Ashdown's agar showing characteristic cornflower head morphology.

self-propulsion. It can grow in a number of artificial nutrient environments, especially betaine- and arginine-containing medium. The optimal temperature for growth is around 40° C in neutral pH or slightly acidic environments (pH 6.8–7.0). The majority of strains are homofermentive; hence, it ferments sugars without gas formation (most importantly, glucose and galactose; older cultures are capable of metabolizing maltose and starch also). Bacteria produce both exotoxins and endotoxin and can remain dormant intracellularly for many years before emerging as an active infection.

B. pseudomallei is not fastidious and will grow on a large variety of culture media (blood agar, MacConkey agar, EMB, etc.). Ashdown's medium (or *Burkholderia cepacia* medium) (Figure 19.1) is generally used for the selective isolation of *B. pseudomallei* (Peacock et al., 2005). Cultures typically become positive in 24 to 48 hours (this rapid growth rate differentiates the organism from *Burkholderia mallei*, which takes minimum 72 hours to grow properly). Colonies are wrinkled with a metallic appearance and possess an earthy odor. On Gram staining, the organism appears as a gram-negative rod with a characteristic safety pin–like appearance (bipolar staining). For environmental specimens, an arabinose test is necessary to differentiate from the nonpathogenic *B. thailandensis*, which has never been isolated from clinical specimens (Chaiyaroj et al., 1999).

Distribution

B. pseudomallei is ubiquitous throughout Southeast Asia, northern Australia, and the South Pacific. It is predominantly distributed in tropical and subtropical area with "hyperendemicity" in the Top End of the Northern Territory of Australia and northeast

Thailand. The true boundaries of its endemicity are ambiguous probably because of the movement of the organism and its ability to travel to and exist in temperate climatic regions (southwest Australia and France), where it may cause sporadic disease outbreaks. *B. pseudomallei* have been introduced to new areas with the export of animals, and shipments of contaminated soil and water could produce the same results. Possible autochthonous melioidosis has also been reported from India, Pacific islands, Central and South America, the Caribbean, Africa, and the Middle East.

Pathogenecity and Virulence Factors

B. pseudomallei is a facultative intracellular pathogen because of its ability to invade, resist serum factors, and survive intracellularly. It can easily be contracted by inhaling dust containing the bacterial cells or by having contact between contaminated soil and cuts or scrapes of the skin. *B. pseudomallei* produces a glycocalyx polysaccharide capsule, which is an important virulence determinant (Steinmetz et al., 1995). This capsule is also referred to as biofilm or "slime," and allows for the formation of microcolonies in a protective environment in which the organism is phenotypically altered, resulting in significant antibiotic resistance (Vorachit et al., 1993). *B. pseudomallei* is considered an "accidental pathogen," that is, an environmental organism that does not need to pass through an animal host to replicate; therefore, human infection is an evolutionary dead end for the parasite (Nandi et al., 2010). Strains associated with human disease differ from those causing disease in animals because it possesses certain genomic islands (Sim et al., 2008). It may have the ability to cause disease in humans because of the DNA it has acquired from other microorganisms (Sim et al., 2008) with a high mutation rate because the organism continues to evolve even after infecting the host (Price et al., 2010). *B. pseudomallei* is capable of polymerizing actin and to spread from cell to the cell where it mediates cell fusion and the formation of multinucleate giant cells (Kespichayawattana et al., 2000). It also expresses a toxin designated as lethal factor 1 (Cruz-Migoni et al., 2011). *B. pseudomallei* is one of the first proteobacteria and has been identified as containing an active Type 6 secretion system. It is the only organism identified containing up to six different Type 6 secretion systems (Shalom et al., 2007). Many cell-associated antigens of *B. pseudomallei* have been reported to be immunogenic in patients with melioidosis, including capsular polysaccharide (CPS), lipopolysaccharide (LPS) (Ho et al., 1997), and flagellin proteins (DeShazer et al., 1997) etc. Capsular polysaccharide also has a role in environmental protection (Kanai and Kondo, 1994), immune system evasion (Puthucheary et al., 1996), and attachment to host epithelial cells (Ahmed et al., 1999).

The pathogenesis of melioidosis is not completely defined because the clinical picture and outcome of infection with *B. pseudomallei* depends on the balance between the host's immune system, the virulence of the infecting strain, and the size and route of administration of the initial inoculum. *B. pseudomallei* acts as an opportunistic pathogen, which implies that host response is critical in determining the outcome of infection. Little is known about the specific immunologic mechanisms responsible for protection, although the cell-mediated immune response is probably important. Interferon-γ plays a key role in controlling melioidosis as reported in inbred mouse. Some in vitro studies have suggested that along with the natural-killer cells, CD8 + T cells, activated by a cytokine-dependent bystander mechanism, are the most important sources of the rapid production of IFN-γ that occurs in the innate response to *B. pseudomallei* and probably other intracellular pathogens (Lertmemongkolchai et al., 2001).

Resistance

Similar to many other saprophytic organisms, *B. pseudomallei* is a resilient bacterium and can survive in a variety of hostile conditions, including nutrient deficiency, acid and alkali pH, disinfectant and antiseptic solutions (including detergents and chlorine), exposure to many antibiotics, and extremes of temperature. It is also well adapted to many different hosts, producing proteases, lipases, lecithinase, catalase, peroxidase, superoxide dismutase, hemolysins, a cytotoxic exolipid, and a siderophore (iron chelators). It is resistant to complement, lysosomal defensins and cationic peptidases and can survive within many eukaryotic cells, including phagocytic cells such as neutrophils and macrophages (White, 2003). On antibiotic sensitivity testing, the organism appears highly resistant (it is innately resistant to a large number of antibiotics, including colistin and gentamicin), which again differentiates it from *B. mallei*, which is in contrast, exquisitely sensitive to a large number of antibiotics.

Clinical Manifestations

Melioidosis usually occur as a subclinical infection, localized infection (such as cutaneous), pneumonia, meningoencephalitis, sepsis, or chronic suppurative infection. It may mimic tuberculosis, with fever, weight loss, productive cough, and upper lobe infiltrate, with or without cavitation. The incubation period is generally 1 to 21 days, although it may extend for up to months or years. In cases that a high dose of bacterium gains entry, symptoms can develop within few hours. More than 50 percent cases of melioidosis present with pneumonia, and without appropriate treatment, case-fatality ratio may reach 90 percent within 48 hours of developing symptoms. Morbidity and mortality of melioidosis are higher in people with underlying diseases, such as diabetes mellitus, renal dysfunction, chronic pulmonary disease, or compromised or weakened immune system; however, HIV infection does not appear to be a major risk factor for developing melioidosis.

Route of Infection

Humans and animals (sheep, goats, and horses) acquire the *Burkholderia* infection by inhalation of dust, ingesting contaminated water, and contact with contaminated soil especially through skin abrasions. The relative contribution of each of the three modes of acquisition (inhalation, ingestion, and inoculation) is not clear. The dose of the inoculum and the route of administration are also responsible for the pattern and severity of disease. Inoculation is now considered the major mode of acquisition. Rice farmers acquire minor wounds in their feet during the planting and harvesting seasons, when farmers spend most of the working day wading in mud and surface water; inoculation, at the time of a snake bite, has also been described (Chaowagul et al., 1989).

Melioidosis can also spread from one person to another by contact with the body fluids of an infected person. The infection can also be epizootic because sheep, horses, goats, and swine can harbor the infection; however, animals are unlikely to be the primary reservoirs of infection. Person-to-person transmission can include both in a sibling of a child with cystic fibrosis (Holland et al., 2002) and through sexual transmission from a returned serviceman to his partner (McCormick et al., 1975). Neonatal cases suggest

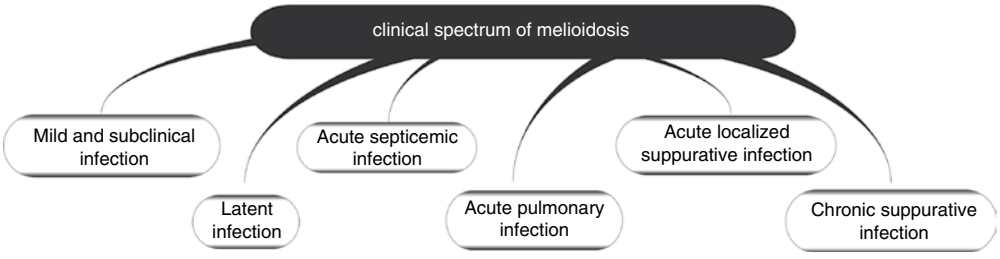

Figure 19.2. Clinical spectrum of melioidosis.

perinatal transmission (Halder et al., 1998), whereas vertical transmission has been proven on only one occasion in humans (Abbink et al., 2001). Transplacental spread has also been documented in goats (Low Choy et al., 2000).

Risk Factors

An important risk factor for melioidosis is the presence of underlying disease, which is generally reported in up to 76 percent of cases (Puthucheary et al., 1992). Patients with diabetes mellitus particularly have a high incidence of melioidosis, with up to 60 percent of patients having preexisting or newly diagnosed type 2 diabetes (Currie et al., 2000). Apart from diabetes, the other risk factors for the development of melioidosis include chronic renal disease, excess alcohol consumption, malignancy, connective tissue diseases, and chronic lung disease and therapy. Although the reason for these specific risk factors is not yet clear, it may be attributed to the effect of these comorbidities on neutrophil function (Currie et al., 2000), which is important in the pathogenesis of melioidosis (Jones et al., 1996).

The use of steroids has also been found to be associated with melioidosis cases, which includes steroid-containing herbal remedies (yaa chud). In Thailand herbal use has been documented in up to 10 percent of Thai patients (Suputtamongkol et al., 1999).

The Clinical Spectrum

The clinical spectrum of melioidosis (Figure 19.2) is extremely broad, and melioidosis has been referred to as "the remarkable imitator" (Poe et al., 1971). It may be recognized as inapparent infection, acute localized suppurative infection, acute pulmonary infection, acute septicemic infection, or chronic suppurative infection. The incubation period has not been defined clearly; however, based on the development of infection after injury it may be as short as 2 days. Clinically inapparent infection may remain latent for a number of years after a person leaves an endemic area and before apparent disease ensures.

Mild and Subclinical Infections

In some patients, infection with *B. pseudomallei* may present minimal symptoms or an asymptomatic stage, resulting in a chronic carrier state. In these patients, the immune system suppress the infection and no clinical disease develops; the patient may become asymptomatic for a longer time periods.

Latent Infections

Melioidosis is an unusual bacterial infection, probably because of its long period of latency (up to 29 years) before the disease becomes clinically apparent (Chodimella et al., 1997). Relapses usually occur during intercurrent stress (e.g., other acute infection, burns or trauma, malignancies) when cellular immunity is likely to be suppressed. Patients with latent melioidosis may be symptom free for decades; for an example, the longest period between presumed exposure and clinical presentation is approximately 62 years (Ngauy et al., 2005).

Acute Septicemic Infections

Most of patients with melioidosis present clinically community-acquired sepsis syndrome, with a short duration (median, 6 days, range, 1 day–2 months) of high fever and rigors, although some showing a less acute, typhoidal illness with a swinging fever, often associated with profound weight loss (Chaowagul et al., 1989). Approximately 50 percent patients have evidence of a primary focus of infection, usually in the lung or skin and subcutaneous tissues. In cases of bacteremia, patients showing complicating pneumonitis symptoms include disorientation, extreme dyspnea, and severe cutaneous pustular lesions on the head, trunk, or extremities. Confusion, stupor jaundice, muscle tenderness, and diarrhea may also be prominent features. Chest examination does not show any signs; or rales and rhonchi or pleural rubs may be heard. In this type of infection, the liver and spleen may be palpable, and there might be the development of arthritis or meningitis. As soon as the septic shock has supervened, the mortality approaches 95 percent with many patients dying within 48 hours of hospital admission.

Acute Pulmonary Infection

It is the commonest form of disease representing a primary pneumonitis or hematogenous pneumonitis as a manifestation of septicemic form. The onset of the disease may be abrupt without prodromal symptoms or more gradual with headache, anorexia, and generalized myalgia with fever in almost all patients. The illness is characterized by a nonproductive cough or productive cough with nonspecific sputum or hemoptysis. Laboratory findings show total leukocyte counts ranging from normal to 20,000/mm^3, mild normochromic, normocytic anemia, and an increase in the concentration of C-reactive protein (>5 mg/ dL). The pneumonia is generally a subacute cavitating pneumonia accompanied by profound weight loss and is often confused with tuberculosis. More severe complications include pneumothorax, empyema, and purulent pericarditis, which ultimately progresses to septicemia.

Acute Localized Suppurative Infection

This type of infection might occur through inoculation of a break in the skin, resulting in a nodule with an area of acute lymphangitis, and regional lymphadenitis, which further may rapidly progress to the acute septicemic form.

Chronic Suppurative Infection

In some patients with melioidosis, secondary abscesses dominate the clinical picture, which involves organs such as skin, brain, lung, myocardium, liver, spleen, bones, joints,

lymph nodes, and even the eye. The presence of an abscess particularly if associated with a splenic abscess is indicative of immediate treatment.

Laboratory Diagnosis

Melioidosis should be considered in the patient with any febrile illness who has been in an endemic area, especially if the presenting features are those of fulminant respiratory failure, with multiple abscesses, or if there is a radiological pattern of tuberculosis, but tubercle bacilli cannot be demonstrated by acid-fast staining. Melioidosis can only be diagnosed by the isolation of *B. pseudomallei* from the blood, urine, sputum, or skin lesions or by detecting and measuring antibodies to the bacteria in the blood. Bacteriological diagnosis can be done by microscopic examination of gram-stained preparations from skin lesions or sputum if showing small gram-negative bacilli with specific bipolar staining. In all suspected cases additional staining with Wright's stain and methylene blue also found to be useful.

Principal clues to spot *B. pseudomallei* in a specimen culture include a sweet putrefactive smell of in fresh culture, wrinkled appearance of older colonies, demonstration of an oxidase-positive, or gram-negative bacillus with bipolar or irregular staining, which is resistant to aminoglycosides and older-generation penicillins and cephalosporins.

Isolation, Culture, and Microscopic Examination

Bacterial identification by culturing is the gold standard for the specific diagnosis of *B. pseudomallei*. Ashdown/Francis medium-containing aminoglycoside can be used to culture nonsterile specimens, whereas blood agar or chocolate agar media is preferred for sterile specimens. On MacConkey's agar media, *B. pseudomallei* appears as pink, rugose colonies with a metallic sheen (Figure 19.3). Throat swab has been reported to have 100 percent specificity with 38 and 47 percent sensitivity in adult and pediatric patients, respectively.

Figure 19.3. Growth of *Burkholderia pseudomallei* on MacConkey agar (appears as pink, rugose colonies with a metallic sheen).

Microscopic examination of exudate will reveal bipolar or unevenly stained gram-negative rods, but it has a low specificity and sensitivity. Direct immunofluorescent microscopy may prove to be helpful in endemic areas, but it is not widely available (Walsh et al., 1994)

Serological Identification

B. pseudomallei can easily be identified serologically by agglutination tests (rapid slide agglutination or tube agglutination test). In endemic areas of melioidosis, enzyme-linked immunosorbent assay (ELISA) is also available, based on monoclonal antitoxin. These tests are further categorized into two categories: one detects antibodies and other detects antigens in the patient's specimen.

Detection of Antibodies

A number of serological tests have been developed for the detection of specific antibodies to *B. pseudomallei* in patient's sample. The main drawback of these antibody assays is that false-positive result are common, and the presence of background antibody in some healthy individuals in endemic areas limits their value in clinical situations. IHA has been the most important serological test for melioidosis for many years, and has remained unchanged since it was first described more than 40 years ago. Despite variable levels of sensitivity and specificity, it still remains the most commonly employed serological test, with titers of 1:40 or greater considered reactive as per the Australian standard. IHA is based on the agglutination of sheep red blood cells in the presence of serum antibodies to polysaccharide and lipopolysaccharide antigens derived from a specific strain of *B. pseudomallei*. Apart from IHA, another important diagnostic technique is immunoglobulin G (IgG) and immunoglobulin M (IgM) ELISA), with IgG ELISA exhibiting a sensitivity of 96 percent and specificity of 97 percent; IgM ELISA has a sensitivity of 74 percent and a specificity of 99 percent (Chenthamarakshan et al., 2001). However, there is a need for the development of an internationally standardized serodiagnostic test for melioidosis because no ELISA test has yet been clinically validated as a diagnostic tool (Peacock et al., 2011).

Detection of Antigens

This is a more logical approach and is superior to antibody detection tests because it indicates the presence of active disease. Many immunological methods have been developed for detecting *B. pseudomallei* antigen, which includes the detection of soluble secreted product in blood and urine, as well as the detection of the whole organism in different specimens, such as in pus, wound, sputum, and throat swabs. Other developed assay systems that have been evaluated to detect *B. pseudomallei* antigen include immunofluorescence assay (Walsh et al., 1994) and latex agglutination test (Smith et al., 1993).

Molecular Assays

The hybridization techniques using specific DNA probes have not shown sufficient sensitivity for *B. pseudomallei* infection. Therefore, the polymerase chain reaction (PCR) technique has been more commonly performed with satisfactory results with a sensitivity of 95.2 percent and specificity of 91.7 percent (Sermswan et al., 2000).

Along with PCR, 16S mRNA sequencing has proven to be useful for the clinical identification (Visca et al., 2001) of *Burkholderia* spp.

Clinical Management

The definitive diagnosis of melioidosis is difficult at the preliminary level, even after the culturing in a microbiology laboratory, because gram-negative septicemia has so many common causes. The *B. pseudomallei* colonies on an agar plate can be helpful after subculturing from blood culture bottles onto solid media, but only after 2 to 3 days of growth. Younger cultures may not suggest *B. pseudomallei*, thereby making a false early diagnosis of melioidosis. By this stage, usually 1 to 2 days after collecting blood or sputum culture, a severely sick patient may be in respiratory distress or even dead. Antibiotic treatment of melioidosis also poses a challenge because the antibiotics that are commonly used for gram-negative septicemia, including gentamicin, quinolones, and third-generation cephalosporins, do not have a reliable effect against *B. pseudomallei* infection (Kenny et al., 1999). Intravenous administration of antibiotics is preferred for the treatment of *B. pseudomallei* septicemia, but there is a tendency for early relapse at around 10 to 14 days after the commencement of intravenous antibiotic therapy (Chaowagul et al., 1993). In some cases, late relapse can occur months or even years after initial septicemic infection. An additional consequence of this capacity for melioidosis to remain dormant is delayed presentation of acute infection even many decades after the initial exposure (Ngauy et al., 2005). Currently no vaccine preparation is available to protect an individual against melioidosis.

Immediate and Continuation Therapy

Some clinical trials have shown a significant reduction in mortality by early intervention with a suitable intravenous antibiotic. *B. pseudomallei* is intrinsically resistant to a large number of antimicrobial agents; an important mechanism involved is that it is able to pump drugs out of the cell and mediates resistance to aminoglycosides (*AmrAB-OprA*), tetracyclines, fluoroquinolones, and macrolides (*BpeAB-OprB*) (Norris et al., 2011). Ceftazidime was the first antibiotic reported to reduce mortality, although theoretically Carbapenems should also be used instead for severe infections (Inglis et al., 2004), and both imipenem and meropenem have been shown to be as good as ceftazidime (Cheng et al., 2004). Some supplementary antibiotic agents might be useful in treating persistent bacteremic infection as well as reducing the risk of early relapse but which agent is most suitable or when it should be introduced it is not yet clear. Various other important measures that are important for treating specific patients with severe, acute infection include correction of metabolic acidosis, ketosis, diabetic control, and oxygenation (Inglis et al., 2001). All these factors actually assist phagocytic cell function and thereby possibly reduce the risk of late relapse; but still there is a lack of consensus on exactly how long to continue intravenous antibiotics and what to continue for oral eradication therapy. These issues will only be resolved through carefully planned clinical trials.

The conventional treatment of melioidosis can be divided into two stages: an intravenous high-intensity phase and an eradication phase to prevent recurrence (Table 19.1). Surgical drainage is usually suggested in cases of prostatic abscesses and septic arthritis

Table 19.1. Antibiotic therapy for culture confirmed melioidosis cases.

Phase	Disease Stage	Antibiotic Therapy
I	Acute stage	In cases with severe septicemia, with or without pneumonia, central nervous system infection and other invasive forms of the disease the treatment recommended is: Either ceftazidime (adult): 2–3 g or 40 mg/kg per dose every 8 hours intravenously for 2–4 weeks *plus* co-trimoxazole (trimethoprim-sulfamethoxazole) 10/50 mg/kg (up to 320/1600 g) every 12 hours *OR* Meropenem 1 g or 25 mg/kg every 8 hours intravenously for ≥2 weeks
II	Eradication stage	1. Trimethoprim-sulfamethoxazole 8/40 mg/kg every 12 hours for ≥12–20 weeks 2. Doxycycline 4 mg/kg per day *plus* trimethoprim-sulfamethoxazole 8/40 mg/kg every 12 hours for ≥12–20 weeks 3. Chloramphenicol 40 mg/kg per day *plus* doxycycline 4 mg/kg per day, *plus* trimethoprim-sulfamethoxazole 8/40 mg/kg every 12 hours for ≥12–20 weeks.

and may also be indicated for parotid abscesses but not usually for hepatosplenic abscesses. In cases unresponsive to intravenous antibiotic therapy, splenectomy has been attempted, but there is only anecdotal evidence to support this practice (Inglis et al., 2001).

Investigation of an Outbreak

While investigating an outbreak it should be kept in mind that the clinical cases of melioidosis should not be confused with other bacterial septicemias. All notified cases must be investigated thoroughly; history of the movement of the cases during the incubation period, including activities or occupational exposure, must be established to determine possible source of infection; persons who are likely to be exposed to the common source must be identified and their health status must be assessed. All the suspected and probable cases of melioidosis should be investigated following a defined pattern (Figure 19.4).

An outbreak can be defined as more than one confirmed cases of melioidosis in the same locality or from a common source of infection within the incubation period (21 days).

Case Definition

Person having some predisposing factors especially diabetes mellitus with:

- Fever and/or
- Pneumonia and/or
- Single or multiple abscesses and other evidence of infections *and*
- History of exposure to high-risk occupational activities, including agriculture, construction site, mining, fresh water recreational area, camping, etc.

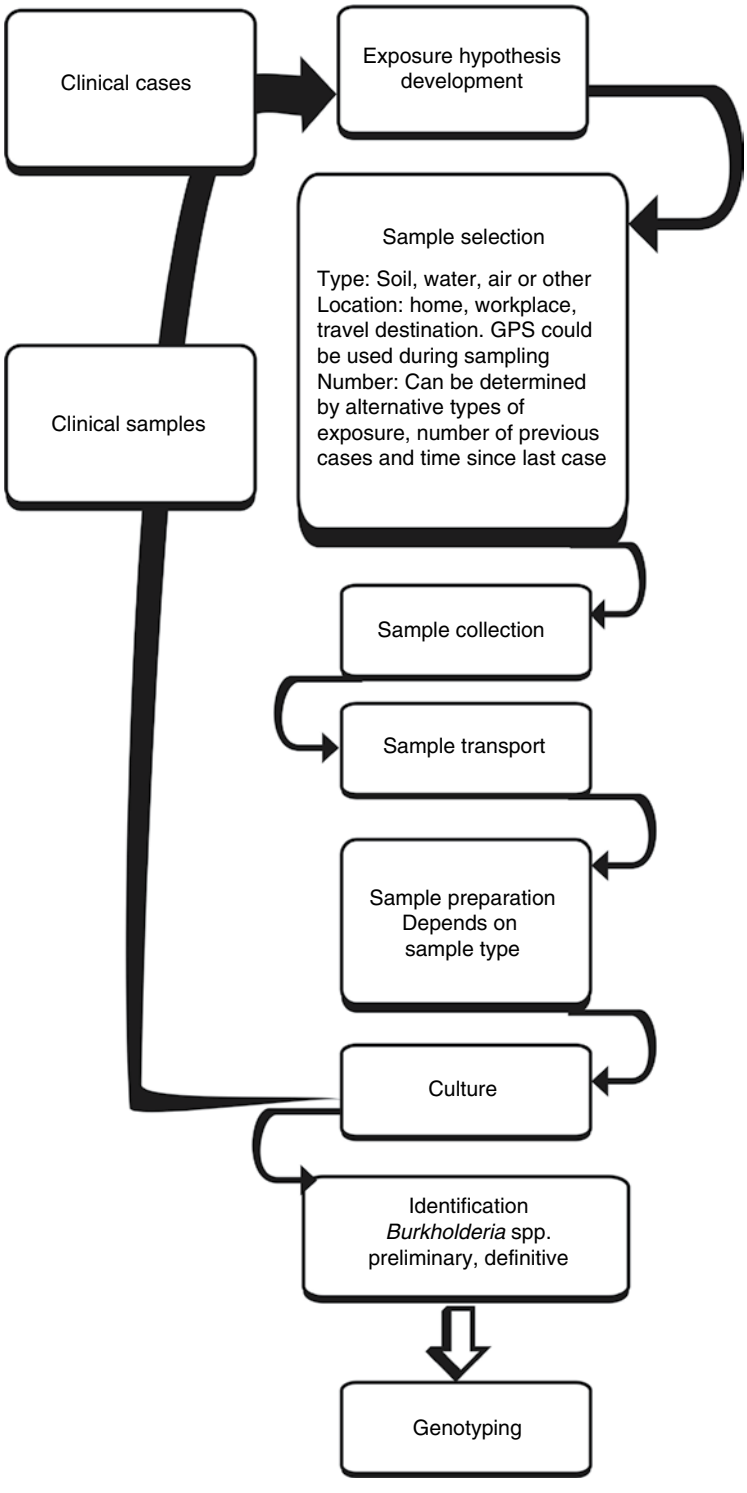

Figure 19.4. *Burkholderia* environmental sample processing flow chart.

Case Classification

- **Suspected case**: A case that is compatible with clinical case definition.
- **Probable case**: A suspected case with indirect fluorescent antibody titer (IFT) IgM ≥1:80
- **Confirmed case**: Any case with positive culture or positive PCR or fourfold rise in serology titer.

Case Notification

A medical practitioner diagnosing the confirmed case should notify the nearest health laboratory office only of laboratory-confirmed cases of melioidosis within 1 week of diagnosis in a usual notification form. The suspected and probable cases need to be notified.

Case Registration, Documentation, and Reporting

All notified case must be investigated, and information should be entered into melioidosis cases registry at a district health office by an assistant environmental health officer. All activities during the outbreak of melioidosis should be adequately documented, and the report should be further disseminated so that further outbreak can be handled more effectively. A progress report of the outbreak must be sent to the state health department of the country on a daily basis. A final report of the outbreak must be sent within 1 month from the date on which the outbreak has been declared over.

Prevention and Control of Melioidosis

Personal Measures

Because of the absence of an effective vaccine for melioidosis, the prospect of comprehensive disease control seems a distant reality; however, the predilection of infection with *Burkholderia* for specific high-risk groups in particular locations provide a guide for environmental control methods that can be used with expected benefit. At a personal level, any person with regular contact with soil or surface water in known endemic locations should be advised to follow personal precautions to reduce the risk of infection. These precautions include simple personal protective measures such as avoiding skin abrasions, cleaning them thoroughly when contaminated with soil or water, and wearing protective equipment such as gloves and suitable clothing for exposure-prone occupations. People with known comorbidities, such as diabetes or renal disease, need to take special care during application of these measures.

Community Measures

At a community level, it is difficult to prevent sporadic infection completely, but repetition of true outbreaks may be avoided by careful chlorination of the drinking water supply (Howard and Inglis, 2005). In cases where animal death is reported in domestic livestock, it should be thoroughly investigated by veterinary pathologists as a possible sentinel event for subsequent human infection.

Environmental Management

In an attempt to manage the environment, a fascinating alternative approach is obtaining data from repeated environmental investigations along with the restoration of native vegetation and removal of chemical fertilizers (urea and superphosphate), both of which are substrates for *B. pseudomallei*. This data is consistent along with the promotion of environmental risk by modern intensive cultivation methods, which are remediable by restoration of native flora. Though this interpretation is provisional, it may be worth considering in rural locations that become hyperendemic or have a persistent association with fatal infection. Specific investigations are currently under way that focus on rice cultivation and rubber plantations because workers in both types of crop cultivation are at increased risk of melioidosis.

Reducing Occupational Risk

Outdoor Occupations

Rural workers having regular exposure to moist soil or surface water are at increased risk of melioidosis, particularly including rice farmers that employ traditional methods of cultivation. This risk could be reduced by mechanization or cheaply by wearing protective footwear. Lowland rice cultivation in flooded paddy fields is probably the highest form of risk for this group; rubber tree tappers have also been shown to be at risk of the disease. Further, *B. pseudomallei* can be found in the soil under rubber trees. Recent reports regarding melioidosis risk of mineworkers suggests that, at a mine site with identifiable *B. pseudomallei* contamination, a combination of careful occupational health measures, dust suppression, and other environmental management measures mitigate infection risk. Possibly, the most unpredictable melioidosis risks are the people engaged in adventure travel, which includes overland trekkers, explorers of the remote tropics, field workers, exploratory mining engineers, eco-tourists, their guides, and military personnel engaged in training expeditions. Because it is difficult to anticipate, quantify, or locate the specific exposure to previously unidentified environments contaminated by *B. pseudomallei* after the event, only general preventive personal measures can be suggested prior to departure. Post-travel serosurveillance may prove to be helpful for such groups or for individuals with unexplained fever. A similar approach may be justified for workers in the power and water supply industries, those who excavate trenches, install pipes and cables; and those involved in ducting.

Laboratory Workers

The occupational risk of melioidosis, especially for diagnostic laboratory workers, is often overlooked. In Australia, *B. pseudomallei* is considered as a biological safety level 2 organism and, therefore, does not require a high level of laboratory biosecurity, but it does not mean that workers can handle it freely on the open bench. Laboratory staff members, who handle live *B. pseudomallei* cultures, may be at risk of infection hence the aerosol-generating procedures with live *B. pseudomallei* cultures should be conducted always in a biological safety cabinet in the laboratory, and the workers involved should use latex or similar protective gloves and wear a laboratory gown in accordance with good microbiology laboratory practice. For practical reasons, it is essential to obtain baseline melioidosis serology prior to commencement of laboratory work with *B. pseudomallei* and can be periodically repeated, on completion of the work, or after potential exposure, according

to the intensity of risk. Seroconversion (with a more than fourfold increase in titer) can be used as an indication of likely exposure, which is proved to be more reliable than a single high titer. The same approach can also be used with specific, high-risk occupational groups, subject to their consent and the availability of specialist interpretive advice from the public health reference laboratory running the serological tests for *B. pseudomallei*.

Public Health Importance

B. pseudomallei has been considered an important potential bioweapon, with increasing funding overseas for research regarding virulence factors and vaccine development (Jeddeloh et al., 2003). It is believed that bioweapons research using *B. pseudomallei* started in the former Soviet Union, although the extent of this effort and the possibility of engineered antibiotic resistant strains remain unknown (Alibek, 1999). Some other countries that showed military interest in *B. pseudomallei* included the United States and possibly Egypt (Kortepeter et al., 2001). The potential risk of *B. pseudomallei* as a bioweapon is still uncertain. Melioidosis has a potentially high mortality rate, and its causative agent has intrinsic antibiotic resistance and a wide host range; however, weaponization using the agent has not been reported. The disease does not spread from person to person, and the susceptibility of immunocompetent individuals after inhalation is not yet clear.

The biosecurity threat of melioidosis may have been overemphasized during the deliberate dispersal of anthrax in the United States during 2001 and has led to some erroneous claims about the potential disease threat. There is no evidence for deliberate use of *B. pseudomallei* as an agent of terrorism or by a malign foreign power. The lack of an appropriate vaccine, the high level of intrinsic antibiotic resistance in bacterium, infection via the respiratory route, and the potential fatal outcome of melioidosis might make it appear attractive to those who intend to do harm. However, the lack of person-to-person spread, the common association of comorbidity with fatal infection, the requirement for an unidentified environmental amplifier, and the unpredictability of sporadic cases of *B. pseudomallei* infection make it a poor candidate. The consequence of *B. pseudomallei*'s listing as a potential bioweapon has been an increased difficulty transporting live cultures of this species between public health laboratories. The appropriate method to control the spread of melioidosis is careful recording of bacterial isolate transfers, defined material transfer agreements, rigorous adherence to import licensing, incorporation in registered culture collections, and controlled access to archived, imported strains. These requirements are unduly restrictive and will place more pressure on the international network of public health laboratories to use standard molecular identification and genotyping methods, which can be applied to *B. pseudomallei* DNA and reducing reliance on transborder shipment of live isolates of *B. pseudomallei* bacterium.

References

Abbink FC, Orendi JM, de Beaufort AJ. 2001. Mother-to-child transmission of Burkholderia pseudomallei. *N Engl J Med* 344:1171–1172.

Ahmed K, Enciso HD, Masaki H, Tao M, Omori A, et al. 1999. Attachment of *Burkholderia pseudomallei* to pharyngeal epithelial cells: A highly pathogenic bacteria with low attachment ability. *Am J Trop Med Hyg* 60:90–93.

Alibek K. 1999. *Biohazard*. New York: Random House.
Apisarnthanarak A, Khawcharoenporn T, Mundy LM. 2012. Flood-associated melioidosis in a non-endemic region of Thailand. *Int J Infect Dis* 16:e409–e410.
Barker A. 2005. Rise in melioidosis rates in NT. Australian Broadcasting Corporation. Accessed February 20, 2013, at http://www.abc.net.au/pm/content/2005/s1285014.htm.
Beeker A, Van de Stadt KD, Bakker K. Melioidosis. 1999. *Neth J Med* 54:76–79.
Ben RJ, Tsai YY, Chen JC, Feng NH. 2004. Non-septicemic Burkholderia pseudomallei liver abscess in a young man. *J Microbiol Immunol Infect* 37:254–257.
Brilhante RS, Bandeira TJ, Cordeiro RA, Grangeiro TB, Lima RA, et al. 2012. Clinical-epidemiological features of 13 cases of melioidosis in Brazil. *J Clin Microbiol* 50:3349–3352.
Chaiyaroj SC, Kotrnon K, Koonpaew S, Anantagool N, White NJ, et al. 1999. Differences in genomic macrorestriction patterns of arabinose-positive (*Burkholderia thailandensis*) and arabinose-negative *Burkholderia pseudomallei*. *Microbiology and Immunology* 43:625–630.
Chan K, Raghuram J, Low J, Kurup A. 2003. Epidemiologic characteristics and prognostic determinants of death in severe melioidosis requiring intensive care. *Crit Care Med* 31:A120.
Chaowagul W, Suputtamongkol Y, Dance DA, Rajchanuvong A, Pattara-arechachai J, et al. 1993. Relapse in melioidosis: incidence and risk factors. *J Infect Dis* 168:1181–1185.
Chaowagul W, White NJ, Dance DA, Wattanagoon Y, Naigowit P, et al. 1989. Melioidosis: A major cause of community-acquired septicemia in Northeastern Thailand. *J Infect Dis* 159:890–899.
Cheng AC, Heng AC, Fisher DA, Anstey NM, Jacups SP, et al. 2004. Outcomes of patients with melioidosis treated with meropenem. *Antimicrob Agents Chemother* 48:1763–1765.
Chenthamarakshan V, Vadivelu J, Putucheary SD. 2001. Detection of immunoglobulins M and G using culture filtrate antigen of *Burkholderia pseudomallei*. *Diagn Microbiol Infect Dis* 39:14–7.
Chodimella U, Hoppes WL, Whalen S, Ognibene AJ, Rutecki GW. 1997. Septicemia and suppuration in a Vietnam veteran. *Hosp Pract* 32:219–221.
Clayton AJ, Lisella RS, Martin DG. 1973. Melioidosis: A serological survey in military personnel. *Mil Med* 138:24–26.
Cottew GS. 1950. Melioidosis in sheep in Queensland. A description of the causal organism. *Aust J Exp Biol Med Sci* 28:677–683.
Crotty JM, Bromich AF, Quinn JV. 1963. Melioidosis in the Northern Territory: A report of two cases. *Med J Aust* 50:274–275.
Cruz-Migoni A, Hautbergue GM, Artymiuk PJ, Baker PJ, Bokori-Brown M, et al. 2011. A *Burkholderia pseudomallei* toxin inhibits helicase activity of translation factor eIF4A. *Science* 334:821–824.
Currie BJ, Fisher DA, Howard DM, Burrow JN, Lo D, et al. 2000. Endemic melioidosis in tropical northern Australia: A 10-year prospective study and review of the literature. *Clin Infect Dis* 31:981–986.
Dance DAB. 1991. Melioidosis: The tip of the iceberg? *Clin Microbiol Rev* 4:52–60.
Dance DA, Smith MD, Aucken HM, Pitt TL. 1999. Imported melioidosis in England and Wales. *Lancet* 353:208.
Dance DA. 2000. Melioidosis as an emerging global problem. *Acta Trop* 74:115–119.
DeShazer D, Brett PJ, Carlyon R, Woods DE. 1997. Mutagenesis of *Burkholderia pseudomallei* with Tn5-OT182: Isolation of motility mutants and molecular characterization of the flagellin structural gene. *J Bacteriol* 179:2116–2125.
Finkelstein RA, Atthasampunna P, Chulasamaya M. 2000. Pseudomonas (*Burkholderia*) *pseudomallei* in Thailand, 1964–1967: Geographic distribution of the organism, attempts to identify cases of active infection, and presence of antibody in representative sera. *Am J Trop Med Hyg* 62:232–239.
Fournier J. 1965. Melioidosis and the Whitmore bacillus. Epidemiological and taxonomic controversies. *Bull Soc Pathol Exot* 58:753–765.
Grosskopf S. 2000. An unusual cause of pneumonia. *Aust Fam Physician* 29:552–553.

Halder D, Zainal N, Wah CM, Haq JA. 1998. Neonatal meningitis and septicaemia caused by Burkholderia pseudomallei. *Ann Trop Paediatr* 18:161–164.
Heng BH, Goh KT, Yap EH, Loh H, Yeo M. 1998. Epidemiological surveillance of melioidosis in Singapore. *Ann Acad Med Singapore* 27:478–484.
Heyse AM, Dierick J, Vanhouteghem H, Ameye F, Baert D, et al. 2003. A case of imported melioidosis presenting as prostatitis. *Infection* 31:60–62.
Ho M, Schollaardt T, Smith MD, Perry MB, Brett PJ, et al. 1997. Specificity and functional activity of anti-Burkholderia pseudomallei polysaccharide antibodies. *Infect Immun* 65:3648–3653.
Holland DJ, Wesley A, Drinkovic D, Currie BJ. 2002. Cystic fibrosis and *Burkholderia pseudomallei*: An emerging problem? *Clin Infect Dis* 35:e138–e140.
Howard K, Inglis TJ. 2005. Disinfection of *Burkholderia pseudomallei* in potable water. *Water Res* 39:1085–1092.
Hsueh PR, Teng LJ, Lee LN, Yu CJ, Yang PC, et al. 2001. Melioidosis: An emerging infection in Taiwan? Emerg. *Infect Dis* 7:428–433.
Inglis TJ, Rodrigues F, Rigby P, Norton R, Currie BJ. 2004. Comparison of the susceptibilities of *Burkholderia pseudomallei* to meropenem and ceftazidime by conventional and intracellular methods. *Antimicrob Agents Chemother* 48:2999–3005.
Inglis TJ, Golledge CL, Clair A, Harvey J. 2001. Case report: Recovery from persistent septicemic melioidosis. *Am J Trop Med Hyg* 65:76–82.
Jeddeloh JA, Fritz DL, Waag DM, Hartings JM, Andrews GP. 2003. Biodefense-driven murine model of pneumonic melioidosis. *Infect Immun* 71:584–587.
John TJ, Jesudason MV, Lalitha MK, Ganesh A, Mohandas V, et al. 1996. Melioidosis in India: The tip of the iceberg? *Indian J Med Res* 103:62–65.
Jones AL, Beveridge TJ, Woods DE. 1996. Intracellular survival of *Burkholderia pseudomallei*. *Infect Immun* 64:782–790.
Kanai K, Kondo E. 1994. Recent advances in biomedical sciences of *Burkholderia pseudomallei* (basonym: *Pseudomonas pseudomallei*). *Jpn J Med Sci Biol* 47:1–45.
Kanaphun P, Thirawattanasuk N, Suputtamongkol Y, Naigowit P, Dance DA, et al. 1993. Serology and carriage of *Pseudomonas pseudomallei*: a prospective study in 1000 hospitalized children in northeast Thailand. *J Infect Dis* 167:230–233.
Kenny DJ, Russell P, Rogers D, Eley SM, Titball RW. 1990. *In vitro* susceptibilities of *Burkholderia mallei* in comparison to those of other pathogenic *Burkholderia* spp. *Antimicrob Agents Chemother* 43:2773–2775.
Kespichayawattana W, Rattanachetkul S, Wanun T, Utaisincharoen P, Sirisinha S. 2000. *Burkholderia pseudomallei* induces cell fusion and actin-associated membrane protrusion: a possible mechanism for cell-to-cell spreading *Infect Immun* 68:5377–5384.
Ketterer PJ, Webster WR, Shield J, Arthur RJ, Blackall PJ, et al. 1986. Melioidosis in intensive piggeries in South-Eastern Queensland. *Aust Vet J* 63:146–149.
Kibbler CC, Roberts CM, Ridgway GL, Spiro SG. 1991. Melioidosis in a patient from Bangladesh. *Postgrad Med J* 67:764–766.
Kortepeter MG, Christopher T, Cieslak R, Culpepper R, Darling J, et al., eds. 2001. *Medical Management of Biological Casualties Handbook*, pp. 23–27. Fort Detrick, MD: US Army Medical Research Institute of Infectious Diseases.
Krishnaswami CS. 1917. Morphia injectors' septicaemia. *Indian Medical Gazette* 52:296–299.
Lee YH, Chen Y, Ouyang X, Gan YH. 2010. Identification of tomato plant as a novel host model for *Burkholderia pseudomallei*. *BMC Microbiol* 10:28.
Leelarasamee A, Bovornkitti S. 1989. Melioidosis: Review and update. *Rev Infect Dis* 11:413–425.
Leelarasamee A, Trakulsomboon S, Kusum M, Dejsirilert S. 1997. Isolation rates of Burkholderia pseudomallei among the four regions in Thailand. *Southeast Asian J Trop Med Public Health* 28:107–113.
Leeuwenburgh I, Driessen JT, van Keulen PH, Stijnen PJ, Verburg GP. 2002. Melioidosis. N*ed Tijdschr Geneeskd* 146:723–725.

Lertmemongkolchai G, Cai G, Hunter CA, Bancroft GJ. 2001. Bystander activation of CD8+ T cells contributes to the rapid production of INF-γ in response to bacterial pathogens. *J Immunol* 166:1097–1105.

Low Choy J, Mayo M, Janmaat A, Currie BJ. 2000. Animal melioidosis in Australia. *Acta Trop* 74:153–158.

Luo CY, Ko WC, Lee HC, Yang YJ. 2003. Relapsing melioidosis as cause of iliac mycotic aneurysm: An indigenous case in Taiwan. *J Vasc Surg* 37:882–885.

McCormick JB, Sexton DJ, McMurray JG, Carey E, Hayes P, et al. 1975. Human-to-human transmission of *Pseudomonas pseudomallei*. *Ann Intern Med* 83:512–513.

Nandi T, Ong C, Singh AP, Boddey J, Atkins T, et al. 2010. A genomic survey of positive selection in *Burkholderia pseudomallei* provides insights into the evolution of accidental virulence. *PLoS Pathog* 6:e1000845.

Ngauy V, Lemeshev Y, Sadkowski L, Crawford G. 2005. Cutaneous melioidosis in a man who was taken as a prisoner of war by the Japanese during World War II. *J Clin Microbiol* 43:970–972.

Norris MH, Propst KL, Kang Y, Dow SW, Schweizer HP, et al. 2011. The *Burkholderia pseudomallei* γasd mutant exhibits attenuated intracellular infectivity and imparts protection against acute inhalation melioidosis in mice. *Infect Immun* 79:4010–4018.

Parry CM, Wuthiekanun V, Hoa NT, Diep TS, Thao LT, et al. 1999. Melioidosis in Southern Vietnam: clinical surveillance and environmental sampling. *Clin Infect Dis* 29:1323–1326.

Peacock SJ, Cheng AC, Currie BJ, Dance DA. 2011. The Use of Positive Serological Tests as Evidence of Exposure to *Burkholderia pseudomallei*. *Am J Trop Med Hyg* 84:1021–1022.

Peacock SJ, Chieng G, Cheng AC, Dance DA, Amornchai P, et al. 2005. Comparison of Ashdown's medium, *Burkholderia cepacia* medium, and *Burkholderia pseudomallei* selective agar for clinical isolation of *Burkholderia pseudomallei*. *J Clin Microbiol* 43:5359–5361.

Poe RH, Vassallo CL, Domm BM. 1971. Melioidosis: The remarkable imitator. *Am Rev Respir Dis* 104:427–431.

Pons R, Advier M. 1927. Melioidosis in Cochin China. *J Hyg* 26:28–30.

Price EP, Hornstra HM, Limmathurotsakul D, Max TL, Sarovich DS, et al. 2010. Within-host evolution of *Burkholderia pseudomallei* in four cases of acute melioidosis. *PLoS Pathog* 6:e1000725.

Puthucheary SD, Parasakthi N, Lee MK. 1992. Septicaemic melioidosis. A review of 50 cases from Malaysia. *Trans R Soc Trop med Hyg* 86:683–685.

Puthucheary SD, Vadivelu J, Ce Cile C, Kum Thong W, Ismail G. 1996. Electron microscopic demonstration of extracellular structure of Burkholderia pseudomallei. *Am J Trop Med Hyg* 54:313–314.

Raghavan KR, Shenoi RP, Zaer F, Aiyer R, Ramamoorthy P, et al. 1991. Melioidosis in India. *Indian Pediatr* 28:184–188.

Rimington RA. 1962. Melioidosis in Northern Queensland. *Med J Aust* 1:50–53.

Sermswan RW, Wongratanacheewin S, Anuntagool N, Sirisinha S. 2000. Comparison of the polymerase chain reaction and serologic tests for diagnosis of septicemic melioidosis *Am J Trop Med Hyg* 63:146–149.

Shalom G, Shaw JG, Thomas MS. 2007. *In vivo* expression technology identifies a type VI secretion system locus in *Burkholderia pseudomallei* that is induced upon invasion of macrophages. *Microbiology* 153:2689–2699.

Sim SH, Yu Y, Lin CH, Karuturi RKM, Wuthiekanun V, et al. 2008. The core and accessory genomes of *Burkholderia pseudomallei*: Implications for human melioidosis. *PLoS Pathog* 4:e1000178.

Smith DM, Wuthiekanun V, Walsh AL, Pitt JL. 1993. Latex agglutination test for identification of *Pseudomonas pseudomallei*. *J Clin Pathol* 46:374–375.

So SY, Chau PY, Aquinas M, Gabriel M, Lam WK. 1987. Melioidosis: A serological survey in a tuberculosis sanatorium in Hong Kong. *Trans R Soc Trop Med Hyg* 81:1017–1019.

So SY, Chau PY, Leung YK, Lam WK. 1984. First report of septicaemic melioidosis in Hong Kong. *Trans R Soc Trop Med Hyg* 78:456–459.

Stanton AT, Fletcher W. 1921. Melioidosis, a new disease of the tropics. *Far Eastern Association of Tropical Medicine: Transactions of the Fourth Congress*. Batavia, Dutch East Indies: Javasche Boekhandel en Drukkerij.

Stanton AT, Fletcher W. 1932. *Melioidosis*, vol. 21. London: John Bale and Danielson Ltd.

Steinmetz I, Rohde M, Brenneke B. 1995. Purification and characterization of an exopolysaccharide of *Burkholderia* (Pseudomonas) *pseudomallei*. *Infect Immun* 63:3959–3965.

Suputtamongkol Y, Chaowagul W, Chetchotisakd P, Lertpatanasuwun N, Intaranongpai S, et al. 1999. Risk factors for melioidosis and bacteremic melioidosis. *Clin Infect Dis* 29:408–413.

Suputtamongkol Y, Hall AJ, Dance DA, Chaowagul W, Rajchanuvong A, et al. 1994. The epidemiology of melioidosis in Ubon Ratchatani, northeast Thailand. *Int J Epidemiol* 23:1082–1090.

Thurnheer U, Novak A, Michel M, Ruchti C, Jutzi H, et al. 1988. Septic melioidosis following a visit to India. Schweiz. *Med Wochenschr* 118:558–564.

Trakulsomboon S, Vuddhakul V, Tharavichitkul P, Na-Gnam N, Suputtamongkol Y, et al. 1999. Epidemiology of arabinose assimilation in *Burkholderia pseudomallei* isolated from patients and soil in Thailand. *Southeast Asian J Trop Med Public Health* 30:756–759.

Tsang TY, Lai ST. 2001. A case of thoracic empyema due to suppurative melioidosis. *Hong Kong Med J* 7:201–204.

Van Peenen PF, See R, Soysa PE, Irving GS. 1976. Seroepidemiological survey of hospital-associated populations in Colombo, Sri Lanka. *Southeast Asian J Trop Med Public Health* 1:16–20.

Visca P, Cazzola G, Petrucca A, Braggion C. 2001. Travel-associated *Burkholderia pseudomallei* infection (melioidosis) in a patient with cystic fibrosis: A case report. *Clin Infect Dis* 32:E15–E16.

Vorachit M, Lam K, Jayanetra P, Costerton JW. 1993. Resistance of *Pseudomonas pseudomallei* growing as a biofilm on silastic discs to ceftazidime and co-trimoxazole. *Antimicrob Agents Chemother* 37:2000–2002.

Walsh AL, Smith MD, Wuthiekanum V, Suputtamongkol Y, Desakorn V, et al. 1994. Immunofluorescence microscopy for the rapid diagnosis of melioidosis. *J Clin Pathol* 47:377–379.

White NJ. 2003. Melioidosis. *Lancet* 361:1715–1722.

Whitmore A. 1913. An account of a glanders-like disease occurring in Rangoon. *J Hyg* 13:1.

Yabuuchi E, Kasako Y, Oyaizu H, Yano I, Hotta H, et al. 1992. Proposal of *Burkholderia* gen nov and transfer of seven species of the genus Pseudomonas homology group II to the new genus, with the type species *Burkholderia capacia* (Palleroni and Holmes 1981) comb nov. *Microbiol Immunol* 36:1251–1275.

Yang S, Tong S, Lu Z. 1995. Geographical distribution of *Pseudomonas pseudomallei* in China. *Southeast Asian J. Trop. Med. Public Health* 26:636–638.

Chapter 20

Biowarfare and Bioterrorism

Introduction

It is important to discriminate between biowarfare and bioterrorism; the use of bioweapons against the military or armed forces is biowarfare, and the use of biological agents against civilians is bioterrorism. In a bioterrorism attack, viruses, bacteria, toxins, or other harmful agents can be deliberately released to spread illness or to cause death in people, animals, or plants. The agents used are generally found in nature, but they could be mutated or altered enough to increase their ability to cause disease, make them resistant to currently available medicines, or to increase their ability to be spread into the environment. These biological agents can be spread through the air, water, or through food materials. Terrorists prefer to use biological agents because they are extremely difficult to detect and do not cause illness for several hours to several days. Some bioterrorism agents, including the smallpox virus, can easily be spread from person to the person and others, such as anthrax, cannot (Centers for Disease Control and Prevention [CDC], 2008). Biological warfare agents are usually more potent than conventional and chemical weapons. During the past century, recent advances in biotechnology have simplified the development and production of such biological weapons; genetic engineering techniques hold the most dangerous potential. Bioterrorism is an attractive plan because biological agents are relatively easy and inexpensive to obtain, can be easily disseminated, and can cause widespread fear and panic beyond the actual physical damage they cause. Military leaders, however, have recognized that, as a military asset, bioterrorism has some limitations; the most important is the difficulty to employ a bioweapon in a unidirectional way, which only affects the enemy and not friendly forces. A biological weapon is particularly useful to terrorists as a method of creating mass panic and disruption to a state or a country. However, technologist Bill Joy has warned of the potential power that genetic engineering might place in the hands of future bioterrorists (Joy, 2007). On the other hand, some biological agents do not readily cause harm to humans but may disrupt the

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

economy of the country; for example foot and mouth disease (FMD) virus, which is capable of causing widespread economic damage and public concern, reported in the 2001 and 2007 FMD outbreaks in the United Kingdom, but it had almost no capacity to infect humans. Another real and current threat to a country's population that governments and private companies cannot ignore is the contamination of food and water supplies for terrorist purpose. Food terrorism is an act or threat of deliberate contamination of food materials for human consumption with biological, chemical, and physical agents or radionuclear materials for the purpose of causing injury or death to civilian populations and disrupting social, economic, or political stability of a particular country. The biological agents that can be used are communicable infectious or noninfectious pathogenic microorganisms: viruses, bacteria, and parasites; the chemical agents may be man-made or natural toxins; the physical agents may include a wide range of objects including glass, needles, and metal fragments; and the radionuclear materials include radioactive chemicals capable of causing injury if present at unacceptable levels. Surveillance, preparedness, and response systems should be sufficiently rapid, efficient, and sensitive to meet the threat of bioterrorism.

Historical Aspects

The use of biological agents in warfare is not a new concept, and history is replete with examples of biological weapon use. Before the 20th century, biological warfare took on three main forms:

1. Deliberate poisoning of food and water with infectious material.
2. Use of microorganisms or toxins in some form of weapon system.
3. Use of biologically inoculated fabrics.

Early History

History of biowarfare started with the Scythian archers who infected their arrows by dipping them in decomposing bodies or in blood mixed with manure as far back as 400 B.C. Persian, Greek, and Roman literature from 300 B.C. quoted examples of the use of animal cadavers to contaminate water wells and other fresh water sources. In 190 B.C., during the Battle of Eurymedon, Hannibal won a naval victory over King Eumenes II of Pergamon by firing earthen vessels full of venomous snakes into the enemy ships. In the 12th century A.D., during Tortona battle, Barbarossa used the bodies of dead soldiers to poison water wells. Another example of biological terrorism was reported in Ancient Rome, when feces were thrown into faces of enemies. This biological terrorism continued on into the 14th century when the bubonic plague was used to infiltrate enemy cities (Figure 20.1) both by instilling the fear of infection in residences, in hopes that they would evacuate, and also by destroying defending forces that would not yield to the attack. The use of disease substances as a weapon in this stage of history exhibited a lack of control aggressors had over their own biological weapons. Primitive medical technology only provided limited means of protection for the aggressor and a battle's surrounding geographical regions. After the battle was over, the inability to contain enemies who escaped death led to several widespread epidemic diseases, affecting not only the enemy forces, but also surrounding regions' inhabitants. Because of the use of such biological

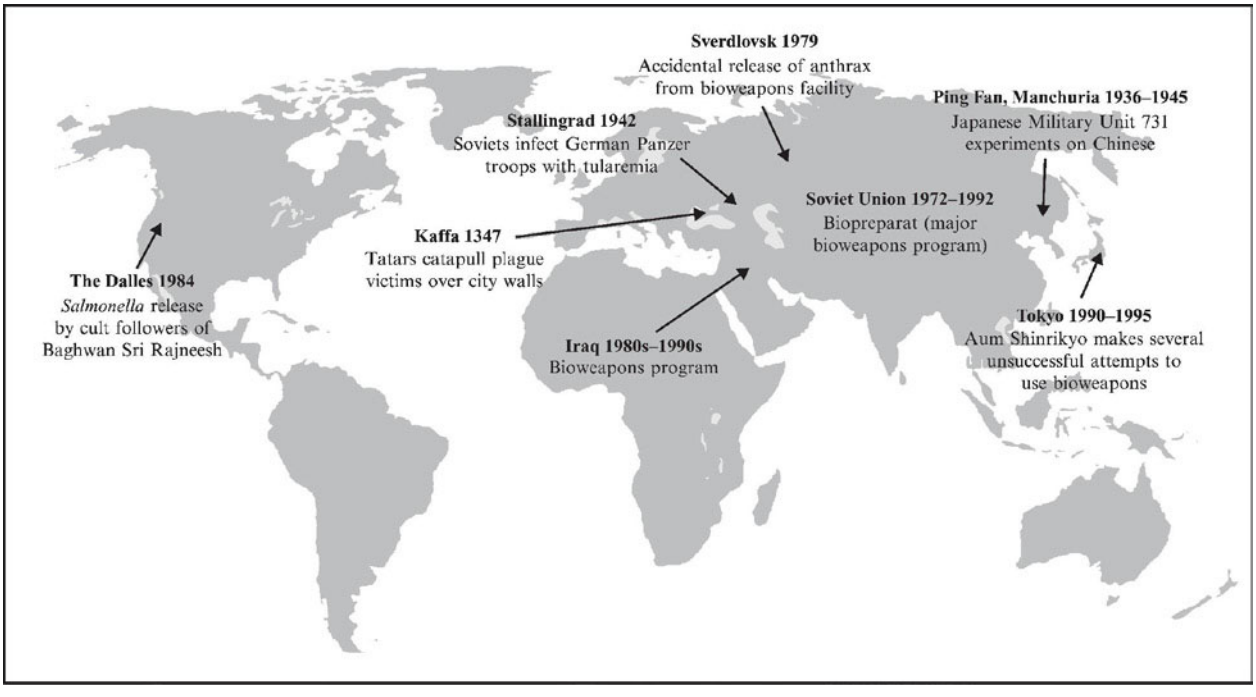

Figure 20.1. Historical incidents involving biological weapons span the globe and range from relatively modest events, such as salmonella poisoning of salad bars in The Dalles, Oregon, in 1984 to the notorious experiments by the Japanese military during the 1930s and 1940s.

weapons, and the apparent lack of medical advancement necessary to defend surrounding regions from them, widespread epidemics such as the bubonic plague quickly moved across all of Europe, destroying a large portion of the population. The victims of biological terrorism in fact, became weapons themselves by spreading diseases. In the 18th century A.D. during the French and Indian War, British forces in North America provided blankets from smallpox patients to American Indians to transmit the disease to the immunologically naïve tribes.

Biowarfare in the 19th Century

In the 19th century, biological warfare became more complex because countries began to develop weapons that were much more effective and much less likely to cause infection. One significant enhancement in the development of biological weapon was the use of anthrax. Anthrax effectiveness was initially limited to victims of large dosages, but this became a weapon of choice because it is easily transferred, has a high mortality rate, and can be easily obtained; also the variants of the anthrax bacterium can be found all around the world. Another important property of anthrax that made it a good biological weapon is its poor ability to spread far beyond the targeted population because it could not be spread from person to person. Another bioweapon reported was yellow fever virus in 1863, when a confederate surgeon was arrested and charged with attempting to import clothes infected with yellow fever into the northern parts of the United States during the Civil War.

Biowarfare in the 20th Century

Biological warfare directed against both animals and humans became more sophisticated during the 1900s. During World War I, the Germans developed anthrax, glanders, cholera, and a wheat fungus as biological weapons. They allegedly spread plague bacterium in St. Petersburg, infected mules with glanders in Mesopotamia, and attempted to do the same with the horses of the French calvary. At the start of World War I, Germany launched a biological sabotage campaign in the United States, Russia, Romania, and France (Gregory and Waag, 1997); Anton Dilger lived in Germany and was sent to the United States in 1915 carrying cultures of glanders, a virulent disease of horses and mules. Dilger set up a laboratory in his home in Chevy Chase, Maryland, where he used stevedores working the docks in Baltimore to infect horses with glanders while they were waiting to be shipped to Britain. Although Dilger was under suspicion as being a German agent, he was never arrested. In 1916, the Russians arrested a German agent with similar intentions. Germany and its allies infected French cavalry horses and many of Russia's mules and horses on the eastern front. These actions hindered artillery and troop movements, as well as supply convoys (Gregory and Waag, 1997). In 1925, the Geneva Protocol was signed by 108 nations, including the five permanent members of the United Nations Security Council; this was the first declared multilateral agreement, which prohibited the use of chemical and biological weapons in warfare but without prohibition for their development or their stockpiling.

US biological weapon development began in 1942 when President Franklin D. Roosevelt placed George W. Merck in charge of the effort to create a development program (Endicott and Hagerman, 1998). Initially they investigated anthrax and botulinum toxin for use as bioweapons, and sufficient quantities of botulinum toxin and

Table 20.1. Bioweapon agents in the US bioweapons program 1945–1969.

	Weaponized	Weapons systems
I	Bacteria	Sergeant missile warhead
	Anthrax	
	Tularemia	
	Brucellosis (*B. suis*)	Spray dispenser for drones
	Q fever	
II	Virus	Wet and dry spray tanks for jet planes
	Venezuelan equine encephalitis	
	Yellow fever	
III	Toxins	Cluster bombs
	Botulinum	
	Staphylococcal enterotoxin	
IV	Antiplant	Bomblet dispenser for long range bombers
	Rice blast	
	Wheat stem rust	
	Rye stem rust	

anthrax cattle cakes were stockpiled to allow limited retaliation if the Germans first used biological agents. The United States continued research on various offensive biological weapons during the 1950s and 1960s. From 1951 to 1954, simulants (*Bacillus globigii, Serratia marcescens*) were released off both coasts of the United States to demonstrate the vulnerability of US cities to biological attacks. This vulnerability was again tested in 1966 when the simulant *B. globigii* was released in the New York subway system. Table 20.1 shows the bioweapon agents enlisted in the US bioweapons program from 1945 to 1969 (Leitenberg, 2001).

In 1957, the British government decided to end its biowarfare capabilities and destroy all its weapon stockpiles; however, biowarfare programs in United States continued until 1969, when President Richard Nixon finally shut down all programs related to the US offensive use of biological weapons (Endicott and Hagerman, 1998). After it was recognized that biological weapons may produce global epidemics and impair the health of future generations, Nixon announced that the United States would confine its future biological research program to defensive measures, such as vaccines and field detectors (Tucker et al., 2009). The most notable development regarding the control of bioweapons following World War II was the Biological Weapons Convention (BWC) of April 10 1972, which went into effect on March 26, 1975. The United States appeared as a signatory nation of this, which addressed the prohibition of the development, production, stockpiling, and destruction of bacteriologic and toxin weapons. The United States had already terminated its offensive biological weapons program for microorganisms in 1969, and in 1970 for toxins; finally US stockpiles of biological weapons were destroyed completely by 1973.

In Chicago, two college students, Allen Schwander and Stephen Pera, were arrested by police because they were planning to poison the city's water supply with typhoid and other bacteria. Schwander had founded a terrorist group, R. I. S. E., and Pera collected and grew cultures for terrorist purpose from the hospital where he worked. (Seth Carus, 2000).

Table 20.2. List of bioweapons developed and weaponized in 1993 by the Soviet Union.

Type of Agent	Diseases They Cause
Bacteria	Anthrax
	Tularemia
	Brucellosis
	Plague
	Glanders and melioidosis
Virus	Small pox
	Venezuelan equine encephalitis
	Japanese encephalitis
	Russian spring-summer encephalitis
Viral hemorrhagic fevers	Filovirus-Ebola
	Filovirus-Marburg
	Filovirus-Lassa fever
	Bolivian hemorrhagic fever
	Argentinean hemorrhagic fever

The Soviet Union (USSR) continued to develop biological weapons from 1950 to 1980 (Table 20.2), and the USSR and its allies were suspected of having used "yellow rain" (trichothecene mycotoxins) during campaigns in Loas, Cambodia, and Afghanistan in 1970. In 1979, an accidental release of anthrax from a weapons facility in Sverdlovsk, USSR, killed around 66 people (Meselson et al., 1994). Table 20.2 lists some bioweapons reported having been researched, developed, and weaponized for terrorists attack by USSR in 1993 (Leitenberg, 2001).

Since the 1980s, terrorist organizations have become great users of biological agents with the most frequent bioterrorism incidents involving contamination of food and water. An example of food terrorism was the intentional contamination of salad bars in 11 restaurants, in the city of The Dalles, Oregon, by followers of the Bhagwan Shree Rajneesh in September and October of 1984 resulting *Salmonella typhimurium* infection in around 751 persons in an attempt to control a local election by incapacitating the local population (Török et al., 1997). Although there were no fatalities, this incident was the first known bioterrorist attack in the United States in the 20th century. Further in 1985, Iraq began an offensive biological weapons program producing anthrax, botulinum toxin, and aflatoxin, and in June 1993 the religious group Aum Shinrikyo released anthrax bacterium in Tokyo, Japan. The eyewitnesses reported a foul odor; but the attack was a total failure, not infecting a single person because the group used the vaccine strain of the bacterium because the spores recovered from the attack were identical to an anthrax vaccine strain given to animals, which were unable to cause a symptomatic response (Takashi et al., 1993).

In 1997, the Defense Against Weapons of Mass Destruction Act came into force, and it directed the US Department of Defense to establish a domestic preparedness program to improve the ability of local, state, and federal agencies to respond to biological incidents. During 1998 and 1999, multiple hoaxes occurred involving the

threatened release of anthrax in the United States, which resulted in decontamination and antibiotic prophylaxis for the intended victims. Around 6000 persons across the United States were affected by these threats. According to the CDC, an intentional release of anthrax by a bioterrorist group in a major US city would result in an economic impact of $477.8 million to $26.2 billion per 100,000 persons exposed (Kaufmann et al., 1997).

Biowarfare in the 21st Century

From September to November 2001, a total of 23 confirmed or suspected anthrax cases related to bioterrorism (including 10 inhalation and 13 cutaneous cases) appeared in the United States. Most of them involved postal workers in New Jersey and Washington, D.C., and the rest occurred at media companies in New York and Florida, where letters contaminated with anthrax were handled or opened. As a result, approximately 32,000 persons with potential exposures had antibiotic prophylaxis initiated to prevent them from anthrax disease. The threat regarding the use of biological agents on military forces and civilian populations is now more likely then at any point in all of history (Williamson, 2011; Linney et al., 2011).

Potential Agents

Criteria for a Successful Biological Warfare Agent

Probably lethality is only one of many characteristics necessary to consider in the development, production, and use of a biowarfare agent. For effective biological warfare, there should be some optimum requirements with respect to the agent, target population, and the aggressor (Spencer and Wilcox, 1993).

The Agent

The agent

- Should be highly potent and consistently produces a desired effect of either death or disease.
- Should be highly contagious and become infective in low doses.
- Have short and predictable incubation time to develop the disease.
- Should be difficult to identify in the target population.
- Is appropriate for mass production, storage, and weaponization (the conversion of the biological agent into a form that can then be used as a weapon).
- Is capable of efficient dissemination and should remain stable during and after dissemination.
- Have low persistence after delivery.
- additionally it should be capable of:
  (a) Infecting more than one kind of target (man and animals both) through more than one portal of entry.
  (b) being disseminated by various means.
  (c) producing desired psychological effects.

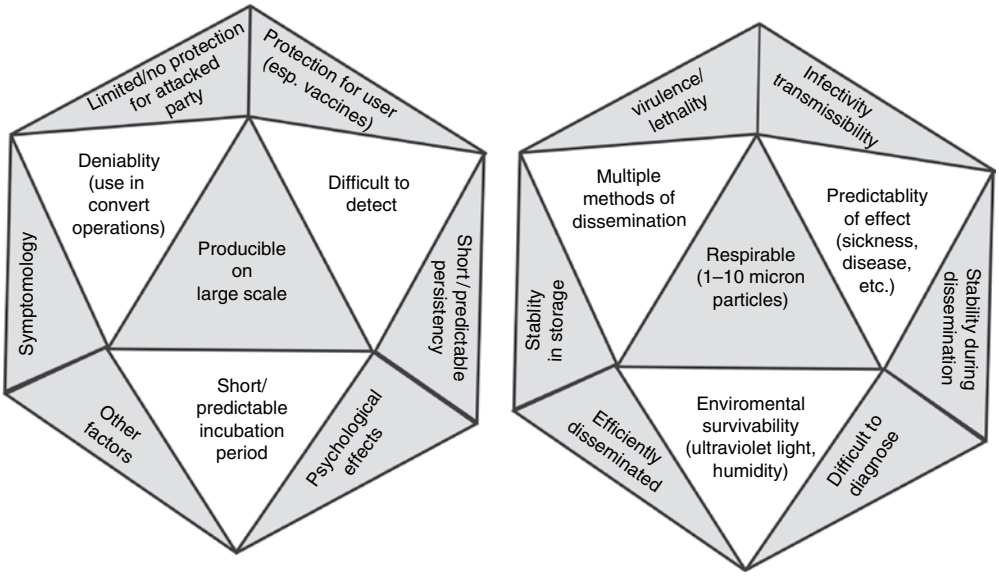

Figure 20.2. Balancing characteristics of a biowarfare agent.

The Target Population

The target population

- Should have little or no natural or acquired immunity against the particular warfare agent.
- Should have little or no access to immunoprophylaxis or therapy.

The Aggressor

Has means to protect or treat own forces and population against the agent.

Figure 20.2 illustrates the various characteristics that should be controlled during the development of a highly effective biowarfare agent. Historically, the accentuation of one characteristic often resulted in the attenuation of one or more other characteristics, possibly even rendering the modified agent ineffective to use as a weapon. However, advances in biotechnology, genetic engineering, and related scientific fields provide increasing potential to control more of these factors, possibly leading to the ability to use biowarfare agents as tactical battlefield weapons. Biotechnology is helpful by providing an increasing number of methods for the protection of country forces and also by shedding new light on methods to kill or incapacitate with unprecedented ferocity.

Generation of Potential Biowarfare Agent

Actually three methods are available to biowarfare developers seeking pathogens characterized by their increased virulence. First, they may use genetic engineering methods to design new infectious or toxic agents. Second they may seek to employ naturally emerging variants without any further modification. Third they may combine the first two methods

to use emerging diseases as a source of virulence factors that can be transferred to known pathogens. Presently, the first may be beyond the capability of scientific knowledge, but the second and third method pose an immediate threat that cannot be ignored.

So many potential types of novel biological agents (microorganisms) could be produced through genetic engineering methodologies, including:

- Benign microorganisms, which are genetically altered to produce a toxin, venom subfraction, or endogenous bioregulator.
- Microorganisms that are resistant to antibiotics, standard vaccines, and therapeutics.
- Microorganisms with enhanced aerosol and environmental stability.
- Immunologically altered microorganisms capable of defeating standard identification, detection, and diagnostic methods.
- Combinations of above items with improved delivery systems.

It is noteworthy that each of these techniques recognizes the extreme lethality of biowarfare agents and tries to exploit this potential by developing methods to deliver and control the agents efficiently on the battlefield. With the advent of various genetic engineering techniques, some biological compounds, including human insulin, growth hormone, and blood clotting factors, can be produced in fermentors containing cultures of microorganisms altered to include the genes that code for the elaboration of these proteins. This technology potentially affords a country with a competent system, access to a pharmaceutical industry, and the military to pursue a biowarfare program and the potential ability to create infectious organisms with novel properties.

The genetic engineering may also improve the ability to use biological weapons probably by enhancing the ability of biowarfare agents to survive under normally hostile environmental conditions, which can be done by splicing a gene from a toxin or other lethal agent with an otherwise nonlethal spore-forming bacteria, which results in the bacterial spore providing increased protection against inactivation from ultraviolet (UV) light (present in sunlight), humidity, heat, or other environmental factors. Another technique involves the microencapsulation of a toxin or virus to protect it from harsh environmental factors. The encapsulating wall is designed as of a respirable size (approximately 1 to 10 μ), which will survive harsh environmental conditions, yet degrade to release the pathogen after being inhaled. The resulting protection against harsh environmental factors could allow a potential aggressor to employ biowarfare agents in what would be otherwise poor conditions; for example, a pathogen may quickly decay in sunlight (Table 20.3). But if there is a technique to improve a pathogen's rate of decay from 5 percent per minute to

Table 20.3. Aerobiological decay.

Rate of Decay (%/min)	Half-Life (min*)
0.5	138
1	69
2	34
5	13
10	6

*Rounded to nearest minute.

0.5 percent per minute, it could survive for more than 2 hours over a target area (rather than a few minutes). Hence, it exposes a greater number of persons and increases the probability that it will have effect on those in the target area.

Another possible approach is to employ modified viral agents so that they do not simply result in the customary symptoms, such as fever or malaise, but some other far more debilitating effect. By such alterations, the cellular machinery of the host body can be used for producing an incapacitating or lethal substance. A hypothetical example is the use of a benign virus, such as vaccinia, as a vector for the genetic instructions for elaboration of a toxic compound (e.g., cobra toxin) within the host cell. Nowadays the vaccinia virus is being used in developing new means of immunization against other infectious organisms. Current researchers can splice into the vector virus genetic instructions to produce a toxin or some other factor, such as "bioregulators," which have harmful physiological or psychological properties; this approach probably offers a means for producing and delivering a detrimental substance from within the body over an extended time period and would make a diagnosis and treatment difficult. Along with the virus vectors, modified bacteria, rickettsia, and fungi also could be used to bring about infectious conditions with novel effects.

Ongoing scientific research on to the various disease organisms also is expected to provide insights for the development of advanced medical defenses against new and emerging biowarfare threats. The examples of potential infectious organisms that are attracting particular attention as biowarfare agents are HIV, the causative agent of AIDS, Hantaviruses (hemorrhagic fever-causing agents, such as *Ebola*), and the "flesh-eating" streptococcus bacteria. The streptococcus infection is not a new medical problem; the particular strain involved is capable of producing a combination of toxins resulting in simultaneous toxic shock and rapid spread of tissue breakdown. Once established, the infection is difficult to control, even with the use of antibiotics. Although the original form of this organism may not have significant potential as an aerosol threat agent, those seeking new infectious agents for military use could investigate its mechanisms of action.

Categories of Biological Warfare Agents

The US Department of Health and Human Services defined bioagents as "select agents" especially to those having "potential to pose a severe threat to public health and safety." Further, the CDC divided these agents into three categories as A, B, or C (Table 20.4) and administers the "Select Agent Program," which regulates the laboratories that may possess, use, or transfer select agents within the United States.

Category A

This category consists of high-priority agents posing a risk to national security. They can be easily transmitted and disseminated, result in high mortality, have potential major public health impact, may cause public panic, and always require special action for public health preparedness.

Anthrax

Anthrax is a noncontagious disease caused by the spore-forming bacterium *Bacillus anthracis*. The bacterium ordinarily produces a zoonotic disease in domesticated and wild animals including goats, sheep, cattle, horses, and swine. Humans become infected

Table 20.4. Categories of potential biological war agents based on Centers for Disease Control classifications.

Category	Characteristics	Disease and Their Causative agent
A	These agents pose the highest risk to national security because 1. These agents can be easily disseminated or transmitted from person to person 2. Have high mortality 3. Have potential to cause public panic and social disruption 4. Require special action for public preparedness.	Anthrax (*Bacillus anthracis*), plague (*Yersinia pestis*), smallpox (Variola major), monkeypox, tularemia (*Francisella tularensis*), botulinum toxin (*Clostridium botulinum*), viral hemorrhagic fevers, such as Marburg and Ebola viruses
B	These pose the second highest risk because 1. They are moderately easy to disseminate 2. Have moderate morbidity and low mortality 3. They require enhancement of diagnostic capacity and surveillance	Brucellosis (*Brucella* spp.), Glanders (*Burkholderia mallei*) and melioidosis (*Burkholderia pseudomallei*), Q fever (*Coxiella burnetii*), psittacosis (*Chlamydia psittaci*), food-borne diseases (enteric pathogens as *Salmonella* spp., *Shigella* spp., *Escherichia coli* and *Staphylococcus aureus)*, water supply threats (cholera [*Vibrio Cholerae*], cryptosporidiosis [*Cryptosporidium parvum*], typhoid [*Salmonella typhoid*]), Venezuelan, Eastern, and Western equine encephalitides (Alpha viruses), toxins (ricin toxin, abrin toxin, staphylococcal enterotoxin B, ε toxin of *Clostridium perfringens*, Mycotoxins)
C	These are emerging pathogens that could be engineered for mass dissemination because they 1. Are easily available 2. Easy to produce and disseminate 3. Have potential for high morbidity and mortality	Nipah virus, Hantaviruses, yellow fever, Severe Acute Respiratory Syndome, H1N1 (influenza), HIV/AIDS, Wes Nile virus, dengue, Kyasanur Forest disease, tick-borne encephalitis, multidrug resistant tuberculosis (*Mycobacterium tuberculosis*)

Rotz LD, Khan AS, Lillibridge SR, Ostroff SM, Hughes JM. 2002. Public health assessment of potential biological terrorism agents. *Emerg Infect Dis* 8:225–230.

by coming in contact with infected animals or contaminated animal products. Infection predominantly occurs through the cutaneous route and rarely via the respiratory or gastrointestinal route. The infection begins with the inoculation of spores through the skin or mucosal surface with an estimated infectious dose of 8,000 to 50,000 spores. These spores are then ingested by tissue macrophages, where they are germinated to the vegetative bacilli, which produces capsules and toxins. Proliferation at the tissue sites stimulates an inflammatory reaction and the produced lethal toxins impair host leukocyte function, leading to the distinctive and pathologic findings such as edema, hemorrhage, tissue necrosis, and a relative lack of leukocytes. When anthrax spores are inhaled, they are ingested by alveolar macrophages, which transport them to the regional tracheobronchial lymph nodes, where they germinate and produce a toxin that will give rise to the characteristic pathologic picture of massive hemorrhagic, edematous, and necrotizing lymphadenitis, and mediastinitis. The bacillus can further spread to the blood, leading to

septicemia and causing hemorrhagic meningitis, death mainly results from respiratory failure, overwhelming bacteremia, septic shock, and meningitis.

The first recorded modern incidence of anthrax in biological warfare were when Scandinavian "freedom fighters" supplied by the German general staff used anthrax against the Imperial Russian Army in Finland in 1916 (Bisher, 2003). Further in 1993, the Aum Shinrikyo used anthrax in Tokyo but with zero fatalities. Anthrax was used in a series of attacks on several United States Senators offices in late 2001; during these attacks, the bacilli were in a powder form, and it was delivered by the mail (Dewan et al., 2002). Afterward, anthrax is one of the few biological agents for which federal employees have been vaccinated. If discovered, early the anthrax disease can be cured by antibiotics therapy, such as ciprofloxacin (Vietri et al., 2009). Anthrax vaccine is also available but requires many injections for stable use. Ease of isolation, stability, and easy transmission property makes use of anthrax as potential biowarfare agent.

Bubonic Plague

Plague is a disease caused by the bacterium *Yersinia pestis*. Rodents are the normal regular host of plague, and the disease is then transmitted to humans by flea bites and occasionally by aerosol causing the disease pneumonic plague. Plague has been the cause of three great human pandemics in the Common Era, in the 6th, 14th, and 20th centuries. Historically, the oriental rat flea (*Xenopsylla cheopis*) has been largely responsible for spreading bubonic plague. When the flea ingests a blood meal of a bacteremic animal (rodent), bacilli can multiply and block the flea's foregut with a fibrinoid mass of bacteria. When this infected flea with a blocked foregut attempts to feed again, it regurgitates clotted blood and bacteria into the victim's bloodstream and thereby passes the infection onto the victim, whether rat or human. The black rat, *Rattus rattu*, has been considered the most responsible for the persistence and spread of plague in urban epidemics worldwide. There are three forms of plague septicemic, bubonic, and pneumonic (see Chapter 6). Patients with the bubonic form of the disease may also develop secondary pneumonic plague, and this complication can further lead to human-to-human spread by the respiratory route and cause primary pneumonic plague. Pneumonic plague is the most severe form of disease and if untreated, has a mortality rate approaching around 100 percent.

The pathogen has a history of use in biological warfare dating back many centuries and is considered a threat because of its ease of culture and ability to remain in circulation among local rodents for a long period of time. This weaponized threat comes mainly in the form of pneumonic plague, the disease that caused the Black Death in medieval Europe.

Smallpox

Smallpox is caused by the variola virus, which is the most notorious and highly contagious member of the poxviruses (family *Poxviridae*, genus *Orthopoxvirus*). The disease principally affects only humans and does not have any external hosts or vectors.

It represents a significant threat as a biowarfare agent because of its easy transmission through the atmosphere and has a high mortality rate (20–40 percent) and secondary spread. The virus remains stable in the environment and can retain infectivity for longer time periods. Following exposure to aerosolized virus, it starts multiplication locally in the respiratory tract. After an incubation period of 7 to 17 days (average 12 days), variola hematogenously (primary viremia) spreads to regional lymph nodes, where it replicates

additionally. It further hematogenously spreads (secondary viremia) to small dermal blood vessels, where it causes skin inflammatory changes (pox). Two types of smallpox have been recognized, variola major and variola minor. Variola major is the most severe form and has a fatality rate of around 30 percent in unvaccinated individuals and 3 percent in previously vaccinated individuals, whereas variola minor is a mild form and produces lethality in only 1 percent of unvaccinated individuals.

It was an important cause of morbidity and mortality in the developing world until 1980, when the World Health Organization (WHO) declared smallpox eradicated, with the last occurrence in Somalia in 1977. However, some samples of Variola are still available in Russian and US laboratories. Although people born before 1970 will have already been vaccinated for smallpox under the WHO program, the effectiveness of vaccination is limited because the vaccine provides a high level of immunity for only 3 to 5 years, but revaccination protection lasts longer. As a biological weapon, smallpox is dangerous because of the highly contagious nature of both the infected victims and their pox and also because of the infrequency with which vaccines are administered among the general population since the eradication of the disease, which would leave most people unprotected in the event of an outbreak.

Monkeypox

The monkeypox virus is a naturally occurring relative of variola originated in Africa. The first case of human with monkeypox was identified in 1970, with subsequently confirmed cases (less than 400). In 2003, an outbreak of 81 human cases occurred in the United States probably because of exposure to imported pets. This outbreak did not report any fatality or secondary human-to-human transmission.

It has been speculated that monkeypox may be weaponized; however, human monkeypox virus is less virulent than smallpox virus. It has a case-fatality rate of 11 percent in humans that had not been vaccinated against smallpox; however, pneumonia resulting from monkeypox has approximately a 50 percent mortality rate. In case of monkey pox, the secondary attack rate is only 9 percent which is far lower than the rate (25–40 percent) observed in smallpox.

Tularemia

Tularemia or rabbit fever is a zoonotic disease caused by the gram-negative, facultative intracellular bacterium *Francisella tularensis*. The disease had been discovered by G. W. McCay in Tulare County, California, in 1911 with the first confirmed human case in 1914. In 1921, Edward Francis described its transmission by deer flies via infected blood and coined the term *tularemia*. The disease can be contracted through contact with the fur, inhalation or ingestion of contaminated water or through insect bites. *F. tularensis* is usually introduced into the host body through breaks in the skin or through the mucous membranes of the eye, respiratory tract, or gastrointestinal tract. Experimentally, the subcutaneous injection of at least 10 virulent organisms and 10 to 50 organisms by aerosol can cause infection in humans. On inoculation, *F. tularensis* is then ingested by and multiplies within macrophages. The host mediates the defense against *F. tularensis* through a T cell-independent mechanism appearing early after infection (<3 days), and a T cell-dependent mechanism appearing later (>3 days) after infection. The disease is typically characterized by fever, localized skin or mucous membrane ulceration, regional lymphadenopathy, and occasionally pneumonia, which are further accompanied by a cutaneous chancrelike ulcers occurring in approximately 60 percent of patients and

considered as the most common sign of tularemia. These ulcers are generally single lesions with heaped up borders of around 0.4 to 3 cm in diameter. There is a change in the location of the lesions because lesions associated with infection acquired from mammalian vectors are usually located on the upper extremities, whereas lesions associated with infection from arthropod vectors usually are located on the lower extremities.

F tularensis has been considered an important biowarfare agent because of its high infectivity on aerosolization. People inhaling an infectious aerosol would experience severe respiratory illness, including life-threatening pneumonia and systemic infection if not treated properly. The causative agent occurs widely in nature and could easily be isolated and grown in large quantities in a laboratory, although manufacturing an effective aerosol weapon would require considerable sophistication.

Botulinum Toxin

It is one of the deadliest known toxins, which is produced by the anaerobic, spore-forming, gram-positive bacillus *Clostridium botulinum* and causes the disease botulism. Botulinum toxins are the most lethal toxins depending on the subtype (A–G) and are 10,000–100,000 times more toxic than chemical neurological inducers. The estimated lethal dose to 50 percent of the exposed population (LD50) is 0.001 mcg/kg in humans and causes death by respiratory failure and paralysis. Once reached, botulinum toxin binds to the presynaptic nerve terminal at the neuromuscular junction and cholinergic autonomic sites preventing the presynaptic release of acetylcholine, blocking neurotransmission, and finally producing muscular weakness and paralysis. The symptoms appear hours to several days after inhalation exposure (usually from 12–36 hours) and can include blurred vision, mydriasis (in 50 percent of cases), ptosis, dysphagia, dysarthria, dysphonia, and muscle weakness followed by some anticholinergic signs and symptoms, such as dry mouth, urinary retention, ileus, and constipation. After 24 to 48 hours, these neuromuscular manifestations progress to symmetric descending paralysis and respiratory failure. Complete recovery requires months because it requires the development of new axons.

The toxin is readily available worldwide probably because of its cosmetic applications in injections. Because botulinum toxin is highly lethal and easy to manufacture and weaponize, it represents a credible threat as a biowarfare agent. As a biowarfare or terrorist agent, exposure is likely to occur following inhalation of aerosolized toxin or ingestion of food contaminated with the preformed toxin or microbial spores. In 1995, Iraq admitted to active research on the offensive use of botulinum toxins and to weaponize and deploy more than 100 munitions with botulinum toxin; this is considered an excellent example of food terrorism. Table 20.5 illustrates the comparative theoretical lethality of a chemical agent (VX) and Botulinum toxin (biological agent) as declared by Iraq following Desert Storm.

Table 20.5. Comparative lethality of Botulinum toxin and VX.

Dose	Botulinum Toxin	Chemical agent (VX)
Lethal dose (LD50)/70 kg	0.14 micrograms	20 milligrams = 20,000
Quantity in Iraqi stockpile	11,800 liters	500 tons†
Theoretical lethal doses*	86 × 1012 (trillion)	23 × 109 (billion)

*By injection
†Approximately 500,000 liters.

Viral Hemorrhagic Fevers

Viral hemorrhagic fevers are caused by four families of viruses including the *Arenaviridae* (Lassa, Argentine, Bolivian, Brazilian, Venezuelan hemorrhagic fevers), *Bunyaviridae* (Rift Valley, Crimean-Congo, Hantavirus), *Filoviridae* (Marburg, Ebola hemorrhagic fevers), and *Flaviviridae* (yellow fever, dengue, Kyasanur forest, Omsk hemorrhagic fevers). The best known of all the viral hemorrhagic fever agents is Ebola virus. This was first recognized in Zaire in 1976 and linked to multiple outbreaks in Africa with a 53 to 92 percent mortality rate. The natural reservoir of Ebola is not known, currently there is no cure, and vaccines are also under development. *Filoviridae* members are usually spread from human to human by infected blood, secretions, organs, or semen. Congo-Crimean hemorrhagic fever usually is tick borne but also can be spread by infected body fluids or the meat of infected animals. Hantavirus is rodent borne, whereas Rift Valley fever and yellow fever diseases are mosquito-borne illness. In Africa, more than 200,000 cases of Lassa fever occur each year with more than 5000 deaths.

All of these agents producing viral hemorrhagic fever are enveloped RNA viruses. They are quite stable at a neutral pH and capable of surviving in blood for long periods, which leads to their infectivity of patients around domestic animal slaughters. These viruses are related to the ecology of their vector, whether rodent or arthropod, which helps in their diagnosis. All of these viral agents cause laboratory infectious hazards by aerosol. The specific viral hemorrhagic fever syndrome developing in patients depends on numerous factors including viral virulence and strain characteristic, route of exposure, infectious dose, and host factors. Mortality also varies depending on the group of viruses as for the *Arenaviridae* it ranges from 15 to 38 percent, for *Bunyaviridae* from 1 to 50 percent, for *Filoviridae* from 0.5 to 92 percent, and for *Flaviviridae* from 0.2 to 50 percent. All viral hemorrhagic fever agents primarily target vascular beds, produce microvascular damage, and enhance vascular permeability. Clinical manifestations may include fever, myalgia, headache, prostration, conjunctival injection, mild hypotension to severe shock, and mucosal and petechial hemorrhages with neurologic, hematopoietic, hepatic, and pulmonary involvement, which are observed in more severe cases. Among the arenaviruses, hemorrhagic or neurologic manifestations are not found associated with Lassa fever, but deafness is commonly observed and it causes the most severe capillary leak syndrome among the hemorrhagic fever viruses and causes massive edema. Argentine and Bolivian hemorrhagic fevers present with pronounced hemorrhagic and neurologic manifestations, whereas Rift Valley fever virus is primarily hepatotrophic in which patients demonstrate hemorrhagic signs. Crimean-Congo virus is the most severely hemorrhagic among all viral hemorrhagic fevers because it causes disseminated intravascular coagulation (DIC). Hantaan virus is associated with pulmonary and renal failure with the characteristic feature of sunburn flush on the head, neck, and upper back. The patients of Ebola hemorrhagic fever show nonpruritic, centripetal, pinhead-sized maculopapular erythematous exanthem (visible mainly in fair-skinned patients), bleeding, and DIC. In flaviviruses, yellow fever virus is primarily hepatotrophic, hematemesis commonly cause black vomit, and patients usually develop clinical jaundice and die from hepatorenal syndrome.

Hence all these viruses are uniformly characterized by an acute generalized febrile illness that includes malaise, prostration, increased vascular permeability, and abnormalities of circulatory system. All these viral agents are highly infectious via the aerosol route; most are stable as respiratory aerosols and could easily be replicated in cell culture, thus making them ideal for use by terrorists. The Soviet Union investigated the use of filoviruses in biological warfare and the Aum Shinrikyo group unsuccessfully attempted

Table 20.6. Category B diseases, their etiological agents, type, and transmissibility.

Disease	Agent	Type of Agent	Zoonoses	Contagious Person to Person
Brucellosis	*Brucella* species	Bacteria	Yes	No
Glanders	*Burkholderia mallei*	Bacteria	Yes	No
Melioidosis	*Burkholderia pseudomallei*	Bacteria	Yes	No
Q fever	*Coxiella burnetii*	Rickettsia	Yes	No
Psittacosis	*Chlamydia psittaci*	Bacteria	Yes	No
Food and water safety threats	*Salmonella* species, *Shigella dysenteriae*, *E. Coli* 0157:H7, *Cryptosporidium parvum*, *Vibrio cholerae, Salmonella typhi*	Bacteria	No	No
Viral encephalitis	Several arboviruses (e.g., VEE, WEE, EEE, SLE)	Virus	Yes	No
Epsilon toxin poisoning	*Clostridium perfringens* epsilon toxin	Bacteria derived	No	No
SEB poisoning	SEB	Bacteria derived	No	No
Ricin poisoning	Ricin toxin from *Ricinus communis*	Plant derived	No	No

SEB, Staphylococcal Enterotoxin B.

to obtain cultures of Ebola virus. The arenaviruses have a reduced case-fatality rate as compared to the disease caused by filoviruses, but these are more widely distributed, mainly in central Africa and South America.

Category B

Category B comprises diseases and agents that are moderately easy to disseminate and have low mortality rates (Table 20.6).

*Brucellosis (*Brucella *spp.)*

Brucellosis is a zoonotic infection of domesticated and wild animals caused by the genus *Brucella*. The organism mainly affects cattle, sheep, goats, and other ruminants, causing abortion, fetal death, and genital infection. Humans are incidentally infected when coming in contact with infected animals, tissues or discharges, blood, urine, or by ingesting unpasteurized milk products and may develop numerous symptoms, in addition to the usual ones of fever, malaise, and muscle pain. The disease often becomes chronic, and victims may relapse, even after taking appropriate treatment. Because of its ease of transmission by aerosol *Brucella* species may be useful as a biowarfare agent.

Brucella spp. is small, nonmotile, nonsporulating, aerobic, gram-negative coccobacilli classified into six species, each to be having a characteristic predilection to infect certain animal species. But only four species, *Brucella melitensis, Brucella suis, Brucella abortus*, and *Brucella canis* can cause disease in humans. The approximate dose of around 10 to 100 bacterial cells are required to cause human disease. Animals may transmit *Brucella* organisms during septic abortion, at the time of slaughter, and in their milk, whereas the disease is rarely transmitted from human to human. *Brucella* species can enter mammalian

host body through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract. The organism can replicate intracellularly as well as extracellularly in host tissue. These are rapidly ingested by polymorphonuclear leukocytes, which usually fail to kill them and then further phagocytized by macrophages of the lymphoid tissue and eventually localize in the lymph nodes, liver, spleen, joints, kidneys, and bone marrow. The host cellular response against brucellosis may range from abscess formation to granuloma formation with caseous necrosis.

There is a wide range of clinical manifestations of brucellosis, and the disease course also varies accordingly. Patients may generally present with an acute, systemic, febrile illness; an insidious, chronic infection; or a localized inflammatory process. The disease outcome may be abrupt or insidious in onset, with an incubation period ranging from 3 days to several months. Patients usually show some nonspecific symptoms such as fever, malaise, sweats, fatigue, anorexia, and muscle or joint aches with the frequent occurrence of neuropsychiatric symptoms such as depression, headache, and irritability. Additionally, focal infection of bones (including the spine), joints (monoarticular or polyarticular), or the genitourinary tract may cause local pain. Sometimes cough and pleuritic chest pain also may be noted. Symptoms usually last 3 to 6 months and occasionally for longer than a year. Brucellosis does not usually cause leukocytosis, and patients may be neutropenic. Among all the *Brucella* spp., *Brucella melitensis* tends to cause more severe, systemic illness. *Brucella suis* is more likely to cause localized suppurative disease with the infection sites including the heart, central nervous system (CNS), and skin. *Brucella* endocarditis is a rare but feared complication as accounts for 80 percent of deaths from brucellosis.

Laboratory-acquired infections emphasize the tremendous potential of *Brucella* spp. as biowarfare agent (Yagupsky and Baron, 2005). A bioterrorism scenario was evaluated using an aerosolized *B. melitensis* agent spread along a line with the prevailing winds and optimal meteorological conditions and assumed that the infectious dose to infect 50 percent (ID_{50}) of a human population would require inhalation of at least 1,000 vegetative cells. Once disseminated, the environmental decay of the organism is estimated at 2 percent per minute.

Glanders and Melioidosis

Burkholderia mallei and *Burkholderia pseudomallei* are the causative organisms of glanders and melioidosis disease, respectively. Although rarely observed in western countries, both of these organisms have unique potential as bioterrorism agents. Although being unique organisms, *B. mallei* and *B. pseudomallei* share many similarities and, therefore, may be considered together in the context of a deliberate-release event. These pathogens are less familiar to medical and laboratory personnel than other selected bioterrorism bacterial agents including *Bacillus anthracis*, *Y. pestis*, and *F. tularensis*. These pathogens share many characteristic features making them nearly "perfect" agents for biological terrorism (Table 20.7).

Glanders primarily affects animals and can be transmitted both from animal to animal and animal to human. Human-to-human transmission rarely occurs in nature. Melioidosis affects both humans and animals. The principal reservoir for *B. pseudomallei* is the contaminated environment (in endemic regions), especially soil and water. Human-to-human, as well as zoonotic, transmission of melioidosis is rare. It is a saprophytic species and is quite capable of surviving in relatively hostile environments for years (Chierakul et al., 2005). Humans and animals acquire melioidosis through percutaneous inoculation, inhalation, or ingestion and most rarely through sexual transmission.

Table 20.7. Key features contributing to the bioterrorism potential of *Brucella mallei* and *Brucella pseudomallei.*

Aspect	Key Features
Epidemiology	• Rare diseases in western countries • Melioidosis is endemic in Southeast Asia and Oceania whereas glanders is rare and mostly occur sporadically
Pathogenesis	• Environmental persistence requires weeks for *B. mallei* whereas years for *B. pseudomallei* • Infection through inhalation, ingestion, or percutaneous inoculation • Low infective doses • Highly variable incubation period
Clinical features	• Acute, subacute or chronic disease; possibility of relapse and late reactivation • Wide spectrum of manifestations; great imitators • High prevalence of severe sepsis and septic shock • Significant morality
Diagnosis	• Lack of experience among both clinicians and laboratory personnel • Identification by routine laboratory methods difficult to impossible • Need for specialized laboratory reagents, equipment, and expertise • Unusual biosafety requirements
Therapy	• Complex protocols using intravenous antimicrobials • Long duration of therapy as requires months • Frequent need for surgical interventions and supportive critical care • Antimicrobial resistant isolates not uncommon
National preparedness	• Species highly accessible • No national reference laboratory • Difficulty in stockpiling relevant antimicrobials • Antimicrobial resistance • No available vaccine • No evidence regarding postexposure prophylaxis • Lack of awareness in medical community

The predominant portal of entry is the percutaneous route, even for patients with pneumonic melioidosis'; pneumonia in such cases, therefore, involves hematogenous dissemination after percutaneous inoculation. However, pulmonary infection may also occur directly via inhalation (Currie et al., 2000). Infection through ingestion mostly involves contaminated water. The pathogenesis of *B. mallei* infection is far more enigmatic, unlike *B. Pseudomallei*; it is not an environmental pathogen and its main reservoir is animals. It has limited surviving capabilities in the environment and has been described to persist only for up to 6 weeks in infected horse stables. It is primarily a disease of equids as including horses, donkeys, and mules but can also affect goats, sheep, dogs, and cats. The exact mode of infection of glanders disease is not at all clear and probably involves several routes of infection including inhalation, percutaneous inoculation, and ingestion. The incubation period is similar to that of melioidosis, ranging from 1 to 5 days in inhalational infection to many months. Chronic glanders are sometimes referred to as "farcy" in which multiple abscesses are produced with the muscles of the arms and legs. Chronic glanders may even lead to abscesses in the liver, spleen, and joints, which is a serious medical condition with a case fatality rate of 60 percent. Relapse and reactivation may also occur in both diseases.

Both *B. pseudomallei* and *B. mallei* have been considered important bioterrorism agents because of their ready worldwide availability and their potency to be transmitted through an aerosol route. A deliberate release of *Burkholderia* could affect hundreds or thousands of people, depending on the method of release. Glanders has several characteristics making it a potential agent for biological warfare and terrorism because very few *Burkholderia mallei* organisms are required to cause disease and an ease of production of the organism. For example in the 1980s, the Soviet Union produced more than 2,000 tons of formulated *B. mallei*. When *B. mallei* cells are inhaled, the disease produces a high mortality rate. Additionally, the diagnosis and treatment of glanders is confounded by the lack of knowledge of this disease among health-care providers and diagnosticians and patients who recover from the disease do not develop a protective immunity; this further provides the possibility of reuse of the agent.

Q FEVER

Q fever or Query fever (*Coxiella burnetii*) is a zoonotic disease caused by a rickettsia-like organism of low virulence but remarkable infectivity (as a single organism may initiate infection). It is designated as *Coxiella burnetii* to recognize the contribution of both Harold Cox and MacFarlane Burnet in the isolation and characterization of the pathogen in 1937 and 1938. The genus *Coxiella* has only single species, *burnetii*, which is extremely infectious but is unable to grow or replicate outside the host cells; however, a sporelike form of the organism is extremely resistant to heat, pressure, and many antiseptic compounds, which allows *C. burnetii* to persist in the environment for longer time periods under harsh conditions. In contrast to this high degree of inherent resilience and transmissibility, the acute clinical disease associated with Q fever is usually a benign, but temporarily incapacitating, illness in humans in which most of the patients recover even without treatment.

The primary reservoir for human infection with *C. burnetii* is livestock, particularly parturient females, and the disease is distributed worldwide. Humans who work in animal husbandry, especially those who assist during parturition, are at risk of acquiring the disease Q fever. The host range of *C. burnetii* is diverse and including a large number of mammalian species and arthropods. Among all these hosts, the human is the only host identified experiencing an illness as a result of infection. Many different strains of *C. burnetii* have been identified worldwide with different clinical manifestations and associated complications. Humans are infected most commonly by contact with domestic livestock, particularly goats, cattle, and sheep, and the risk of human infection is increased substantially when they are exposed to these animals at parturition. During gestation, *C. burnetii* proliferates in the placenta, which facilitates the aerosolization of large numbers of the pathogen during parturition. The organism survives on inanimate surfaces, such as straw, hay, or clothing and allows the further transmission to individuals who are not in direct contact with infected animals. Human infection with *C. burnetii* is usually the result of inhalation of infected aerosols; however, it may also occur after consumption of unpasteurized dairy products because the organism survives in dairy products. Following infection, host cells phagocytize the organisms, which will further disseminate as a result of free circulation of an organism in the plasma on the surface of the cells and are then carried by circulatory macrophages. At the initial portal of entry, a little host reaction occurs, either in the lung following inhalation of aerosol or in the skin following a tick bite. Q fever develops without the formation of a primary infectious focus in the area of the tick bite, and the organism does not generally infect the vascular

endothelium, which is common with other rickettsial pathogens. The lipopolysaccharide layer present on the surface of *C. burnetii* cell protects the pathogen from host microbicidal activities. In human beings the incubation period varies from 2 to 40 days (mean 15 days), which is correlated inversely with the magnitude of the inoculum because a higher inoculum also increases the severity of the disease. In humans, the disease may be manifested by asymptomatic seroconversion, acute illness, or chronic disease with the chronic frequency (usually endocarditis) probably less than 1 percent of the total infected population.

For cases with acute Q fever, no characteristic illness is described, and manifestations may vary considerably between locations where the disease is acquired. The onset of symptomatic Q fever may be abrupt or insidious with common signs and symptoms of fever, chills, headache, diaphoresis, malaise, myalgias, fatigue, and anorexia. Arthralgias are relatively uncommon and cough often occurs later in the illness. Chest pain occurs in a minority of patients and sometimes nonspecific, evanescent skin eruptions have also been reported without any characteristic rashes. Most of the patients appear mildly to moderately ill with the fluctuating temperature, peaking at 102.2 to 104° F (39–40° C), and is biphasic in approximately 25 percent of patients. The fever generally lasts less than 13 days but has also been reported to last longer in older adults. Some patients reported encephalopathic symptoms, headache, hallucinations (visual, auditory), expressive dysphasia, facial pain resembling trigeminal neuralgia, diplopia, and dysarthria. Patients with acute Q fever may show a clinical picture resembling acute hepatitis with elevations of aminotransferases which are two- to threefold higher than the upper normal limits. In 10 to 15 percent of patients with acute Q fever, there are an elevated bilirubin levels but with normal white blood cell count. The erythrocyte sedimentation rate is elevated in 33 percent of patients presented with mild anemia or thrombocytopenia. Chronic infection with *C. burnetii* usually is manifested by infective endocarditis, which is considered the most severe complication of Q fever. Additionally, hepatitis, infected vascular prostheses, aneurysms, osteomyelitis, pulmonary infection, cutaneous infection, and an asymptomatic form of the disease have been reported.

The potential of *C. burnetii* as a biowarfare agent is directly related to its infectivity. It has been estimated that 50 kg of dried *C. burnetii* would produce casualties at a rate equal to that of similar amounts of anthrax or tularemia organisms. Because of its highly infectious nature, high stability in the environment, and aerosol route of transmission, *C. burnetii* is considered as a potential agent for biowarfare and bioterrorism. Although the overall mortality associated with the disease is low, it is considered a debilitating agent and was extensively researched in the US bioweapons program. WHO estimated that, if *C. burnetii* were aerosolized in a city of approximately 5 million people, there would be 125,000 ill and 150 deaths.

Psittacosis

Psittacosis is a zoonotic disease caused by the bacterium *Chlamydophila psittaci* (formerly *Chlamydia psittaci*). The term *avian chlamydiosis* is used when birds are infected with *C. psittaci*, which is also called as parrot fever and ornithosis. Actually the word *psittacosis* is used when the disease is carried by any species of bird belonging to the *Psittacidae* family, whereas *ornithosis* is used when other birds carry the disease. Psittacosis was first reported in Europe in 1879 (Potter et al., 1983). Psittacosis causes flulike symptoms, which can lead to severe pneumonia and other associated health problems, but the disease is rarely fatal. As with most of these diseases, humans are an incidental, dead-end host for

C. psittaci. Most of the human cases have been found associated with transient exposure to infected pet birds and poultry or free ranging birds or their droppings. Human infection starts with the inhalation of viable *C. psittaci* aerosolized from the dried feces, guano, or respiratory tract secretions of infected birds (Smith et al., 2005) because *C. Psittaci* is found in the feces and nasal discharges of an infected birds. The organism can remain infectious for up to several months in moist and cool conditions as found in guano. Other means of transmission include mouth-to-beak contact and direct transmission from infected bird feathers and tissues but person-to-person and food-borne transmission have not been documented.

C. psittaci is an obligate intracellular bacterium undergoing several transformations during its life cycle. It exists as an elementary body (EB) in between hosts is not biologically active, but is resistant to environmental stress and capable to survive outside of the host. An infected bird sheds this EB, which is then inhaled into the lungs of a new host. From there it is taken into the host cells through the process of phagocytosis by forming an endosome where they are transformed into reticulate bodies (RB), which replicate within the endosome. These regulatory bodies use host cell components to replicate, convert back to EB, are released back into the lung, and subsequently caused death of the host cell. These newly generated EBs released from the dead cells are now able to infect new cells, either in the same host or another one. Thus the life cycle of *C. Psittaci* is an interesting dichotomy, including both a reticulate body, which can replicate but is unable to cause new infection, and an EB, which is able to infect new host but can not replicate. *C. Psittaci* is highly resistant to drying. EBs retain viability in canary feed for 2 months, in poultry litter for up to 8months, and in straw and on hard surfaces for 2 to 3 weeks (Johnston et al., 1999). The incubation period for psittacosis ranges from 1 to 4 weeks, but most of patients develop symptoms after 10 days. The clinical picture ranges from mild to severe systemic illness with pneumonia, occurring most commonly in older adults. Patients usually complain of fever, chills, headache, malaise, myalgia, nonproductive cough, difficulty in breathing, and rash on the trunk of the body. Some patients become lethargic, have sluggish speech, and other organ systems become involved, which can lead to more serious conditions such as heart complications, hepatitis, arthritis, conjunctivitis, encephalitis, and respiratory failure.

C. psittaci had previously been component of several state-funded bioweapons research programs. The characteristics that may make it a potential bioweapon include its stability in the environment, ease for aerosolization, and worldwide prevalence. Because of this threat, the United States ended large-scale commercial importation of psittacine birds in 1993 with the implementation of Wild Bird Conservation Act, however, importation on a limited basis still continues. Although bird smuggling is rare, it remains a potential source of parrot fever and psittacosis.

Food-Borne Diseases

Food borne diseases (including enteric pathogens as *Salmonella* spp., *Shigella* spp., *Escherichia coli*, and *Staphylococcus aureus)* are the important causes of illness and death especially in less-developed countries, killing approximately 1.8 million people annually. In developed countries, these are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of dollars in medical care and loss of productivity. New and emerging food-borne pathogens and resulting food-borne diseases are driven by several factors, such as pathogen evolution, changes in agricultural and food manufacturing practices, and changes to the human host status. There are growing

concerns that terrorists could use these food-borne pathogens to contaminate food and water supplies in attempts to incapacitate thousands of people and disrupt economic growth (Fratamico and Bayles, 2005).

***Salmonella* spp.** Among all the food borne pathogens, *Salmonella* serotypes continue to be a prominent threat to food safety worldwide. Human infections are commonly acquired through the consumption of undercooked food products derived from livestock or domestic fowl. The second half of the 20th century saw the emergence of *Salmonella* serotypes that became associated with new food sources (i.e., chicken eggs) and the emergence of *Salmonella* serotypes with resistance against multiple antibiotics (Andrews and Baumler, 2005).

***Shigella* spp.** *Shigella* species are members of the *Enterobacteriaceae* family comprising gram-negative, nonmotile rods. Based on structure and biochemical properties of O-antigen, the genus is divided into four subgroups as *Shigella dysenteriae* (subgroup A), *Shigella flexneri* (subgroup B), *Shigella boydii* (subgroup C), and *Shigella sonnei* (subgroup D). Classical symptoms of *Shigella* infection include mild to severe diarrhea with or without blood, fever, tenesmus, and abdominal pain with some associated complications such as seizures, toxic megacolon, reactive arthritis, and hemolytic uremic syndrome. The bacterium is transmitted by the fecal-oral route, commonly through contaminated food and water with an infectious dose ranges from 10 to 100 organisms. *Shigella* spp. employs a sophisticated pathogenic mechanism to invade colonic epithelial cells of the host, man and higher primates, and has the ability to multiply intracellularly and spread from cell to adjacent cell via actin polymerization. *Shigella* spp. had been considered one of the leading causes of bacterial food-borne illnesses and can spread quickly within a population (Lampel, 2005).

***Escherichia* coli** *Escherichia coli* is the most thoroughly studied bacterial species in the microbial world. It is a commensal of human and animal intestinal tracts with low virulence potential. Many strains of *E. coli* (mainly *E. coli* 0157:H7) are now known as pathogens, inducing serious gastrointestinal diseases and even death in humans. There are six major categories of *E. coli* strains that cause enteric diseases in humans:

1. Enterohemorrhagic *E. coli* (EHEC), which causes hemorrhagic colitis and hemolytic uremic syndrome.
2. Enterotoxigenic *E. coli* (ETEC), which induces traveler's diarrhea.
3. Enteropathogenic *E. coli* (EPEC), which causes a persistent diarrhea in children living in developing countries.
4. Enteroaggregative *E. coli* (EAEC), which provokes diarrhea in children.
5. Enteroinvasive *E. coli* (EIEC) which is biochemically and genetically related to *Shigella* species and can induce diarrhea.
6. Diffusely adherent *E. coli*, which causes diarrhea and is distinguished by a characteristic type of adherence to mammalian cells (Smith and Fratamico, 2005).

Staphylococcus aureus *S. aureus* is a common cause of bacterial food-borne intoxication worldwide. Symptoms of the disease include vomiting and diarrhea that occur shortly after ingestion of S. aureus enterotoxin-contaminated food, which accounts for the short incubation time. These staphylococcal enterotoxins are superantigens having adverse

effects on the immune system. The genes that code for an enterotoxin are accessory genetic elements in S. aureus, meaning not all strains of this organism are enterotoxin-producing and are found on prophages, plasmids, and pathogenicity islands in different strains of S. aureus. Expression of the enterotoxin genes is often under the control of global virulence gene regulatory systems (Stewart, 2008).

Water Supply Threats

Vibrio Cholerae *V. cholerae* causes an infection of the small intestine called cholera. The bacterium is a motile, gram-negative, nonsporulating rod of which two serogroups have been identified as causing symptoms in humans: O1 and O139. Transmission occurs primarily by drinking or eating water or food that has previously been contaminated by the feces of an infected person (even an asymptomatic patient). Worldwide it affects 3 to 5 million people and causes 100,000 to 120,000 deaths in a year as of 2012 (WHO, 2012). The primary symptoms of cholera are profuse painless diarrhea and vomiting of clear fluid, which start suddenly, 1 to 5 days after ingestion of the bacteria (Sack et al., 2004). The diarrhea and vomiting is so severe it can lead to rapid dehydration and electrolyte imbalance and death in some cases. The diarrhea in this case is frequently described as "rice water" in nature and having a fishy odor. An untreated cholera infected person may produce 10 to 20 liters of diarrhea a day with fatal results. For every symptomatic person, around 3 to 100 people acquire the infection but still remain asymptomatic (King et al., 2008). Cholera is also referred to as "blue death" because of a patient's skin turning a bluish gray hue from extreme loss of fluids (McElroy and Townsend, 2009).

Diarrheal fluids are highly infective although; the organism can easily be killed by desiccation. It does not remain viable in pure water but will survive up to 24 hours in sewage and as long as 6 weeks in water-containing organic matter. *V. cholerae* can also withstand freezing for 3 to 4 days, and because of this tolerance, it could be considered as an effective biological weapon if major drinking water supplies were heavily contaminated. Hence terrorists would intentionally use this agent to contaminate food or water supplies.

Cryptosporidium Parvum *C. parvum* parasite is responsible for a water- or food-borne infection of the disease called cryptosporidiosis. The disease is transmitted through the fecal-oral route and involves person-to-person transmission (e.g., while changing diapers caring for an infected person or engaging in certain sexual behaviors) and animal-to-person transmission. It is highly infective, requiring less than 10 organisms, and presumably 1 organism, can initiate an infection. All persons are considered to be susceptible, although people with good immune status may be asymptomatic. Individuals with impaired immunity and children aged 1 to 5 years are most likely to get the disease. Asymptomatic infections are common and become a source of infection for others. The major symptom in humans is profuse and watery diarrhea, preceded by anorexia and vomiting in children and usually accompanied with cramping abdominal pain. General feeling of malaise, fever, anorexia, nausea, and vomiting occur less often. Symptoms often wax and wane but remit in less than 30 days in most of the immunologically stronger people. In most of the immunocompromised patients, cryptosporidiosis usually causes chronic diarrhea; however, it rarely causes lung and biliary tract disease. The disease is rarely lethal in healthy people, but in persons with severely weakened immune status, chronic gastrointestinal illness or more disseminated disease can lead to severe complications and death. Secondary transmission results from an exposure to the stool of infected individuals, both patients with acute infection and asymptomatic carriers. The infectious

stage (oocysts) appears in the stool at the onset of symptoms and becomes infectious immediately upon excretion. These infectious oocysts continue to be excreted in the stool for several weeks after symptoms get resolve and may remain infective outside the body for up to 2 to 6 months in a moist environment. Oocysts are highly resistant to chemical disinfectants generally used to purify drinking water.

In a terrorist attack, *C. parvum* would most likely be disseminated through the intentional contamination of food or water supplies.

Salmonella typhi *S. typhi* causes a potentially fatal multisystemic illness called typhoid fever or enteric fever. The clinical presentation of typhoid includes fever, malaise, diffuse abdominal pain, and constipation. This becomes a grueling illness if untreated, which may progress to delirium, obtundation, intestinal hemorrhage, bowel perforation, and death within 1 month of onset. Survivors may get permanent neuropsychiatric complications. *S. typhi* has no nonhuman vectors and is principally transmitted through the oral route via food or beverages handled by an individual who chronically sheds the bacteria through stool or, less commonly, urine or via sewage-contaminated water or shellfish, especially in the developing world (Earampamoorthy and Koff, 1975) and hand-to-mouth transmission after using a contaminated toilet and neglecting hand hygiene. It is highly infectious as a small inoculum containing only 100,000 organism causes infection in more than 50 percent of healthy volunteers (Levine et al., 2001). Following infection *S, typhi* resists the low pH of the digestive tract and finally gains entry through the distal ileum. The bacterium has specialized fimbriae that adhere to the epithelium of the Peyer's patches of the intestine, the main relay point for macrophages traveling from the gut into the lymphatic system. The bacterium has Vi capsular antigen that masks pathogen-associated molecular patterns (PAMPs), such as flagella and lipopolysaccharides, and thereby avoids a neutrophil-based inflammatory reaction. The bacteria then induce host macrophages to attract more macrophages at the site. It co-opts the macrophages cellular machinery for their own reproduction (Ramsden et al., 2007) as it is carried through the mesenteric lymph nodes to the thoracic duct and the lymphatics and then to the reticuloendothelial tissues of the liver, spleen, bone marrow, and lymph nodes. Once established there, the *S. typhi* bacteria pause, continue to multiply, and increase their progeny until some critical density is reached. Afterward, the bacteria induce macrophage apoptosis, breaking out into the bloodstream to invade the rest of the body (Parry et al., 2002). The gallbladder is then infected either through bacteremia or by the direct extension of *S. typhi* infected bile resulting, the organism re-enters the gastrointestinal tract in the bile and reinfects Peyer's patches. Bacteria that do not reinfect the host are typically shed in the stool and are then ready to infect other hosts (Parry et al., 2002).

S. typhi has been considered a potential bioterror agent because food or water supplies can be intentionally contaminated with the bacterial inoculums. It has already been used by the followers of Rajneesh to contaminate salad dressings in 10 United States restaurants.

Viral Encephalitis Viral encephalitis is an inflammation of the brain caused by a virus. Many viruses are capable of causing infections that lead to viral encephalitis, which include, but are not limited to, enterovirus, herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, adenovirus, rubella, measles, and many of the arbo viruses. All of the encephalitis-causing viral agents that could be useful as bioterror agent belong to arboviruses, requiring transmission by an insect or an arachnid. The vectors involved in the transmission are either culicine mosquitoes or ticks. These encephalitis causing arthropod-borne viruses are also involved in zoonoses and maintain complex life cycles involving a nonhuman primary

Table 20.8. Summary of clinical description of viral encephalitides.

Disease	Clinical Description
Viral encephalitides	1. The majority of human infections is asymptomatic or may result in a nonspecific flulike syndrome. 2. Onset may be insidious or sudden with fever, headache, myalgias, malaise, and occasionally prostration. 3. Infection may lead to encephalitis, with a fatal outcome or permanent neurologic sequelae. 4. Only a small proportion of infected person progress to frank encephalitis.

vertebrate host and a primary arthropod vector. Humans and domestic animals develop clinical illness but usually are dead-end hosts because they do not develop significant viremia to contribute to the transmission cycle. Many encephalitis-causing arboviruses have a variety of different vertebrate hosts and some of them are transmitted by more than one vector. In most of the diseases, the infection is asymptomatic or may result in a nonspecific flulike syndrome (Table 20.8). Onset may be insidious or sudden with fever, headache, myalgia, malaise, and occasionally prostration. Infection may, however, lead to encephalitis with a fatal outcome or permanent neurologic sequelae.

Many think that most of these encephalitis-associated viruses could be used by aerosolization during a bioterrorist attack. It has been suggested that the virus could be transferred from blood to the central nervous system through the olfactory tract. Another possible route of contamination is the use of vector-borne transmission such as infected mosquitoes or ticks. Weaponization of alphaviruses, especially the equine encephalitis viruses, would be considered the easiest one.

Eastern equine encephalitis (EEE), western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE) are alphaviruses causing encephalitis in both human beings and equines. All of these viruses are transmitted to humans by mosquito bites. These viruses are also transmittable by aerosol as already reported in laboratory accidents, and these highly infectious particles have caused more laboratory-acquired disease than any other arbovirus (Smith et al., 1997). These viruses would lend themselves well for weaponization for the following reasons:

- They replicate to very high titers.
- They can be manipulated easily in unsophisticated systems because they are relatively stable and highly infectious for humans by aerosolization.
- Strains that produce incapacitating or lethal infections do still exist.
- Multiple serotypes exist, making the production of adequate appropriate vaccine difficult (Smith et al., 1997).
- The infectious dose for inhalation is extremely low for some viruses (1 pfu for VEE), but is unknown for all individual viruses.
- Some suggest that these viruses could be easily modified by genetic manipulation (Smith et al., 1997).

Although these viruses usually cause similar clinical illness, the consequences and evolution of associated individual diseases varies. Person-to-person transmission of these agents has never been reported, despite the theoretical possibility (e.g., VEE from nasopharynx).

Eastern Equine Encephalitis EEE virus was first identified in the 1930s and currently appears in focal locations along the eastern seaboard, the Gulf Coast, and some inland Midwestern locations of the United States. Although small outbreaks of human disease have occurred in the United States, equine epizootics are a common occurrence during the summer and autumn. Birds living in swamps are the natural reservoir of this virus. The disease is transmitted between birds by the mosquito *Culiseta melaneura* and to horses and humans by *Aedes* mosquitoes. Human cases are usually preceded by an outbreak in horses with an incubation period ranging from 4 to 15 days after the bite of an infected mosquito. Symptoms range from a mild flulike illness to encephalitis, coma, and death. The symptoms usually start with sudden onset of high fever, chills, vomiting, general muscle pains, and headache of increasing severity. Fever may persist up to 11 days before the onset of neurological disease. In children it frequently causes generalized facial or periorbital edema. Patients sometimes progress to more severe symptoms including confusion, somnolence, delirium, stupor, disorientation, dysphasia, paresis, ataxia, myoclonus and cranial nerve palsies, seizures, and coma, and finally death. It has been estimated that 1 out of 23 infected patients developed neurological symptoms such as encephalitis (Golfield et al., 1968). Despite the development of a rapid and neutralizing humoral immune response, the virus is not completely eradicated from the central nervous system and continues the progressive neuronal destruction and inflammation (Smith et al., 1997). EEE is the most severe of the arboviral encephalitides, with case fatality rates of 50 to 70 percent observed mainly in young children and the elderly. It has also been found associated with frequent neurological sequelae in survivors, requiring permanent institutional care (seizures, spastic paralysis, and cranial neuropathies).

EEE was one of more than a dozen agents that the United States had researched as potential biological weapons before the nation suspended its biological weapons program.

Western Equine Encephalitis WEE was first isolated in California in 1930 from the brain of a horse with encephalitis and remains an important cause of encephalitis in horses and humans in North America, mainly in western parts of the United States and Canada. Seroprevalence rates among people living in or near endemic WEE areas is about 100 percent (Smith et al., 1997), indicating that most of the human cases are asymptomatic or present as mild, nonspecific illness. Human cases are usually reported in June or July with an incubation period ranging from 5 to 10 days. WEE is less neuroinvasive than EEE, but having identical signs and symptoms to those noted in cases of EEE. Patients with clinically apparent illness usually have a sudden onset of fever, malaise and headache, followed by nausea, vomiting, and anorexia, which are further intensified and are followed by altered mental status, somnolence, weakness, meningismus, and delirium that can finally progress into coma. Children, especially 1 year old are more severely affected than adults and may be left with permanent sequelae, seen in 5 to 30 percent of young patients.

Venezuelan Equine Encephalitis VEE is an important veterinary and public health problem as observed in Central and South America. Occasionally, large regional epizootics and epidemics can occur resulting in thousands of equine and human infections. Mosquitoes can transmit many different strains of VEE to humans and cause disease. A large epizootic that began in South America in 1969 reached Texas in 1971 during which 200,000 horses died, and several thousand human infections were reported. Another VEE epidemic occurred in autumn 1995 in Venezuela and Colombia with an

estimated 90,000 human infections. The human infection is less severe than with EEE and WEE viruses with rare fatalities and an incubation period ranging from 28 hours to 6 days (Smith et al., 1997). Patients generally complain of a constant and often debilitating headache, and it may last up to 6 months. They may also present with high fever, photophobia, myalgia, sore throat, erythematous pharynx, conjunctival injection, vomiting, diarrhea, and malaise, mimicking a flulike illness. Encephalitis is usually confined to children and it has been estimated that less than 0.5 percent of adults and 4 percent of children develop encephalitis (Sanmartin, 2002). Lethargy, somnolence, confusion, seizures, ataxia, paralysis, and coma are other possibilities. There is complete neurological recovery that takes 1 to 2 weeks. Laboratory findings are the same as those reported in EEE and WEE. During the first 3 days, the VEE virus can easily be isolated either from the serum or the nasopharynx, but the treatment is only symptomatic. Specific diagnosis can only be accomplished with different serologic testings. A live-attenuated (TC-83 and C-84) and an inactivated vaccine for humans (C-84) are available, but they both are associated with numerous side-effects (Smith et al., 1997). Only the live-attenuated vaccine could be useful in the case of a deliberate release of aerosolized VEE viruses (Smith et al., 1997).

If a bioterrorism attack occurs using a viral encephalitis agent, experts feel the most likely agent for weaponization would be VEE virus. Historical military data also report that this virus was extensively studied for weaponization by the United States, United Kingdom, and Soviet Union. The virus particles can easily be aerosolized in an effective form, infecting 100 percent of exposed persons to cause a rather incapacitating syndrome. The infective dose of VEE for humans is considered to be 10 to 100 organisms, making it a militarily effective biowarfare agent. In 1969, WHO estimated that, if 50 kg of virulent VEE particles were aerosolized and disseminated efficiently over a city with 5 million people, 150,000 people would be exposed in a 1-km area downwind from the release in approximately 5 to 7 minutes resulting around 30,000 illnesses and 300 deaths.

Toxins

Toxins (ricin toxin, abrin toxin, staphylococcal enterotoxin B, toxin of *Clostridium perfringens*, mycotoxins) are produced by many bacteria, snakes, shellfish, molds, mushrooms, and plants. Most of them are highly lethal while some would serve mainly to incapacitate the victims and strain resources.

Ricin Toxin Ricin is one of the most lethal and easily produced plant toxins produced by the castor bean plant (*Ricin communis*). Although it is a deadly toxin, it has been used mostly for good purposes. The plant grows to a height of 8 feet and has a brightly colored, variegated leaf pattern (Figure 20.3), which allows people to use it as an ornamental plant or border marker. For many years castor beans were harvested to make castor oil, which acts as a strong laxative, and ricin is a part of the waste mash produced when castor oil is made. The toxin is present in the entire plant but is concentrated only in the beans. When extracted and purified, it can be formulated into a powder, mist, or pellet, is water soluble, and is a stable substance not affected greatly by extremes in temperature.

Toxins are neither infectious nor contagious; therefore, there is no person-to-person-to-person transmission. Three routes of exposure are known to exist: inhalation, ingestion, and injection. Ricin irreversibly blocks protein synthesis at the cellular level by inhibiting a cell defense mechanism known as unfolded protein response (UPR). Proteins synthesized by a cell need to have their long molecular chains folded in a precise pattern; the UPR causes proteins that do not fold, or that fold incorrectly, to be degraded and removed

Figure 20.3. Castor bean plant and castor beans (*Ricinus cummunis*).

from the place in a cell where folding occurs, known as the endoplasmic reticulum (ER). When the toxic ricin A protein enters a cell, it prefers a reverse pathway, being transported to and unfolded in the ER. At this point, the UPR initiates a cell stress response degrading the unfolded proteins and acting as the cell's first line of defense. A piece of the ricin A protein molecule, however, signals the ER to shut down its UPR and the cell's stress response, which is needed for survival. This poisoning with ricin toxin is critically dose dependent and as little as 500 micrograms of ricin through injection would be enough to kill the average adult; however, lethality through inhalation or ingestion requires a greater amount of the toxin. The incubation period also depends on how it enters the victim: if inhaled, poisoning takes about 8 hours; if ingested poisoning occurs in hours to days because of the digestive processes and slow absorption through the gut; if injected under the skin, onset could be immediate, depending on the location and dose of toxin injected. Symptoms can appear within hours and include weakness, difficulty in breathing, nausea, vomiting and diarrhea, cough, muscle aches, and chest pain. Pulmonary edema occurs about 18 to 24 hours after inhalation of the toxin and death can result within days from low blood pressure, severe dehydration, severe respiratory distress that leads to respiratory failure, hypoxemia, and eventually, failure of organs including the liver and kidneys. The people who survive severe ricin poisoning may still have permanent or long-lasting organ damage. The seeds of the castor beans are attractive and, therefore, useful in costume jewelry. Unfortunately, they look good enough to eat, especially for small children, and they often become a victim of castor bean poisoning. Within a few hours of ingestion, these children report severe intestinal cramps along with nausea, vomiting, and headache and are followed by diarrhea, gastrointestinal bleeding, and dilation of the pupils. Ultimately victims will experience vascular collapse and die within 3 days.

Ricin toxin is feared as a bioterror agent because of its extreme ease of production, wide availability, potency (high toxicity), and unavailability of antidotes. Because of these features, ricin was considered for production and formulation by the United States during their biological weapons research program. For an example, in a classic case of international espionage in 1978, ricin was used to assassinate Georgi Markov, a Bulgarian defector in which a small metal pellet containing ricin crystals was injected into Markov's calf muscle by a specially engineered weapon disguised as an umbrella and resulted in

Markov's death 3 days after the incident. Although this act was executed by the Bulgarian government, the technology used to commit the act was supplied by the Soviet Union.

Abrin Toxin Abrin is a toxic protein (toxalbumin) found in the seeds of a plant called the *Abrus precatorius* (lucky bean, rosary pea or jequirity pea). Abrin is similar in structure and properties to, but far more deadly than, ricin. It is highly toxic, with an estimated human fatal dose of 0.1 to 1 μg/kg, and has caused death after accidental and intentional poisoning. Abrin can be extracted from jequirity beans by a relatively simple and cheap procedure, which satisfies one criterion of a potential warfare agent, but the lack of large-scale production of jequirity seeds suggests its unavailability for ready mass production for weapons and contrasts with the huge cultivation of *Ricinus* seeds for castor oil production. At the cellular level, abrin inhibits protein synthesis, thereby causing cell death. Abrin poisoning can also be explained by abrin-induced endothelial cell damage, which causes an increase in capillary permeability with consequent fluid and protein leakage and tissue edema (the so-called vascular leak syndrome). Most of the reported human poisoning cases involve the ingestion of jequirity beans, which predominantly cause gastrointestinal toxicity. Abrin is actually a lectin composed of two polypeptide chains (A and B) connected by a disulfide bridge which is similar to that of botulinum toxin, tetanus toxin, cholera toxin, diphtheria toxin, and insulin (Balint, 1974). The seeds of abrus are harmless like castor when ingested whole because the hard outer shell resists digestion; however, chewing or crushing of the seed before swallowing will release the toxins. Polypeptide chains (B) of abrin mediate the binding to the intestinal cell membrane and the other chain (A) enters the cytoplasm. Inside the cell, the A chain acts on the 60S ribosomal subunit, preventing binding of elongation factor 2, thus inhibiting protein synthesis and leading to cell demise (Barri et al., 1990). The fatal dose of abrin is reported to be just 2 to 3 seeds for an average adult (Hart, 1963).

Abrin is considered as an available toxin for weaponizing because of easy cultivation of the source, *A. precatorius*, and the uncomplicated preparation of the pure toxin. For nations or terrorists that lack the money to spend on nuclear weapons and other high-tech killing instruments, toxin warfare offers horrific appeal. Toxin weapons are cheap, easy to make, and simple to conceal. If effectively used, they could cause massive injuries and make many people suffer even at smaller amounts (Patocka, 1998).

Staphylococcal Enterotoxin B Staphylococcal enterotoxin B (SEB) is an extracellular product produced by coagulase-positive *Staphylococci (S. aureus)*, which is one of the best-studied and, therefore, best-understood toxins and is the most common causes of food poisoning in humans, which has been produced by some countries as a biological weapon. The toxin markedly causes a distinguished clinical syndrome when exposed through a nonenteric route. The enterotoxin is heat stable and generally produced during overgrowth of staphylococcal organisms as occurs with poorly handled food. The effects of the toxin are mediated by its interactions with the host's own immune system because it is a super antigen that acts by stimulating cytokine release and inflammation. The toxin directly binds to the major histocompatibility complex (MHC) and subsequently stimulates large numbers of T lymphocytes to release various cytokines (e.g., tumor necrosis factor, interleukin-1 and interleukin-2, interferon), which actually mediate the toxic effects of SEB. Signs and symptoms of the disease begin in 2 to 12 hours after inhalation and 2 to 10 hours after ingestion. Mild-to-moderate inhalation exposure to the toxin produces nonspecific systemic illness, characterized by fever, chills, headache,

nausea, vomiting, dyspnea, chest pain, myalgias, and a nonproductive cough. Severe exposures can lead to a toxic shock syndrome leading to death. Oral exposure results in nausea, vomiting, and diarrhea, but fever, chills, and myalgias may also be present and ocular exposure may result in conjunctivitis.

In a biowarfare or terrorist situation, the toxin is likely to be acquired through inhalation of an SEB-containing aerosol because SEB is quite stable as an aerosol and requires a low dose (0.004 μg/kg) to incapacitate individuals. In a terrorist attack, SEB could also be used to contaminate food or small-volume water supplies.

Epsilon (ε) Toxin of *Clostridium Perfringens* ε-toxin is one of the 12 protein toxins produced by *C. perfringens*, a gram-positive, anaerobic, spore-forming rod. There are five strains of *C. perfringens* designated as A through E, and ε-toxin is produced by toxin types B and D strains (Sterne and Warrack, 1964). The toxin is a pore-forming protein that causes potassium and fluid leakage from cells. It is the etiological agent of dysentery in newborn lambs but is also found to be associated with enteritis and enterotoxemia in goats, calves, and foals. It is considered to be a potential biowarfare or bioterrorism agent by the CDC and reported as the most potent clostridial toxin after botulinum and tetanus neurotoxins (Rood, 1997). Although, the exact mode of action of ε-toxin is still unclear, it has been reported that ε-toxin can increase vascular permeability in the brains, kidneys, and intestines (Rood, 1998). Because of its rapid onset of disease leads to mortality, treatment has not been useful except in rare cases; therefore, vaccines have been considered as the traditional choice for prevention (Rosskopf-Streicher et al., 2004).

Mycotoxins Mycotoxins are naturally occurring secondary metabolites produced by various species of fungi that typically afford the organism survival benefit. Approximately 300 mycotoxins are produced by around 350 species of fungi, and many of these toxins are pathogenic to animals and humans. The T-2 mycotoxin (trichothecene mycotoxin) is the only mycotoxin known to have been used as a biological weapon. It is a low molecular weight (250–500 dal) nonvolatile highly toxic compound produced by filamentous fungi (molds) of the genera *Fusarium*, *Aspergillus*, *Myrotecium*, *Trichoderma*, *Cephalosporium*, *Stachybotrys*, and others. T-2 toxin is relatively insoluble in water but is highly soluble in acetone, ethyl acetate, chloroform, ethanol, methanol, and propylene glycol; vaporizes when heated in organic solvents; and is extremely stable to heat and ultraviolet light inactivation. It is extracted from fungal cultures as a yellow-brown liquid that further evaporates into a yellow crystalline product giving a "yellow rain" appearance. These toxins could be completely inactivated by a 3 to 5% sodium hydroxide solution and heating at 900° F(482.2° C) for 10 minutes or 500° F (260° C) for 30 minutes.

T-2 and other mycotoxins may enter the body through the skin, digestive, or respiratory epithelium and are fast-acting potent inhibitors of protein and nucleic acid synthesis. They mainly affect rapidly proliferating tissues such as the bone marrow, skin, mucosal epithelium, and germ cells. During a successful biowarfare attack with T-2 toxin, the toxin can adhere to and penetrate the skin, be inhaled, or can be ingested. Clothing would also be contaminated and serve as a reservoir for further toxin exposure. The symptoms starts within minutes of exposure include burning skin pain, redness, tenderness, blistering, and progression to skin necrosis with leathery blackening and sloughing of large areas of skin in lethal cases. Nasal contact with the toxin is manifested by nasal itching and pain, sneezing, epistaxis, and rhinorrhea; pulmonary/tracheobronchial toxicity by dyspnea, wheezing, and cough; and mouth and throat exposure by pain and

blood-tinged saliva and sputum. Acid reflux, nausea, vomiting, and watery or bloody diarrhea with abdominal cramps occur along with gastrointestinal toxicity. When it enters into the eye, it may cause eye pain, tearing, redness, foreign body sensation, and blurred vision within minutes. Systemic toxicity is manifested by weakness, prostration, dizziness, ataxia, and loss of coordination that are followed by tachycardia, hypothermia, and hypotension, especially in fatal cases. Death may occur within minutes, hours, or days depending on the host's immune status and the dose of toxin. The pathophysiology of T-2 mycotoxin is multifactorial; it stimulates breakage in DNA, chromosomal abnormalities, and inhibition of protein synthesis, which seems to be the primary cause of symptoms in intoxicated patients.

Because of its ease of large-scale production and ease to disperse by various methods including dusts, droplets, aerosols, smoke, rockets, artillery mines, portable sprays, mycotoxins have an excellent potential for weaponization. Strong evidence suggests that T-2 toxin has been used as a biowarfare agent in southwest Asia and Afghanistan and as an assassination tool this can be used as a food- or water-borne poison. It is the only biologically active toxin that is effective through dermal exposure and respiratory and gastrointestinal portals.

Category C

Category C agents are emerging pathogens that could be engineered genetically for mass dissemination because of their availability, ease of production and dissemination, high mortality rate, or ability to cause a major health impact. Recently emerging human diseases present a unique challenge to public health officials and infectious disease specialists. Some of these agents have been with humans for millions of years, lurking in a dark corner of the environment, waiting for an opportunity to jump from their natural transmission cycle to a human host or may represent something totally new. Regardless of their origin, an emerging pathogen must quickly be characterized by molecular biologists and microbiologists to get rid of the diseases caused by them. Category C agents may be exploited by the terrorist groups because they might take an advantage of the emergence of one of these special pathogens intentionally to introduce an emerging disease into an area, thereby causing, fear, panic, and social disruption in a community or a large area of a country.

Nipah Virus

Nipah virus (NiV) infection is an emerging infectious viral infection of public health importance in the Southeast Asia region. This virus along with Hendra virus comprises a new genus designated Henipavirus in the subfamily *Paramyxovirinae* (Reynes et al. 2005). It is a newly emerging paramyxo virus isolated during a large outbreak of viral encephalitis in Malaysia and has been considered as a potential bioterrorism agent. The outbreak caused widespread panic and fear because of high mortality and the inability to control the disease at the initial level. This is a highly virulent virus, which is believed to introduced into pig farms by fruit bats, spread easily among pigs, and is then transmitted to humans who came into close contact with infected animals. It causes considerable social disruptions and tremendous economic loss to an important pig-rearing industry. The virus was also transmitted from pigs to other animals such as dogs, cats, and horses. It is an extremely pathogenic organism with a case mortality of around 40 percent and has been classified as an organism in Biosafety Level-4. It usually causes acute infection

but also can give rise to clinical relapse months and years after infection. The virus can easily be produced in large quantities in cell culture, making it an important biologic weapon. It should also be possible to stabilize NiV as an aerosol with the capacity for widespread dispersal. Besides infecting humans, the virus can also infect livestock, domestic animals, and wildlife and hence is likely to cause additional panic to the population. As the complete NiV genome has been characterized, genetic manipulation of the virus can be easily achieved; this is probably helpful to use it as an effective biological weapon.

Hantaviruses

The original Hantaan virus was actually researched as part of a bioweapons program, mostly to know more about the pathogen. The American Sin Nombre virus, a more virulent version of Hantaan virus appeared to have the possibility of being weaponized. Concerns have grown recently over the ability of terrorists or others to harness the Hantavirus as a bioweapon given its extreme lethality; however, these concerns should be evaluated on the basis of its history, the characteristics of the virus, and whether it is efficient enough to be a part of a coordinated biological attack. These analyses reveal that the Hantvirus would not be the first choice to weaponize because of its limitations.

Rather than directly using these emerging diseases to cause microorganisms in biowarfare, their genetic material could be appropriated as a source of DNA coding for particular traits as per the requirement. Virulence factors could be isolated from these agents and inserted into existing pathogens with known epidemiological properties to create more potent disease variations. For an example the Sin Nombre virus may represent nature's version of this phenomenon, which acquired an unidentified virulence factor, probably from another microorganism, that altered its disease expression. Biowarfare developers may use this type of hybridization to create weaponizable agents, taking advantage of the propensity of viruses to integrate foreign genetic material and express production of new proteins.

Yellow Fever

Yellow fever is a fatal viral infection caused by a flavivirus that is transmitted by mosquitoes in tropical regions. It has both an urban cycle and a sylvatic cycle that relies on monkeys as carriers. When a mosquito bites, it introduces the virus into the bloodstream through the saliva. It can then transport throughout the body and reproduce itself in a variety of the body cells usually the liver, kidneys, and blood vessels. The mild cases resemble influenza, but serious cases develop a high temperature and may have a series of aftereffects, such as internal bleeding, kidney failure, and meningitis. Hepatitis is one of the classic features of yellow fever, the reason for the yellow coloring of the skin (jaundice), and the name of the disease. Yellow fever can cause sudden epidemics with the mortality rate of approximately 50 percent. Although a safe, efficient vaccine has been available against yellow fever for the last 60 years, epidemics still occur, constituting a health risk in tropical regions.

Severe Acute Respiratory Syndrome

Severe acute respiratory syndrome (SARS) is a serious form of pneumonia caused by a member of the corona virus family (the same family that can cause the common cold) and was first identified in 2003. The first case of SARS was reported in China, and within 6 weeks other SARS cases were documented in 27 countries around the world. It causes acute respiratory distress (severe breathing difficulty) and sometimes death. It was initially

considered as mild infectious disease, but by late 2002 it had turned into a new and global epidemic with the potential of becoming a pandemic of overwhelming proportions.

The SARS corona virus could also be an intentionally designed to use as a bioweapon by the projects that have been undertaken by all the major militaries in the world. Alternatively, it could have developed because of the horizontal transfer, a kind of microbiological collateral damage that occurs when unplanned genetic mutations occur in the open, out of the control of the original designer.

H1N1 (Influenza)

Influenza A virus has been responsible for widespread human epidemics because it is readily transmitted from humans to humans by aerosol. Even though, it has been evident in various societies for a long period of time, it is still a developing and challenging disease. There are a number of considerations that make influenza virus a cause of concern to be employed in a bioterrorist attack, including the capacity of the virus to be transmitted in human populations and the severe pandemics caused in the past involving high mortality with the introduction of a new influenza virus containing novel surface antigenic components to which the population has no prior immunity (antigenic shift). The other important thing is the availability of new technology that can be used to alter deliberately the genetic composition of influenza virus making it highly potential for spread in the population.

To make it as a potential bioterrorism agent, one negative feature is its extreme transmissibility, which would make it difficult to target a specific population, and a newly introduced virus would spread unpredictably.

Multidrug Resistant Tuberculosis

Multi-drug-resistant tuberculosis (MDR-TB) is caused by certain strains of *Mycobacterium tuberculosis* that are resistant to isoniazid and rifampicin, the two most powerful first-line anti-tuberculosis (TB) drugs. The disease develops during treatment of fully sensitive TB, most commonly when doctors given inappropriate treatment or patients miss doses (interrupted antibiotics course), resulting in insufficient levels of drugs in the body to kill 100 percent of mycobacterial population present. This might happen for a number of reasons because patients may feel better and halt their antibiotic course, drug supplies may run out or become scarce, or patients may forget to take their medication on time. MDR-TB is usually spread from person to the person as readily as drug-sensitive TB and in a similar manner. MDR-TB strains are often potent and less transmissible; thereby outbreaks occur more readily in people with weakened immune status (e.g., patients with HIV). These MDR-TB strains can also be used by terrorists group as bioterrorism agent.

Apart from these, some other viral agents also have been placed in category C biowarfare agent including HIV/AIDS, West Nile virus, dengue, Kyasanur Forest disease, tick-borne encephalitis, and hemorrhagic fever, and all impose a great threat as potential bioterrorism agents.

Epidemiological Clues

It is impossible to determine the objectives of a bioterrorism executor in advance, whether the intent is to kill, incapacitate, or obtain visibility; or how a biological agent may be dispersed, whether through the air, in contaminated food or water, or by direct

inoculation. In a bioterrorism attack, usually the total casualties may be small and, therefore, unrecognized as intentionally infected, especially when the biowarfare agent is also a common cause of disease in the affected community. In addition, depending on the agent's incubation period, individuals may seek care from different care providers or travel to different parts of the country before they become ill and seek medical care. Because of the probability of these situations, it is essential for health-care providers to be aware of potential clues that may be tip-offs or red flags of something unusual. Although sometimes these clues may occur with natural outbreaks and do not necessarily signal a biowarfare attack, they should at least heighten suspicion that an unnatural event has occurred (Wiener and Barrett, 1986).

- **Clue 1: An unusual event with large numbers of casualties**. Although the reporting of biowarfare or bioterrorism may elicit images of massive casualties, this may not actually occur with a real event. Various examples of illness that spread naturally have caused massive casualties. Nevertheless, the type of large outbreak receiving particular attention is one in which no plausible natural explanation for the cause of the infection exists.
- **Clue 2: Higher morbidity or mortality than is expected**. In any community if the clinicians are observing illnesses that are causing higher morbidity or mortality than what is typically reported for a specific disease, this may indicate an unusual event. A perpetrator may have modified an agent making it more virulent and effective, and if the illness is normally sensitive to certain antibiotics but displays resistance, then resistance may have been purposefully engineered. There may also be a possibility that individuals could be exposed to a higher inoculum than they would normally receive with natural spread of the agent, thereby causing higher morbidity or mortality.
- **Clue 3: Outbreak is of an uncommon disease**. Most of the infectious diseases have predictable population and their infectivity are distributed based on environment, host, and vector factors, yet unnatural spread may occur if a disease outbreak is uncommon in a certain geographical area. In such cases, concern should be heightened if the naturally occurring disease requires a vector for spread and the competent vector is missing. Similarly a disease such as yellow fever, which is endemic to parts of South and Central America and sub-Saharan Africa, occurred in the United States without any known travel, it would be a concern. Natural outbreaks occurred in new geographical locations including the West Nile virus in New York City in 1999 (Fine and Layton, 2001). In such cases, it is important for a health-care provider to consider whether the occurrence of these uncommon diseases is natural.
- **Clue 4: Point-source outbreak is seen**. An outbreak curve should be developed for each and every outbreak demonstrating the timeline of dates when patients developed illness. These timelines can have different features depending on whether individuals are exposed at the same time from a single source or over time and whether the illness has been propagated by the person-to-person spread. With an intentional bioterrorism event, a point-source outbreak curve would be seen (Steele et al., 2000) in which individuals would be exposed at a similar point in time. The typical point-source outbreak curve shows a relatively quick rise in cases, a brief plateau, and then an acute and a sudden drop (Figure 20.4). The epidemic curve may be slightly compressed probably because infected individuals were exposed more closely in time (i.e., within seconds to

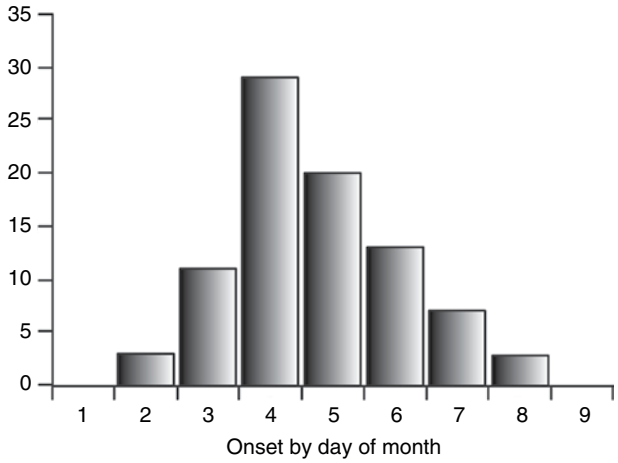

Figure 20.4. Typical point-source outbreak epidemic curve.

minutes of each other) from an aerosol release, compared with individuals becoming ill after eating a common food over a period of minutes to hours. There is also the possibility that the inoculum may also be greater than what is typically seen with natural spread, thus yielding a shorter incubation period than expected.

- **Clue 5: Multiple epidemics occur at same time**. A perpetrator can develop and release a single biowarfare agent or similarly multiple perpetrators can do so with a single agent at different locations; this leads to the occurrence of simultaneous epidemics at the same or different locations with the same or multiple organisms. In such cases, an unnatural source must be considered. There may also be a consideration that a mixture of biological organisms with different disease incubation periods could be combined, and would, therefore, cause serial outbreaks of different diseases in the same population.
- **Clue 6: There are lower attack rates in protected individuals**. This clue is particularly important to military personnel. If certain military units wore military-oriented protective posture (MOPP) gear or respiratory protection (such as high-efficiency particulate air [HEPA]-filtered masks) or stayed in a HEPA-filtered tent and showed lower illness rates than nearby groups that were unprotected, this might indicate that a biological agent has been released via aerosol.
- **Clue 7: Outbreaks of dead animals also occur**. Historically, dead and diseased animals have been used as sentinels of human disease. The storied use of canaries in coal mines to detect the presence of noxious gases is one example. Because most of the biological agents that could be used for biowarfare and bioterrorism are zoonoses, death of a local animal may indicate the release of a biological agent that might also infect humans. This had been observed during the West Nile virus outbreak in New York City in 1999, when many of the local crows, along with the exotic birds at the Bronx Zoo, developed fatal disease (Ludwig et al., 2002; Steele et al., 2000).
- **Clue 8: Reverse or simultaneous spread of outbreak**. Zoonotic diseases exhibit a typical pattern of an epizootic outbreak occurring first among a susceptible animal population, followed by cases of human illness. When Sin Nombre virus appeared in the desert

southwest of the United States (Zaki et al., 1995), the environmental factors increased food sources and caused the field mouse (*Peromyscus maniculatus*) population to surge. The proliferating field mice encroached on human habitats, and the virus spread among the mice, causing a persistent infection in them and subsequent excretion of the virion in their urine (Netski et al., 1999). Humans became infected from these mice. If human disease precedes animal disease or human and animal disease occurs simultaneously, then unnatural spread should be considered.

- **Clue 9: Disease has unusual manifestation**. Approximately 95 percent of worldwide anthrax cases appear as cutaneous illness, therefore, a single case of inhalational anthrax may likely be an unnatural event. The same logic may also be applied to case reports of a disease such as plague, where the majority of naturally occurring cases are the bubonic, and not the pneumonic form.
- **Clue 10: Disease displays a downwind plume pattern**. The geographic locations where cases report can be charted on a geographic grid or map. If these reported cases are found to be clustered in a downwind pattern, this suggests an aerosol release.
- **Clue 11: There is direct evidence of intentional spread**. The final clue might be the most obvious and the most useful determining that there was intentional cause of illnesses, if a preparatory leaves a signature.

Laboratory Diagnosis

Recently observed bioterrorist events emphasized the need to detect and identify potential bioterrorism agents immediately that might be used further by terrorists groups. Rapid and accurate identification of these agents are important to confirm the occurrence of a bioterrorism event and to determine suitable measures that should be implemented to protect public health. For the detection, diagnosis, and reporting of biological threat agents an organized system has been designed by the CDC and designated as Laboratory Response Network (LRN), to provide structured guidance for the detection, diagnosis, and reporting of biological threat agents (Snyder and Check, 2001). The LRN comprises four laboratory levels (A-D) based on testing capabilities and their handling of various bioterror agents. Level A laboratories are standard clinical laboratories, which handle specimens for likely biothreat agents as part of classic diagnostic analysis on hospital cultures and are the first line of detection and familiar with likely agents, such as *B. anthracis*, *Y. pestis*, and *F. tularensis*, and conduct diagnostic analyses as directed by the CDC. Level B laboratories are typically public health laboratories that can confirm identification of suspicious isolates. Level C laboratories are either typing laboratories or public health laboratories that preferentially use various molecular methods and typing procedures further to identify or confirm the identification of species and strains of bioterrorism agents. Level D laboratories are capable of doing high-level characterization of bioterrorism isolates under tightly controlled conditions and are located at the CDC and US Army Medical Research Institute of Infectious Diseases.

Accurate and definitive identification of biological threats require an integrated approach (Figure 20.5) because no single identification technology is sufficient to identify biological threats probably because of the microbial diversity, an often confusing clinical presentation, the close antigenic and genetic relatedness of some biological agents, and the possibility of misidentification. In the near future, first responders, medical care providers, and diagnostic laboratories will require a combination of overlapping diagnostic approaches tied together by a robust information management system.

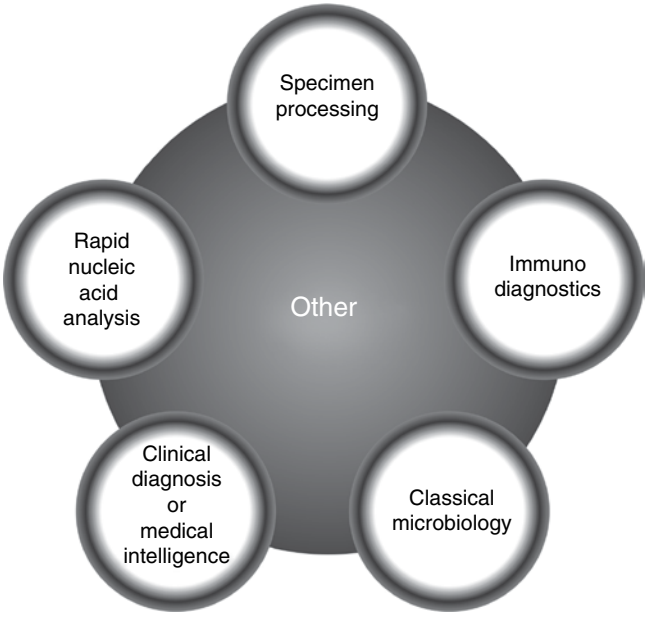

Figure 20.5. Integrated Diagnostic System presenting the requirement for overlapping technologies with interactive information systems to obtain desirable laboratory results.

Challenges to Detection

The prospect of biological terrorism presents many challenges for detection and diagnosis of biothreat agents. Some of these challenges are unique to bioterrorism and others are common for all testing situations. Ideally, detection techniques should be capable of rapidly detecting and confirming all the potential biothreat agents, including modified or previously uncharacterized agents, directly from complex matrix samples, with no false results. This requires that the instrument should be portable, user friendly, and capable of simultaneous testing of multiple agents. Although several detection platforms exhibit many of the desired characteristics, no one system satisfies all of the required criteria.

Detection assays must be highly sensitive and specific, capable of even detecting low concentrations of target agents without interference from background materials. Although many chemical detectors can detect chemical agents at levels that pose a risk to human health, biological detectors lack sensitivity and thereby can only rarely detect microorganisms directly from samples at or below human risk levels. Generally, nucleic acid-based detection systems are found to be more sensitive than antibody-based detection systems. For example, the polymerase chain reaction (PCR) assay can detect 10 or fewer microorganisms in a short period of time (Bell et al., 2002; Fode-Vaughan et al., 2003; Ibekwe and Grieve, 2003), but it requires a clean sample and is unable to detect protein toxins and other non-nucleic acid-containing analytes including prions. Furthermore, cultures of the target organism are not sometimes available for archiving and additional tests after PCR analysis.

Along with the sensitivity, high specificity is also important when detecting biothreat agents to minimize background signals and false-positive results from complex samples and

uncharacterized mixtures of organic and inorganic materials. Specificity depends on both by humics and other background particles and high concentrations of competing antigens and DNA. The high sensitivity of PCR can also be a major weakness because contaminating or carryover DNA can also be amplified, resulting in false-positive results.

Another important requirement for detection platforms is reproducibility. Detection platforms that do not providing reproducible results are considered unreliable and may exacerbate a terrorist event. Detection platforms must be capable of detecting a variety of biothreat agents in samples. This is vital capability because suspect samples may contain toxins, bacteria, viruses, or other types of analytes. In some cases, known biothreat agents may have been deliberately altered through genetic, antigenic, or chemical modifications and may represent new or uncommon variants of known microorganisms and make detection of a biothreat agent difficult. Some of the conventional biothreat agents are difficult to detect in complex sample matrices even without any modifications. Most of the common samples such as human specimens (blood and stool), powder, food, water, and even air present challenges for detection assays. Anticoagulants, leukocyte DNA, and heme compounds present in blood samples inhibit PCR assay (Garcia et al., 2002); similarly lipids present in ground beef and high numbers of background bacteria present in stool specimens interfere with immunoassays. For these reasons, target analytes usually must be isolated or purified from such samples prior to analysis and identification. However, this requires hours or days to detection protocols and often cannot be performed in the field. Further it is difficult to culture some viable microbes or they may require specific nutritional requirements for culture.

Collection and handling of samples is also an important consideration in biodetection. Problems associated with sampling include the type of material, the collection procedure, and the transportation of collected samples. Generally, air- and water-borne samples must first be concentrated from large volumes to detect low levels of target analytes; additionally all the air-borne samples must also be extracted to a liquid because most detection platforms process only liquid samples. The percent recovery and its efficiency from various concentration and extraction procedures may vary and affect detection limits. This is dependent on the sample size, number, and distribution, as well as the transport time and method, particularly while dealing with the fastidious, living microbes that may require specific environmental and nutritional conditions for survival.

Sample Matrix Processing

Sample Processing

Conventional culture and staining techniques are considered as the gold standard for isolation, detection, and identification of target bioterrorism agents. These culture and isolation techniques are based on the ability of healthy bacterial cells to multiply in nutrient-rich medium containing selective and differential agents that inhibit the growth of nontarget organisms and thereby differentiating target from nontarget organisms. However, these are lengthy assays taking days for preliminary results. Some of the rapid detection methods, such as DNA hybridization, nucleic acid amplification, antibody agglutination, and enzyme immunoassays, can replace the selective and differential culturing methods, but most of these rapid detection methods are suitable only when the biothreat agent is present in large numbers with the absence of interfering substances. In most of the cases, rapid detection methods require various steps to concentrate the

target biothreat agent and purify the target analyte from the sample matrix. For example, PCR and nucleic acid sequence-based amplification (NASBA) enrich a single specific DNA or RNA sequence up to 106-fold in 20 minutes to a few hours and theoretically have a sensitivity of a single bacterial cell. These molecular methods provide rapid and specific detection but are limited by small sample volumes (5 μL). However, substances, such as bile salts, polysaccharides, heme, and humic acids in sample matrices, inhibit enzymatic reactions, which are required for nucleic acid amplification procedures (Radstrom et al., 2004).

Methods for Sample Processing

Ideally the method to separate, concentrate, and purify the target biothreat agent should be universal and also usable for all samples for all types of target analytes. Additionally, the sample preparation method should be capable of rapidly removing the sample matrix, which could inhibit detection capabilities and concentrating the analyte. The majority of the current sample preparation methods do not fulfill this criteria. The sample preparation procedure is usually limited to specific types of samples, which is generally time consuming and sometimes labor intensive. In addition, the concentration or purification method should ideally maintain cell viability, which would allow the culturing of the target organism for confirmation of viability, further characterization and archived as evidence in the case of a criminal investigation. Many sample preparation methods are currently under investigation, including chemical, physical, and biological manipulation of the sample (Benoit and Donahue, 2003; Radstrom et al., 2004).

Centrifugation

Centrifugation has been conventionally used to concentrate and recover microorganisms from liquid samples. The centrifugation times may vary from 30 seconds to 1 hour depending on the type of sample and the number of washing steps. During this process, undesired sample debris may also be concentrated, and the process cannot be easily automated. For example, *E. coli* O157:H7 cells have been directly recovered from a ground beef-buffer suspension through a 5-minute differential centrifugation step that separated the suspension into three distinct layers. The middle layer containing the majority of the target cells was used for rapid detection of *E. coli* O157:H7 using an evanescent wave fiberoptic biosensor (De Marco and Lim, 2002). Buoyant density gradient centrifugation has been used to separate and concentrate *Yersinia enterocolitica* in meat fluids from pork (Wolffs et al., 2004), which removed all the dead cells, and the concentrated samples contained only viable cells that were then directly used for PCR. This has also been successfully used to separate and concentrate bacteria from food in a 1-minute procedure (Lindqvist et al., 1997).

Filtration

Filtration methods separate microorganisms on the basis of cell size. Although liquid samples can be rapidly forced through different pore size filters, sample debris can clog filters and retain bacteria and further removal of bacteria from filters can also be difficult. The commercially available Iso-Grid (Neogen Corp., Lansing, MI) is a dual filtration method developed for food products (Payne and Kroll, 1991) in which prefiltered food is passed through a 0.45-μm filter, which is then placed on an agar plate and bacteria captured on the filter are grown and detected using selective and

differential media. Iso-Grid filtration for *E. coli* O157:H7 has been approved as AOAC Official Method 997.11 (AOAC International, 2003).

Another example is FTA filters (Whatman, Springfield, KY), which have been developed for rapid isolation of nucleic acids from environmental, clinical, or food samples. In this, samples are directly added to the filter, which can then be washed, and the nucleic acid remains bound to the filter. The filter is then ready for processing or can be stored for a longer time at room temperature. FTA filters have already been used for the detection of *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus megaterium* spores using nested PCR.

Dielectrophoresis

Dielectrophoresis is based on the intrinsic dielectric properties of bioparticles to enable separation of particles in nonuniform electric fields. This process employs a nonuniform electric field consisting of positive and negative dielectrophoretic forces, which are generated by microelectrodes in a small chamber. Different microbial cells and other components in the sample can be separated based on the conductivity of each particle. Particles are released from regions near the electrodes when the dielectrophoretic response of each particle changes from attraction to repulsion from such regions. Different bacterial species have different cell wall structures and compositions, and these differences give rise to large differences in particle conductivities. The particle conductivity is also influenced by the changes in the physiological state of the cell. Dielectrophoresis has been incorporated into a micro-fabricated bioelectronic chip and used to separate *E. coli* from the sample (Cheng et al., 1998).

Immunomagnetic Separation

In this method antibodies are immobilized on the surface of magnetic particles (beads) and then used to purify and concentrate targeted biothreat agents, which can bind target cells, toxins, or other molecules found in samples. The magnetized particles are then collected and separated using a magnetic field. Sample debris and nontarget organisms or molecules are removed by washing and the particles are released after removing the magnetic field is removed. The remaining solution contains mainly concentrated cells or molecules of interest that have bound to the antibody attached to the magnetic particle; thereby this antibody-based method of cell separation is referred to as *immunomagnetic separation*. Immunomagnetic separation technique has been used to isolate and concentrate *Salmonella* spp. and *E. coli* O157:H7 from stool and food samples (Karch et al., 1996; Rijpens et al., 1999).

Nucleic Acid Extraction

The sensitivity, reproducibility, and accuracy of any nucleic acid detection method depend on the efficiency of extraction and purification of nucleic acid (either DNA or RNA) from cells or spores of potential bioterror agents. Some of the substances in complex sample matrices can inhibit hybridization as well as enzymatic reactions, degrade the nucleic acid, and reduce the efficiency of cell or spore lysis. In addition, spores must be disrupted (through sonication) prior to nucleic acid extraction, and the nucleic acid must also be concentrated in large samples, often over 1,000-fold, into a smaller volume appropriate for nucleic acid analysis. Commercially there are many kits available for purifying nucleic acid (Chua, 2004) For example, the Cepheid GeneXpert and Biothreat agent detection system (Cepheid, Sunnyvale, CA) integrates sample preparation, PCR

technique, and detection into a disposable cartridge. An instrument automatically processes the cartridge and allows the sample preparation in fewer than 5 minutes and detection (four-color real-time PCR) in 25 minutes without any special skills. Using this method, large volumes (100 µL to 5 mL) of raw sample can be handled, and up to four targets can be detected simultaneously per cartridge. This system automatically sonicates and purifies the sample followed by the addition of PCR reagent to the extracted nucleic acid, and finally the mixture is dispensed into a PCR tube (McMillan, 2002). Various samples such as swab extracts, serum, cerebrospinal fluid, urine, bone marrow, sputum, tissue, and whole blood samples have been tested with this system.

Sample Matrix Processing

Sample matrices can be of different compositions and, therefore, may require different processing methods.

Food

The vast variety of food types making it difficult to develop a universal sample preparation method for food samples. Currently available methods rely on the time-consuming enrichment culture for the growth of the target microorganisms in the sample, whereas more rapid methods such as immunomagnetic separation require specialized equipment as well as skilled labor. Obstacles while preparing food samples for testing using rapid detection protocols include the requirement that food volumes need to be large (25 g or mL) and homogenous. Various nutritional components of food such as lipids, polysaccharides, acid, and salts will inhibit enzymatic reactions required for certain rapid detection approaches such as PCR. Most of the large samples contain low concentrations of the target pathogen, which will also decrease sensitivity of detection. In conventional methods, solid food is usually diluted in a buffer solution and is then blended or homogenized to obtain a homogenous mixture, which is then further purified or cultured prior to testing. A homogenous mixture must still be prepared even while using a rapid detection technique. The target microorganism can then be concentrated from the mixture. Unfortunately, the most effective technique for obtaining large numbers of target organisms in food is culture enrichment methods. Rapid effective separation and concentration of target pathogens in large samples remains a barrier to the rapid detection of microorganisms in foods.

Water

Microorganisms and their by-products present in drinking water, and recreational waters are usually too dilute for direct measurements. All the water samples generally must first be concentrated to obtain the sufficient number of target microorganisms necessary for microbiological analysis. Detection of microorganisms or their toxins from water samples is difficult because of poor recovery and because of the presence of inhibitory substances. Target microorganisms must be isolated and concentrated from the water samples. Concentration is performed using ultrafiltration technique in which parasites, bacteria, viruses, and some high-molecular-weight biotoxins are retained on the filter (Morales-Morales et al., 2003). The accumulated material on the filter is removed, collected, and analyzed. However, some other potential interferents such as chlorine, salts, humic acids, and indigenous bacteria may also accumulate on the filter and inhibit rapid detection.

Human Specimens

Blood Blood contains heme, erythrocytes, and other biologically active components, which can interfere with antibody and nucleic acid-based assays. Removal of these components and separation of the target pathogen from such a complex mixture is a major hurdle that must be overcome while processing blood samples for rapid detection. Instead of removing the sample matrix, Spectral Diagnostics, Inc. (Canada), took advantage of the components present in blood by developing a rapid whole-blood elimination test for gram-negative bacterial infection for patients admitted in the intensive care unit. The US Food and Drug Administration had approved the test to identify patients at risk for developing severe sepsis on admission to intensive care units. The test is directly performed on whole blood, and it does not require separation of the target bacteria from the blood. If endotoxin is present, it reacts with antiendotoxin antibody in the test; this binding reaction primes the patient's neutrophils, resulting in an enhanced respiratory burst in the presence of zymosan. Zymosan is an insoluble preparation of yeast cells that has been shown to activate macrophages via toll-like receptor 2 (TLR-2). The magnitude of the priming is proportional to the concentration of the endotoxin/antiendotoxin antibody complex (Romaschin et al., 1998).

Liu et al. (2004) reported an integrated miniaturized biochip device that automatically integrates sample preparation with PCR and DNA microarray detection. This automated device mixes the blood sample with immunomagnetic separation beads in a sample storage chamber using air bubbles in a phenomenon designated as microstreaming. The target pathogen bound to the immunomagnetic separation beads is then pumped to a PCR chamber, where the pathogen is concentrated using the magnetic element of the device and separated from whole blood after a washing step. The sample is then ready for the direct addition of PCR reagents to the PCR chamber.

Urine It is unlikely that biothreat agents would need to be detected in the course of a urinary tract infection; however, urine contains yet-unidentified substances inhibitory to PCR, and many components such as bacterial contaminants commonly found in the urine can interfere with detection results. For example, Western blot results of urine samples used to detect the presence of protease-resistant prion proteins could be misinterpreted as bands with a molecular weight similar to prion proteins can be detected from these Western blots (Furukawa et al., 2004).

Stool Specimens Stool specimens contain many natural substances including bile salts and complex polysaccharides that will inhibit PCR and other enzymatic reactions. Additionally, there will be large numbers of background bacteria that will interfere with immunoassays and other affinity-based detection techniques. Immunomagnetic separation and the MagNaPure system are techniques that are commonly used to isolate and concentrate bacteria and nucleic acid from stool specimens. The QIAamp DNA stool purification kit (QIAGEN, Inc., Valencia, CA), intended for Taq DNA polymerase inhibitor removal, has already been tested for detection of Shiga-toxin-producing *E. coli* by PCR directly from cattle fecal samples (Gioffre et al., 2004).

Nasal and Throat Swab Specimens Donaldson et al. (2004) described a method in which throat swab specimens spiked with vaccinia virus were resuspended in the buffer and directly detected using an evanescent wave fiberoptic biosensor. Vaccinia virus-specific antibodies attached to the biosensor's fiberoptic waveguide and were used to separate and capture virus particles from other cells and debris in the specimens.

Powders and Soil

Powders were one of the most common nonclinical specimens especially submitted to designated laboratories during the threat of *B. anthracis* spores (CDC, 2001). Luna et al. (2003) developed a rapid and accurate method to check powder and other environmental samples harmless for the safe extraction and identification of DNA. They have extracted DNA from ≤10 *B. anthracis* spores and then detected using PCR technique. Samples were prepared for PCR by germinating spores by heat shock (68 ° F [80 ° C] for 2 minutes), which was followed by 30 minutes sonication and 20 minutes autoclaving at 249.8 ° F (121 ° C). Further the DNA was purified using the MagNaPure instrument.

An evanescent wave fiberoptic biosensor method has also been developed to test powders for *B. anthracis* spores (Tims and Lim, 2004). Talc-based baby powder, corn starch–based baby powder, confectioner's sugar, baking soda, and *Bacillus thuringiensis*–based pesticide spiked with *B. anthracis* spores had been successfully processed by this method. This method employs the resuspension of powder in buffer and tested directly by the biosensor. Some other kits have also been developed to expedite extraction of total DNA from natural microbial communities present in soils and sediments.

Aerosols

Two major sized particles are present in infected aerosols (>5 μm and 1–5 μm in diameter). Particles that 1 to 5 μm diameter behave like gases (Cox and Wathes, 1995) and can be collected by processing large volumes of air and passing the air through a filter or impinging the particles from the collected air into a liquid or semisolid sample (Stetzenbach et al., 2004). Larger particles (>5 μm in diameter) settle from the atmosphere and bind to various surfaces. Impaction samplers collect culturable air-borne bacteria and fungi by depositing air-borne particles onto a surface of semisolid agar media, which further are cultured to detect the presence of collected microorganisms. Although these samplers are quite capable of collecting small numbers of bacteria (approximately 20 CFU), their usefulness is limited at higher levels of bacteria (>104 CFU) and by sampling time.

Northrop Grumman (Arlington, VA) has introduced the existing GeneXpert technology into its high-volume Biohazard Detection System for screening mail at US Postal Service facilities; it directly collects air samples above the cancellation equipment and concentrates air samples for 1 hour by absorbing and concentrating air-borne particles into a sterile water base. In 2003, followed by extensive testing, Northrop Grumman was awarded a production contract to install and manage these systems at US Postal Service sorting centers nationwide (Jaffer, 2004).

Detection and Identification of Biological Threat Agents

Numerous methods have been used for the detection and identification of human threat agents, which rely on cultivation and biochemical assays and take around 24 hour to 1 month when performed by a well-trained clinical technician. Although these methods are reliable, they generally cannot be employed in the field or are not capable of providing real-time detection and identification of biothreat agents during a bioterrorism event. With advancing bioterrorism incidences, there is a critical need to develop more rapid and accurate methods to detect and identify bio threat agents.

Table 20.9. Examples of biothreat agents and identification tests for Laboratory Response Network level A laboratories.

	Suitability of Test for Bacterium			
Screening Test or Medium	*B. anthracis*	*Y. pestis*	*F. tularensis*	*Brucella* spp.
Gram stain	+	+	+	+
Catalase test	+	+	+	+
Oxidase test		+	+	+
Urease test		+	+	+
India ink	+			
Wright-Giemsa stain		+		
Beta-lactamase			+	
XV or *S. aureus*[a]			+	+
Motility medium	+			
5% sheep blood agar	+	+	+	+
Chocolate agar	+		+	+
MacConkey agar	+	+	+	+
Phenyl ethyl alcohol agar	+			
Tryptic soy broth	+			
Thioglycollate broth	+		+	
Brain heart infusion agar		+		
Eosin methylene blue agar[b]		+	+	
Thayer-Martin agar[c]			+	+

[a]XV factors or *S. aureus* for satellite growth.
[b]Alternative to MacConkey agar.
[c]Alternative to chocolate agar.

Manual Biochemical Tests

When infected or exposed individuals seek medical assistance, local clinics and hospital laboratories are the first facilities likely to encounter biothreat agents (Snyder and Check, 2001). The available microbial identification systems for the routine diagnosis in clinical laboratories are not designed or optimized for the detection of biothreat agents. Thus, screening hierarchies have been established using standard microbiological as well as biochemical tests to reduce the potential for false alarms and rule out biothreat agents as quickly as possible at the clinical laboratory level. Table 20.9 summarizes a series of biochemical tests used for the screening of *B. anthracis*, *Y. pestis*, *F. tularensis*, and *Brucella* spp.

Automated Biochemical Tests

As we have already discussed, most of the commercially available biochemistry-based clinical microbe identification systems are not developed specifically for identification of

biothreat agents; however, some commercial systems that use pattern recognition databases or libraries have recently begun to offer biothreat update packages. The patterns generated by these types of systems are typically based either on the bacterium's ability to metabolize specified compounds or on gas-liquid chromatography of cellular components.

Substrate Usage Patterns

Some automated identification systems use pattern recognition or fingerprinting systems based on metabolized substrates and carbon sources or susceptibility toward various antimicrobial agents such as VITEK (bioMérieux, Hazelwood, MO) and MicroLog (BiOLOG, Hayward CA). A turbidometrically controlled aliquot of a pure bacterial suspension is then added to analysis or identification cards or plates. These cards or plates contain numerous wells with different types of biochemical substrates and can be purchased in many different configurations depending on the requirements of the laboratory. The inoculated cards or plates are then incubated for a defined time period and read using an automated system.

Fatty Acid Profile

The Microbial Identification System (MIDI Inc., Newark, DE) converts cellular fatty acids from pure cultures of bacteria to fatty acid methyl esters and uses a gas chromatographic technique for separation and identification. Samples are harvested, saponified, and methylated followed by their extraction and washing. The resulting organic phase is used for identification and pattern recognition software is used to identify isolates from the chromatograph reading. This method has been used to identify and differentiate *Bacillus* spores (Song et al., 2000), *Burkholderia* spp. (Inglis et al., 2003), *Francisella* spp. (Bernard et al., 1994), and *Yersinia* spp. (Leclercq et al., 2000).

Each of these biochemical methods is inherently time consuming because each requires pure isolates to attain accuracy. Additionally, well-trained and experienced technicians are required to identify the agent accurately and subsequently handle further testing. Although automated systems are initially expensive, once a pure isolate has been obtained, the benefits of such systems include the ability to perform multiple biochemical tests simultaneously, the speed of obtaining results after minimal incubation times, and minimal advanced technician interaction. These systems generally are more convenient than manual procedures but typically not feasible for field applications and their usefulness may be limited by the extent of their databases, which must be updated on a regular basis.

Immunological Detection Devices

Various immunoassays has been used and developed for the detection of infectious diseases, drugs, toxins, and contaminants in the medical, pharmaceutical, and food industries. In addition, immunological detection has been successfully employed for the detection of biothreat agents, including bacterial cells, spores, viruses, and toxins and based on the fact that any compound capable of triggering an immune response can be targeted as an antigen. Most of these immunoassays rely on four basic components regardless of the application and underlying technology:

1. The antigen to be detected
2. The antibody or antiserum used for detection

3. The method to separate bound antigen and antibody complexes from unbound reactants.
4. The detection procedure.

The efficiency of any given immunoassay is dependent on two major factors: the efficiency of antigen-antibody complex formation and the ability to detect these complexes (Andreotti et al., 2003).

Typically, most of the immunoassays test for only one analyte per assay. This suggests that multiple simultaneous or sequential assays must be performed to detect more than one analyte in a sample or specimen. Gradual advances in assay design and in matrix format have resulted in development of multiplex assays, which can be performed on multiple samples simultaneously by automated systems. However, the specificity of immunoassays is limited by antibody quality, and their sensitivity (detection limits ~105 CFU) is typically lower than that of PCR and other DNA-based assays. With the improvements in antibody quality and in assay parameters, it might be possible to increase sensitivity and specificity of an immunoassay.

Many different immunoassay formats are available commercially for a wide variety of detection needs (Table 20.10).

Commercially available sandwich methods have expanded to include newer innovations of biotechnology, principally in detection capabilities. These immunoassays can be performed with a wide variety of substrates and labels (fluorescent, chemiluminescent, and electrochemiluminescent), as well as on multiple platform types (biosensors, flow cytometry, microarray, and lateral flow diffusion devices). Many currently available systems use different combinations of these advances.

Solid-Support Platforms

LUMINEX XMAP

Luminex xMAP (Luminex Corp, Austin, TX) technology uses the basic "sandwich" assay format, but the capture antibody is coated onto the surface of a polystyrene bead rather than using a microwell plate. These beads are processed and then separated for analysis via flow cytometry (Vignali, 2000). The beads or microspheres are spectrally unique and color coded into different sets, which can be differentiated by the Luminex100 analyzer. Each type of bead can be labeled with different antibodies, which enables multiple analyses to be simultaneously performed in the same well. During the assay, the beads are maintained in solution, thereby permitting liquid phase binding of target and separation spectrally by a dual-laser detection system. The laser detects excitation of internal bead dyes and reporter dyes of any captured targets and its signal intensity is directly proportional to the amount of target analyte in the sample. The xMAP technology had been incorporated for detection of exposure to bio threat agents in humans (Biagini et al., 2004).

BV TECHNOLOGY

The BV M-Series technology (BioVeris Corp., formerly ORIGIN-Igen Corp., Gaithersburg, MD) uses electrochemiluminescence method to detect reporter molecules used in the sandwich assay. The paramagnetic beads are the support structure in this process to capture antibodies. On capturing the target, the reporter antibodies are then labeled with BV-TAG, Ru(bpy)32+ and are passed through the system; any target analyte captured on

the beads is labeled. The beads are passed via a flow cell over a magnet on the surface of an electrode, resulting in capture by the magnet and are thus separated from unbound label and remaining matrix components. Electrical potential is then applied to the electrode that excites the BV-TAGs, which then emit light that can further be detected and processed. This technology has been mainly used for clinical diagnosis but is being applied to detect bio threat agents such as *E. coli* O157 (Shelton and Karns, 2001), *Bacillus* spores, toxins and toxoids (Gatto-Menking et al., 1995), *Yersinia* spp. (Yu, 1996) and *Salmonella enterica* serovar Typhimurium (Yu and Bruno, 1996).

Bio-Detector

The Bio-Detector (Smiths Detection, Edgewood, MD) employs enzyme-linked immunosorbent assay (ELISA) principles on a tape format in a portable rugged housing. Liquid samples are injected and separated for detection of various biothreat agents. During the reaction, each subsample is mixed with biotin and fluorescein-labeled antibodies and with streptavidin, which attaches to the biotin label. When these labeled antibodies attach to the targets within the subsample, they are filtered and captured by biotin-coated biotape in different locations for each subsample. The streptavidin serves as a bridge between the biotin-tagged targets and the biotin-coated tape. A solution of antifluorescein antibody, previously conjugated to the enzyme urease, is filtered through the tape and tags the bound target. The tape is then positioned over the sensor, where it is covered in a solution of urea; in cases where the tape has trapped a targeted agent, the urease reacts with the urea and causes a change in pH. The amount of pH change is directly proportional to the amount of target present. The sensor processes this signal and determines the presence and quantity of target agent present.

Delfia

The dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA; Perkin-Elmer Life Sciences, Akron, OH) system is one of the examples of a format based on time-resolved fluorescence. This technique relies on lanthanide chelate labels having long fluorescence decay times and allows measurements of fluorescence without interference from background signals. Typically, it is incorporated into a standard ELISA microplate assay format, and after the reaction, the label is disassociated from the detection antibodies using a low pH enhancement solution. The free molecules rapidly form new stable and highly fluorescent chelates, which can be read by the system. The DELFIA system has been used to detect *F. tularensis*, *Clostridium botulinum* toxin, SEB, and *E. coli* O157:H7 (Peruski et al., 2002, Yu et al., 2002).

Lateral Flow Platforms

Lateral flow devices have been basically developed for rapid field assay formats but are quickly becoming incorporated in clinical laboratory settings. These tests are usually one-time use, disposable cartridge tests in the form of either immunochromatographic line assays or enzyme immunoassays generating detectable colored end-products, which give negative or positive test result. These assays use antibodies mounted on a paper strip or membrane as the capture antibody and use capillary flow to move colloidal gold or colored microparticle-labeled antigen detection antibody complexes in the fluid phase toward the capture antibody. A positive result is obtained from the capture of the labeled antigen-antibody complex with a second immobilized antiantibody

Table 20.10. Commercially available biothreat detection systems.

Type	Test Format	Test Name	Manufacturer	Targets[a]
Biochemical	FAME-GC	Sherlock Bioterrorism Library	MIDI, Inc.	*B. anthracis, Y. pestis, Brucella* spp., *F. tularensis, B. mallei, B. pseudomallei*
	Substrate utilization	MicroLog Dangerous Pathogen Database	BiOLOG	*B. anthracis, Y. pestis, Brucella* spp., *F. tularensis, B. mallei, B. pseudomallei*
		Vitek	bioMérieux	*Y. pestis, V. cholerae, C. botulinum*
		API Series	bioMérieux	*B. anthracis, C. botulinum, Y. pestis, Salmonella* spp.
Antibody	Microarray	NanoChip	Nanogen	Pathogens, SEB, *V. cholerae* toxin B
	ELISA	xMap Technology	Luminex	Pathogens
		BV Technology	BioVeris Corp.	*B. anthracis, E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., *C. botulinum* toxins A, B, E, & F, ricin, SEB
		Quick ELISA *B. anthracis*-PA kit	Immunetics Inc.	*B. anthracis*
		RAPTOR	Research International	*B. anthracis*, *F. tularensis*
				Y. pestis, *Brucella* spp., *V. cholerae*, ricin, SEB, *E. coli* O157:H7, *Salmonella* spp.
		Bio-Detector	Smiths	Assorted organisms
	Smart Tickets	Instant Check	EY Laboratories	Ricin
		BioThreat Alert	Tetracore	*B. anthracis, F. tularensis, Y. pestis, C. botulinum, Brucella* spp., ricin, SEB
		Redline Alert	Tetracore	*B. anthracis*
		SMART-II	New Horizons Diagnostic, Inc.	*B. anthracis*, *F. tularensis*, *Y. pestis*, *C. botulinum*, *V. cholerae*, ricin, SEB, *E. coli* O157:H7, *Salmonella* spp.
		Biowarfare Agent Detection Device (BADD)	Osborne Scientific	*B. anthracis*, *C. botulinum*, ricin
		RAMP	Response Biomedical Corp.	*B. anthracis*, *C. botulinum*, *Y. pestis*, ricin, smallpox
		HHA & HHMA	ANP Tech	*B. anthracis*, *C. botulinum*, *Y. pestis*, smallpox

	TRF	DELFIA	Perkin-Elmer	Pathogens
DNA	Q-PCR	GeneXpert/Smart Cycler	Cepheid	*B. anthracis*
		RAPID	Idaho Technology	*B. anthracis, F. tularensis, Y. pestis, C. botulinum, E. coli* O157:H7, *Salmonella* spp., *Brucella* spp., *Listeria* spp.
		LightCycler detection kit	Roche	*B. anthracis*
		BioSeeq	Smiths Detection	Pathogens
		B. anthracis PCR kit	Takara Mirus Bio	*B. anthracis*
		O157:H7; StxI; StxII	Applied Biosystems Inc.	*E. coli* O157:H7
		RealArt PCR kits	Artus	*B. anthracis, Salmonella* spp., dengue virus orthopox virus, other viruses
		PathAlert detection system	Invitrogen	*B. anthracis*, *Y. pestis*, *F. tularensis*, smallpox
		Certified Lux primer set	Invitrogen	*B. anthracis*, *Y. pestis*, *F. tularensis*, smallpox, *C. botulinum*
Other	Mass spectrometry	TEEMmate	JEOL	Spores
	Bioluminescence	Profile-1	New Horizon Diagnostic, Inc.	Pathogens plus spores

ELISA, enzyme-linked immunosorbent assay; FAME, fatty acid methyl ester; GC, gas chromatography; HHA, handheld assay; HHMA, handheld microarray assay; NA, not available to public; SEB, staphylococcal enterotoxin B.
[a]Examples of targets detected, not a complete accounting.

(typically an anti-immunoglobulin G for the detection antibody host) and the formation of a line or pattern (Murray et al., 2003). Positive and negative control agents are also tested. The negative control is an indicator that the test worked correctly and that the sample was passed through the testing field. These devices are commonly referred to as dipstick tests, and the public is familiar with their use.

Although lateral flow device assays are easier to perform and more rapid than classic instrument-based immunoassays, they typically are not as sensitive and can give false-positive results. However, such devices prove to be useful in rapid preliminary screening of samples for a biothreat agent, and any positives should be further confirmed by more sensitive tests such as PCR.

Nucleic Acid Detection via Quantitative PCR

Quantitative real-time PCR (Q-PCR) involves gene amplification with simultaneous detection of amplified products based on changes in reporter fluorescence (Heid et al., 1996). There are two available formats of Q-PCR, nonspecific detection and specific detection. Nonspecific detection uses DNA-intercalating dyes that fluoresce when bound to DNA (e.g., SYBR green; Molecular Probes Inc., Eugene, OR). With the amplification of DNA, the dye is intercalated into the product. After amplification, a melting curve is performed, and with the DNA dissociation, the fluorescent signal decreases proportionally. This is useful for optimizing PCR conditions and checking specificity of primer designs. For specific detection, the change in fluorescence relies on the use of dual-labeled fluorogenic probes containing both a reporter fluorescent dye and a quencher dye. An increase in fluorescence indicates that the probe has been hybridized to the target DNA, and the quencher dye is no longer able to mask the signals of the fluorescent dye.

The main Q-PCR format used for the detection of biothreat agents is the specific target detection, and many primer and probe combinations are available from a variety of companies for this purpose. Many of these specific-target configurations rely on mechanistic variants in the primer/probe construction and combinations including TaqMan probes (double-dye probes) (Holland et al., 1991), locked nucleic acid probes (Koshkin et al., 1998), black hole quenchers (Johansson and Cook, 2003), lux primers (Nazarenko et al., 2002), and scorpion primers (Whitcombe et al., 1999). Each type uses a slightly different method of separating the fluorophore from the quencher, is commercially available, and can easily be customized. As the fluorescent reporter dyes attach to these primers and probe combinations can have different emission spectra, Q-PCR method can be used to detect several targets simultaneously using different reporter dyes for different targets. Some of the common reporter dyes include 6-carboxyfluorescein, 6-carboxytetramethylrhodamine, Cy5, Cy3, Rox, Texas Red, rhodamine, fluorescein, and Oregon Green.

Typically these Q-PCR thermocyclers incorporate intricate software to monitor the progression of the reactions. At Ct value (the point when the product signal is detected above the background) and in the exponential phase, the software can quantitate and compare the test signal to standards. More abundant the target, the faster it is detected above background and the lower the Ct value. This Ct value can be either qualitative or quantitative, depending on the standards and the needs of the user. With the progress in current technology, these instruments are becoming smaller, faster, and more sensitive, making them highly sensitive methods for detecting biothreat agents.

Q-PCR Thermocycler Platforms

Although many Q-PCR technologies are available, a few are more focused toward pathogens, especially biothreat agents. Most of these systems are based on sophisticated molecular techniques that perform best in a laboratory setting such as hospitals, monitoring offices, and research laboratories. An example is the GeneXpert Q-PCR DNA detection system (Cepheid, Sunnyvale, CA), which incorporates the standard Q-PCR features of the Smart Cycler system (Cepheid) with disposable automated testing cartridges that can process samples and perform amplifications followed by product detection (Belgrader et al., 2000). This is an automated process that removes the human variable and speeds the processing time by eliminating the need to extract DNA from samples prior to analysis. A GeneXpert test cartridge specific for *B. anthracis* is available, and other targets are under development.

These Q-PCR instruments are useful in a laboratory, but recent bioterrorism events have underscored the need for fieldable Q-PCR platforms. For such purpose, many biothreat agent and pathogen detection kits are available in freeze-dried format for RAPID detection (Higgins et al., 2003; Van Kessel et al., 2003).

Polymerase Chain Reaction Reagent Kits for Biothreat Agents

There are innumerable combinations of probes and primers in the literature databases that can be used for the detection of biothreat agents. Recently several companies have begun to make PCR kits in various formats for detecting these agents (*see* Table 20.8). These kits eliminate the need for extensive primer/probe design and facilitate the rapid detection and monitoring programs. The kits consist of all the reagents along with the prepared controls and only lack the sample DNA to be tested.

Bioluminescence Detection

Bioluminescence has been widely used in clinical, food, and environmental settings for monitoring incidences of microbial contamination. The best example of bioluminescence is the luciferin-luciferase reaction occurs in the presence of adenosine triphosphate (ATP), which is based on the principle of that the amount of ATP in a sample correlates proportionally to the biomass. Because ATP is found in all living cells, care should be taken during sample processing to eliminate ATP contamination from nontarget sources (plant cells, mammalian cells, and nontarget bacteria). For such purposes, different strengths of detergents are used to lyse nontarget cells (e.g., mold, pollen, somatic cells) in the sample, so that only the intended cells are monitored (Cutler et al., 1996).

Developing Technologies

Several sensor technologies have been developed for bioterrorism defense, which can be divided into a few broad groups depending on the way of recognizing and responding to a particular biological threat agent. These groups are designated as biochemical, immunological, nucleic acid, cell or tissue, and chemical or physical technologies. Each of these recognition systems must be paired with a transducer that can transform the response into a signal that can be analyzed. Detection platforms combining a biological recognition system and a physical transducer are termed *biosensors*. There is a variety of transducers that can be paired with a given biological recognition system to generate an analyzable signal, including electrochemical, optical, mass, thermal, and high frequency.

Biochemical Detection

Biochemical systems for recognition of biological targets usually measure the products and enzymatic activity associated with microbial metabolism. The methods employed are generally not as specific as an antibody or nucleic acid-based methods because the targeted product or enzyme may also be present in some other organisms.

An example is electronic nose device that detects the metabolic products of organisms by using a transducer, such as a cantilever, acoustic wave, or conducting polymer, which has been coated with a chemical that reacts with specific volatile organic compounds or gases to create a sensing element (Deisingh and Thompson, 2004). Additionally an array of sensors, each specific for different vapors or gases, can also be constructed and used to detect multiple analytes. This technology has been used by researchers for detecting and identifying volatile organic compounds produced by specific bacteria or fungi. Electronic nose devices require complex pattern recognition software to interpret the results, which can be rapid and sensitive but not specific, because the compounds produced by microorganisms can fluctuate with energy or carbon source and with environmental conditions. Furthermore, different organisms can produce similar volatile products leading to false detection.

Conducting polymers (organic polymers that can conduct electricity) can also be used to detect biologically produced chemicals. These polymers can be doped with enzymes, antibodies, or other biomolecules, which allow them to specifically capture target biological compounds.

Some biosensors have already been reported that detect the presence of a bacterial toxin based on inhibition of enzyme activity. A sensor for anatoxin-a, a toxin released by cyanobacteria, detected an inhibition of acetyl cholinesterase activity using an electrochemical transducer (Villatte et al., 2002).

Immunological Detection

This involves the use of antibody-based and similar affinity probes that are under development. This type of detection technique uses the specificity of the immune system to target agents of interest. Shape-recognition technologies can be used to detect a range of potential threat agents, including viruses, bacteria, toxins, and bioregulators. Substantial research has been concentrated on improving antibody sensitivity and specificity by generating recombinant antibodies, antibody fragments, and phage probes. Other types of shape recognition probes are also under investigation that includes receptors, aptamers, and peptide ligands.

Antibodies and Fragments

Conventionally prepared antibodies are the predominant affinity probe used in shape-recognition–based technologies. However, other forms of antibodies, including mono- and divalent antibody fragments, such as Fab and F(ab)2, respectively, and single-chain variable regions, have also been explored to check any advantages they might offer in sensitivity, specificity, or durability compared to conventional antibodies. Such fragments may be further modified using recombinant technology in an attempt to improve the binding kinetics and make them more favorable as probes.

Aptamers and Peptide Ligands

Aptamers and peptide ligands are the alternatives to antibodies. Aptamers are small DNA fragments or RNA ligands that can recognize a target by shape, not by sequence, and they are generated using combinatorial methods. Aptamers have been used to detect

ricin toxin in a bead-based biochip sensor (Kirby et al., 2004) and cholera toxin, SEB, and *B. anthracis* spores in an electrochemiluminescence assay (Bruno and Keil, 1999, 2002). Two variations of aptamers are available including ribozymes/autocatalytic RNA aptamers that can be engineered to generate a signal after target capture (Hesselberth et al., 2003), and photoaptamers/DNA aptamers that have been modified to bond covalently to a bound target while exposed to ultraviolet light (Smith et al., 2003).

Flow Cytometry

Biosensors that employ shape-based recognition for capturing and detecting specific biothreat agents are being developed. An example of this is the flow cytometer, which serves as the detector for the Autonomous Pathogen Detection System (APDS) developed at Lawrence Livermore National Laboratory. It is based on proprietary Luminex technology, in which Luminex color-coded beads are conjugated to antibodies binding to specific target agents (McBride et al., 2003a, b). Each differently coded bead is labeled with a target-specific antibody, providing extensive multiplex capabilities. The APDS has been used to detect the threat agent *Bacillus globigii*, *Erwinia herbicola*, MS2, and ovalbumin singly and in mixtures (McBride et al., 2003a) and is also able to detect simultaneously *B. anthracis* and *Y. pestis* in air (McBride et al., 2003b).

Biochip Arrays

Target detection using biochip technology is under investigation. One approach uses dielectrophoresis to concentrate target agents and then detects them using an electric-field-driven immunoassay (Huang et al., 2001). Dielectrophoresis and electric fields are used to direct the assay components and targets to the proper position on the array; the resulting fluorescence is visualized microscopically. This method has been tested with the agents *E. coli* O157:H7 and *B. globigii* spores (Huang et al., 2001; Yang et al., 2002) and SEB and cholera toxin B (Ewalt et al., 2001; Yang et al., 2002).

Another tested method uses a sensor array constructed on a complementary metal oxide semiconductor integrated circuit to detect targets on a microarray (Moreno-Bondi et al., 2003). This method uses an optical detector that can interrogate all spots on the array simultaneously or individually and has been designed for detection of *B. globigii* spores in the air by integrating a portable air sampler with an on-chip ELISA (Stratis-Cullum et al., 2003).

Microarrays

Identification of bacteria using rRNA was tested using *E. coli* in a mix containing *Bordetella bronchiseptica* (Gau et al., 2001). After single-stranded DNA capture of rRNA from the bacteria on a self-assembled monolayer, the bacteria were tagged with another single-stranded DNA probe labeled with fluorescein dye, which is followed by the addition of an antifluorescein peroxidase-labeled antibody to amplify the signal. This peroxidase activity is then detected by amperometric detection. The authors reported detection of approximately 103 *E. coli* cells by this method.

Many biochip technologies have also been tested for the detection of nucleic acids. The electric-field-driven assay of immunological detection has also been used to detect bacterial DNA. Bacteria are concentrated by dielectrophoresis, lysed, and the obtained DNA is denatured by heat denaturation. The DNA is then amplified using strand displacement method and is analyzed using an on-chip electric-field-driven hybridization assay.

Another antibody-based detection chip technology for DNA detection uses capillary array electrophoresis, and laser-induced fluorescence method to detect PCR-amplified DNA (Song et al., 2003). For this purpose, DNA from an enterotoxigenic strain of *E. coli* was used. This method was comparable to gel analysis for identifying specific amplicons with greater speed and multiplexing capabilities on a microarray platform.

Tissue and Cell-Based Detection

Tissue and cell-based detection systems use the intrinsic response of a specific cell type toward a toxic or infectious foreign substance to identify a biothreat agent. In such devices, the cells constituting the sensor produce a potential signal that can be measured by an electrode or optical detector (Kovacs, 2003). The detector cell may either originate from a specific unicellular organism or a tissue type, such as nerve or heart cells, and may be primary or immortalized. Potential applications of such approach for the detection of biothreat agents have been described (Stenger et al., 2001; Kovacs, 2003).

Biosensors in which the cells originated from neurological and cardiac tissues are being explored for the detection of harmful substances including biological threat. An example incorporates genetically engineered cardiomyocytes, which responds selectively to specific functional activity, (Aravanis et al., 2001; DeBusschere and Kovacs, 2001).

Chemical and Physical Detection

Biosensors are also based on physical and chemical properties and respond to specific characteristics intrinsic to the target analyte. Such technologies include mass spectrometry, Raman spectrometry, and intrinsic fluorescence/luminescence. These methods do not require additional biological reagents; however, affinity probes may be required in target capture and to increase specificity.

Clinical Management

Bioterrorism and its aftereffects can impose heavy demands on the public health-care system, which will be called on to handle the consequences. This requires an effective public health-care system with strong disease surveillance, rapid epidemiological survey and laboratory investigation, improved and efficient medical management, and information, education, and communication (IEC) to counter any act of covert or overt bioterrorist attack.

Initial sequence of events

1. Dispersion of the agent in a patent or covert way.
2. Primary contagion can take many forms (inhalation, food, contact, etc.) and can be abrupt or gradual.
3. Incubation period, the longer the larger number of victims until focus is detected.
4. Transmission from person to person, if the agent showing this capability it requires measures of isolation that are scarce and costly.
5. Increased mobility of the population favors dissemination, requiring quarantine in some cases.

Procedure to Be Followed

There is a series of procedure to be followed following a bioterrorism attack as (Flowers et al., 2002; Mothershead et al., 2002; Woods, 2005):

1. Reporting of suspected biological incident to the health authorities.
2. Protect and alert own staff.
3. Carry out epidemiological surveillance and screening in health centers and rule out other hypotheses, such as related emerging diseases.
4. Define the population at risk.
5. Make an initial assessment of the extent and spread of the outbreak.
6. Attempt to control the initial dispersion of the agent and the population.
7. Decide on resources, and whether these are sufficient; establish operational measures, prealert other resources.
8. Implement a system of informing the public with clear recommendations.
9. Elaborate prevention and surveillance measures to contain the outbreak.

10-Step Method for Clinical Management

There is a 10-step method for the clinical management of bioterror events (Flowers et al., 2002; Mothershead et al., 2002; Woods, 2005).

1. **Maintain a certain degree of suspicion**. Unlike the conventional terrorism attack, an attack with biological weapons is not necessarily obvious. It has an incubation period, which favors a dispersed distribution of victims and complicates the task of locating the original focus. Moreover, in most of the diseases caused by these agents, early treatment is vital; it is, therefore, essential to maintain a certain level of suspicion for early detection.
2. **Apply self-protection measures**. Self-protective measures should be applied. Usually M-40 series of masks are indicated (one-piece silicon-sealed rubber with two rigid lenses and a filter device), but these masks are often not available. An alternative of this is the surgical mask, which offers adequate protection against aerosols containing biological agents. Empirical chemoprophylaxis should be recommended before and after an exposure along with vaccination of the staff, once the agent has been identified.
3. **Assess the patient and use the ABCD of advanced life support**. This includes a physical examination, with special emphasis on respiratory, neuromuscular, and dermatologic examination, as well as study of history and detailed epidemiological data reflecting recent travel food consumed in public, exposure to vectors, occupation and leisure activities, nearby people having similar symptoms, etc.
4. **Begin proper decontamination, if applicable**. In public health centers, clothing should be timely changed and the patient cleaned with a normal disinfectant. In an outbreak, decontamination solutions, such as the hypochlorite, can be used, which are effective against most of the agents.
5. **Establish a diagnosis**. For diagnostic purposes, clinical and epidemiological criteria should be considered together with laboratory testing. Laboratory samples can be obtained from nasal and oropharyngeal swabs for PCR and culturing purposes; blood and sputum for culture, serum for serological tests, biopsies, or scrapings of

lesions, and environmental samples from sites suspected to harbor the agent. Before the laboratory results, the physician should establish a suspected clinical and epidemiological diagnosis, initiate preventive measures and alert the epidemiological surveillance services.

6. **Provide appropriate treatment**. In cases with suspected diagnosis, empirical therapy should be initiated without waiting for lab results. Wide-spectrum antibiotic should be selected to which the suspected agent has the least resistance (e.g., tetracyclines and fluoroquinolones). Prophylactic treatment should also be considered for the population at risk (Dewan et al., 2002).
7. **Control the infection**. Standard precautionary measures provide adequate protection against most of the diseases. However, special treatment measures are required against some diseases such as smallpox, pneumonic plague, and certain hemorrhagic fevers. The source of the disease should be ensured to be inactive.
8. **Alert the authorities**. If a physician detects a suspected outbreak he or she must report it immediately to epidemiological surveillance systems.
9. **Assist in epidemiological investigations and treat psychological effects**. Epidemiological investigations should be assisted, the victim and their families supported, psychological treatment provided, and the sight of the general population managed.
10. **Maintain continuous education and disseminate knowledge**. Most health-care personnel lack experience required to handle such situations and have theoretical knowledge only. Therefore, it is essential to maintain adequate training and preparation in this field despite the low probability of such an attack, given its potential impact and serious consequences.

Biosurveillance

Biosurveillance deals with the detection of real-time disease outbreak. Currently existing surveillance systems for bioterrorism-related diseases vary widely to the methods used to collect the surveillance data, surveillance characteristics of the data collected, and analytical methods used to determine the occurrence of a potential outbreak. Traditional methods for collecting the surveillance data were manual reporting of suspicious and clinicians, hospitals, and laboratories dealing with the disease notifying public health officials (Teutsch and Churchill, 1994). Some of the recent innovations in disease surveillance may improve the timeliness, sensitivity, and specificity of the bioterrorism-related outbreak detection and include surveillance for syndromes (group of diseases) rather than specific diseases and the automated extraction and analysis of routinely collected clinical, administrative, pharmaceutical, and laboratory data.

After a bioterrorism attack, the public health and medical communities are the first-line response. These people are the ones who must first detect that the incident has actually occurred, identify the specific biological agent, decontaminate the area (if required), determine the likelihood of secondary transmission, identify the exposed population, and provide preventive measures and treatments. First responders are emergency department doctors and nurses, infection control practitioners, epidemiologists, laboratory experts, public health officials, and hospital administrators, and they can save many lives through rapid detection, accurate diagnosis, and speedy treatment. Hence, one of the challenges is to ensure that first responders are capable of performing their tasks successfully. To combat biological warfare successfully, unprecedented cooperation is required between the federal

government, state and local agencies, and the medical community. The federal government (Health and Human Services [HHS], the Federal Bureau of Investigation []FBI), Federal Emergency Management Agency [FEMA], and others) plays a key leadership role by supporting state and local planning efforts through funding, expertise, training, and developing an infrastructure for detecting biological attacks and delivering mass medical care as required. The medical response plans for managing the bioterrorism consequences must be well integrated and coordinate with other emergency response systems. Because most countries and people do not have enough experience with a biological attack, the public health systems would be challenged to undertake emergency management of bioterrorism. Some special measures should be needed for patient care and hospitalization, obtaining laboratory confirmation regarding the identity of the biological agent, providing vaccine or antibiotics to a large population, and identifying and possibly quarantining patients. Rapid and accurate surveillance detection and epidemiologic investigation by the first responders is a key factor in minimizing sufferings and loss of life in a bioterrorism attack. Many limitations of the public health departments in conducting disease detection and surveillance and epidemiologic investigations have caused many public health experts to raise concerns about the adequacy of the country's infectious diseases surveillance network and its ability to function in the midst of a biological attack.

Local Disease Surveillance and Detection by Physicians

The current system of medical response to a bioterrorism attack in the United States emphasizes the critical role of the first responders, the local emergency care systems, during the initial period of a biological attack. Information regarding the occurrence of any biological attack (the detection) is the first challenge faced by first responders because biological agents lend themselves to clandestine dissemination in the air, food, or water supply (Siegrist, 1999). The release would most likely be unannounced by the attacker and would most certainly be remain undetected. As an example, an aerosol release would produce a cloud that would be invisible, odorless, and tasteless. Depending on the type of biological agent used, no one would know until days or weeks later that anyone had been infected. The information would come long after a considerable amount of damage had been done, and probably most of the victims of an unannounced biological attack will delay seeking medical care because most of the biological agents used in such attacks will manifest flulike symptoms in the early stages of infection (Hendricks, 2001). After an attack, the severely ill patients would be admitted to intensive care units, and medical wards, because of the harried pace of the medical personnel, may not notice the influx of patients currently being treated in multiple emergency facilities came from the same geographic area.

Despite the emphasis on emergency physicians as the early response team, the medical community may not be capable of identifying the reason people are falling ill until days or even weeks later, after hospitalization, and when laboratory results are available and by this time, many lives would have been lost.

Local Laboratory Surveillance and Capacity

The next impediment to detection that probably hinders a rapid response by the first responders occurs in the laboratory. As soon as a clinical specimen is delivered to the laboratory, diagnosis may be hindered for several reasons (Garrett, 1994). First, microbes that are well adapted and growing in the lungs or intestines can be difficult to grow in

artificial media in a petri dish. Second, microbiologists find difficulty in growing cultures when samples are not taken with precision and properly prepared and stored. Before receiving cultures in the laboratory, they are handled by clinicians and delivery service personnel who might not be trained in the appropriate procedures for taking, preparing, and storing cultures, which usually happens when medical personnel are working with a large influx of patients. Third, microbiologists routinely perform a series of time-consuming tests for ordinary diseases before they start testing for exotic ones. Fourth, if a presented disease has only seen in textbooks by them, the technicians are likely to restart the test, often requesting that a new culture be drawn from the patient. At some point, the cultures that are difficult to identify go into the stack of unknowns to be scrutinized by a pathologist, who may request additional diagnostic assays (Franz et al., 1997). Fifth, some microbiologists may not be well skilled with how to plate and test for biological warfare agents; for example, some agents such as anthrax require the use of special medium.

Problems with Current Epidemiological Investigation

A major goal of public health departments is prompt identification and then suppression of infectious diseases. The national concept of operations against an early bioterrorism response relies heavily on local, state, and federal health organizations being able to detect a biological attack through surveillance by first responders and their reporting of a possibly uncommon disease. Surveillance systems for collecting, analyzing, and interpreting reports of bioterrorism cases and trained staffs to monitor for disease outbreak are the foundation of public health epidemiology (National Research Council, 2001). They are also the core of the problems associated with the current epidemiologic investigation capabilities. Surveillance systems that rely on voluntary disease reporting from health-care providers are termed *passive surveillance systems*, which are notorious for their poor sensitivity, lack of timeliness, and minimal coverage. Because the passive system is inexpensive to implement, it comprises the majority of surveillance systems in place at local, state, and federal levels, but the quality of information in passive surveillance systems is greatly limited, making them not suitable to the needs of bioterrorism surveillance. The CDC oversees a large number of passive disease surveillance systems based on collaboration with state and local health departments, which in turn depend on physician-initiated reports of specific diseases or information from state health laboratories; the National Notifiable Disease Surveillance System is probably the best known in this category. CDC and state epidemiologists compile and periodically review a list of 50 diseases, which currently includes anthrax, smallpox, plague, hemorrhagic fevers, and botulism. According to state laws, clinicians, hospitals, and laboratories should report cases involving any of these diseases—many states have legal penalties against a health-care provider who does not report, but the penalties are seldom imposed—and the reliability of passive surveillance systems is low because physicians or hospitals often fail to make the initial report or do not report in a timely manner.

Active surveillance requires a staff to actively search for and identify new cases; further it provides more timely and accurate information than the passive systems but must have sufficient numbers of adequately trained epidemiologists to collect, compile, analyze, and interpret the data to determine the source of the biological agent. Sentinel Surveillance Networks (SSNs) is an example of active surveillance system. To detect and characterize an outbreak caused by a covert release of a biological agent can be difficult, but it may also be

startlingly obvious. A reporting of an anthrax case in an area of the country where anthrax has never reported or in an individual without any obvious risk factors for the disease would raise the suspicions of the public health epidemiologist. Although intentional infection would not necessarily be the first explanation investigated, additional case reports would eventually lead to a serious consideration of this possibility. The time duration it takes to reach this point can determine if there is a small casualty count or mass casualties. In a case with a biological attack, lost time may quickly translate into lost lives. Therefore, it is a critical infrastructure resource and expertise problem of national importance that there should be a sufficient number of adequately trained epidemiologists at both the local and state levels. The CDC trains a cadre of persons and designates them as Epidemic Intelligence Service (EIS) officers, available to assist state and local epidemiological response.

Efforts to Enhance Surveillance and Detection

Detection and identification of biological agents, either in the environment or in victims, is important. Local health officials, disease emergency planners, state public health officials, and the CDC are striving to find more expedient ways to identify and respond to a biological attack.

At the local level, several surveillance concepts are implemented for the early detection of suspicious disease outbreaks merely by auditing fluctuations in the number of patients admitted to hospitals; the numbers are derived from the activity levels of the emergency management systems (EMS). In 1994, the CDC launched three complementary programs to help rebuild the US public health infrastructure for surveillance and response to infectious diseases that will prove useful in a condition of a bioterrorism incident; these include the Epidemiology and Laboratory Capacity (ELC) program, the Emerging Infections Programs (EIP), and provider-based sentinel networks. The motive of the ELC program is to help large health departments in developing the core capacity to meet the infectious disease threats of the future by providing technical tools, training, and financial resources. ELC activities include the development of innovative systems for early and rapid detection and investigation of disease outbreaks and ensuring electronic reporting of surveillance data. The motive of the EIP is to conduct population-based surveillance and research to address new hurdles in dealing with the infectious diseases, public health, and to enhance laboratory and epidemiologic capacity. The EIP also evaluates certain diseases of unknown origin. The US Department of Defense also conducts global biosurveillance through several programs, including the Global Emerging Infections Surveillance and Response System (Pellerin, 2011).

Food Surveillance

There is a possibility of deliberate contamination of the nation's food supply, and the economic and psychological implications of an attack on the food supply are sobering. Proper monitoring of food contamination and surveillance of food-borne diseases requires a coordinated multidisciplinary approach with the participation of stakeholders from all sectors of the "farm-to-fork" range including the public and private health sector. A wide range of pathogenic microorganisms can deliberately be introduced into the food chain at any point: from livestock feed via the on-farm production site; at the slaughterhouse or packing plant; in manufacturing, processing, and retailing of food; catering; and home preparation. Because pathogenic microbes can be transmitted by so many possible routes throughout production,

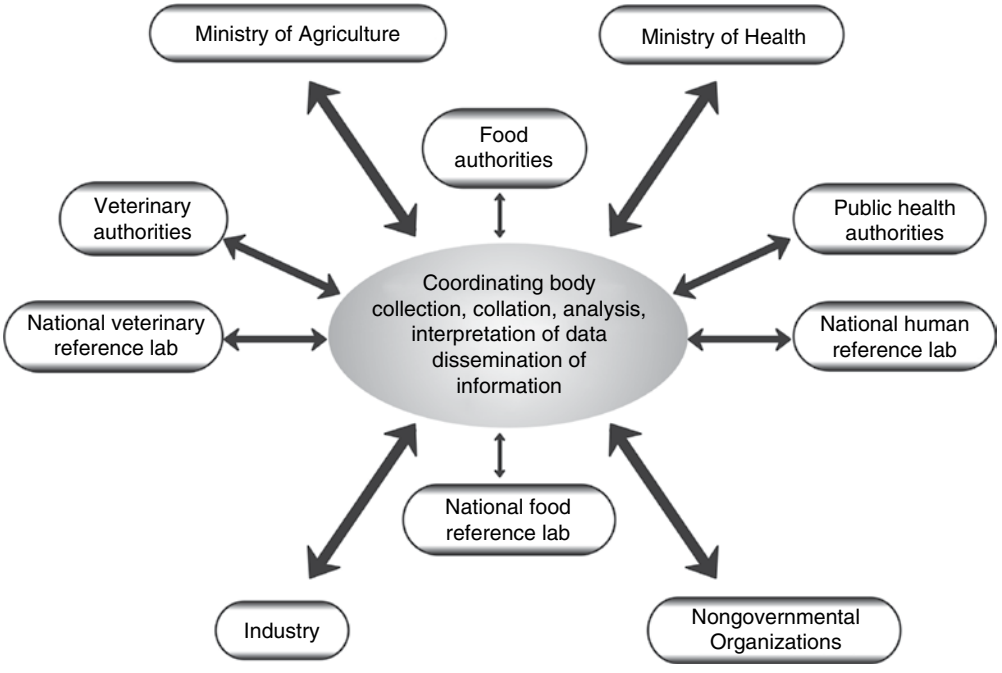

Figure 20.6. Collection, collation, analysis, and interpretation of surveillance data and the subsequent dissemination of information to all the major stakeholders in food safety.

isolated actions (e.g., decontamination of animal feed) will in most cases not ensure lasting consumer protection. To effectively manage the problem of food terrorism activities, special measures should be considered at all levels of production, which in turn requires a coordinated surveillance and response effort from all major stakeholders in food safety.

The food industry whether small or large is responsible for the quality and the safety of their products and is, therefore, a major stakeholder in food safety. Production processes may be monitored through various certification programs (ISO), process control schemes, or HACCP (Hazard Analysis Critical Control Points)–based control programs. The data generated by these control activities can constitute an important contribution to national surveillance programs. Also during an outbreak investigation, additional sampling is required to trace-back human infection to the point of contamination in the food-production chain, which can be achieved through the close cooperation between the private and public sector. The main stakeholders in food safety representing the government are the ministries of health and of agriculture/food. The agencies that are responsible for the legislative, technical, and practical implementation of food safety programs are actually run under the guidance of these ministries, and each agency often has a dedicated reference laboratory associated with it. The access to surveillance data often goes through these reference laboratories. These organizational structures often run independent of each other and to get a comprehensive view of the national food safety status, the two ministries and their respective agencies and reference laboratories should work in association with each other (Figure 20.6).

Apart from these governmental organizations, some nongovernmental organizations including consumers, food industry workers, or environmentalists are other stakeholders

of food safety (*see* Figure 20.6). Although these organizations seldom are directly involved in the generation of data, they can influence the implementation of food safety initiatives and serve as a driving force behind the initiation of surveillance efforts.

Integration of surveillance activities at the national level facilitates optimization and cost efficiency while generating and using surveillance data. The challenge is to optimize the sensitivity of the surveillance system along with the cost minimization. For an example:

- Integration of surveillance components between links of a production chain (e.g., to investigate possible associations between the levels of food-borne pathogens in food animals and in food products).
- Integration of different surveillance programs of the same production animal; for an example, using the same serum samples for the detection of antibodies against both *Salmonella* and porcine reproductive and respiratory syndrome (PRRS).
- Integration of different surveillance programs for different production animals; for an example, estimating the relative contribution of the main reservoirs to the total number of human cases of food-borne illness.
- Integration of national surveillance programs for rapidly recognizing and reporting international outbreaks (e.g. EnterNet, OzFoodNet and Global SalmSurv).

The integration of food-borne disease surveillance activities can be achieved through the communication, collaboration, coordination, and the central storage of data. Communication can be maintained between major stakeholders through regular meetings and direct, informal contact between veterinary and public health workers. Collaboration consists mainly of the routine exchange of data and participation in any outbreak investigation and response. Outbreak control activities and the sharing of information should be coordinated within and between programs. Managing a central database consisting of all surveillance data allows for coherent analyses of the relation between food-borne-pathogen reservoirs and disease in time and space. These four components ensure the optimal use of data that have been generated.

Investigation of an Outbreak

When studying any outbreak, it is important to understand the basic goals of an investigation (Figure 20.8). Any outbreak should be investigated first to find the source of the disease, which should be identified and eliminated quickly, even if the exposure source has dissipated so that ameliorative care can be offered and case interviews can be conducted. Case identification assists in preventing additional cases, especially with a transmissible infectious disease.

With notification of any outbreak, whether natural or human-originated, there should be some standard steps to follow in an outbreak investigation (Figure 20.7), although these steps may not always occur in order (Reingold, 2000). The first step is preparation involving the availability of all necessary response elements (personnel, equipment, laboratory capabilities) and establishing communications in advance with partners in the investigation. In cases of ongoing outbreak, the second step is to investigate, verify the diagnosis, and decide whether an outbreak exists. At the initial stage of an outbreak, its significance and scope are often not known; therefore, existing surveillance information and heightened targeted surveillance efforts are used to determine whether reported

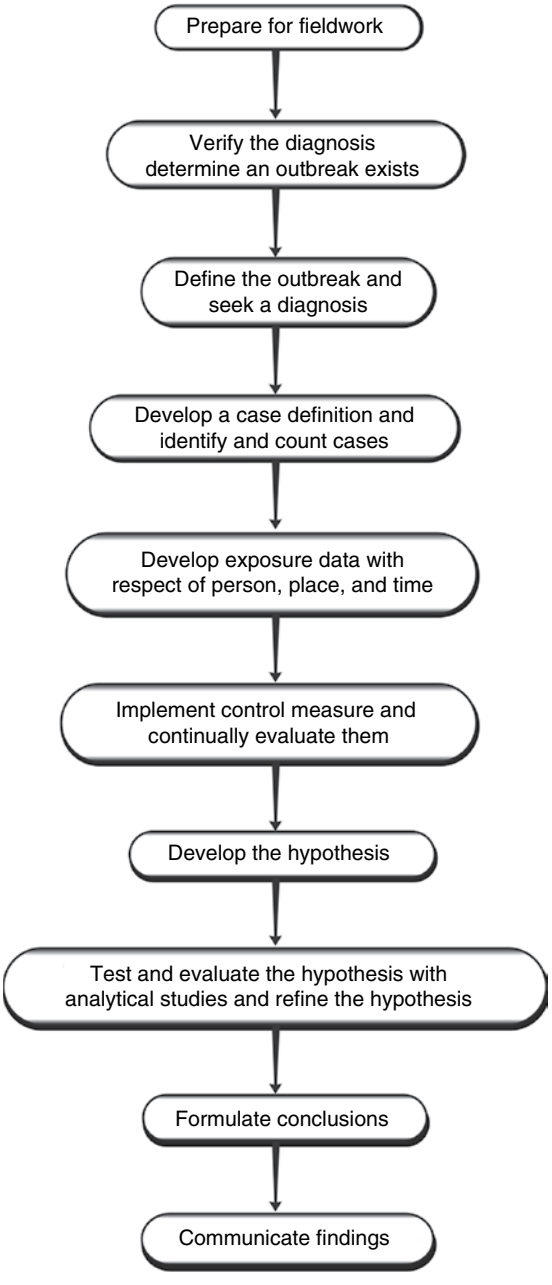

Figure 20.7. 10 steps in an outbreak investigation.

items are cause for concern. The third step is to define the outbreak clearly and seek a definitive diagnosis based on historical, clinical, epidemiological, and laboratory information and then establish differential diagnosis. The fourth step is to establish a case definition that includes all the clinical and laboratory features shared by all the ill

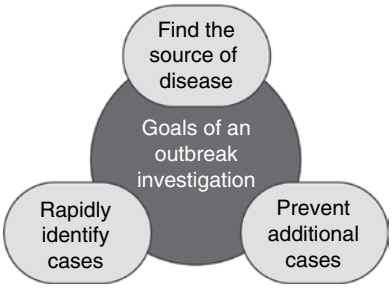

Figure 20.8. Goals of an outbreak investigation.

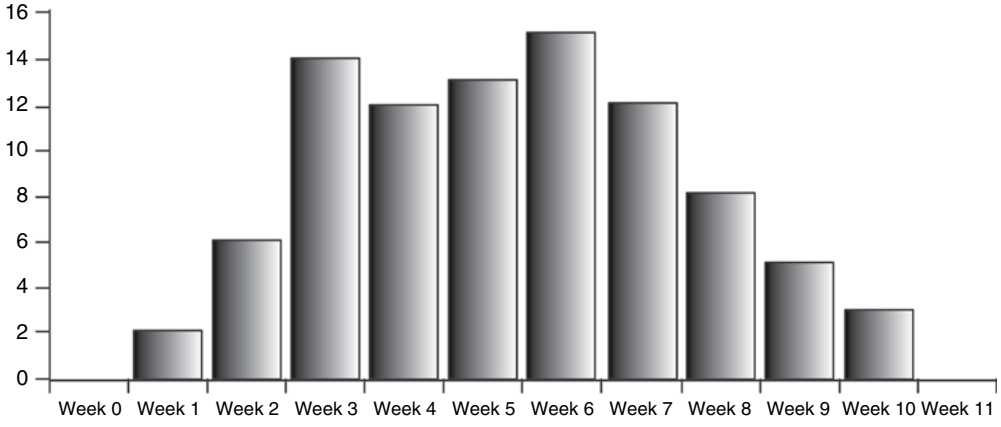

Figure 20.9. Typical continuous common-source outbreak epidemic curve.

individuals. The case definition enables the investigator to count all outbreak cases and compare between cases and non-cases. To generate information regarding symptoms, it may not be sufficient to look at health-care facilities only, but it will likely also be necessary to interview the ill persons and their family members, as well as coworkers, classmates, or others with whom they have close contact. It is particularly important to maintain a roster of potential cases while obtaining this information. Commonly during an investigation, there may be a risk of double- or even triple-counting of cases because they may be reported more than once through different means. Key information needed from each ill person includes date of illness onset, signs and symptoms, recent travel, ill contacts at work, home, or school, animal exposures, and treatments provided. Using this information, an epidemic curve can be constructed, which may provide information as to when a release may have occurred, especially if the disease is known, and an expected exposure date based on the typical incubation period, known ill contacts, or geographic risk factors.

Different modes of disease spread may have typical features comprising an epidemic curve. If the agent is transmitting person to person, successive waves of illness may be seen as one group of individuals infects a follow-on group, which in turn infects another, and so on (Figure 20.9). With time duration and additional cases, the successive waves of illness may overlap sometimes with each other. In cases in which there is a common vehicle for disease transmission (such as a food or water source) that remains contaminated, it

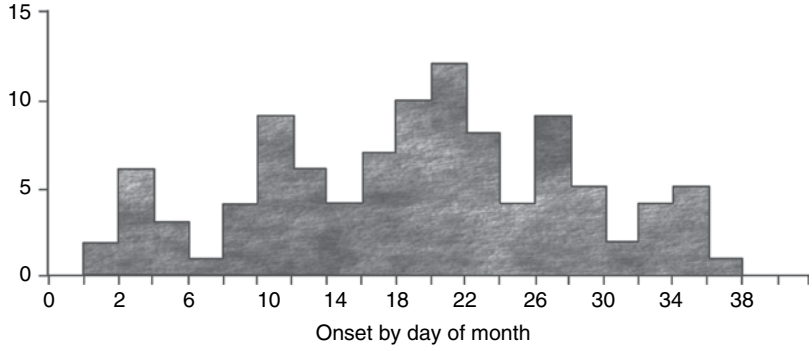

Figure 20.10. Typical propagated (secondary transmission) outbreak epidemic curve.

might be possible to see a longer illness plateau (a continuous common source curve [Figure 20.10] than is seen with a point source of infection).

The fifth step is developing the exposure data with respect to person, place, and time. Cases need to be identified and counted and once identified, exposures based on person, place, and time can be determined. Information obtained from those individuals who would likely have had similar exposures but do not appear ill can also help in determining the potential cause and method of transmission of an agent. Information can either be obtained informally or formally with a case control study (in which investigators start with individuals, with and without disease, and compare their potential exposures or risk factors for disease). The sixth step is implementation of control measures and continuously evaluates. Control measures should be implemented as soon as possible and then modified if required as additional case information becomes available. The seventh step is developing a hypothesis based on the characteristics of the disease, the ill persons, and environmental factors. This includes how the disease occurs, how it is spreading, and the potential risk to the uninfected people. The eighth step involves testing and evaluating the hypothesis using analytical studies and refines the hypothesis. Once developed, the hypothesis should be tested formally with analytical studies to ensure it fits with the known facts. The ninth step is to formulate and declare a conclusion about the nature of the disease and its exposure route. In the final tenth step, findings can be communicated through the media or medical literature, depending on the urgency of notification of the public and medical community.

These different steps may always not occur in sequence. It is necessary to implement control measures with incomplete information, especially if an outbreak is fast-moving or has a high morbidity or mortality rate.

Preparedness and Containment

Containment involves the use of safe methods while managing infectious materials in the laboratory environment. Its purpose is to reduce or completely eliminate exposure of laboratory workers and the environment to potentially hazardous microbial agents. There are two levels of containment: the primary containment is the protection of personnel and the immediate laboratory environment, and secondary containment is the protection of the environment external to the laboratory. Hence, the three most important topics in laboratory safety are:

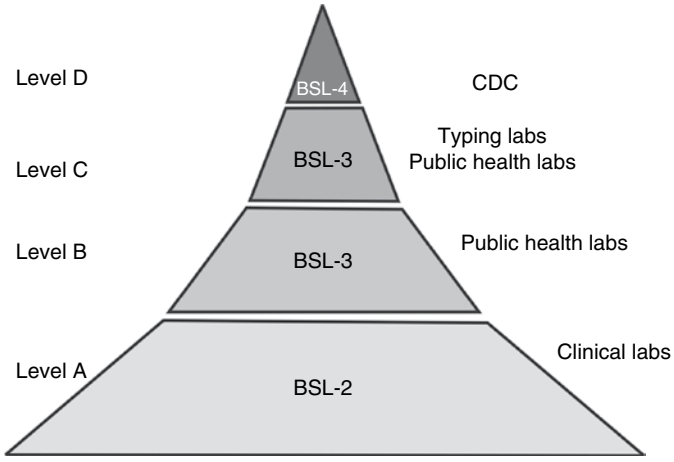

Figure 20.11. Laboratory Response Network laboratory levels. CDC, Centers for Disease Control. Centers for Disease Control and Prevention and National Institutes of Health. 1999. *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. Washington, DC: US Department of Health and Human Services, Public Health Service.

1. Laboratory practice and techniques.
2. Safety equipment.
3. Design of facilities.

Laboratory personnel must always be aware of the potential hazards while dealing with clinical specimens. As a result, following standard microbiological practices and techniques is the most important factor in containment, and each laboratory should develop a biosafety manual. To minimize or eliminate risk exposure to certain agents, laboratory personnel must be continually trained to ensure awareness of diagnostic and preventive measures.

Safety equipments are the primary barriers against biological materials, including biological safety cabinets (BSCs), enclosed containers, and other engineering controls. These may also include items for personal protection, such as hand gloves, face masks, and safety glasses, which are often used in combination with a BSC. There are currently three types of BSC: open-fronted class I and II BSCs offer protection to laboratory personnel and to the environment; class II BSCs additionally provide protection from external contamination; and the gas-tight class III BSC provides the highest attainable level of protection to personnel and environment.

The secondary barriers will depend on, the transmission risk of specific agents (CDC and NIH, 1999) and the design and the construction of the laboratory facility are part of these secondary barriers. They can contribute to the laboratory workers' protection, as well as protect the community environment from infectious agents because they might accidentally be released from the laboratory.

A detailed description of biosafety level practices and classification of BSCs is given in the 4th edition of the *Biosafety in Microbiological and Biomedical Laboratories*, which is also available on the CDC's Web site. Biosafety Level-1 represents a basic level of containment without any recommendations for special primary or secondary barrier precautions (Figure 20.11). In these laboratories, workers deal with the microorganisms that do not cause

disease in healthy adults but may behave as opportunistic pathogens in very young or older patients and also in immunocompromised hosts. Biosafety Level-2 laboratories deal with the moderate-risk organisms, such as hepatitis B virus and *Salmonella*, which may cause disease of varying severity. Biosafety Level-3 laboratory requires all work be performed in a BSC, and more emphasis is placed on primary and secondary barriers; organisms include *M. tuberculosis* and *C. burnetii*. Exotic agents posing the risk of life-threatening diseases, which may be transmitted via aerosol, and for which there is no available vaccine or therapy, must be handled in a Biosafety Level-4 facility. The laboratory workers must be completely isolated from aerosolized infectious materials while working in a class III BSC. Most of the highly infectious viral agents are processed in this last type of laboratory.

For bacterial warfare agents, activities concerning specimen handling and diagnosis of suspected cultures should be done in at least a level-2 facility. Bacterial culture modification and large quantity production and other activities, which might lead to aerosol formation, should be avoided and should always be referred to a level-3 laboratory.

References

Andreotti PE, Ludwig GV, Peruski AH, Tuite JJ, Morse SS, et al. 2003. Immunoassay of infectious agents. *BioTechniques* 35:850–859.

Andrews HL, Baumler AJ. 2005. *Salmonella* species. In: Fratamico PM, Bhunia AK, Smith JL, eds., *Foodborne Pathogens: Microbiology and Molecular Biology*, pp. 327–339. Norfolk, England: Horizon Scientific Press Ltd.

AOAC International. 2003. *The Official Methods of Analysis of AOAC International*, 17th ed. Gaithersburg, MD: AOAC International.

Aravanis AM, DeBusschere BD, Chruscinski AJ, Gilchrist KH, Kobilka BK, et al. 2001. A genetically engineered cell-based biosensor for functional classification of agents. *Biosensor Bioelect* 16:571–577.

Balint GA. 1974. Ricin: The toxic protein of castor oil seeds. *Toxicology* 2:77–102.

Barri ME, El Dirdiri NI, Abu DH, Idris OF. 1990. Toxicity of *Abrus precatorius* in Nubian goats. *Vet Hum Toxicol* 32:541–545.

Belgrader P, Okuzumi M, Pourahmadi F, Borkholder DA, Northrup MA. 2000. A microfluidic cartridge to prepare spores for PCR analysis. *Biosensor Bioelect* 14:849–852.

Bell CA, Uhl JR, Hadfield TL, David JC, Meyer RF, Smith TF, et al. 2002. Detection of *Bacillus anthracis* DNA by Light Cycler PCR. *J Clin Microbiol* 40:2897–2902.

Benoit PW, Donahue DW. 2003. Methods for rapid separation and concentration of bacteria in food that bypass time-consuming cultural enrichment. *J Food Prot* 66:1935–1948.

Bernard K, Tessier S, Winstanley J, Chang D, Borczyk A. 1994. Early recognition of atypical Francisella tularensis strains lacking a cysteine requirement. *J Clin Microbiol* 32:551–553.

Biagini RE, Sammons DL, Smith JP, Page EH, Snawder JE, et al. 2004. Determination of serum IgG antibodies to Bacillus anthracis protective antigen in environmental sampling workers using a fluorescent covalent microsphere immunoassay. *Occup Environ Med* 61:703–708.

Bisher J. 2003. During World War I, terrorists schemed to use Anthrax in the cause of Finnish independence. *Military History* 17–22.

Bruno JG, Kiel JL. 1999. *In vitro* selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. *Biosensor Bioelect* 14:457–464.

Bruno JG, Kiel JL. 2002. Use of magnetic beads in selection and detection of biotoxin aptamers by electrochemiluminescence and enzymatic methods. *BioTechniques* 32:178–183.

Centers for Disease Control and Prevention (CDC). 2001. Evaluation of *Bacillus anthracis* contamination inside the Brentwood Mail Processing and Distribution Center B, District of Columbia. *Morb Mortal Wkly Rep* 50:1129–1133.

Centers for Disease Control and Prevention (CDC). 2008. Bioterrorism overview. Accessed February 20, 2013, at http://www.bt.cdc.gov/bioterrorism/overview.asp.
Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH). 1999. *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. Washington, DC: US Department of Health and Human Services, Public Health Service.
Cheng J, Sheldon EL, Wu L, Uribe A, Gerrue LO, et al. 1998. Preparation and hybridization analysis of DNA/RNA from *E. coli* on microfabricated bioelectronic chips. *Nat Biotechnol* 16:541–546.
Chierakul W, Winothai W, Wattanawaitunechai C, Wuthiekanun V, Rugtaengan T, et al. 2005. Melioidosis in 6 tsunami survivors in Southern Thailand. *Clin Infect Dis* 41:982–990.
Chua J. 2004. A buyer's guide to DNA and RNA prep kits. *Scientist* 18:38–39.
Cox C, Wathes C. 1995. Bioaerosols in the environment. In: Cox C, Wathes C, eds., *Bioaerosols Handbook*, pp. 11–14. Boca Raton: Lewis Publishing.
Currie BJ, Fisher DA, Howard DM, Burrow JN, Lo D, et al. 2000. Endemic melioidosis in tropical northern Australia: A 10-year prospective study and review of the literature. *Clin Infect Dis* 31:981–986.
Cutler CN, Dorsa WJ, Sirogusa GR. 1996. A rapid microbial ATP bioluminescence assay for meat carcasses. *Dairy Food Environ Sanit* 16:726–736.
DeBusschere BD, Kovacs GTA. 2001. Portable cell-based biosensor system using integrated CMOS cell-cartridges. *Biosensor Bioelect* 16:543–556.
Deisingh AK, Thompson M. 2004. Biosensors for the detection of bacteria. *Can J Microbiol* 50:69–77.
DeMarco DR, Lim DV. 2002. Detection of Escherichia coli O157:H7 in 10- and 25-gram ground beef samples with an evanescent-wave biosensor with silica and polystyrene waveguides. *J Food Prot* 65:596–602.
Dewan PK, Fray AM, Laserson K, Tierne BC, Quinn C P, et al. 2002. Inhalational anthrax outbreak among postal workers, Washington, D.C. *Emerg Infect Dis* 8:1066–1072.
Donaldson KA, Kramer MF, Lim DV. 2004. A rapid detection method for Vaccinia virus, the surrogate for smallpox virus. *Biosensor Bioelect* =20:322–327.
Earampamoorthy S, Koff RS. 1975. Health hazards of bivalve-mollusk ingestion. *Ann Intern Med* 83:107–110.
Endicott SL, Hagerman E. 1998. World War Two origins. In: *The United States and Biological Warfare*, p. 27. Bloomington: Indiana University Press.
Ewalt KL, Haigis RW, Rooney R, Ackley D, Krihak M. 2001. Detection of biological toxins on an active electronic microchip. *Anal Biochem* 289:162–172.
Fine A, Layton M. 2001. Lessons from the West Nile viral encephalitis outbreak in New York City, 1999: Implications for bioterrorism preparedness. *Clin Infect Dis* 32:277–282.
Flowers LK, Mothershead JL, Blackwell TH. 2002. Bioterrorism preparedness. II: The community and emergency medical services systems. *Emerg Med Clin North Am* 20:457–476.
Fode-Vaughan KA, Maki JS, Benson JA, Collins MLP. 2003. Direct PCR detection of *Escherichia coli* O157:H7. *Lett Appl Microbiol* 37:239–243.
Franz DR, Jahrling PB, Friedlander AM, McClain DJ, Hoover DL, et al. 1997. Clinical Recognition and Management of Patients Exposed to Biological Warfare Agent. *JAMA* 278:400–401.
Fratamico PM, Bhunia AK, Smith JL, eds. 2005. *Foodborne Pathogens: Microbiology and Molecular Biology*. Norfolk, England: Horizon Scientific Press Ltd.
Furukawa H, Doh-ura K, Okuwaki R, Shirabe S, Yamamoto K, et al. 2004. A pitfall in diagnosis of human prion diseases using detection of protease-resistant prion protein in urine. *J Biol Chem* 279:23661–23667.
Garcia ME, Blanco JL, Caballero J, Gargallo-Viola D. 2002. Anticoagulants interfere with PCR used to diagnose invasive aspergillosis. *J Clin Microbiol* 40:1567–1568.
Garrett L. 1994. *The Coming Plague: Newly Emerging Diseases in a World out of Balance*, pp. 174–191. New York: Farrar, Straus and Giroux.

Gatto-Menking DL, Yu H, Bruno JG, Goode MT, Miller M, et al. 1995. Sensitive detection of biotoxoids and bacterial spores using an immunomagnetic electrochemiluminescence sensor. *Biosensor Bioelect* 10:501–507.

Gau J-J, Lan EH, Dunn B, Ho C-M, Woo JCS. 2001. A MEMS based amperometric detector for E. coli bacteria using self-assembled monolayers. *Biosensor Bioelect* 16:745–755.

Gioffre A, Meichtri L, Zumarraga M, Rodriguez R, Cataldi A. 2004. Evaluation of a QIAamp DNA stool purification kit for Shiga-toxigenic *Escherichia coli* detection in bovine fecal swabs by PCR. *Rev Argent Microbiol* 36:1–5.

Golfield M, Welsh J, Taylor B. 1968. The 1959 outbreak of eastern encephalitis in New Jersey: The inapparent infection disease ratio. *Am J Epidemiol* 87:32–38.

Gregory B, Waag D. 1997. Military medicine: Medical aspects of biological warfare. Office of the Surgeon General, Department of the Army, Library of Congress 97-22242. Accessed May 22, 2009, at http://www.bordeninstitute.army.mil/published_volumes/biological_warfare/BW-ch06.pdf.

Hart M. 1963. Hazards to health-Jequirity bean poisoning. *N Engl J Med* 268:885–886.

Heid C, Stevens J, Livak K, Williams P. 1996. Real time quantitative PCR. *Genome Res* 6: 986–994.

Hendricks M. 1999. Rx against terror. *Johns Hopkins Magazine* (February 1999). Accessed February 9, 2001, at http://www.jhu.edu/jhumag/0299web/germ.html.

Hesselberth JR, Robertson MP, Knudsen SM, Ellington AD. 2003. Simultaneous detection of diverse analytes with an aptazyme ligase array. *Anal Biochem* 312:106–112.

Higgins JA, Cooper M, Schroeder-Tucker L, Black S, Miller D, et al. 2003. A field investigation of *Bacillus anthracis* contamination of U.S. Department of Agriculture and other Washington, D.C., buildings during the anthrax attack of October 2001. *Appl Environ Microbiol* 69:593–599.

Holland P, Abramson R, Watson R, Gelfand D. 1991. Detection of specific polymerase chain reaction product by utilizing the 5′ to 3′ exonuclease activity of Thermus aquaticus DNA polymerase. *Proc Natl Acad Sci USA* 88:7276–7280.

Huang Y, Ewalt KL, Tirado M, Haigis R, Forster A, et al. 2001. Electric manipulation of bioparticles and macromolecules on microfabricated electrodes. *Anal Chem* 73:1549–1559.

Ibekwe AM, Grieve CM. 2003. Detection and quantification of Escherichia coli O157:H7 in environmental samples by real-time PCR. *J Appl Microbiol* 94:421–431.

Inglis TJJ, Aravena-Roman M, Ching S, Croft K, Wuthiekanun V, Mee BJ. 2003. Cellular fatty acid profile distinguishes *Burkholderia pseudomallei* from avirulent *Burkholderia thailandensis*. *J Clin Microbiol* 41:4812–4814.

Jaffer AS. 2004. Biohazard detection system deployment resumes. *US Postal Service Postal News* No. 38. Accessed February 20, 2013, at http://www.lunewsviews.com/usps/pcr.htm.

Johansson MK, Cook RM. 2003. Intramolecular dimers: A new design strategy for fluorescence-quenched probes. *Chem Eur J* 9:3466–3471.

Johnston WB, Eidson M, Smith KA, Stobierski MG. 1999. Compendium of chlamydiosis (psittacosis) control. Psittacosis Compendium Committee, National Association of State Public Health Veterinarians. *J Am Vet Med Assoc* 214:640–644.

Joy B. 2007. *Why the Future Doesn't Need Us: How 21st Century Technologies Threaten to Make Humans an Endangered Species*. New York: Random House.

Karch H, Janetzki-Mittmann C, Aleksic S, Datz M. 1996. Isolation of enterohemorrhagic *Escherichia coli* O157 strains from patients with hemolytic-uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. *J Clin Microbiol* 34:516–519.

Kaufmann AF, Meltzer MI, Schmid GP. 1997. The economic impact of a bioterrorist attack: Are prevention and postattack intervention programs justifiable? *Emerg Infect Dis* 3:83–94.

King AA, Ionides EL, Luckhurst J, Bouma MJ. 2008. Inapparent infections and cholera dynamics. *Nature* 454:877–880.

Kirby R, Cho EJ, Gehrkem B, Bayer T, Park Y S, et al. 2004. Aptamer-based sensor arrays for the detection and quantitation of proteins. *Anal Chem* 76:4066–4075.

Koshkin AA, Singh SK, Nielsen P, Rajwanshi VK , Kumar R, et al. 1998. LNA (locked nucleic acids): Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation, and unprecedented nucleic acid recognition. *Tetrahedron* 54:3607–3630.

Kovacs GTA. 2003. Electronic sensors with living cellular components. *Proc IEEE* 91:915–929.

Lampel KA. 2005. *Shigella* spp. In: Fratamica PM, Bhunia AK, Smith JL, ed., *Foodborne Pathogens: Microbiology and Molecular Biology*, p. 341. Norfolk, England: Caister Academic Press.

Leclercq A, Guiyoule A, El Lioui M, Carniel E, Decallonne J. 2000. High homogeneity of the *Yersinia pestis* fatty acid composition. *J Clin Microbiol* 38:1545–1551.

Leitenberg M. 2001. Biological weapons in the 20th century: A review and analysis. *Crit Rev Microbiol* 27:267–320.

Levine MM, Tacket CO, Sztein MB. 2001. Host-Salmonella interaction: Human trials. *Microbes Infect* 3:1271–1279.

Lindqvist R, Norling B, Lambertz S. 1997. A rapid sample preparation method for PCR detection of food pathogens based on buoyant density centrifugation. *Lett Appl Microbiol* 24:306–310.

Linney AC, George Kernohan W, Higginson R. 2011. The identification of competencies for an NHS response to chemical, biological, radiological, nuclear and explosive (CBRNe) emergencies. *Int Emerg Nurs* 19:96–105.

Liu RH, Yang J, Lenigk R, Bonanno J, Grodzinski P. 2004. Self-contained, fully integrated biochip for sample preparation, polymerase chain reaction amplification, and DNA microarray detection. *Anal Chem* 76:1824–1831.

Ludwig GV, Calle PP, Mangiafico JA, Raphael BL, Danner DK, et al. 2002. An outbreak of West Nile virus in a New York City captive wildlife population. *Am J Trop Med Hyg* 67:67–75.

Luna VA, King D, Davis C, Rycerz T, Ewert M, et al. 2003. Novel sample preparation method for safe and rapid detection of *Bacillus anthracis* spores in environmental powders and nasal swabs. *J Clin Microbiol* 41:1252–1255.

McBride MT, Gammon S, Pitesky M, O'Brien TW, Smith T, et al. 2003a. Multiplexed liquid arrays for simultaneous detection of simulants of biological warfare agents. *Anal Chem* 75:1924–1930.

McBride MT, Masquelier D, Hindson BJ, Makarewicz AJ, Brown S, et al. 2003b. Autonomous detection of aerosolized *Bacillus anthracis* and *Yersinia pestis*. *Anal Chem* 75:5293–5299.

McElroy A, Townsend PK. 2009. *Medical Anthropology in Ecological Perspective*, 5th ed. Boulder: Westview Press.

McMillan W. 2002. Real-time point-of-care molecular detection of infectious disease agents. *Am Clin Lab* 21:29–31.

Meselson M, Guillemin J, Hugh-Jones M, Langmuir A, Popova I, et al. 1994. The Sverdlovsk anthrax outbreak of 1979. *Science* 266:1202–1208.

Morales-Morales HA, Vidal G, Olszewski J, Rock CM, Dasgupta D, et al. 2003. Optimization of a reusable hollow-fiber ultrafilter for simultaneous concentration of enteric bacteria, protozoa, and viruses from water. *Appl Environ Microbiol* 69:4098–4102.

Moreno-Bondi M, Alarie J, Vo-Dinh T. 2003. Multi-analyte analysis system using an antibody-based biochip. *Anal Bioanal Chem* 375:120–124.

Mothershead JL, Tonat K, Koenig KL. 2002. Bioterrorism preparedness. III: State and federal programs and response. *Emerg Med Clin North Am* 20:477–500.

Murray P, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. 2003. *Manual of Clinical Microbiology*, 8th ed. Washington, DC: ASM Press.

National Research Council. 2001. Chemical and biological terrorism: Research and development to improve civilian medical response. Committee on Research and Development Needs for Improving Civilian Medical Response to Chemical and Biological Terrorism Incidents. Accessed February 9, 2001, at http://www.nap.edu/readingroom/books/terrorism.html.

Nazarenko I, Lowe B, Darfler M, Ikonomi P, Schuster D, Rashtchian A. 2002. Multiplex quantitative PCR using self-quenched primers labeled with a single fluorophore. *Nucleic Acids Res* 30:e37.

Netski D, Thran BH, St. Jeor SC. 1999. Sin Nombre virus pathogenesis in *Peromyscus maniculatus*. *J Virol* 73:585–591.

Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. 2002. Typhoid fever. *N Engl J Med* 347:1770–1782.

Patocka J. 1998. Toxicological characteristic of ricin (in Czech). *Voj Zdrav Listy* 67:166–168.

Payne MJ, Kroll RG. 1991. Methods for the separation and concentration of bacteria from foods. *Trends Food Sci Technol* 2:315–319.

Pellerin C. 2011. *Global Nature of Terrorism Drives Biosurveillance*. Washington, DC: American Forces Press Service.

Peruski AH, Johnson III L H, Peruski Jr LF. 2002. Rapid and sensitive detection of biological warfare agents using time-resolved fluorescence assays. *J Immunol Methods* 263:35–41.

Potter ME, Kaufmann AK, Plikaytis BD. 1983. Psittacosis in the United States, 1979. *Morb Mortal Wkly Rep* 32:27.

Radstrom P, Knutsson R, Wolffs P, Lovenklev M, Lofstrom C. 2004. Pre-PCR processing: Strategies to generate PCR-compatible samples. *Mol Biotechnol* 26:133–146.

Ramsden AE, Mota LJ, Münter S, Shorte SL, Holden DW. 2007. The SPI-2 type III secretion system restricts motility of Salmonella-containing vacuoles. *Cell Microbiol* 9:2517–2529.

Reingold A. 2000. Outbreak investigation-a perspective. *Epidemiol Bull PAHO* 21:2–7.

Reynes JM, Counor D, Ong S, Faure C, Seng V, et al. 2005. Nipah Virus in Lyle's Flying Foxes, Cambodia. *Emerg Infect Dis* 11(7):1042–1047. Accessed March 25, 2013, at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3371782/.

Rijpens N, Herman L, Vereecken F, Jannes G, De Smedt J, De Zutter L. 1999. Rapid detection of stressed *Salmonella* spp. in dairy and egg products using immunomagnetic separation and PCR. *Int J. Food Microbiol* 46:37–44.

Romaschin AD, Harris DM, Ribeiro MB, Paice J, Foster D M, et al. 1998.A rapid assay of endotoxin in whole blood using autologous neutrophil dependent chemiluminescence. *J Immunol Methods* 212:169–185.

Rood JI. 1997. *The Clostridia: Molecular Biology and Pathogenesis*. San Diego: Academic Press.

Rood JI. 1998. Virulence genes of *Clostridium perfringens*. *Annu Rev Microbiol* 52:333–360.

Rosskopf-Streicher U, Volkers P, Noeske K, Werner E. 2004. Quality assurance of *C. perfringens* epsilon toxoid vaccines—ELISA versus mouse neutralisation test. *Altex* 21 Suppl 3:65–69.

Rotz LD, Khan AS, Lillibridge SR, Ostroff SM, Hughes JM. 2002. Public health assessment of potential biological terrorism agents. *Emerg Infect Dis* 8:225–230.

Sack DA, Sack RB, Nair GB, Siddique AK. 2004. Cholera. *Lancet* 363:223–233.

Sanmartin C. 2002. Diseased hosts and Man *Venezuelan Encephalitis*. In: Schultz CH, Mothershead JL, Field M. eds., *Bioterrorism Preparedness I: The Emergency Department and Hospital. Emerg Med Clin North Am* 20:437–455.

Seth Carus W. R. I. S. E. 2000. In: Tucker J, ed., *Toxic Terror: Assessing Terrorist Use of Chemical and Biological Weapons*, pp. 55, 69. Cambridge: MIT Press.

Shelton DR, Karns JS. 2001. Quantitative detection of *Escherichia coli* O157 in surface waters by using immunomagnetic electrochemiluminescence. *Appl Environ Microbiol* 67:2908–2915.

Siegrist DW. 1999. The threat of biological attack: Why concern now? *Emerg Infect Dis* 5(4):505–508.

Smith D, Collins BD, Heil J, Koch TH. 2003. Sensitivity and specificity of photoaptamer probes. *Mol Cell Proteomics* 2:11–18.

Smith JF, Davis K, Hart MK, Ludwig GV, McClain DJ, et al. 1997. Viral encephalitides. In: Sidell FR, Takafuji ET, Franz DR, eds., *Textbook of Military Medicine, Medical Aspects of Chemical and Biological Warfare*, p. 579. Washington DC: Borden Institute.

Smith JL, Fratamico PM. 2005. Diarrhea-inducing *Escherichia coli*. In: *Foodborne Pathogens: Microbiology and Molecular Biology*, pp. 357–382. Norfolk, England: Caister Academic Press.

Smith KA, Bradley KK, Stobierski MG, Tengelsen LA. 2005. Compendium of measure to control *Chlamydophila psittaci* (formerly *Chlamydia psittaci*) infection among humans (psittacosis) and pet birds, 2005. *J Am Vet Med Assoc* 226:532–539.

Snyder JW, Check W. 2001. Bioterrorism threats to our future: The role of the clinical microbiology laboratory in detection, identification, and confirmation of biological agents. Washington, DC: ASM Press.

Song JM, Mobley J, Vo-Dinh T. 2003. Detection of bacterial pathogen DNA using an integrated complementary metal oxide semiconductor microchip system with capillary array electrophoresis. *J Chromatogr* B783:501–508.

Song Y, Yang R, Guo Z, Zhang M, Wang X, Zhou F. 2000. Distinctness of spore and vegetative cellular fatty acid profiles of some aerobic endospore-forming bacilli. *J Microbiol Methods* 39:225–241.

Spencer RC, Wilcox MH. 1993. Agents of biological warfare. *Rev Med Microbiol* 4:138–143.

Steele KE, Linn MJ, Schoepp RJ, Komar N, Geisbert TW, et al. 2000. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet Pathol* 37:208–224.

Stenger DA, Gross GW, Keefer EW, Shaffer KM, Andreadis JD, Ma W, et al. 2001. Detection of physiologically active compounds using cell-based biosensors. *Trends Biotechnol* 19:304–309.

Sterne M, Warrack GH. 1964. The Types of *Clostridium perfringens*. *J Pathol Bacteriol* 88:279–283.

Stetzenbach LD, Buttner MP, Cruz P. 2004. Detection and enumeration of airborne biocontaminants. *Curr Opin Biotechnol* 15:170–174.

Stewart GC. 2008. *Staphylococcus aureus*. In: Fratamico PM, Bhunia AK, Smith JL, eds., *Foodborne Pathogens: Microbiology and Molecular Biology*, pp. 273–284. Norfolk, England: Horizon Scientific Press.

Stratis-Cullum DN, Griffin GD, Mobley J, Vass AA, Vo-Dinh T. 2003. A miniature biochip system for detection of aerosolized Bacillus globigii spores. *Anal Chem* 75:275–280.

Takashi H, Keim P, Kaufmann AF, Keys C, Smith KL, Taniguchi K, et al. 1993. *Bacillus anthracis* bioterrorism incident, Kameido, Tokyo, 1993. Accessed February 20, 2013, at http://wwwnc.cdc.gov/eid/article/10/1/03-0238.htm.

Teutsch SM, Churchill RE. 1994. *Principles and Practice of Public Health Surveillance*. New York: Oxford University Press.

Tims TB, Lim DV. 2004. Rapid detection of *Bacillus anthracis* spores directly from powders with an evanescent wave fiber-optic biosensor. *J Microbiol Methods* 59:127–130.

Török TJ, Tauxe RV, Wise RP, Livengood JR, Sokolow R, et al. 1997. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA* 278:389–395.

Tucker JB, Mahan ER. 2009. *President Nixon's Decision to Renounce the U.S. Offensive Biological Weapons Program*. Washington, DC: National Defense University Press.

Van Kessel JS, Karns JS, Perdue ML. 2003. Using a portable real-time PCR assay to detect Salmonella in raw milk. *J Food Prot* 66:1762–1767.

Vietri N J, Purcell BK, Tobery SA, Rasmussen SL, Leffel EK, et al. 2009. A short course of antibiotic treatment is effective in preventing death from experimental inhalational anthrax after discontinuing antibiotics, *J Infect Dis* 199:336–341.

Vignali DA. 2000. Multiplexed particle-based flow cytometric assays. *J Immunol Methods* 243:243–255.

Villatte F, Schulze H, Schmid R, Bachmann T. 2002. A disposable acetylcholinesterase-based electrode biosensor to detect anatoxin-a(s) in water. *Anal Bioanal Chem* 372:322–326.

Whitcombe D, Theaker J, Guy SP, Brown T, Little S. 1999. Detection of PCR products using self-probing amplicons and fluorescence. *Nat Biotechnol* 17:804–807.

Wiener SL, Barrett J. 1986. Biological warfare defense. In: Weiner SL, Barrett J, eds., *Trauma Management for Civilian and Military Physicians*, pp. 508–509. Philadelphia: Saunders.

Williamson HM. 2011. Disaster management mobile protocols: A technology that will save lives. *Am J Disaster Med* 6:55–64.

Wolffs P, Knutsson R, Norling B, Radstrom P. 2004. Rapid quantification of *Yersinia enterocolitica* in pork samples by a novel sample preparation method, flotation, prior to real-time PCR. *J Clin Microbiol* 42:1042–1047.

Woods, JB, ed. 2005. *USAMRIID's Medical Management of Biological Casualties Handbook*. Fort Detrick, MD: U.S. Army Medical Research Institute of Infectious Diseases.

World Health Organization (WHO). 2012. Fact sheet No. 107. Accessed March 25, 2013, at http://www.who.int/mediacentre/factsheets/fs107/en/index.html.

Yagupsky P, Baron EJ. 2005. Laboratory exposures to brucellae and implications for bioterrorism. *Emerg Infect Dis* 11:1180–1185.

Yang JM, Bell J, Huang Y, Tirado M, Thomas D, et al. 2002. An integrated, stacked microlaboratory for biological agent detection with DNA and immunoassays. *Biosensor Bioelect* 17:605–618.

Yu H. 1996. Enhancing immunoelectrochemiluminescence (IECL) for sensitive bacterial detection. *J Immunol Methods* 192:63–71.

Yu H, Bruno JG. 1996. Immunomagnetic-electrochemiluminescent detection of *Escherichia coli* O157 and *Salmonella typhimurium* in foods and environmental water samples. *Appl Environ Microbiol* 62:587–592.

Yu LSL, Reed SA, Golden MH. 2002. Time-resolved fluorescence immunoassay (TRFIA) for the detection of *Escherichia coli* O157:H7 in apple cider. *J Microbiol Methods* 49:63–68.

Zaki SR, Greer PW, Coffield CS, Goldsmith CS, Nolte KB, et al. 1995. Hantavirus pulmonary syndrome: pathogenesis of an emerging disease. *Am J Pathol* 146:552–579.

Chapter 21
Antimicrobial Resistance

Introduction

Antibiotics have revolutionized how patients with bacterial infections are treated; they have further contributed to reducing the mortality and morbidity from bacterial diseases. They are also an essential tool for modern medicine and several surgical procedures, such as transplantation, chemotherapy for cancer, and even the orthopaedic surgery, could not be performed without the administration of potent antibiotics. Antibiotic resistance is currently the greatest challenge to the effective treatment of infectious diseases globally. It is a type of resistance in which a microorganism is able to survive exposure to a specific antibiotic. The drug resistance can be acquired by a spontaneous or induced genetic mutation in bacteria. Genes conferring drug resistance can be transferred between bacteria horizontally by any of the methods of genetic recombination, including conjugation, transduction, or transformation. Thereby, a gene for antibiotic resistance, which had evolved via natural selection, may be shared, and an evolutionary stress, such as exposure to antibiotics, then selects for the antibiotic resistant trait. Most of the antibiotic resistance genes reside on plasmids (an extra chromosomal material), facilitating their easy transfer. In case a bacterium carries several resistance genes against different antibiotics, it is called multidrug resistant (MDR), or informally, a superbug or superbacterium.

Genes for resistance to antibiotic drugs, like the antibiotics themselves, are ancient (D'Costa et al., 2011). However, the increasing prevalence of drug-resistant bacterial infections commonly observed in clinical practice stems from antibiotic use both within human medicine and veterinary medicine. The use of antibiotics can increase selective pressure in a population of bacteria and allow the resistant bacteria to thrive and the susceptible bacteria to die off. As resistance toward antibiotics becomes more common, there arises a greater need for alternative treatments. However, despite a discovery of new antibiotic therapies, there has been a continued decline in the number of newly

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

approved drugs (Donadio et al., 2010). Antibiotic resistance poses a significant problem, and it is also important to understand international patterns of resistance with globalization booming. The excessive use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria. Although initially there were few pre-existing antibiotic-resistant bacteria before the widespread use of antibiotics, evolutionary pressure from their use has played a role in the development of multidrug resistance varieties and the spread of resistance against more than one drug between bacterial species.

People infected with drug-resistant organisms are more likely to have longer hospital stays and may die as a result of infection. In case the drug of choice for treating their infection does not work, these patients require treatment with second or third choice drugs, which may be less effective, more toxic, and more expensive. This suggests that patients with an antimicrobial-resistant infection may suffer more and pay more for treatment.

Global Scenario

Drug resistance actually follows the evolutionary laws of Darwin, which states that the excessive use of antimicrobials creates a selection pressure on microorganisms and as a result, weaker organisms are killed and stronger organisms adapt and survive. When pathogenic microorganisms can multiply beyond some critical mass in the presence of antimicrobials, treatment outcome is compromised; this phenomenon is referred as *antimicrobial resistance* (AMR). AMR has been an underappreciated threat to public health in nations around the globe. It was initially observed as only being a human medical problem, especially in hospital-acquired infections, and usually only in critically ill and patients who were severely immunocompromised. Today, AMR has reached the point that the general population is considered to be at risk, bringing about an era where many common bacterial infections are becoming increasingly difficult to treat. One of the major contributing factors to this changing trend is the spillover of AMR from antibiotic use in poultry and livestock. AMR has become a global concern because geographic borders among countries and continents have become less distinct probably as a result of increasing global trade, expanding human and animal populations, societal advances, and technological developments. With this increasing global connectivity, there is now rapid transport of infectious agents and their AMR genes, which may consequently exert an impact throughout the world. With the increasing observations of AMR, on World Health Day (WHD) 2011, a six-point policy package was declared in which countries were called upon to:

1. Commit to a comprehensive, financed national plan with accountability and civil society engagement.
2. Strengthen surveillance and laboratory capacity.
3. Ensure uninterrupted access to essential medicines of assured quality.
4. Regulate and promote rational use of medicines in animal husbandry and to ensure proper patient care.
5. Enhance infection prevention and control.
6. Foster innovations and research and development of new tools.

Historical Observations

As early as half a century ago, just few years after the discovery of penicillin (in 1929 by Sir Alexander Fleming), scientists observed the emergence of a penicillin-resistant strain of *Staphylococcus aureus*, a normal common bacterial flora of the human body. Afterward, more than 95 percent of *S. aureus* isolates globally found are resistant to various types of penicillins. An initial response to penicillin resistance was the development of methicillin, a semisynthetic penicillin (Bennett and Geme, 1999). The period of late 1940s and early 1950s lead to the discovery and introduction of broad-spectrum antibiotics such as streptomycin, chloramphenicol, and tetracycline; thus began the age of antibiotic chemotherapy. These antibiotics were effective against a wide range of bacterial pathogens including gram-positive and gram-negative bacteria and intracellular parasites including the tubercle bacilli. Some synthetic antimicrobial agents, such as the sulfa drugs (sulfonamides) and antituberculosis drugs, such as para-aminosalicylic acid (PASA) and isoniazid (INH), were also discovered used widely. This is further followed by the resistant strains of dysentery causing *Shigella*, a major cause of premature death in developing countries as well as *Salmonella* spp. By 1953 during *Shigella* infection outbreaks in Japan, a strain of the dysentery bacillus was isolated that was resistant to multiple drugs and exhibited resistance to chloramphenicol, tetracycline, streptomycin, and sulfanilamide. By the late 1980s, methicillin resistant *S. aureus* had been observed in many hospitals and was difficult to treat (Bennett and Geme, 1999). Until recently, vancomycin was considered as an appropriate drug for the treatment of infections caused by multidrug-resistant *Enterococci*, but vancomycin resistance began to emerge in the mid-1980s, which had increased more than 20-fold from 1989 to 1995 (Gaynes and Edwards, 1995). Another community-acquired pathogen, *Neisseria gonorrhoeae*, showed significant changes in antibiotic resistance. For a number of years, penicillins were the drug of choice to treat gonorrhea, but in 1976, the plasmid-mediated β-lactamase enzyme of *Escherichia coli* was found in *N. gonorrhoeae* isolates in Africa and Asia (Neu, 1992). Among all the drug-resistant pathogens, the most important is the emergence of multidrug-resistant tuberculosis (MDR-TB), which is observed in almost every country, especially in patients coinfected with HIV. MDR-TB has appeared among health-care workers and in general populations in various countries, such as Africa, Asia, and Eastern Europe. Similarly penicillin-resistant *Pneumococci* are also spreading rapidly and resistant species of malarial parasites are affecting and killing millions of children and adults each year. In 1990, almost all *Vibrio cholerae* strains in New Delhi, India, were found to be sensitive to the first-line drug treatments, such as furazolidone, ampicillin, co-trimoxazole, and nalidixic acid; thus, most of these first-line drugs are not effective during cholera epidemics now. Although most of these drugs are still active against infection, the lengthening shadow of resistance indicates that many of them may not be effective for longer time periods.

Global Threat

Nature is the reservoir of antimicrobial drugs and superbugs; however, the overuse or misuse of these drugs has increased their residual levels in the environmental reservoirs, leading to the emergence of superbugs or the multidrug-resistant microbes. Hospitals are the breeding grounds of almost all the drug-resistant bacteria, probably as a result of poor hospital hygiene, selective pressure by overuse of particular antibiotic, and mobile genetic elements that encode for bacterial resistance mechanisms. At any given period,

approximately 25 to 30 percent patients receive systemic antibiotics to treat infections or as prophylaxis (Eickoff, 1992). This creates enormous selective pressure, which further leads to the emergence and spread of antibiotic-resistant bacteria. Most intensive care units (ICU) throughout the world are also facing the problem of rapid spread and the emergence of antibiotic-resistant bacteria. Both gram-negative and gram-positive bacteria have been reported as important causes of hospital-acquired infections (Hanberger et al., 1999; Sieradzki et al., 1999). Although resistance mechanisms may require months or years to develop completely(Davies, 1996), once established, a single resistance mechanism can often allow a bacterium to resist multiple drugs. It still remains unclear whether resistance is reversible, and thus whether drug effectiveness is a renewable or nonrenewable resource (Witte, 2000). The greatest long-term threat of AMR is that the resistant strains erode drug efficacy over time, allowing the spread of those drug-resistant pathogens within a hospital or specific community and then to a nation at large or across national boundaries because of travel and migration within a country. All these things probably contribute to the growth of that nation's resistance problem.

Drug-Resistant Organisms

Antimicrobial-resistant organisms (AROs) or drug-resistant organisms (DROs) are bacteria or other organisms that developed a resistance to certain antimicrobial drugs used to treat infection caused by these microbes. The result is that the particular drug is no longer able to control the infection caused by that specific microorganism. A number of pathogens are resistant to one or more antimicrobial drugs used for treatment, including tuberculosis bacteria, the viruses causing influenza, the parasites causing malaria, and the fungi causing infections. Some common examples of drug-resistant organisms are listed in Table 21.1.

Table 21.1. Drug-resistant organisms.

Group of microbes	Drug-resistant organisms	
1	Bacteria	• Methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA) • Glycopeptide-intermediate *Staphylococcus aureus* (GISA) • Vancomycin-intermediate *Staphylococcus aureus* (VISA) • Vancomycin-resistant *Staphylococcus aureus* (VRSA; not present in nature but can emerge or evolve from VISA) • Vancomycin-resistant *Enterococci* (VRE) • Extended-spectrum β-lactamases (ESBLs; are resistant to cephalosporins and monobactams) • Penicillin-resistant *Streptococcus pneumoniae* (PRSP) • Multidrug resistant *Klebsiella* species and *Escherichia coli* • *Neisseria gonorrhoeae* strains resistant to penicillins, tetracyclines, spectinomycin, and fluoroquinolones • Fluoroquinolone-resistant *Neisseria meningitides* and *Salmonella typhi* strains • Multidrug-resistant tuberculosis (MDR-TB)
2	Viruses	Influenza
3	Fungi	Fluconazole resistant *Candida*
4	Parasites	Malaria parasites (*Plasmodium falciparum* and P. vivax)

Bacterial Agents

Bacterial resistance to antibiotics poses a serious challenge to the prospect of chemotherapy. Rational use of antibiotics is most desirable, but it cannot provide a permanent solution to the problem (Sengupta and Chattopadhyay, 2012). There are so many bacterial agents that show resistance to various antimicrobial drugs:

Methicillin-Resistant Staphylococcus Aureus (MRSA)

Some strains of the *S. aureus* bacterium are resistant to the action of methicillin antibiotic, and other related β-lactam antibiotics (e.g., penicillin and cephalosporin). Actually MRSA has evolved a resistance not only to β-lactam antibiotics, but to several other classes of antibiotics. These organisms are usually subcategorized as hospital-associated MRSA (HA-MRSA) or community-associated MRSA (CA-MRSA), depending on the circumstances of acquiring the disease. HA-MRSA is mostly found among patients undergoing invasive medical procedures or those having weakened or compromised immune systems and being treated in hospitals and health-care facilities, such as nursing homes and dialysis centers. MRSA commonly causes serious and potentially life-threatening infections, including bloodstream infections, surgical site infections, or pneumonia. The most common sources of transmission of HA- MRSA are the asymptomatic patients that already have an MRSA infection or carry the bacteria on their bodies without showing symptoms (colonized). The other mode of transmission is through the hands of health-care workers, which have been contaminated with MRSA bacteria while coming in contact with infected or colonized patients.

CA-MRSA infections usually occur in healthy persons that have not been recently (within a year) hospitalized or undergoing a medical procedure such as dialysis, surgery, catheters. Approximately 75 percent of CA-MRSA infections are localized to skin and soft tissue, such as abscesses, boils, and other pus-filled lesions and can be treated effectively. However, CA-MRSA strains display enhanced virulence, spreading more rapidly and causing more severe illness as compared to the traditional HA-MRSA infections. CA-MRSA can also affect vital organs, leading to widespread infection, toxic shock syndrome, and pneumonia. Two-thirds of the 85 percent of MRSA infections in hospitals or other health-care exposures occurred among people who were no longer hospitalized. MRSA infection is particularly common in people older than age 65. Incidence rates among blacks were twice that of the general population, and lowest rates were observed among children older than the age of 4 and teens.

Vancomycin-Resistant Staphylococcus aureus

Some strains of *S. aureus* become resistant to the glycopeptide antibiotic vancomycin. Vancomycin or similar glycopeptide antibiotic (teicoplanin) was usually a treatment of choice in infections associated with MRSA, which resulted the emergence of three classes of vancomycin-resistant *S. aureus*: vancomycin-intermediate *S. aureus* (VISA), heterogenous vancomycin-intermediate *S. aureus* (hVISA), and high-level vancomycin-resistant *S. aureus* (VRSA) (Appelbaum, 2007). Among all these three vancomycin resistant strains, high-level vancomycin resistance is rarely reported (Gould, 2010). VISA was first reported in Japan in 1996 and has since been found in hospitals in the United Kingdom, France, the United States, Asia, and Brazil. It is also termed glycopeptide-intermediate *S. aureus* (GISA), which indicates resistance to all glycopeptide antibiotics. These bacterial

strains possess a thick cell wall, which reduces the ability of the antibiotic vancomycin to diffuse into the division septum of the cell, required for effective vancomycin treatment (Howden et al., 2010).

Vancomycin-Resistant Enterococci

Enterococci make up a significant part of the normal bacterial flora of the digestive tract and female genital tract of healthy human beings and normally do not pose any threat to healthy people. Infections appear to occur commonly in people, in hospitals, or other health-care facilities. Physicians commonly use vancomycin to treat infections, and on exposure, some bacteria will acquire resistance to the antibiotic. The infections caused by these resistant strains could be fatal, particularly those caused by strains of vancomycin-resistant *enterococci* (VRE). VRE first reported in Europe in 1986 and then in United States in 1989. According to the Centers for Disease Control and Prevention (CDC) during 2004, VRE caused approximately one of every three infections in hospital intensive-care units. *Enterococci* infections are more common in elderly persons, particularly those in long-term care facilities and skilled nursing homes because they are more likely to experience infection risk factors, such as exposure to medical instruments.

Extended-Spectrum β-Lactamase-Producing Members of Enterobacteriaceae

Extended-spectrum β-lactamase (ESBL) is a plasmid-associated β-lactamase that has recently been found to be produced by the members of *Enterobacteriaceae* family. ESBL can hydrolyze penicillins, many narrow spectrum cephalosporins, many extended-spectrum cephalosporins, oxyimino-cephalosporins (cefotaxime, ceftazidime), and monobactams (aztreonam). ESBL is mainly produced by *Klebsiella* spp., predominantly *Klebsiella pneumoniae*, and *E. coli*, but it has also been found throughout the *Enterobacteriaceae* family. As ESBL enzymes production is mediated by plasmids, the genes encoding these enzymes are easily transferable among different bacteria. Along with the DNA encoding ESBL enzymes, most of these plasmids carry genes conferring resistance to several non-β-lactam antibiotics; consequently most ESBL isolates are quite resistant to many classes of antibiotics. The most frequent coresistances found in ESBL-producing organisms are against aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole-trimethoprim. Treatment of these multiple DROs is a therapeutic challenge for all the health-care workers.

ESBL-producing strains have been isolated from abscesses, blood, catheter tips, lung, peritoneal fluid, sputum, and throat cultures, and they apparently showed a worldwide distribution. The risk factors for colonization and infection with organisms harboring ESBL are admission to an intensive care unit, recent surgery, instrumentation, prolonged hospital stay, and antibiotic exposure, especially to extended-spectrum β-lactam antibiotics, which exerts a selective pressure for the emergence of ESBL-producing strains. Lower gastrointestinal tract is the main reservoir for these organisms. Nursing home patients are more likely to possess an ESBL-producing strain because they are treated empirically with antibiotics, and these ESBL producing organisms are then transmitted from patient to patient via the hands of hospital staff. Thereby the spread of ESBL-producing strains can only be minimized by good infection control practices, especially by good hand washing technique.

Penicillin-Resistant Streptococcus pneumoniae

Streptococcus pneumoniae, also called pneumococcus, is a common flora of noses and throats of healthy children and adults. It is most frequently observed in young children, the elderly, or in people having an existing severe illness or other health conditions such as chronic lung, heart, or kidney disease. Other persons at risk are those addicted to alcohol, persons with diabetes, and persons with weakened immune systems. The bacterium causes pneumonia, meningitis, or a bloodstream infection, which can be treated by administering penicillin, but there are a growing number of pneumococcus bacteria that cannot be killed by penicillin. The patients infected with these penicillin-resistant bacteria now are required to be treated with other, more expensive antibiotics. This resistance to penicillin actually arises from mosaic mutation of penicillin binding protein (PBP) genes due to interspecies recombination of homologous genes.

Penicillin-resistant *S. pneumoniae* (PRSP) is also found to be resistant to other common antibiotics, such as macrolide, tetracycline, co-trimoxazole, chloramphenicol, and clindamycin. This limits the choice of antibiotics to third-generation cephalosporin and vancomycin together with rifampicin in case of critical infections including meningitis.

Apart from these drug-resistant bacteria, some species of *Klebsiella* and *E. coli* have been found to be resistant to a wide range of antimicrobial drugs. Similarly *N. gonorrhoeae* strains are also resistant to penicillins, tetracyclines, spectinomycin, and fluoroquinolones antibiotics (Sparling et al., 1975; Vijaylakshmi et al., 1982; Deguchi et al., 1996; Galimand et al., 2000). Resistance to fluoroquinolone antibiotic has also been found in *Neisseria meningitides* and *Salmonella typhi* strains (Galimand et al., 2000; Turner et al., 2006), and the most common example are tuberculosis strains (MDR-TB), which are reported to be resistant to multiple antibiotic drugs.

Drug-Resistant Viruses

Viruses are typically not thought to be alive, and therefore, cannot be killed by antiviral drugs. Viruses spread the infection by using the host cell machinery, which would allow them to replicate and infect nearby healthy cells. Viruses are, therefore, different from bacterial cells in that they are obligatory intracellular parasites, which cannot survive without the help of host cells. The spread of virus can be slowed or even stopped through various antiviral drugs preventing them from replicating and generating new virus copies. Viruses generally use small strands of DNA or RNA to replicate, which can undergo mutations, and as a result, their replication is not delayed or stopped by these antiviral drugs and antiviral drug resistance is generated. Hence antiviral resistance indicates that a virus has changed in a manner that the antiviral drug is less effective in treating or preventing illnesses.

Influenza Virus

The hallmark of influenza virus is its ability to change continuously. These viruses often change from one season to the next and can even change within the course of one flu season. As an influenza virus replicates (making copies), its genetic makeup may change in a way resulting the virus becomes resistant to one or more of the antiviral drugs used to treat or prevent influenza. Currently two classes of drugs are available against influenza virus, the neuraminidase (NA) inhibitors oseltamivir (brand name Tamiflu) and zanamivir (brand name Relenza), which impair the efficient release of virus from infected

cells. Apart from these, other drugs include M2 protein inhibitors amantadine (several brand names) and rimantadine (brand name Flumadine) that target the M2 protein of viral particle, which is required for the uncoating of the virus inside the cell. The NA inhibitors are effective against all NA subtypes of influenza, whereas the M2 inhibitors are effective only against influenza A virus (Moscona, 2005). The key factor that affects the epidemiology of drug resistance in influenza is the rate at which treatment generates resistance de novo. M2 inhibitor–resistant mutants have been reported to occur in about 30 percent of treated patients (Harper et al., 2005). Until recently, it seemed that de novo resistance rarely occurred against NA inhibitors. Resistance against zanamivir was found only in one child who was immunocompromised (Gubareva et al., 1998), whereas resistance against oseltamivir was found in 0.7 to 4 percent of adults (Jackson et al., 2000; Gubareva et al., 2001) and in 4 to 8 percent of children (Jackson et al., 2000; Roberts, 2001). However, a study found resistant isolates in 18 percent of children treated with oseltamivir (Kiso et al., 2004).

HIV Antiviral Resistance

HIV is a rapidly mutating virus that mutates (high replication and mutation rate approximately one mutation per replication) through the human immune system, making it difficult for the body to combat. Anti-HIV drugs typically work to stop virus replication through a number of different ways, such as through the enzymes protease or retrotranscriptase inhibitors. These inhibitor drugs are usually mixed together into a cocktail to slow down the replication process of HIV, but to date, no complete cure for HIV has been found.

Drug-Resistant Fungi

The problem of antiobiotic resistance in pathogenic fungi is of special importance. Many mutant fungi have been isolated, demonstrating resistance to a wide range of antifungal antibiotics. This broad-spectrum drug tolerance in fungi is similar to the multidrug resistance that occurs in organisms ranging from bacteria to humans (Ling, 1997). The other important feature is the availability of a limited number of antifungal drugs, which makes this an acute problem in the eradication of fungal infections by chemotherapeutic agents. A level of multidrug resistance has also been observed in nonpathogenic yeast *Saccharomyces cerevisiae*, and its multidrug-resistant phenotype is referred to as pleiotropic drug resistance (PDR) and genes influencing this phenotype are typically designated PDR loci. Among the pathogenic fungi, *Candida* is the major fungal agent causing bloodstream infections in humans and candidemia is the fourth most common nosocomial infection (Slavin et al., 2004); *Candida albicans* represents the primary *Candida* species associated with the disease (Pfaller et al., 2001) and has been the most intensively studied organism in terms of multidrug resistance. The second most common *Candida* species associated with human infections is *Candida glabrata* (a haploid species) (Pfaller et al., 2001), which has been reported to acquire tolerance against commonly used antifungal agents, such as azoles (Pfaller and Diekema, 2007). Other than *Candida*, the filamentous fungi, *Aspergillus fumigatus* has been observed as a primary human pathogen (Richardson, 2005), which has high morbidity and an intrinsically high resistance to many of the standard antifungal agents (Pfaller and Diekema, 2007).

Fungal drug resistance is an important problem because of the availability of a limited number of antifungal compounds (Kontoyiannis and Lewis, 2002). Understanding the

regulation and function of multidrug resistance pathways in fungi is still in progress, but its importance continues to grow with the increasing number of worldwide patients who are immunocompromised and their increasing reliance on chemotherapy to control fungal infections

Drug-Resistant Parasites

Parasitic diseases always present a unique challenge to physicians trying to treat them. The parasitic microbes usually evade the immune system of the human body; thus, the human host can control the parasitic disease but not eliminate it completely. It is difficult to design efficient vaccines against these parasites; hence, drugs are currently the only way to prevent or treat parasitic diseases. There are many ways through which different parasites encounter an antiparasitic drug. Some parasites, such as *Entamoeba histolytica* and *Toxoplasma* have significant reservoirs other than humans; these parasites mostly cause chronic diseases and the patient becomes asymptomatic for longer time periods. Hence, the parasite population is treated with drugs relatively infrequently and rather late during the patient's illness. These factors slow the speed of selection by reducing the probability for the parasite to encounter the drug. *E. histolytica* and *Trichomonas vaginalis* infections are usually treated using metronidazole but patients with *E. histolytica* are often asymptomatic and the infection is rarely treated with drugs, thereby, metronidazole resistance has not evolved rapidly, whereas *T. vaginalis* is a developed world pathogen and is commonly treated by drugs, in this case, metronidazole resistance proved to be an important clinical problem (Land and Johnson, 1999). Important parasites causing malaria disease, *Plasmodiuum falciparum* and *Plasmodium vivax*, exclusively infect humans and cause an acute illness with a high fever and discomfort, which is usually treated immediately. The ratio of parasite population exposed to the drug treatment varies greatly and depends on the endemicity. But overall, the exposure of these parasites to drugs is far higher than that in other parasites, and thereby the selection of resistant populations is also correspondingly higher. The best known example is drug resistance against chloroquine; it has dramatically reduced the usefulness of the drug, which was the drug of choice for the treatment of nonsevere or uncomplicated malaria and for chemoprophylaxis. Malaria parasites also show resistance to other quinine drugs, such as amodiaquine, mefloquine, and halofantrine, and to antifolate combination drugs including sulfadoxine in association with pyrimethamine.

Causes of Drug Resistance

Previously it was observed that antibiotic resistance probably occurs because of the failure of prescribed drug regimens, but now it has been accepted that human errors also contribute to the development of antibiotic-resistant bacteria.

Misuse of Antibiotics

Misuse of antibiotics occurs in medicine, agriculture, and household products; examples include erroneous antibiotic prescriptions for nonbacterial infections and the addition of antibiotics to livestock feed and cleaning agents, which helped in creating a reservoir of antibiotic-resistant bacteria. Indiscriminate and often unnecessary use of antibiotic drug

is considered the most significant human contribution to the acceleration of antibiotic resistance. In the United States alone, between 160 and 260 million courses of antibiotics are prescribed each year, and an estimated 75 percent of these are administered for treating respiratory infections. However, between one-third and one-half are unnecessary and are prescribed to people who have viral infections that are not treatable with antibacterial medication. Most physicians acknowledge that they prescribe antibiotics to patients simply because the patients demand them. Because every application of antibiotics encourages the growth of resistant strains of pathogens, misuse of antibiotics accelerates the problem of drug resistance.

In addition, most of the doctors in hospitals prescribe broad-spectrum antibiotics even in cases where a more targeted drug that only kills the specific bacterium causing an infection would be enough. The advantage of using these broad-spectrum antibiotics for the patient is that they wipe out a wider range of susceptible microbial species, but the downside is that this goes even further in clearing the playing field for the toughest, most resistant strains that are left behind.

Antibiotic resistance may also be developed when patients fail to take the full course of a prescription of antibiotic medication. Actually this happens because the weakest germs are killed within the first few days, which often eliminates the symptoms of the infection. Thus, people stop taking the drugs, leaving behind bacteria. The result is the creation of stronger bacteria that can resist the first few days of medication, whereas had the patient taken the full course of the medicine, it might have killed them completely. Another method by which humans accelerate AMR is the use antibiotics in farm animals. Approximately, half of all the antibiotics produced for use in the United States are used on farm animals, mostly at low doses for a longer period to encourage growth, but this low dose allows more resistant strains of bacteria in animals to outsurvive the weaker ones. Some of these resistant bacteria, including strains of *Salmonella*, *Shigella*, and *E. coli*, can be further transferred to people in improperly prepared foods. Thus, when people develop bacterial illness, the strains of these bacteria become resistant to the drugs used to control them.

Anomalous Combinations

Sometimes anomalous combinations help in perpetuating drug-resistant microbes. For example, one study on Rhesus monkeys reported that mercury in dental amalgam fillings fostered a 61 percent increase in antibiotic-resistant bacteria and just on removing the amalgam fillings, drug-resistant bacteria dropped 58 percent. Another example is in which a *S. aureus* strain acquires vancomycin resistance genes through cohabitation with the vancomycin-resistant bacteria, *Enterococcus faecalis*, in the wound of a hospitalized patient. The reason behind this is the exchange of genetic material between bacterial species; the mere coexistence of these two particular bacteria helped to bring about drug resistance in *S. aureus*.

Enhanced Transmission of Resistance Factors

Antibiotic resistance may also be developed through the enhanced transmission of resistance factors or the increased efficiency with which resistance genes are exchanged. The contributing factors include the survival of patients with chronic disease, an increased number of individuals who are immunocompromised, substandard hospital hygiene, more international travel, and improper health-care administration.

The Reservoir Hypothesis

According to this hypothesis, antibiotic-resistant bacteria have evolved because of the selective pressures applied by antibiotic drugs. The hypothesis states that each antibiotic has a threshold level that is required to induce, confer, and maintain antibiotic resistance. With a decline in the populations of susceptible bacteria from antibiotic treatment, naturally resistant bacteria begin to thrive and create a reservoir of antibiotic-resistant bacteria.

Antibiotic resistance can also be amplified in certain settings, including schools, hospitals, chronic care facilities, and other environments because there are a lot of people with less effective immune systems carrying a lot of bacteria. Therefore, there are more chances of individual strains swapping their resistance genes. Finally, bacterial resistance accelerates as a result of the global transportation system with people and goods spreading microbes around the world. Most antibiotic drugs can be purchased without a prescription, and thereby, are often taken improperly, which can also lead to resistant strains that then spread worldwide.

Mechanisms of Drug Resistance

Most of the pathogens develop resistance to antimicrobials through the process of natural selection in which a microbial population is exposed to an antibiotic and more susceptible organisms will be eliminated, leaving behind only those resistant to the antimicrobial agent. Further these organisms can either pass on their resistance genes to their offspring during the replication process or to other related bacteria through the genetic recombination, whereby plasmids carrying the genes jump from one organism to another. This is a natural, unstoppable phenomenon that is exacerbated by the abuse, overuse, and misuse of antimicrobials for the treatment of human illness and in animal husbandry, aquaculture, and agriculture.

Antibiotic resistance may either be an inherent trait of the organism (e.g., a particular type of cell wall structure), especially in bacteria, that renders it naturally resistant, or it may be acquired by mutation in its own genetic material or by resistance-conferring nucleic acid from another source.

Inherent (Natural) Resistance

Bacteria may inherit genes providing resistance to a particular antibiotic. It may lack a transport system for an antibiotic or may lack the target for the antibiotic molecule. Sometimes the bacterial cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic as is observed in most cases of gram-negative bacteria. Enzymatic inactivation of the antibiotic is the most common mode of resistance during which an existing cellular enzyme is modified to react with the antibiotic in such a way that it is no longer able to affect the organism. An alternative strategy employed by many bacteria is the alteration of the antibiotic target site (Figure 21.1).

Acquired (Adaptive) Resistance

Organisms may develop several mechanisms to acquire resistance to different antibiotics. This can be done either through the modification of existing genetic material or the acquisition of new genetic material from another source.

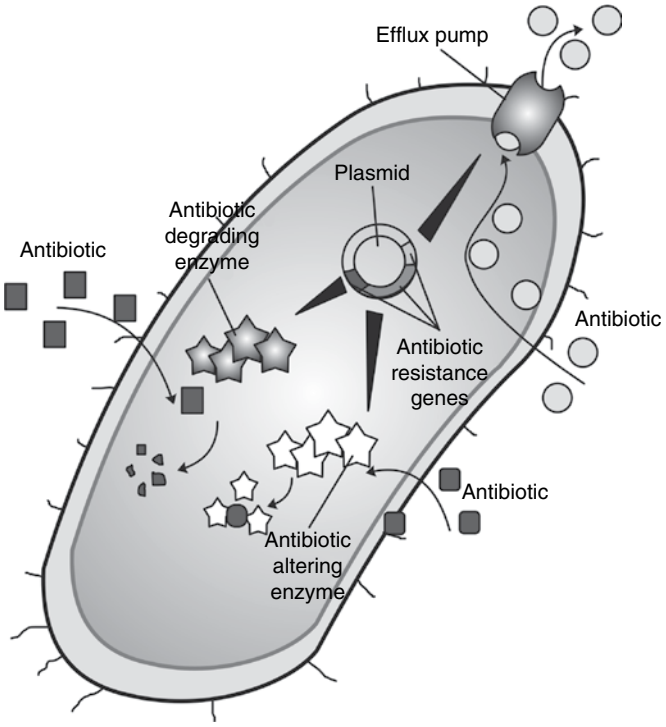

Figure 21.1. Antibiotic resistance mechanisms in bacterial cells.

Antibiotic resistance can be acquired through various routes as:

1. Some organisms either do not absorb antibiotic or may inactivate or modify the drug. The best known example is enzymatic deactivation of penicillin G in some penicillin-resistant bacteria through the production of β-lactamases.
2. By reducing drug accumulation, through decreasing drug permeability, or increasing active efflux (pumping out) of the drugs across the cell surface.
3. Degradation of antibiotic by microbe.
4. Some microbes may alter the usual molecular target for the antibiotics, and the drug becomes ineffective (e.g., the alteration of PBP the binding target site of penicillins in MRSA and other penicillin-resistant bacteria).

By altering metabolic pathway as observed in some sulfonamide-resistant bacteria, which do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides, instead, like mammalian cells, they use preformed folic acid.

Hence bacteria develop resistance either by restricting the entry of the drug molecule within them or quickly expelling them out of their systems (*see* Figure 21.1). Efflux pumps help in eliminating or expelling the antimicrobial drug which is inside. These efflux pumps are basically variants of the bacterial membrane pumps that are responsible for the exchange of nutrients and movement of waste outside the cell. For an example, *E. coli* and

Shigella possess an active efflux pump as a mechanism of antibacterial resistance; similarly *S. aureus* uses an efflux pump for developing resistance against macrolide class of antibiotics. Some gram-negative bacteria are capable of changing the structure of porins (proteins channels), which restrict the entry of the antimicrobial agent (e.g., *Pseudomonas* resistance to imipenem or cilastatin). Fluoroquinolones resistance in *Pseudomonas aeruginosa* and *E. coli* actually is attributed to their decreased ability to reach the target site in sufficient concentration so as to elicit a response (Hooper, 2001). Another major mechanism to acquire resistance is the alteration or elimination of drug target; the best known example of this phenomenon is alteration in the PBPs by which *Streptococcus pneumoniae* and *S. aureus* become resistant to penicillin and methicillin, respectively. There are four different ways by which this newly resistant organism will deal with antibiotic treatments.

1. Can produce enzymes that destroy the antibiotic molecule before it exerts its effect.
2. The protein might be so altered that the antibiotic molecule can no longer associate with it.
3. An organism can use alternative metabolic pathways and abandon the one targeted by the antibiotic.
4. Bacteria can produce protein channels and pumps that help rid the molecule of antibiotic molecules.

Antibiotic resistance can also occur through various mutations in the microbial genome including point mutations, deletions, inversion, etc. When such mutations occur, the antibiotic will kill or inhibit all susceptible organisms, allowing the antibiotic resistant organism to grow and multiply through selection procedure. Resistance has a fitness cost for the microorganism, but mutations occur rapidly and accumulate to overcome this fitness cost; thus, many types of resistance may never disappear in bacterial population. Gene sequences conferring resistance in a drug-resistant microbe can be spread either vertically or horizontally.

- **Vertical gene transfer (VGT)**: The frequency of spontaneous mutation for antibiotic resistance is approximately 10^{-8} to 10^{-9}, meaning that one in every 10^{8}- 10^{9} bacteria in an infection will develop resistance through the process of mutation. For an example in *E. coli* bacterium, streptomycin resistance has been estimated to be acquired at a rate of approximately 10^{-9} while exposed to high concentrations of streptomycin. Once the resistance genes have developed, they are then directly transferred to all the bacterial offspring's during DNA replication by the process known as VGT or vertical evolution. This process follows the Darwinian laws of natural selection in which the wild types (nonmutants) are killed, and the resistant mutant is allowed to grow and flourish in the selective environment.
- **Horizontal/Lateral gene transfer (HGT/LGT)**: This is another mechanism involved in the acquisition of antibiotic resistance. It is a process in which genetic material contained in small packets of DNA can be transferred between bacteria of the same species or even between different species. Three mechanisms of HGT are available, which are equivalent to the three processes of genetic exchange in bacteria. These are conjugation, transformation, or transduction (Figure 21.2). Conjugation is the transfer of genetic material from one bacterium to another by means of a conjugation tube. This occurs when there is direct cell-cell contact between two bacteria (which need not be closely related) and involves the transfer of small pieces of DNA called *plasmids*. This is considered to be the main mechanism of HGT. Conjugation usually has a

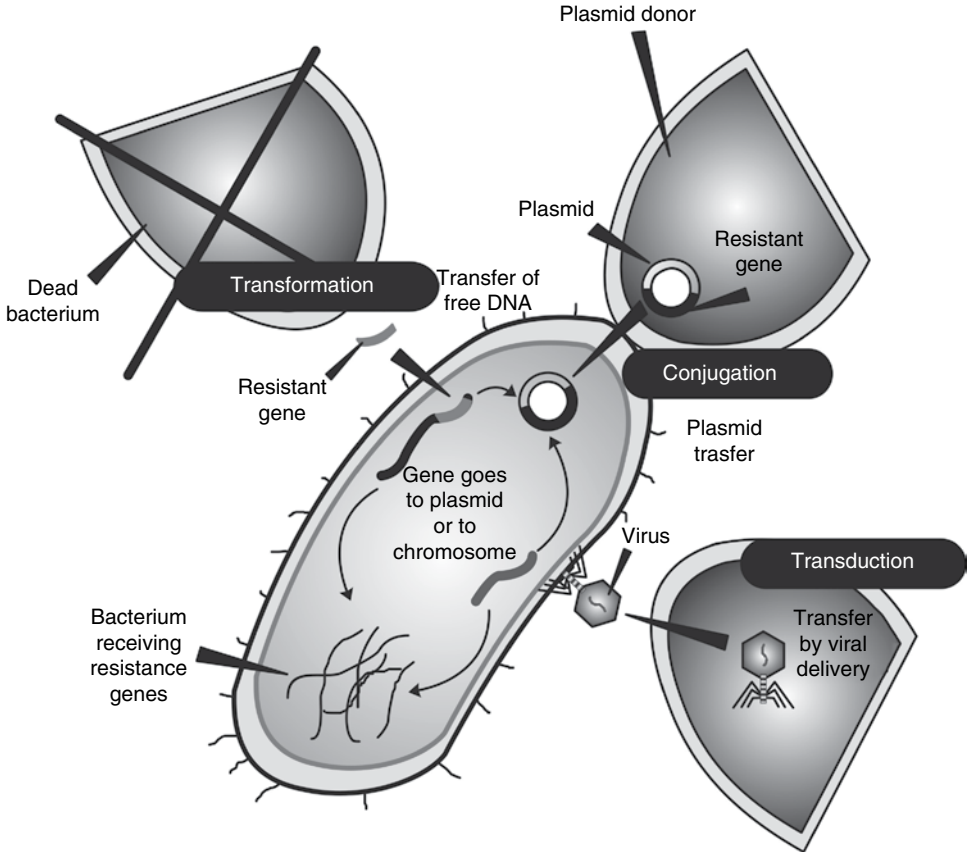

Figure 21.2. Methods of genetic recombination in bacteria.

broader range, which crosses even the gram barrier by the cell-to-cell contact via conjugative elements. Transformation involves the active uptake of naked DNA molecule from one bacterium to another. In this process, parts of DNA are taken up by the bacteria from the external environment, which normally presents in the external environment, probably because of death and lysis of another bacterium. The transformation capacity is found in naturally competent cells having the ability to take up naked DNA from the environment and remodel their DNA sequences by the formation of mosaic genes (Bachi, 2002). In the transduction process, the genetic material is transferred from one bacterium to another by means of a bacteriophage. It has a narrow host range and remains restricted to a particular species only.

Host Factors

Generally healthy people are at low risk of becoming colonized or infected with a DRO because casual contact (such as hugging and touching) with a person harboring a resistant strain is not considered as a risk factor for acquiring the agent. However, it is still

recommended that people wash hands when visiting a person who is infected or colonized to prevent the organism from spreading, especially in a hospital. There are many host factors that increase the risk of colonization and infection. If a person has:

1. A pre-existing severe illness.
2. An underlying disease or presence of some special condition such as chronic renal disease, insulin-dependent vascular disease, dermatitis, or skin lesions.
3. Previous exposure to a large number of antimicrobials.
4. An invasive procedures such as dialysis and catheterization.
5. Repeated and continuous contact with the health-care system.
6. Prolonged stay in a hospital.
7. Previous colonization by any DRO.
8. Receiving immune-suppressing medication or is elderly.

Health-Related and Economic Hazards

Excessive use of antibiotic drugs creates a selection pressure and results in the generation of a large pool of resistant genes (Cars and Norberg, 2004), which places an increased burden on society in terms of high morbidity, mortality, and cost (Roger et al., 2003). An important consequence of AMR in health-care facilities and community-associated infections is the need to change prescribing practices to newer and more costly medicines (World Health Organization [WHO], 2012). Patients infected with any DROs are more likely to have ineffective therapy, longer duration of hospital stay, and need treatment with broad-spectrum antibiotics, which are more toxic and also more expensive (Cars and Norberg, 2004). Thus, it increases the therapy cost for an individual patient because of the need for more costly second-line drugs, longer duration of hospital stay, increased need for intensive care and diagnostic testing, and higher incidences of complications and expenses incurred by use of isolation precaution (Cars and Norberg, 2004). Briefly, antibiotic resistance increases the health-care cost and the severity of disease, which although serious enough in the industrialized world, is often more severe in developing countries.

Because of the antibiotic resistance, infections more seriously affect a person's health, and sometimes a treatable infection turns fatal. In most of the developed countries, including the United States, with the greater access to advanced medical care and pharmaceuticals, a second, third, or fourth type of drug has to be used in a condition of antibiotic resistance because the principal drug against a particular bacterium no longer works. These advanced drugs sometimes have more side effects, are usually in shorter supply, and are much more expensive.

It is difficult to quantify the exact consequences of an antibiotic resistance. Sometimes the drugs fail, sometimes they do not work quite as well as they used to; thus, a patient gets sick and stays sick longer but then recovers. These effects are often observed outside a hospital, nursing home, or other facility where accurate surveillance records can be kept, but a pattern of chilling statistics comes from many sources. For an example, *S. aureus* is a common bacterial flora of human body and can cause minor infections to life-threatening diseases like pneumonia. Previously penicillin was used to treat the patients, but in the 1950s, within 10 years after penicillin hit the market, *S. aureus* had developed resistance to penicillin. As a result, drug companies developed methicillin in

the 1960s and then by the 1980s, *S. aureus* became resistant to methicillin, and physicians switched to vancomycin. Vancomycin is a broad-spectrum drug, but in 1997, the first cases of vancomycin-resistant *S. aureus* were reported in three geographically separate locations and were followed by many more cases. Then in 2000, the first revolutionary new type of antibiotic linezolid was developed, offering promise in the fight against *S. aureus* and other multidrug resistant bacteria, but within a year doctors observed first case of linezolid-resistant *S. aureus*.

Health officials are particularly worried about these drug-resistant pathogens in hospitals and nursing homes because these are the places where a combination of factors raises the risk. Patients who are hospitalized usually have weakened immune systems or are undergoing chemotherapeutic treatments that impair their immune response and are at risk of serious illness or death from infections. Similarly, a significant percentage of people in hospitals are elderly, with compromised immune systems because of the age. In addition, patients who have undergone surgery are more susceptible to any kind of bacterial infection simply because open or healing wounds are another potential route of nosocomial infection. Also, hospitals are the locations where a lot of people carrying infections bring various strains of bacteria within them.

Another place where exposure to drug-resistant bacteria is a serious concern is day-care centers, especially for infants, because there is a combination of children with still-developing immune systems. Here if, one or two people are sick at any given time, it increases the chances of spread of bacteria, accelerating the spread of resistance traits.

Two categories of health-related hazards have been found to be associated with antimicrobial agents.

Infections That Would Otherwise Not Have Occurred

Along with eliminating the disease-causing pathogens, antimicrobial agents also disturb the normal microbial flora of human and animal body, exposing individuals to an increased risk of certain infections. Individuals receiving antimicrobial therapy are, therefore, at increased risk of becoming infected with antibiotic-resistant pathogens as demonstrated in case-control studies of persons infected with drug-resistant *Salmonella* spp. In these cases, persons exposed to antimicrobial agents for unrelated reasons, such as treatment of an upper respiratory tract infection, are at increased risk of infection with resistant *Salmonella* strains. This can be expressed in the form of an "attributable fraction," which is defined as the proportion of *Salmonella* infections that would not have occurred in case if the *Salmonella* strains were not resistant. In a review of this attributable fraction of the more than 1 million *Salmonella* and more than 1 million *Campylobacter* infections each year in the United States, it was estimated that drug resistance in *Salmonella* and *Campylobacter* may result in about 30,000 additional *Salmonella* infections leading to about 300 hospitalizations and 10 deaths and an estimated additional 18,000 *Campylobacter jejuni* infections, which leads to about 100 hospitalizations (Barza and Travers. 2002).

Increased Frequency of Treatment Failures and Increased Severity of Infection

The frequency of treatment failures and increased severity of infection could be manifested by prolonged duration of illnesses, increased frequency of bloodstream infections, increased hospitalization of the patients, or increased mortality. The evidence obtained

from the human health consequences resulting from AMR provide data of the human health impact of the AMR among bacteria isolated from humans as a result of the use of antimicrobial agents in food animals. It should also be noted that the survival of resistant bacteria frequently appears not to be impaired by carrying these resistance genes. An additional important issue is the acquisition of resistance traits by various nonpathogenic, commensals in food animals and these bacteria may then be ingested by or transferred to people.

Laboratory Diagnosis

If antibiotics are used specifically and for the required duration, they are least likely to generate resistance and this requires advanced diagnostics. Clinicians should have accurate information on existing infection, its etiology, the antibiotic susceptibility of the pathogen, and on the clearance of the infection. This requires the introduction of the rapid test to detect pathogen from clinical samples. The accurate diagnosis of AROs varies widely with the type of microorganism and the severity of infection.

Diagnosis of Bacterial Agents

A number of antimicrobial susceptibility testing (AST) methods are available for the determination of bacterial resistance to antimicrobial agents. The selection of a particular method is based on many factors such as practicality, flexibility, automation, cost, reproducibility, accuracy, and availability of facilities. Standardization of AST methodologies, which are used in epidemiological surveillance of AMR becomes critical if obtained data are to be compared among national or international surveillance or monitoring programs of the World Organisation for Animal Health (OIE) member countries. These AST methods should provide reproducible results in day-to-day laboratory use and the data be comparable with those results obtained by an acknowledged gold standard reference method.

Requirements

There are certain requirements for the standardization of AST methods and comparability of AST results:

1. Using the standardized AST methods and the harmonization of AST test parameters, which also includes choice of antimicrobial agents and subsequent interpretive criteria.
2. AST methods, with their critical specifications and interpretive criteria, should be clearly defined, documented in detail, and used by all participating laboratories.
3. All AST methods should generate accurate and reproducible data, which could further be used.
4. All the data should be reported qualitatively as well as quantitatively.
5. National or regional designated laboratories should be established for the coordination of AST methodologies, their interpretations, and appropriate operational techniques used to ensure accuracy and reproducibility (quality controls).
6. Involved microbiological laboratories should implement and maintain a formal quality management program to ensure accuracy.

7. Finally it requires a specific bacterial reference/quality control strains for determining intra- and interlaboratory quality control, quality assurance, and proficiency testing.

Selection of Antimicrobials for Testing and Reporting

Because of the availability of a large number of antimicrobial agents, it is quite difficult to select appropriate antibiotic. Hence, selection decision should be made by the appropriate bodies and organizations. Antimicrobials belonging to the same class may have similar in vitro activities against selected bacterial pathogens. Thereby, a representative antimicrobial should be selected that predicts susceptibility to other members of the same class. Whereas some microorganisms can be intrinsically resistant to particular antimicrobial classes, it is unnecessary and misleading to test certain agents for activity in vitro. This type of intrinsic resistance has to be determined for these organisms via either the scientific literature or through testing procedures. Finally, the number of antimicrobials to be tested should be limited; this ensures the relevance and practicality of AST. It is recommended to review periodically those microorganisms that are currently predictably susceptible to certain antimicrobial agents to ensure that emergent, unexpected resistance is detected. Emerging resistance may also be suspected in cases of poor response to a standard antimicrobial treatment regime.

Antibacterial Susceptibility Testing Methods

AST methodology may be selected on the basis of the ease of performance, flexibility, adaptability to automated or semi-automated systems, cost, reproducibility, reliability, accuracy, the organisms and the antimicrobials of interest, and the availability of suitable validation data for the range of organisms to which susceptibility to be tested. The following two AST techniques have been shown consistently to provide reproducible and repeatable results if performed correctly: Disk diffusion technique and broth and agar dilution technique.

Disk Diffusion Method

It involves the diffusion of an antimicrobial agent of a specified concentration from disks, tablets, or strips into the solid culture medium, which has already been seeded with the pure culture of selected inoculum. This is based on the determination of an inhibition zone, which is proportional to the bacterial susceptibility to the particular antimicrobial agent present in the disk. The diffusion of the antimicrobial agent into the seeded culture media created a gradient of the antimicrobial and as the concentration of the antimicrobial becomes so diluted that it can no longer inhibit the growth of the test bacterium; the zone of inhibition is demarcated. The diameter of the created inhibition zone around the antimicrobial disk is related to minimum inhibitory concentration (MIC) for that particular bacterium and antimicrobial combination. This zone of inhibition correlates inversely with the MIC of the test bacterium; the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organism. However, this actually depends on the concentration of antibiotic in the disk and its diffusibility. Disk diffusion technique is straightforward to perform, reproducible, and does not require any expensive equipment. It has so many advantages:

- Low cost.
- Ease in modifying test antimicrobial disks whenever required.

- Could be used as a screening test against large numbers of bacterial isolates.
- Could identify a subset of isolates used for further testing by other methods, such as determination of MICs.

The manual measurement of zones of inhibition is time-consuming; hence some automated zone-reading devices are available that can easily be integrated with laboratory reporting and data-handling systems.

Broth and Agar Dilution Methods

Dilution methods determine the lowest concentration of the assayed antimicrobial agent that inhibits the growth of the bacterium being tested (MIC, usually expressed in mg/mL or mg/L). However, the MIC does not always represent an absolute value and the "true" MIC is a point between the lowest test concentration that inhibits the growth of the bacterium and the next lower test concentration. Antimicrobial susceptibility dilution methods are considered more reproducible and quantitative than disk diffusion methods. However, antibiotics are usually tested in doubling dilutions, producing inexact MIC data. It is, therefore, essential that such laboratories use quality control organisms to assure accuracy and standardization of their procedures.

Broth Dilution

In the broth dilution technique, bacterial suspension of a predetermined optimal or appropriate concentration is tested against varying concentrations of an antimicrobial agent (usually serial twofold dilutions) in a liquid medium of predetermined, documented formulation. These tests can be performed either in tubes containing a minimum volume of 2 mL (macrodilution) or in smaller volumes using microtitration plates (microdilution). Commercially available microtiter plates containing prediluted antibiotics are also used by the clinicians for this purpose. Because most of the broth microdilution antimicrobial test panels are prepared commercially, this method is less flexible than agar dilution or disk diffusion in adjusting to the changing needs of the surveillance or monitoring programs. Additionally these antimicrobial plates and other required equipment may be costly; this methodology may not be feasible for some laboratories.

Agar Dilution

In this method, varying concentrations of an antimicrobial agent are incorporated into an agar medium, usually using serial twofold dilutions, followed by the application of a defined bacterial inoculum on to the surface. The results obtained are often considered as the most reliable for the determination of a MIC for the test bacterium and the antimicrobial combination. This method is usually recommended as a standardized AST method for fastidious organisms, such as anaerobes and *Campylobacter* and *Helicobacter* species. The advantages are:

1. Capable of testing multiple bacteria, except bacteria that swarm, on the same set of agar plates at the same time.
2. It has the potential to improve the identification of MIC endpoints and to extend the antibiotic concentration range.
3. There is a possibility to semi-automate the method by using an inoculum-replicating apparatus. For this purpose, commercially produced inoculum replicators are available that can transfer between 32 and 36 different bacterial inocula to each agar plate.

Agar dilution methods also have certain disadvantages:

1. If not automated, they are quite laborious and require substantial economic and technical resources.
2. Once the plates have been prepared, they have to be used within a week.
3. The endpoints are usually difficult to read, and the purity of the inoculum cannot be easily verified.

Other Bacterial Antimicrobial Susceptibility Testing and Specific Antimicrobial Resistance Tests

AST for bacterial isolates can also be performed using commercially available gradient strips that diffuse a predetermined antibiotic concentration. However, gradient strips can be expensive, and they show MIC discrepancies when testing certain bacteria/antimicrobial combinations compared with agar dilution results.

Regardless of the AST method used, all the procedures should be documented in detail to ensure accurate and reproducible results, and appropriate reference organisms should be tested every time while performing AST to ensure accuracy and validity of the data. The choice of an appropriate AST method will ultimately depend on the growth characteristics of the bacterium. In some special cases, novel test methods and assays may be considered more appropriate for detecting particular resistance phenotypes. For example, chromogenic cephalosporin-based tests (e.g., nitrocefin) may provide reliable and rapid results for β-lactamase determination in some bacteria (NCCLS, 2003). Similarly inducible clindamycin resistance in *Staphylococcus* spp. can be detected using a disk diffusion method employing standard erythromycin and clindamycin disks in adjacent positions and measuring the resultant zones of inhibition designated as D-zone (Zelazny, 2005).

Diagnosis of Viruses

To diagnose resistant viruses, clinical specimens are collected either as a part of routine patient care through sentinel surveillance or during outbreak investigations. Success of viral diagnosis depends largely on the quality of the specimen and also the storage and transport conditions prior to laboratory processing. For the isolation of, influenza viruses in cell culture and for the direct detection of viral antigens or nucleic acids specimens should be collected within 3 days of the onset of clinical symptoms. An acute-phase serum specimen (isolated from 3–5 mL whole blood) should be collected after the onset of clinical symptoms within 7 days, whereas convalescent phase serum specimen can be collected 2–4 weeks later.

For the diagnosis of viruses, the useful procedure is isolation of virus from the specimen, which amplifies the amount of virus in the original specimen, thereby producing a sufficient quantity for further antigenic and genetic characterization and for drug-susceptibility testing. Cell culture has been considered the gold standard for the isolation of viruses; for example, influenza virus can preferentially be isolated using Madin-Darby canine kidney (MDCK) cells, which allows virus identification through immunofluorescence antibody. Viruses can also be isolated using an embryonated chicken egg, which is an economic method for the isolation.

Apart from isolation, several serological methods also proved to be useful for the diagnosis of new strains of viruses, including microneutralization tests (MNT), hemagglutination test

(HT), hemagglutination inhibition tests (HIT), neuraminidase test (NAT), blotting techniques, immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), radio immuno assay (RIA), and immunohistochemistry (IHC). Along with these serological tests, molecular diagnostic techniques such as reverse transcription-polymerase chain reaction (RT-PCR) is also useful for viral diagnosis.

Diagnosis of Fungi

Accurate and rapid identification of the agent is essential for the containment or resolution of the disease. The two basic approaches to identification include the direct demonstration of the causative agent and the demonstration of agent specific antibodies. As with the bacteria, several broth dilution and disc diffusion techniques are used to confirm and diagnose resistance in fungi. Because different fungi show distinct morphology, the direct demonstration of an agent can be effective for its identification. Moreover, because a number of fungal agents have a unique morphology, the direct examination of a clinical specimen through microscopy can often provide a definitive diagnosis. The best known example is the observation of broad-based budding yeast cells in the sputum of the patient with pneumonia, who had lived in an area of endemic for blastomycosis, and is considered diagnostic for *Blastomyces dermatitidis*. Similarly, the presence of encapsulated yeast cells in an Indian ink preparation of the cerebrospinal fluid (CSF) of a patient with meningitis indicates the presence of *Cryptococcus neoformans*.

Most of the fungal diseases show nonspecific signs and symptoms; difficulty in colonization to distinguish it from invasive disease; negative blood cultures; and patients are often unable to undergo invasive diagnostic procedures. These circumstances led to the strategy of initiating empirical therapy, especially in patients who are high risk. A number of tests have been applied to several body fluids, but at the simplest level, the clinician must be familiar with the appearance and colony characters of various fungi. Apart from culturing methods, other methods include antibody- and antigen-based assays, metabolite detection, and molecular identification, including polymerase chain reaction (PCR) identification of fungal DNA from body fluid samples using conserved or specific genomic sequences. PCR technique can be made specific by genus-specific probes, with 100 percent sensitivity and reasonable specificity. In infection with the fungi *Candida* (candidiasis), the number of sites of colonization correlates with invasion whereas in infection with *Cryptococcus* spp. (cryptococcosis), pronase treatment of serum has reduced false-positive and false-negative results and improved reproducibility of titers. In coccidioidomycosis, serology is the exemplar for all mycology and gene probes has accelerated diagnosis by culture. In histoplasmosis, the antigenuria tests provide high sensitivity and specificity, which has dispelled the chronic confusion in interpreting antibody test results.

Along with the serological and molecular techniques several fungal stains including periodic acid Schiff and Gomori's methenamine silver are also proven to be useful in demonstrating fungi in clinical specimens. Some other tests also have been developed to determine the presence of fungal antigens in fluid specimens. For example, the latex agglutination test, which is used to detect the polysaccharide antigen of *C. neoformans* in spinal fluid. Similarly, RIA has been developed for detection of *Histoplasma* antigens in urine and serum specimens. Immunofluorescent staining can also be used for the detection of fungal antigens.

Fungal Susceptibility Testing

To treat an invasive type of fungal infection, the drug is largely selected on the basis of species to be identified. Fungi less commonly show acquired resistance to various antifungal agents as compared to bacteria, although emergence of resistance has been described in conditions where a large population of fungal pathogens is exposed to antifungal drugs for longer period of time (Anderson, 2005; Verweij et al., 2009). The development of drug resistance depends on several factors, including the target of the antifungal agent, its mode of action (i.e., fungistatic/fungicidal), and the type of fungal agent (Anderson, 2005). Resistance to azole compounds is reported in patients with oral pharyngeal candidiasis and in patients with aspergilloma (*Aspergillus* infection) that had been repeatedly treated with azole compounds for a long period of time (Johnson et al., 1995; Howard et al., 2006). Whereas, azole resistance appears to be less frequent in candidiasis and in invasive aspergillosis (Dannaoui et al., 2004; Pfaller and Diekema, 2007), although the azole resistance in invasive aspergillosis was reported in some Dutch patients (Verweij et al., 2007; Snelders et al., 2008), and in these cases, resistant isolates appeared to be as virulent as azole-susceptible isolates (Snelders et al., 2008). Interpretative breakpoints have been established for the drug fluconazole, especially on the basis of distribution of MICs, pharmacodynamic and pharmacokinetic properties of the drug, and its clinical efficacy (Rodriguez-Tudela et al., 2007). The dose-to-MIC ratio of fluconazole was predicted efficacious both in patients with oral pharyngeal candidiasis and in patients with candidemia who were non-neutropenic. A dose-response relationship has also been found in other studies, in patients with candidemia who were non-neutropenic, and these data will help to optimize dosing of antifungal agents (Clancy et al., 2005; Pai et al., 2007). These experiences are establishing such correlations for mold-active antifungal agents and opportunistic molds.

Diagnosis of Resistant Parasites

In clinical practice, susceptibility of the parasite to antiparasitic drug is rarely tested. However, the emergence of resistance to various parasitic drugs and the potential for development of resistance to the already scarce alternative drugs necessitate antiparasitic drug sensitivity studies to be introduced in clinics. This can also be helpful in studying the mechanism of resistance and in the screening of newer, more effective antiparasitic drugs. For an example, promastigotes, the flagellar forms of *Leishmania* parasite, have been used to study drug sensitivity and screen for newer antileishmanial compounds. For this purpose, promastigotes in their logarithmic growth phase are added to the serial dilution of drug in 96-welled microtiter plates and then incubated at 71.6 to 77° F (22–25° C) for 2 to 7 days until the late log phage. The growth can be monitored by cell counting and ED50 can be extrapolated by plotting the parasitic counts against drug concentration. Although this assay offers the advantage of ease of culture, speedy detection, and ease of quantification, the results obtained do not necessarily correlate with its in vivo leishmanial activity. This can be resolved by studying sensitivity of the intracellular amastigote form in vitro by maintaining these forms in any of the following cell lines:

1. Mouse peritoneal monocyte macrophage.
2. Human peripheral blood monocyte-macrophage cell line.
3. Dog sarcoma and hamster peritoneal cell lines.

Although these are standard methods for in vitro assessment of drug-resistance mechanisms and the results correlate with the clinical response, these methods are technically demanding and expensive. The infected macrophage can be directly determined by observing under an inverted microscope or after harvesting the cell with methanol and staining with Giemsa stain before performing microscopic examination. The infection can also be quantified by flow cytometry techniques using lipophosphoglycan (LPG) monoclonal antibody probes, which rapidly analyzes a large number of cells.

PCR-based detection methods have also been successfully used to demonstrate the presence of parasite DNA in clinical samples, normally by the amplification of genes encoding the surface antigens. By targeting and subsequently determining the DNA sequence of genes considered being associated with the development of drug resistance, such methods can rapidly provide information about the drug resistant status of the parasite. The existing PCR procedure can be coupled to a direct functional analysis of the amplified sequence, which would enable the rapid identification of any resistance allele, whether previously known or novel technology of this type could be applied to the analysis of primary clinical samples and would represent a valuable advance in the ability to both quickly diagnose potentially life-threatening infections and further to investigate the origin and prevalence of such parasites.

Managing Antimicrobial Resistance

Diagnosing infections always remains a problem in the management of AROs, particularly in cases of patients who are immunocompromised. The CDC published a *Guideline for Isolation Precautions in Hospitals* (Garner, 1996), which was designed especially for acute care facilities and acknowledged that facilities will also modify the recommendations according to their needs and circumstances and as directed by federal, state, or local regulations.

Precaution Ally Use of Hand Antisepsis

Many of the diseases associated with antimicrobial-resistant pathogens can be efficiently managed by applying standard precautions along with the use of suitable antiseptic solutions. As the colonization with resistant organisms is often unrecognized, and other infectious organisms may also spread in the community, standard precautions alone would not prove to be sufficient for managing AMR. These standard precautions would probably need to be followed by the use of hand antisepsis and wearing appropriate gloves.

AROs are principally transmitted via the contaminated hands of people. Thus, the single most effective means of reducing the potential for the transmission of resistant organism are hand antisepsis both before and after contact with residents with the pathogen, which destroys or removes transient microorganisms from the hands. This can be accomplished by cleaning hands with antimicrobial soap or by using a waterless alcohol-based hand antiseptic solution (Garner and Favero, 1985; Larson, 1995). The use of a waterless alcohol-based hand antiseptic should be strongly recommended for all the residents because such products are as effective as antimicrobial soaps, may increase hand washing compliance, and are not harmful to hands (Boyce et al., 2000). If hands are not visibly soiled, a waterless alcohol-based product is to be applied in a sufficient amount to wet and cover hands and nails thoroughly; hands should then be rubbed together and

allowed to dry. However, if hands are visibly soiled with organic material or debris (feces, dirt), hands should first be cleaned with antimicrobial soap and running water prior to using a waterless product.

Isolated Placement

Patients with resistant microbes should not be placed in an isolated chamber. They can simply be placed with appropriate roommate/roommates, which are either a patient with the same pathogen or resident, who:

1. Has intact skin without significant open wounds or breaks in skin.
2. Has no invasive devices, indwelling vascular or urinary catheters, or drainage devices (as tracheostomy or tracheal tubes, chest tubes, gastrostomy tubes, Broviac Hickman catheters, intravenous lines, etc.).
3. Is not significantly immunocompromised (probably as a result of transplantation surgery, neutropenia, acute or chronic infection, undergoing steroids or chemotherapy).
4. Is not colonized or infected with another microorganism whether susceptible or resistant.

Group Activities

The following factors should be considered while considering group activities.

- An important component in group activities is hand antisepsis. Residents with resistant organisms should clean their hands with suitable antimicrobial soap or a waterless alcohol-based hand antiseptic solution prior to leaving their room and whenever they again become contaminated when out of their room.
- The dry wound dressings of patients with resistant organisms that contain any drainage should be cleaned. They are instructed to wear clean clothes or a clean cover gown when leaving their rooms.
- In some rare cases, patients are restricted to move in the community; for example, the patients that may be shedding large numbers of pathogens and can transmit them to other healthy or susceptible persons.

Environmental Cleaning

While managing antimicrobial resistance, a patient's room and surroundings should be properly cleaned and appropriate hygienic conditions maintained. Before undergoing any recreational or physical therapy instrument, hands of the patient should be disinfected thoroughly. In situations in which a patient with a resistant microbe shares a bathroom with a roommate not having the same organism, the bathroom should be cleaned and disinfected using standard facility procedure. Commodes may be useful for certain patients, but it should not be shared with roommates who do not have an infection. If tubs and showers are to be shared, they should be cleaned and disinfected after every use by the patient. Laundry items, especially bed linens and towels from residents with uncontained stool, urine, or other secretions should be handled properly to minimize any contamination. Hand gloves and long-sleeved gowns should be worn while changing the beds of residents with uncontained stool, urine, or other secretions/excretions, and all

soiled laundry should be placed directly in a moisture-resistant container and should not be left on the floor or other room surfaces.

Contact Precautions

Although standard precautions and hand antisepsis are sufficient for managing most persons with resistant organisms, contact precautions may be indicated for residents with resistant microbes who are potentially more likely to shed pathogens into their environment. These include the use of gloves, gowns, patient care equipments masks, eye protection, and face shield.

Education

Two sessions of education should be performed, one for staff and one for educating resident, family, and visitors.

Staff Education

AMR can be managed by continuous education programs for staff that are in direct contact with residents or items in their environment. Staff workers responsible for making decisions regarding the care of residents should receive complete information about resistant organisms.

Resident, Family, and Visitor Education

Residents, their families, and visitors should be educated about the AMR and the precautions to be taken, including hand antisepsis and methods to limit environmental contamination with stool, urine, and respiratory secretions.

Surveillance

Surveillance is an important component of all the infection control programs and should include the regular review of all microbiology culture and susceptibility data to detect MRSA, VRE, and other epidemiologically important resistant microorganisms. Although residents known to have MRSA and VRE usually only represent a portion of residents with resistant microbes, screening residents is not indicated as a routine infection control measure.

- A complete list of residents with resistant microbes should be maintained.
- Surveillance of cultures obtained for clinical reasons could establish baseline infection rate for a facility. High rates of endemic infection (more than one case per 1,000 resident-care days) or an outbreak (more than three infections in 1 week or twice the number of infections per month as observed for 3 consecutive months) may be identified by surveillance (Pugliese and Weinstein, 1998).
- Surveillance data could be used in educational programs to reinforce infection control practices and to prioritize infection control activities.
- Culture and susceptibility data can also be used to choose "empiric therapy," which is an antimicrobial treatment prescribed on the basis of a presumed diagnosis before obtaining culture and susceptibility results. Culture and susceptibility data will provide

essential information on antimicrobial susceptibility and resistance patterns in a particular facility, which can guide physicians in the selection of the most appropriate empiric therapy for residents with infections.

Antimicrobial Use

The selective and appropriate use of antimicrobial agents is the most important method of controlling AMR because resistance usually develops through the use and overuse of antimicrobials (called as antimicrobial pressure). When bacteria are exposed to any of the antimicrobial agents, susceptible species are killed, and those that are resistant survive, become predominant, and may then further be transmitted to other persons. Overuse of antimicrobials is a basic problem in all health-care settings, although empiric antimicrobial therapy may be indicated in some situations. It is important that physicians should be aware of culture and susceptibility results so that applicable changes can be made in the treatment. Broad-spectrum antimicrobials often used for empiric therapy should be replaced by narrow-spectrum antimicrobials in cases when culture and susceptibility results are known. The use of narrow-spectrum antimicrobials that target specific microorganisms will lessen the so called antimicrobial pressure.

Prevention and Control

To prevent developing AMR, health-care providers should prescribe the appropriate medication for a patient's illness and avoid overusing or misusing medicines. People should strictly follow the medication prescribed to them and always avoid the medicine prescribed for another person. A patient should communicate with the physician providing the correct understanding of disease symptoms, which helps the physician to determine appropriate antimicrobial drug. People should not store antibiotics for the next time, and if the doctor has prescribed more than the required dose, after completing the prescribed course of treatment the leftover medications should be discarded and not shared with another person. Healthy lifestyle habits, such as proper diet, exercise, good sleeping patterns, and proper hygiene are useful in preventing illness. Some recommendations that combat the development of antibiotic resistance in bacteria and other microorganisms include:

1. Biotechnology and pharmaceutical companies must constantly research, develop, and test new antimicrobial agents to maintain a pool of effective drugs in the market.
2. Antibiotics should never be used as growth-promoting substances in farm animals. For many years, antibiotics were given to farm animals as feed additives to promote animal growth and to prevent infections rather than curing them. Their use contributes to the emergence of antibiotic-resistant bacteria that threaten human health and decreases the effectiveness of the same antibiotics used to combat human infections.
3. Always use the right antibiotic in an infectious situation, determined by antibiotic sensitivity testing.
4. Unnecessary antibiotic prescriptions should be avoided always because these are the causes for an enhanced rate of resistance development. This condition usually happens while treating viral infections. Because antibiotics do not affect viruses, the opportunity for indigenous bacteria (normal flora) to acquire resistance is developed, which can further be transferred to pathogens.

5. Antibiotic prescriptions should be completed because unfinished antibiotic prescriptions may leave some bacteria alive or may expose them to subinhibitory concentrations of antibiotics for a prolonged period of time. This has been seen with *Mycobacterium tuberculosis* bacteria, which infect the lungs causing tuberculosis. Because of the slow growing nature of the infection, treatment programs last for months or even years, which led to many cases on unfinished prescriptions, and 5 percent of strains now observed are completely resistant to all known treatments.
6. Existing strategies to combat antibiotic resistance in the pharmaceutical industry are not that effective; thereby pharmaceutical companies should develop new, less costly strategies for the antibiotics.

Future Perspectives of Antimicrobial Therapy

The selection of multidrug-resistant bacteria has occurred throughout history; however, drug-resistant bacteria have been encountered antibiotics that are nothing more than recapitulations of previous drugs. Hence there is an urgent need for new avenues of therapeutic treatment and a new era of prophylactic (preventative) treatment, which can be done by applying following approaches:

- **Bacterial interference**: Bacterial interference is also referred to as bacterio-therapy. This therapy employs the deliberate inoculation of nonpathogenic/commensal bacteria in the host body to prevent infection by pathogenic strains. When a pathogenic bacteria infect, these nonpathogenic bacteria compete with the infectious bacteria for nutrients and adhesion receptors or spur attacks through secretion of antimicrobial compounds. Thus, infectious bacteria are no longer able to propagate and establish an infection within the host's body. This treatment has had promising results in gut, urogenital tract, and wound sites infections. The major advantage of this therapy is that infection can be avoided without stimulating the host's immune system and decreases the selection for antibiotic resistance as observed with probiotic usage.
- **Bacteriophage therapy**: Bacteriophages are the viruses that infect bacteria; they use the bacterial replicating machinery and direct the host bacterial cell to make viral proteins of their own. Therapeutically, they were previously used as a prophylaxis against cholera, typhoid fever, and dysentery. This practice was abruptly stopped with the introduction of synthetic antibiotics after World War II. Now with the emergence of multidrug-resistant bacteria, bacteriophage therapy again appears useful. This is quite attractive because phage particles are narrow-spectrum agents possessing an inherent mechanism to not only infect bacteria but also their specific strains. Other pathogens may also be targeted through the manipulation of the phage DNA.
- **Bacterial vaccines**: Bacterial vaccine is also an interesting idea especially with the advent of complete genomic sequencing and understanding of virulence regulatory mechanisms. Genomics allows scientists to scan an entire bacterial genome for specific sequences, which can be used to stimulate a protective immune response against some specific bacterial strains. This approach expedites the drug discovery process and also provides a more rational, target-based approach. The best targets are essential bacterial genes, that are common to many species of bacteria, that can code for proteins with the ability to gain accesses through lipid membranes, and that possess no homology to

human genes. Regulatory genes that control virulence protein production are excellent vaccine candidates that prime the human immune system or inhibit virulence production. This technology will inevitably yield superior vaccine candidates.

- **Cationic peptides**: Cationic peptides are natural compounds possessing both hydrophobic and hydrophilic properties. They are found throughout nature in the immune systems of bacteria, plants, invertebrates, and vertebrates and are not the usual synthetic drugs; however, they do exhibit antibacterial effects. Cationic peptides stimulate several mechanisms of action involving interaction with the bacterial cell membrane leading to cell death. From a therapeutic point of view, these proteins proved to be important because they have coevolved with commensal bacteria yet have maintained the ability to target pathogenic bacteria.
- **Cyclic D, L-a-peptides**: These are synthetic cell membrane disruptors, which are amphipathic in nature and are composed of alternating D and L amino acids forming cyclic structures. They are engineered to target gram-positive, as well as gram-negative bacterial membranes. These peptides can self-assemble into flat ring shaped conformations forming nanotubes, which can specifically target and puncture the bacterial cell membranes causing rapid death of affected bacterial cell.

References

Anderson JB. 2005. Evolution of antifungal-drug resistance: Mechanisms and pathogen fitness. *Nat Rev Microbiol* 3:547–556.

Appelbaum PC. 2007. Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J Antimicrob Agents* 30:398–408.

Bachi BR. 2002. Resistance mechanism in gram positive bacteria. *Int J Med Microb* 292:27–35.

Barza M, Travers K. 2002. Excess infections due to antimicrobial resistance: The attributable fraction. *Clin Infect Dis* 34:126–130.

Bennett J, Geme III JW. 1999. Bacterial resistance and antibiotic use in the emergency department. *Pediatric Clinic of North America.* 46:1125–1143.

Boyce JM, Kelliher S, Vallande N. 2000. Skin irritation and dryness associated with two hand-hygiene regimens: Soap and water hand washing versus hand antisepsis with an alcoholic gel. *Infect Control Hosp Epidemiol* 21:442–448.

Cars O, Norberg P. 2004. Antibiotic resistance. The faceless threat, the global threat of antibiotic resistance: Exploring roads towards concerted action [Background document]. A multidisciplinary meeting at the Dag Hammarskjold foundation, Uppsala, Sweden, May 5–7, 2004.

Clancy CJ, Yu VL, Morris AJ Snydman DR, Nguyen MH. 2005. Fluconazole MIC and the fluconazole dose/MIC ratio correlate with therapeutic response among patients with candidemia. *Antimicrob Agents Chemother* 49:3171–3177.

D'Costa VM, King CE, Kalan L, Morar M, Sung WW, et al. 2011. Antibiotic resistance is ancient. *Nature* 477:457–461.

Dannaoui E, Meletiadis J, Tortorano AM, Symoens F, Nolard N, et al. 2004. Susceptibility testing of sequential isolates of *Aspergillus fumigatus* recovered from treated patients. *J Med Microbiol* 53:29–34.

Davies J. 1996. Bacteria on the rampage. *Nature* 383:219–220.

Deguchi T, Yasuda M, Nakano M, Ozeki S, Kanematsu E, et al. 1996. Uncommon occurrence of mutations in the *gyrB* gene associated with quinolone resistance in clinical isolates of *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 40:2437–2438.

Donadio S, Maffioli S, Monciardini P, Sosio M, Jabes D. 2010. Antibiotic discovery in the twenty-first century: current trends and future perspectives. *Journal of Antibiotics* 63:423–430.

Eickoff TC. 1992. Antibiotics and nosocomial infections. In: Bennett JR, Branchmann PS, eds., *Hospital Infections*, 3rd ed., pp. 229–333. Boston: Little Brown and Company.
Galimand M, Gerbaud G, Courvalin P. 2000. Spectinomycin Resistance in *Neisseria* spp. Due to Mutations in 16S rRNA. *Antimicrob Agents Chemother* 44:1365–1366.
Garner JS, Favero MS. 1985. *Guideline for Handwashing and Hospital Environmental Control*. Atlanta: US Department of Health and Human Services, Public Health Service, Centers for Disease Control.
Garner JS. Hospital Infection Control Practices Advisory Committee (HICPAC). 1996. Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol* 17:53–80.
Gaynes R, Edwards J. 1995. Nosocomial vancomycin resistant enterococci in the United States, 1989–1995: The first 1000 isolates. *Infect Control and Hosp Epidemiol* 17:18.
Gould IM. 2010. VRSA-doomsday superbug or damp squib? *Lancet Infect Dis* 10:816–818.
Gubareva LV, Kaiser L, Matrosovich MN, Soo-Hoo Y, Hayden FG. 2001. Selection of influenza virus mutants in experimentally infected volunteers treated with oseltamivir. *J Infect Dis* 183:523–531.
Gubareva LV, Matrosovich MN, Brenner MK, Bethell RC, Webster RG. 1998. Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. *J Infect Dis* 178:1257–1262.
Hanberger H, Garcia-Rodriguez JA, Gobernado M, Goossens H, Nilsson LE, et al. 1999. Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 European countries. *JAMA* 281:67–71.
Harper SA, Fukuda K, Uyeki TM, Cox NJ, Bridges CB, et al. 2005. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 54(RR 8):1–40.
Hooper DC. 2001. Emerging mechanisms of fluoroquinolone resistance. *Emerg Infect Dis* 7:337–341.
Howard SJ, Webster I, Moore CB, Gardiner RE, Park S, et al. 2006. Multi-azole resistance in *Aspergillus fumigatus*. *Int J Antimicrob Agents* 28:450–453.
Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. 2010. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin Microbiol Rev* 23:99–139.
Jackson HC, Roberts N, Wang ZM, Belshe RB. 2000. Management of influenza: Use of new antivirals and resistance in perspective. *Clin Drug Invest* 20:447–454.
Johnson EM, Warnock DW, Luker J, Porter SR, Scully C, et al. 1995. Emergence of azole drug resistance in Candida species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. *J Antimicrob Chemother* 35:103–114.
Kiso M, Mitamura K, Sakai-Tagawa Y, Shiraishi K, Kawakami C, et al. 2004. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet* 364:759–765.
Kontoyiannis DP, Lewis RE. 2002. Antifungal drug resistance of pathogenic fungi. *Lancet* 359:1135–1144.
Land KM, Johnson PJ. 1999. Molecular basis of metronidazole resistance in pathogenic bacteria and protozoa. *Drug Resist* 2:289–294.
Larson EL. 1995. APIC Guideline for handwashing and hand antisepsis in health care settings. *Am J Infect Control* 23:251–269.
Ling V. 1997. Multidrug resistance: molecular mechanisms and clinical relevance. *Cancer Chemother Pharmacol* 40:S3–S8.
Moscona A. 2005. Neuraminidase inhibitors for influenza. *N Engl J Med* 353:1363–1373.
NCCLS. 2003. *Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement*. NCCLS document M100-S12. Wayne, PA: NCCLS.
Neu HC. 1992. The crisis in antibiotic resistance. *Science* 257:1064–1073.

Pai MP, Turpin RS, Garey KW. 2007. Association of fluconazole area under the concentration-time curve/MIC and dose/MIC ratios with mortality in nonneutropenic patients with candidemia. *Antimicrob Agents Chemother* 51:35–39.

Pfaller MA, Diekema DJ, Jones RN, Sader HS, Fluit AC, et al. 2001. International surveillance of bloodstream infections due to Candida species: Frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. *J Clin Microbiol* 39:3254–3259.

Pfaller MA, Diekema DJ. 2007. Epidemiology of invasive candidiasis: A persistent public health problem. *Clin Microbiol Rev* 20:133–163.

Pugliese G, Weinstein A, eds. 1998. Issues and controversies in prevention and control of VRE. *Etna Communications* 50:15–16.

Richardson MD. 2005. Changing patterns and trends in systemic fungal infections. *J Antimicrob Chemother* 56(Suppl. 1): i5–i11.

Roberts NA. 2001.Treatment of influenza with neuraminidase inhibitors: Virological implications. *Philos Trans R Soc Lond B Biol Sci* 356:1895–1897.

Rodriguez-Tudela JL, Almirante B, Rodriguez-Pardo D, Laguna F, Donnelly JP, et al. 2007. Correlation of the MIC and dose/MIC ratio of fluconazole to the therapeutic response of patients with mucosal candidiasis and candidemia. *Antimicrob Agents Chemother* 51:3599–3604.

Roger FG, Greenwood D, Norbby SR, Whitley RJ. 2003. *Antibiotic and Chemotherapy: The Problem of Resistance*, 8th ed., pp. 25–47. Edinburgh: Churchill Livingstone.

Sengupta S, Chattopadhyay MK. 2012. Antibiotic resistance of bacteria: A global challenge. *Resonance* 17:177–191.

Sieradzki K, Roberts RB, Haber SW, Tomasz A. 1999. The development of vancomycin resistance in a patient with methicillin- resistant *Staphylococcus aureus* infection. *N Engl J Med* 340:517–523.

Slavin M, Fastenau J, Sukarom I, Mavros P, Crowley S, Gerth WC. 2004. Burden of hospitalization of patients with candida and Aspergillus infections in Australia. *Int J Infect Dis* 8:111–120.

Snelders E, van der Lee HAL, Kuijpers J, Rijs AJMM, Varga J, et al. 2008. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 5:e219.

Sparling PF, Sarubbi FA Jr, Blackman E. 1975. Inheritance of low-level resistance to penicillin, tetracycline, and chloramphenicol in *Neisseria gonorrhoeae*. *J Bacteriol* 124:740–749.

Turner AK, Nair S, Wain J. 2006. The acquisition of full fluoroquinolone resistance in Salmonella *typhi* by accumulation of point mutations in the topoisomerase targets. *J Antimicrob Chemother* 58:733–740.

Verweij PE, Mellado E, Melchers WJ. 2007. Multiple-triazole-resistant aspergillosis. *N Engl J Med* 356:1481–1483.

Verweij PE, Snelders E, Kema GHJ, Mellado E, Melchers WJG. 2009. Azole-resistance in Aspergillus fumigatus: A side-effect of environmental fungicide use? *Lancet Infect Dis* 9:789–795.

Vijaylakshmi K, Gopalan KN, Gopalkrishnan B, Mohanram CC. 1982. The first case of β-lactamase strain of *Neisseria gonorrhoeae* from Madras. *Ind J Sex Trans Dis* 3:13.

Witte W. 2000. Selective pressure by antibiotic use in livestock. *Int J Antimicrob Agents* 16(Suppl 1):S19–S24.

World Health Day. 2011. Policy briefs. Accessed January 9, 2012, at http://www.who.int/world-health-day/2011/policybriefs/en/index.html.

World Health Organization (WHO). 2012. The evolving threat of antimicrobial resistance. Accessed February 24, 2013, at http://hesp-news.org/2012/08/28/the-evolving-threat-of-antimicrobial-resistance-options-for-action/.

Zelazny AM, Ferraro MJ, Glennen A, Hindler JF, Mann LM, et al. 2005. Selection of strains for quality assessment of the disk induction method for detection of inducible clindamycin resistance in staphylococci: A CLSI collaborative study. *J Clin Microbiol* 43:2613–2615.

Chapter 22

Conventional Methods for Mosquito Control

Mosquito: Habits and Attractants

Mosquitoes are important insect pests that represent a significant nuisance or threat to public health. Mosquitoes belong to the family *Culicidae* (Latin word *culex*, means midge or gnat), which contains small, midge-like flies. Except for a few species that harmless or even useful to humanity, most are harmful because they transmit many serious diseases. The word *mosquito* is derived from the Spanish and Portuguese word for a little fly (Brown, 1993). Physically they resemble crane flies (family *Tipulidae*) and chironomid flies (family *Chironomidae*) but differ functionally because the females of many mosquito species are blood-sucking pests and vectors of dangerous diseases, whereas crane flies and chironomid flies usually do not transmit any disease. Many species of mosquitos do not suck blood and, therefore, do not transmit diseases. Also in the bloodsucking species, only the female mosquitoes suck blood. Furthermore, even among mosquitoes that are carrier of important diseases, neither all species of mosquito, nor or all strains of a given species transmit the same kinds of diseases. Further, they do not all transmit the diseases under the same circumstances because each has different habits. For example, some of these species attack people in houses, and others prefer to attack in the forest area. Therefore, while managing mosquito-borne illnesses, it is difficult to decide which species, even which strains of mosquito, is responsible for transmitting a particular type of disease. More than 3,500 species of mosquitoes have been described from various parts of the world and among them, many mosquitoes that routinely bite humans act as vectors for various infectious diseases that affect millions of people per year (Molavi, 2003). Others are not routine human biters, but when their habitat is disturbed, may become disastrous agents for zoonosis of new diseases, for example, deforestation (Wilcox and Ellis, 2006). Quite similar to flies, mosquitoes also go through four stages in their life cycle: egg, larva, pupa, and adult or imago. In most of the species, females lay their eggs in standing or stagnant water bodies, some near the water's edge, and others attach their

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

eggs to the aquatic plants. Each mosquito species has its own ecological adaptations; for example, some are generalists, some breed in lakes, some in temporary puddles and some breed in marshes. Some acclimatize to the saltiness of salt water (Wigglesworth, 1932).

Mosquito: Habits

Habits of a mosquito vary depending on the genus and species to which it belongs. Some species are active during the day time, whereas some are at night. Most species of *Culex* and *Anopheles* are active at night and rest by day. The *Culex tritaeniorhychnus*, the classical vector of Japanese encephalitis, mainly attacks pigs, cattle, and humans at night and especially 1 hour after dark, whereas *Aedes albopictus*, the primary vector of dengue fever, usually attacks humans and animals during the daytime. In contrast, *Anopheles jeyporiensis*, a local malaria vector, most actively feeds between 11:00 P.M. and 2:00 A.M. This diversity in feeding habit has an important advantage to the survival of mosquito species because it reduces competition for a food source between different mosquitoes. *Culex* rests with its body parallel to the supporting surface, whereas *Anopheles* rests with the head down and the body at an angle to the support. Female mosquitoes usually make a high-pitched buzzing sound by vibrating the tiny appendages covering their spiracles (breathing holes) in the thorax. Most of the mosquitoes lay their eggs in water, but in arctic regions, these eggs are laid in snow, which are further hatched with the melting of the snow. Some species lay their eggs singly and others lay eggs in groups of 100 to 300 found floating on the surface of water (e.g., *Culex*). Within a few days, these eggs hatch as larvae commonly referred to as *wrigglers* or *wigglers* because of the way they move in seeking food and in rising to the surface to breathe. Immediately after a few more days, these larvae develop into pupae (also called as *tumbler* because of how it moves), with an extremely large head and thorax. Pupae do not eat, acquire complete maturation in 2 or 3 days, and rise to the surface, split its skin from end to end, shed it, and then fly away. It requires around 10 days for an egg to develop into a mature adult mosquito. The male mosquito is harmless, and its proboscis is just for sucking to feed on plant juices and pollen (i.e., it helps in the pollination of several flower species). Whereas female proboscis is adapted for piercing as well as sucking and can easily penetrate the skin of reptiles, birds, and mammals. The proboscis contains saw-tipped cutters and two tubes; one tube is adapted to inject saliva after the piercing of the skin by the cutters, and the other tube is principally used to suck blood. The saliva dilutes the blood and makes it easier for sucking. Females actually require some protein present in the blood of these animals to nourish their eggs before they are laid. The female mosquito requires at least one blood meal before laying a batch of eggs. Mosquitoes detect their blood source mainly by the carbon dioxide concentration, heat, and body odor given out by the host animal. The higher the concentration of carbon dioxide, the greater the amount of heat or the stronger the odor released by an animal and more attractive the animal will be to a host-finding mosquito. Although it depends on the species of mosquito, bloodsucking females may prefer to feed the blood of amphibians, reptiles, birds, mammals, particularly humans, and become the vectors for many potential diseases including dengue, Japanese encephalitis, yellow fever, and malaria. Despite the fact that most of the female mosquitoes require blood for reproduction, exceptions do exist. For example, both the adults (male and female) of the genus *Toxorhynchites* feed solely on nectar; similarly, mosquitoes that belong to the genus *Malaya* feed on regurgitated food from the mouth of ants. Another important

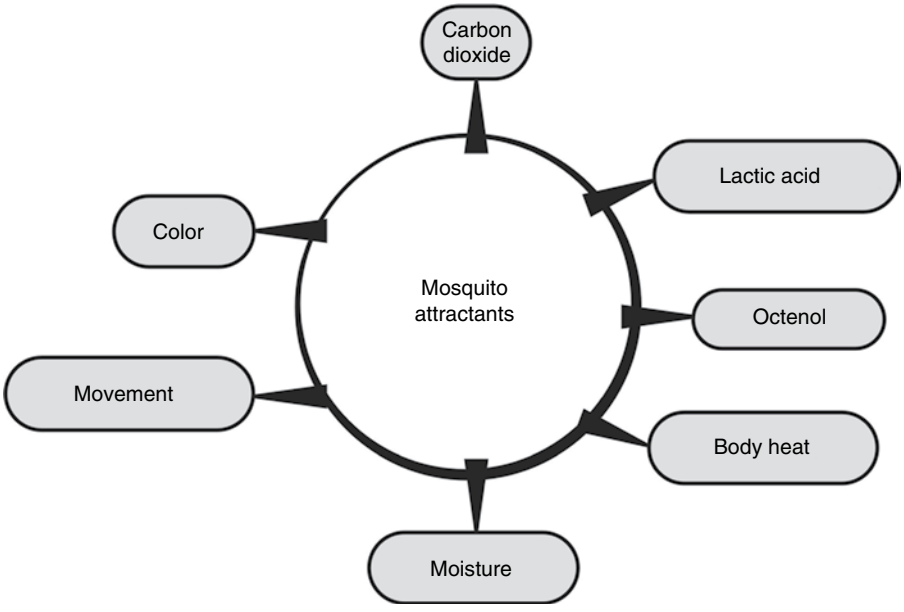

Figure 22.1. The various mosquito attractants in host.

habit is that some mosquitoes, such as *Aedes albopictus*, mainly feed outdoors and others, such as *Aedes aegypti*, prefer indoor feeding.

Mosquito: Attractants

How bloodsucking (hematophagous) insects find their way to feed on human or animal hosts is not currently known. There are many attractant compounds that allow mosquito species to find and reach toward a suitable host (Figure 22.1). Their vision enables them to see the host and their thermosensors on their antennae and mouthparts enable them to find the capillaries from which they feed. These mosquitoes pierce the blood vessel and inject their saliva that contains an anticoagulant compound, which prevents the host's blood from clotting in their mouthparts and gut. They have a pumplike structure in their head that literally sucks blood from the host; mosquitoes will feed until their abdomen is fully distended. The female then moves to a nearby protected area and rests for 1 or 2 days during, which she digests the blood and absorbs the proteins required for the development of eggs. On ingesting a blood meal, mosquitoes reached a state of engorgement and stop seeking a host until after they produce eggs. Sometimes a female mosquito may feed on more than one host to ingest enough blood to produce each batch of eggs. This multihost-feeding process helps in the transmission of viral and protozoan disease.

1. **Carbon Dioxide**: Carbon dioxide released during respiration from human and animal hosts helps mosquitoes to find their way. A burning candle or other fire is another source of carbon dioxide. Mosquitoes smell with their antennae, and their sense of smell is extremely sensitive toward carbon dioxide levels in the air. They orient their

flight pattern upwind to follow air currents containing a filamentous plume of carbon dioxide emanating from a host.
2. **Lactic Acid**: Lactic acid is usually emitted from the skin surface while exercising or consuming particular foods, which is used as an attractant by some mosquitoes.
3. **Octenol**: It is an alcohol, which is exhaled by the human and animals along with the carbon dioxide during respiration. It is also described as "cow's breath in a can" and is a potent attractant for the mosquitoes. Mosquitoes and other biting insect possess extremely sensitive receptors that can detect this chemical from almost 100 feet away.
4. **Body heat**: Mosquitoes possess sophisticated heat sensors through which they can easily detect body heat radiated by the circulation of blood in animals and humans. The exact supporting body temperature is largely depended on the type of mosquito.
5. **Moisture**: During movement and breathing, people exhale water vapor and perspiration, which attracts mosquitoes because it could mean possible blood source or possible breading site.
6. **Movement**: As with the movement of the host body, there is the change in light waves around them; mosquitoes can locate this change within 30 feet and direct their movement accordingly toward the host.
7. **Color**: Most of the dark colors attract mosquitoes, so to avoid mosquito biting one should avoid wearing dark color clothes.

Actually only one source is not sufficient for a mosquito to reach at their suitable host, it requires the combination of more than one attractants. For example, to attract mosquitoes the best combination is carbon dioxide, lactic acid, and octenol.

Environmental Management

Environmental management for mosquito control should include the planning, organization, carrying out, and monitoring of activities for the modification or manipulation of environmental factors, with the aim of preventing or minimizing mosquito breeding and reducing human-mosquito-parasite contacts. When these activities result in the long-lasting or permanent changes in land, water, or vegetation, they are designated as environmental modification (Figure 22.2), whereas if these activities have a temporary effect and need a repetition, they are designated as environmental manipulation.

Environmental Modification

Environmental modification mainly includes drainage, filling, land leveling, and transformation of impoundment margins (e.g., ditches to restrain livestock). Usually these modifications are permanent. But for them to be effective, they require proper operation and adequate maintenance. Some environmental modification activities that are useful for controlling the mosquito population are detailed here.

Removal or Destruction of Breeding Sites

Large breeding sites, such as swamps, ponds, water bodies, and small water collections (hoof-prints, abandoned cans, jars, and tires) can help mosquitos to lay their eggs and complete their life cycle because they serve as mosquito breeding grounds. Therefore, these breeding sites should be removed, destroyed, or covered to deny access to mosquitoes.

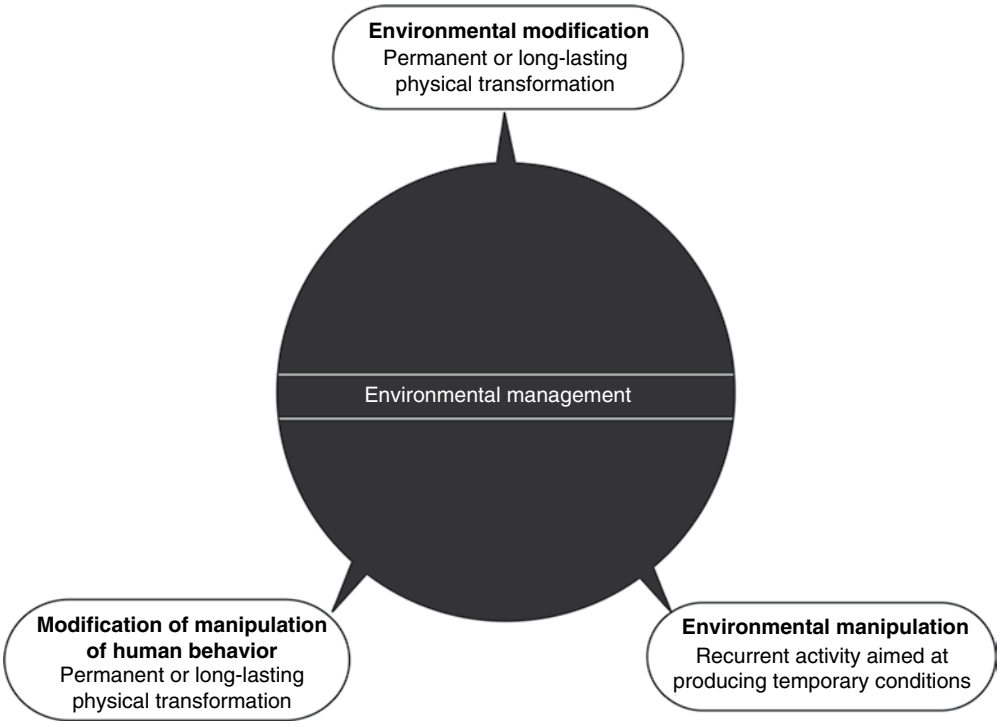

Figure 22.2. Environmental management for mosquito control.

Filled Up Breeding Sites

Mosquito breeding sites can be covered with soil, stones, rubble, ash, or rubbish, which is considered as the permanent control measure. It is mainly suitable for reducing those breeding sites that do not require much filling material, such as small depressions, water holes, borrow pits (a hole that has been excavated by people as a source of soil, stones, or dirt), and abandoned ditches or pools. Waste materials can be used for these purposes, and large areas are filled at little cost by making use of waste soil and stones left over from a construction project.

Drainage of Water

Proper drainage system for water reduces the breeding of mosquitoes. This can be performed by constructing open waterways and dykes with tidal gates, subsoil drainage and pumping; however, leakages, obstructions, and small pools/puddles of residual water in drainage ditches afford suitable breeding sites for mosquitoes. Drainage system planning and construction is complicated and requires expertise manpower, but some small-scale drainage works can be performed by the community members to control mosquito species.

Open earth drainage ditches (Figure 22.3) should be constructed to prevent the accumulation of rainwater in depressions in the ground and to dry out marshy areas, borrow pits, ground pools, and other accumulations of surface water. These ditches carry

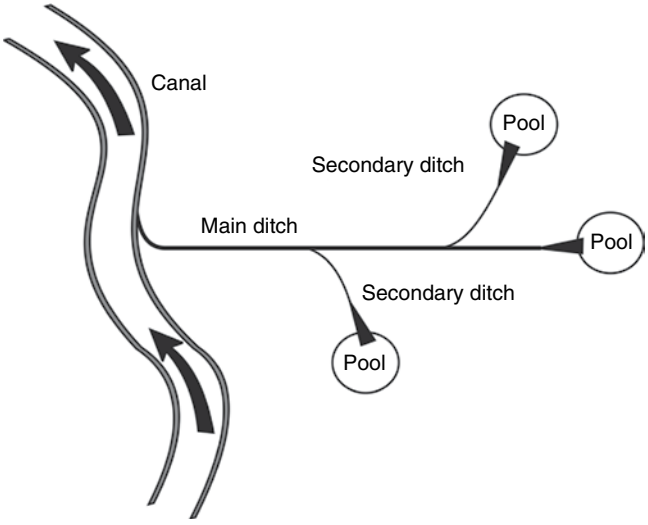

Figure 22.3. Open earth drains for larval control.
Source: *Communicable Diseases Module: 9. Malaria Prevention: Environmental Management and Larviciding for Vector Control*. Accessed March 25, 2013, at http://labspace.open.ac.uk/mod/oucontent/view.php?id=439267&section=1.4.2.

the water away to an appropriate, lower-lying outlet, such as a river, stream, pond soak-away pit, or main drainage ditch. They should follow the natural flow of water along the surface, and a main ditch may have several lateral or secondary ditches to collect water that does not readily drain into the main ditch.

Tree Plantation

Eucalyptus trees should be planted to dry marshy areas and other plots of land with high water. This purpose employs the plantation of rapidly growing species that absorb a lot of water and eventually dry the land by allowing water to evaporate through their leaves. For optimum evaporation activity, they should be planted with adequate spaces between them.

Environmental Manipulation

Environmental manipulation can be performed by increasing the flow of streams, regulating reservoir water level, removing vegetation, and adding shading.

Closing, Screening, or Covering Breeding Sites

All the possible breeding sites, especially in small enclosed habitats (e.g., drinking water storage containers and wells), should be covered, making them inaccessible to mosquito species (Figure 22.4). In some cases removable covers as mosquito-proof lids or wire mesh screening, can be used. Open wells should be closed using slabs, an iron sheet, or grass.

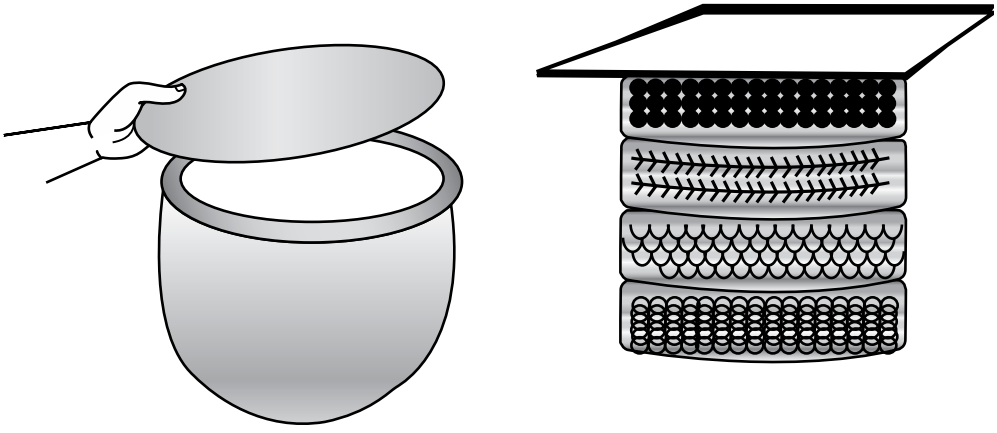

Figure 22.4. Potential mosquito breeding sites in small containers can be covered. *Source*: *Communicable Diseases Module: 9. Malaria Prevention: Environmental Management and Larviciding for Vector Control*. Accessed March 25, 2013, at http://labspace.open.ac.uk/mod/oucontent/view.php?id=439267&section=1.4.2.

Flushing

Flushing is an increasing water flow in streams, which can be employed in small streams where there is a continuous and abundant supply of water flowing slowly enough to permit mosquito breeding in quiet places along the margins. A periodic discharge of a large volume of water with heavy flow washes away the eggs, larvae, and pupae from the edges or strands them on the banks. This can be done through the collection of water, which is accomplished by constructing a small dam upstream of the area where breeding occurs. The dam site should be at a point where the stream or channel is narrow and the banks are high and should have a hand-operated gate to release the water at least once a week. The method requires some initial investment, but it is long lasting and requires little maintenance. This can be initiated by health extension practitioners by motivating the community members to construct a small dam and water release system wherever such a measure is feasible to control mosquito breeding in the village.

Shading of Ponds and Stream Banks

Mosquitoes usually prefer breeding sites, which are partly or fully exposed to sunlight; hence mosquito population can also be controlled by planting shrubs and trees along the banks of streams and covering ponds with iron sheets or local materials.

Removal of Water Plants

Mosquitoes can also be controlled by removing small vegetation from water bodies. These plants provide a safer place for the developing larvae, which helps them hiding from predators (e.g., fish) and also protect them from wave movement and currents. Community members can help in this regard by removing vegetation from small breeding sites, such as borrow pits and ponds using rakes (Figure 22.5). This method may

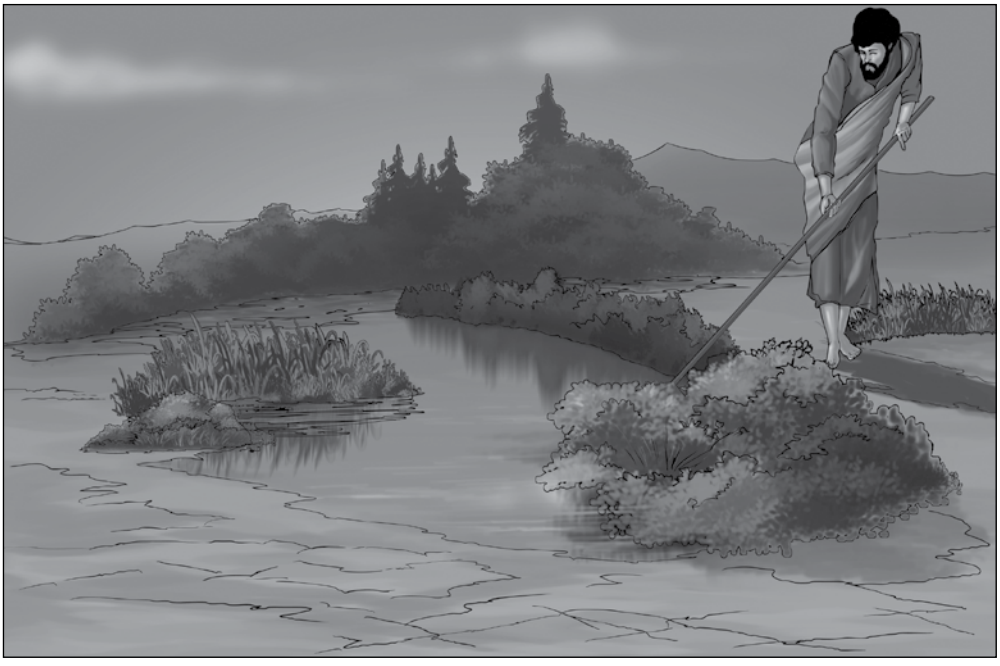

Figure 22.5. Removing water plants.
Source: *Communicable Diseases Module: 9. Malaria Prevention: Environmental Management and Larviciding for Vector Control*. Accessed March 25, 2013, at http://labspace.open.ac.uk/mod/oucontent/view.php?id=439267&section=1.4.2.

also remove resting places for adult mosquitoes and promote evaporation and the drying up small water accumulations, which makes the breeding sites more visible for control purposes.

Straightening and Steepening Shorelines

Shorelines of streams, ditches, and ponds can be made steeper to reduce the availability of shallow places, which are suitable for breeding of mosquitoes and to increase the speed of the flowing water.

Manipulation of Human Behavior

Fact sheets, posters, school poster contests, media contacts, and formal presentations can all be employed to let residents know about the municipality activities combating the mosquito population and to solicit their cooperation.

Source Reduction

Citizens should be encouraged to help control mosquitoes by reducing backyard sources. Mosquitoes can breed in the backyards, water gardens, and swimming pools; these sources can be reduced by removing excess vegetation, using fish and *Bacillus thuringiensis* (Bti), and also by maintaining water quality.

Exclusion

The use of mosquito screens on all of the doors and windows of homes and other buildings is an obvious means of excluding mosquitoes. Similarly, all the doors and windows should always be snug fitting along all four edges.

Personal Protection

Citizens should be educated how to protect them from mosquitoes at a personal level. The most effective and easiest method of personal protection is to avoid places and times of high mosquito densities (e.g., during calm, warm, and humid evenings) if possible. If not persons can be protected by minimizing the exposed skin surfaces by wearing a hat or head net, long trousers, and a long-sleeved shirt. Some mosquitoes, however, will still bite through lightweight clothing, but the number of bites received is definitely reduced if most areas of the body are covered. When mosquito densities become high in a particular geographical area, there is a risk of pathogen transmission, which can be avoided by applying a mosquito repellent.

Antilarval Measures

Basis of Mosquito Larviciding

The appropriate time to start a mosquito management program is before the emergence of adult mosquitoes. Mosquitoes are most efficiently and economically destroyed when they are in the larval stage of their cell cycle and are concentrated in their breeding site. Preventing the larvae from becoming adult mosquitoes also minimizes the area that would have to be treated to control mosquito populations. It prevents the development of a health problem associated with mosquitoes and reduces the potential environmental impacts of the adult mosquito control program. This can be done by inspecting pools as soon as the mosquito eggs starts hatching to quantify the numbers of larvae present and thereby determining the effectiveness of a larvicide agent. Larviciding methods can reduce overall use of insecticides in a mosquito control program by reducing or eliminating the need for ground or aerial application of insecticides to kill adult mosquitoes.

Monitoring Mosquito Breeding Sites and Larval Populations

Mosquito monitoring is a prerequisite to an effective, efficient, and environmentally sound mosquito control program. Monitoring is preferentially used to:

- Define the nature and extent of the mosquito problem within the targeted control zone, based on larval and adult mosquito survey program.
- Determine major sources of mosquitoes just outside of the control zone.
- Calculate the amount of larvicide needed to treat the total surface area of the infested pools.
- Give direction for daily mosquito control operations.
- Determine the proximity of environmentally sensitive areas, which could include any fish and wild life habitat, to the larval breeding sites.
- Determine which larval breeding sites can be easily eliminated.
- Evaluate the effectiveness of mosquito control operations.

- Generate the data needed to comply with provincial or territorial larvicide-use permits
- Evaluate the potential for transmission of mosquito-borne pathogens so that control them.

The first step in determining if the mosquito must be monitored on a routine basis is to establish the species causing problems (i.e., regardless of whether they are a nuisance or vector species). Once determining the key target species, the jurisdiction involved can be surveyed, with a special emphasis on the typical breeding sites of these species. The survey should include the trained professionals collecting both adults and the larvae. Information obtained from these surveys can be used to determine the abundance and seasonal distribution of each species and its relative importance as a target for control efforts. Because mosquito collection methods differ in their effectiveness for sampling different species, more than one collection method should be used to determine accurately the relative abundance of all of the species in a particular geographical area. Both an inventory of mosquito breeding site and a larval surveillance system should be developed by these skilled persons. The mosquito-breeding site inventory is actually a permanent collection of descriptions of all breeding sites, whereas a larval surveillance system describes the numbers of mosquitoes breeding at each site at the time when each site was sampled. Inventory and survey data can provide a record of mosquito breeding activity over time and thereby assisting in the decision to either apply larvicides to the site or eliminate it (recognizing the need to protect fish and fish habitat).

Monitoring Environmental Factors

To maximize the usefulness of mosquito surveillance data, key weather events (e.g., rainfall) must be recorded. Rainfall will direct when breeding sites will be flooded and when they will need to be inspected for mosquito larvae. Because rainfall is highly localized, it is important to record rainfall amounts from as many locations as possible. The data obtained will allow locating the area most likely to have mosquito hatching. These rainfall data can also be coupled with temperature records and one can predict, almost to the day, when the adult female mosquitoes will emerge, and as a result, larviciding will start in the areas having the highest rainfall.

Characteristics of a Good Larvicide

A larvicide chemical should be economically feasible and highly effective. It should have following properties:

- Highly toxic to mosquito larvae.
- Rapid and persistent action.
- Good dispersal qualities in the spray tank and in the source of mosquito.
- Easily approachable at low cost.
- Safe and convenient to handle, transport, and apply.
- Effective in all weather conditions.
- Primarily effective against larvae and possibly against eggs, pupae, and adults.
- Effective in all types of water sources, such as brackish, polluted, acidic, alkaline etc.
- Nontoxic to nontarget resources, such as man, food, plants, poultry, domestic animals, and fishes (both food and larvicidal).

- Good penetration power.
- Effective at low doses.

Mosquito Larviciding

Mosquito larvae can be controlled either by employing environmental methods or by applying biological or chemical larvicides.

Biological Methods

Biologically living organisms are used to control mosquito larvae. They are important in protecting the public from mosquitoes and include the use of larvivorous fish *Gambusia affinis*, guppies, and Bti.

Mosquitofish

The first preferred biological control is the mosquitofish (*Gambusia affinis*). When introduced to a mosquito breeding source, it quickly adapts, multiplies, preys on mosquito larvae, and is capable of controlling mosquitoes in a given area. It is a live-bearing US fish of muted silver and light olive green color and is a predator of mosquito larvae and can be applied in many diverse aquatic habitats. Additionally it is able to lighten or darken its body color depending on the surroundings. The fully grown female *Gambusia* fish is usually less than 2½ inches in total length and the male is typically less than 1½ inches.

Guppies

Guppies (*Poecilia reticulate*) have been used for biological control of mosquito since World War I and have been introduced throughout the world from the areas of tropical South America to which it is native. In many areas, the guppy has provided good mosquito control, especially in highly polluted sources, such as sewage pools, dairy lagoons, chicken ranch ditches, and slightly acidic sources. Unlike the mosquito fish, the guppy's ability to reproduce or control mosquitoes is not reduced by low levels of dissolved oxygen and hence is considered more effective.

Bacillus Thuringiensis Subspecies *Israelensis*

Bti is a naturally occurring bacterial species that produces a toxin that is lethal to mosquitoes. When Bti is added to the water, mosquito larvae ingest it; these ingested Bti spores contain a crystallized toxin that is dissolved by the larva's alkaline stomach fluids. The toxin then ruptures some of the stomach cells of the mosquito larva, causing death. Bti is also the active ingredient present in microbial larvicides. Further the effectiveness of Bti as larvicide depends on the mosquito species, weather conditions, the formulation of the product, and water quality (organic content). Although products containing Bti may be expensive, they have great potential to control mosquitoes with minimal impact on most other organisms that might be present in typical larval breeding sites. This is probably the reason that microbial larvicides are well suited for use in an integrated mosquito management program. Bti is also beneficial because it has a minimal impact on most of the beneficial organisms residing in habitats (Merritt, 1989; Becker and Margalit, 1993).

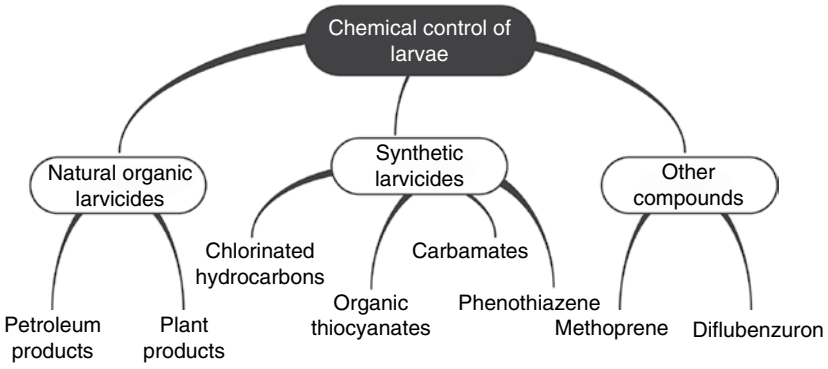

Figure 22.6. Chemical control of mosquito larvae.

Chemical Methods

There are many types of chemical methods to help deal with mosquitos (Figure 22.6).

Natural Organic Larvicides

- **Petroleum products**: These are contact poisons because their volatile toxic fraction penetrates the trachea and stable fraction interferes with air intake by mosquito larvae. Petroleum products are frequently used as solvents for other larvicides.
- **Plant products**: The best examples in this category are pyrethrum and rotenone, which are naturally occurring larvicides obtained from plant materials. Pyrethrum is a contact poison extracted from the genus *Chrysanthemum*, whereas rotenone acts both as a stomach poison and contact poison, derived from the roots of leguminous plants. Pyrethrum is also the oldest known and safest commercially available insecticide.

Synthetic Larvicides

- **Chlorinated hydrocarbons**: As the name suggests, these compounds consist of chlorine in combination with carbon and hydrogen. They show varying levels of activity depending on their chemical structure. These are central nervous system poisons, although their exact mode of action is still unknown. These compounds are quite stable and long lasting and accumulate in the fatty tissues of humans, animals, fishes, and other living organisms, which makes their use limited as larvicides. For example DDT, aldrin, lindane, etc.
- **Organic thiocyanates**: The solution of organic thiocyanates in kerosene or fuel oils is used as larvicide, especially in thermal fog generators, because of their heat stability (e.g., lethane and thanite).
- **Carbamates**: They are poor larvicides and are only effective at higher doses (100 g/ha), and therefore are expensive for most applications.
- **Phenothiazine**: It is used for larviciding restricted places, preferentially for killing *Anopheles* larvae.

Other Compounds

- **Methoprene**: Methoprene is an analogue of juvenile hormone acting as a growth regulator. It is an amber-colored liquid with a faint fruity odor, which is essentially nontoxic to humans when ingested or inhaled. Acting as a growth regulator, it mimics the juvenile hormone of insects required for a pupa to develop into a complete adult.

Hence methoprene-treated larvae are then unable to form into a mature adult. Methoprene is also referred to as biochemical pesticide because it is not directly toxic to target larva and prevents maturity of insect by interfering with its life cycle.

- **Diflubenzuron**: It is chitin-synthesis inhibitor. During molting phase of their life cycle, an insect requires chitin as the hard component of the new outer skin of the larva. A failure to synthesize larval chitin halts molting, leading to various physiological problems, and ultimately, the death of the mosquito. Hence it is also called as an insect growth inhibitor or regulator.

Chemical Adulticides

Adulticiding is the most visible form of mosquito control activities. It is also considered a last method in an integrated pest management (IPM) of mosquito control. Before starting the procedure, the threshold dose of any adulticide chemical must be determined first and is based on the biology of mosquito species to be controlled. The appropriate equipment is then chosen, and the chemical will be delivered to the adult mosquito through the most appropriate method.

Basis of Mosquito Adulticiding

When mosquito larviciding process has failed to control the larvae population and adult mosquitoes started blowing in from outside the larviciding zone, this situation requires the application of mosquito adulticides.

The number of mosquitoes collected in mosquito light traps as well as the number of mosquitoes caught during landing counts normally determines the need for an adulticiding program for the control of nuisance mosquito species. Before the application of an adulticide, a mosquito monitoring program should be conducted to detect an increase in the mosquito population. Alternatively, the imminent risk of disease transmission in a particular area may require immediate adulticiding, even when the mosquito population is below the normal numerical threshold for taking such action.

Mosquito Adulticides

To control adult mosquitoes is more difficult because they can spread out and move. Adulticides are broad-spectrum pesticides (Figure 22.7) and have the potential to affect nontarget organisms. To minimize the potential for environmental impact of these adulticiding chemicals, the applicator needs to understand the methods and equipment used and the potential risks involved.

Organophosphates

Organophosphates (OPs) are neurotoxic compounds inhibiting enzymes involved in the transmission of normal nerve impulse, working as both larvicide and an adulticide. Nerve impulses travel along neurons by way of electrical signals; however, at the junction between two neurons (a synapse) and between a neuron and a muscle (neuromuscular junction), the impulse is transmitted in the form of a neurotransmitter. One example of this neurotransmitter is acetylcholine, participating in the autonomic nervous system, neuromuscular junctions, and parts of the central nervous system. The enzyme cholinesterase breaks down and inactivates this acetylcholine in milliseconds. OPs inhibit

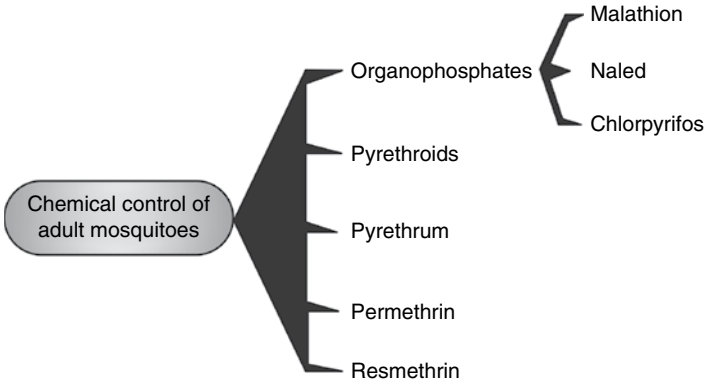

Figure 22.7. Chemical control of adult mosquitoes.

cholinesterase activity, resulting in the continuous transmission of nerve impulse by acetylcholine and causing paralysis and eventually death of the insect.

- **Malathion**: Malathion is used for both ground and aerial adulticiding operations. It is a contact stomach poison but also has an effect on the respiratory system and is extensively used to control major arthropod disease vectors (Family *Culicidae*), ecto-parasites of animals, household insects, and for the protection of stored grain products. Similar to the OPs, malathion also exhibits lowest mammalian toxicity, but because it has been used for a long time, it results in the development of localized resistance.
- **Naled**: Naled is also a contact and a stomach poison exhibiting respiratory action. It is actually used to control spider mites, aphids, and other insects feeding on many crops. It is also used in animal houses and in public health to control insects such as flies, ants, fleas, cockroaches, and extensively for the control of mosquitoes. However, this chemical rapidly breaks down in the environment and is highly corrosive; therefore, it requires special consideration in handling and equipment design.
- **Chlorpyrifos**: It is a nonsystemic contact and stomach poison with respiratory action. It is used to control and eliminate fleas, insects, termites, pests, as well as mosquitoes. It is a broad-spectrum product and is used inside the house mainly for pets and also in the manufacture of termite treatment and in cockroach pesticides. It is also useful in controlling pest population in vegetation field and from there it can reach inside the human or animal body, which could be harmful. Chlorpyrifos and its more toxic metabolite, chlorpyrifos oxon, altered firing rates in the locus coeruleus, which indicates that the pesticide may be involved in Gulf War syndrome (Cao et al., 2011).

Pyrethroids

Pyrethroids are synthesized organic compound that shows structural similarity to natural insecticide pyrethrins, produced in the flower heads of some plants belonging to the family *Asteracae* (e.g., *Chrysanthemums*). Pyrethroids are highly toxic to insect pests at low doses (often one order of magnitude less than OPs). Synthetic pyrethroids have been chemically altered making them more stable and safer to mammals. Pyrethroids are axonic poisons causing paralysis because they keep the sodium channels open in the neuronal membranes of an organism. These channels consist of a membrane protein

having a hydrophilic interior with a tiny hole referred to as voltage-gated sodium channel. Normally, this protein opens to cause stimulation of the nerve and closes to terminate the nerve signal. Pyrethroids bind to this gate, preventing it from closing normally, which results in continuous nerve stimulation. Finally the control of the nervous system is lost, producing uncoordinated movement and ultimately mortality. These became more commonly used against bed bugs after DDT was banned. As of 2010, a super strain of bedbugs evolved nerve cell mutations impervious to pyrethroids, which caused a bed bug pandemic because of ineffective treatment methods (Cappiello, 2009).

Pyrethrum

Pyrethrum has a nonsystemic contact action exhibiting similar mode of action to that of pyrethroids. It is used to control a wide range of insects and mites in public health and agriculture, generally after combining with a synergist that inhibits detoxification by the insect. Pyrethrum is a naturally occurring compound and environmentally acceptable because it breaks down rapidly in sunlight and has few negative residual effects.

Permethrin

Permethrin is a nonsystemic insecticide having a contact and stomach action. This compound is effective on a broad range of pests and is also one of the least expensive adulticiding compounds. It has good residual activity on treated plants, without any phototoxicity and exhibits low mammalian toxicity but high toxicity to aquatic organisms.

Resmethrin

Resmethrin can be used for both ground and aerial adulticiding. It is a nonsystemic insecticide with contact action and is a potent contact insecticide effective against a wide range of insects, which is often used in combination with more persistent insecticides. It has rapid mosquito knockdown properties along with low mammalian toxicity. It is a photolabile compound so does not persist long but is highly toxic to aquatic organisms and relatively expensive also.

Adulticiding Procedures

When a source reduction or larviciding program fails to provide adequate control of adult mosquito populations, two procedures may be followed to reduce adult mosquito levels: residual adulticide treatments and ultralow volume (ULV) adulticide treatments. First, residual adulticide treatments may be made to mosquito resting sites, which can kill the mosquitoes resting in these areas for up to 24 to 48 hours after treatment, depending on the residual activity of the insecticide which is used. Second, ULV adulticide spraying may be carried out in recreational and residential areas, especially when objective data indicate a significant nuisance or disease threat. A typical sequence of events should be followed (Figure 22.8).

Residual Treatment

Perifocal treatment through the means of hand-operated compression sprayers has both adulticiding and larviciding effects. Indoor residual spraying (IRS) is the application of long-acting chemical insecticides on the walls and roofs of all houses

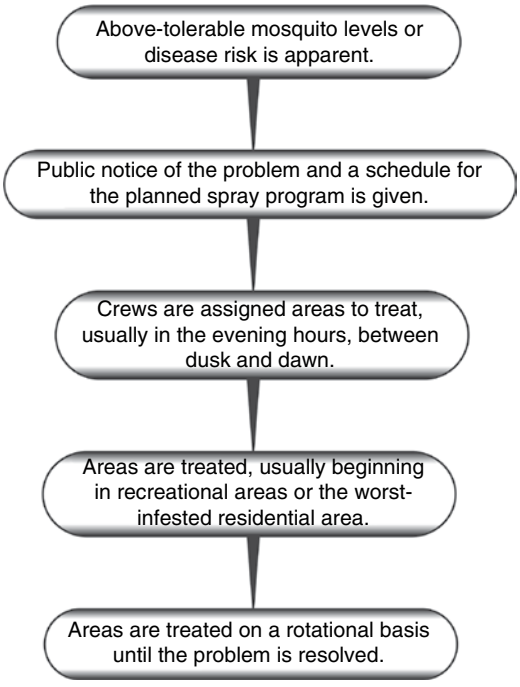

Figure 22.8. Steps of adulticiding.

and domestic animal shelters in a given area to kill all the adult vector mosquitoes that land and rest on these surfaces.

Space Sprays

This type of spraying is recommended in emergency situations to suppress an ongoing epidemic or to prevent an incipient one. The objective of space spraying is the massive, rapid destruction of the adult vector population in a particular area. If space spraying is used early in an epidemic and on a large scale, the intensity of transmission may be reduced, which would give time for the application of other vector control measures that provide longer term control, including larviciding and community-based source reduction. The efficiency of space spraying is dependent on many factors, such as the method of release, fog types, droplet size, application rate, climatic conditions, and size of the target area.

Repellents

Repellents are substances that help people avoid mosquito bites, but they do not kill mosquitoes, only preventing them from biting (Figure 22.9). Repellents can be originated from plant sources (citronella, neem, eucalyptus, marigold, etc.) or they may be purely chemical substances (DEET, diethyl benzamide, etc.). Both of these chemical- and plant-derived repellents are available in the form of creams, lotions, sprays, wipes, roll-on

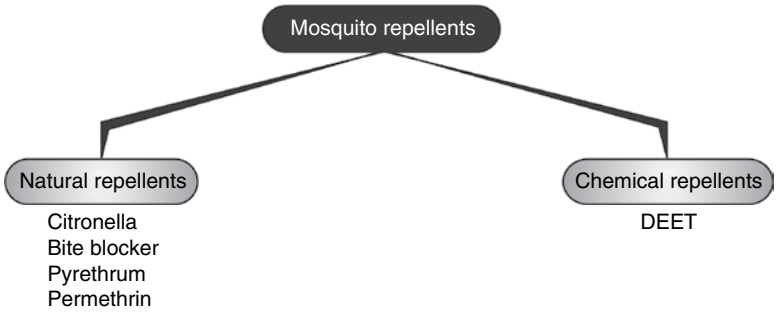

Figure 22.9. Mosquito repellents.

sticks, foams, liquid vaporizers, sprays, coils, and mats. Skin repellents that contain natural repellent herbs, such as aloe vera, eucalyptus, tea tree oil, lavender, vanilla, citronella, and other helpful herbs, can also be used (Garrett, 2012). Nowadays electronic repellents are also widely applicable, which use ultrasound and electromagnetic waves to repel mosquitoes and other insects.

Natural Repellents

Natural repellents are volatile plant oils, which when used in higher concentration and frequent reapplication, effectively repel mosquitoes. Thousands of plants have already been tested as potential sources of insect repellents (Sukumar et al., 1991), For example, citronella oil, lemon eucalyptus oil, cinnamon oil, castor oil, rosemary oil, lemongrass oil, cedar oil, peppermint oil, clove oil, geranium oil, and other possible oils from verbena, pennyroyal, lavender, pine, cajeput, basil, thyme, allspice, soybean, and garlic (Brown and Hebert, 1997) have been used to repel mosquitos.

- **Citronella**: This is an active ingredient commonly found in natural or herbal insect repellents especially common in the United States. It is also registered with the US Environmental Protection Agency as an insect repellent. Citronella oil originated from the grass plant *Cymbopogon nardus* and has a lemony scent; it is an effective repellent but provide short protection against mosquitoes. To repel mosquitoes in the backyard, however, citronella candles are used effectively.
- **Bite blocker**: It is also a plant-based repellent that was released in the United States in 1997. Bite blocker is a combination of soybean oil, geranium oil, and coconut oil.
- **Pyrethrum and Permethrin**: Pyrethrum is a powerful insecticide derived from the crushed and dried daisy (*Chrysanthemum cinerariifolium*) flowers (Casida and Quisted, 1995). Permethrin is a human-made synthetic pyrethroid, which does not repel insects but works as a contact insecticide, causing nervous system toxicity that leads to death or "knockdown" of the insect. The chemical is effective against mosquitoes, flies, ticks, and chiggers. Permethrin exhibits low toxicity for mammals, is poorly absorbed by the skin, and is rapidly inactivated by ester hydrolysis. It can be directly applied to clothing or other fabrics such as tent walls (Schreck, 1991) or mosquito nets (Lines et al., 1987) but not to skin. The spray form is nonstaining, nearly odorless, and highly resistant to degradation by heat or sun and maintains its potency for at least up to 2 weeks, even through several launderings (Schreck et al., 1978).

Natural repellents do not always imply safe usage because many people are sensitive to plant oils, and some natural insect repellents are actually toxic when consumed. Therefore, although natural repellents provide an alternative to synthetic chemicals, instructions given by the manufacturer should be followed before using these products.

Chemical Repellent

N, N-Diethyl-3-Methylbenzamide

DEET was first discovered by scientists at the US Department of Agriculture and was patented by the US Army in 1946. Then in 1957 it was subsequently registered for use by the general public; since then it remains the gold standard of currently available insect repellents. It is a broad-spectrum repellent, which is effective against mosquitoes, biting flies, chiggers, fleas, and ticks. Broadly the mosquitoes have three sets of receptor cell types that are sensitive to carbon dioxide, lactic acid, and temperature, respectively. All these receptors must be stimulated simultaneously at the same time to evoke the blood feeding response of a mosquito. DEET inhibits the lactic acid receptors, and as a result, the mosquito cannot smell lactic acid and cannot identify the person as their host. DEET confuses the mosquito and prevents the blood feeding response. In a behavioral test, DEET had a strong repellent activity in the absence of body odor attractants, such as 1-octen-3-ol, lactic acid, or carbon dioxide, in which male and female mosquitoes showed the same response (Syed and Leal, 2008; Fox and Wiessler, 2011). DEET has also been found to inhibit the activity of the central nervous system enzyme, acetylcholinesterase, in both insects and mammals (Corbel et al., 2009)

Insecticide-Impregnated Bed Nets and Screens

Mosquito control is a serious concern, especially in developing countries where mosquito-borne illnesses are prevalent. The use of insecticide alone is not potent enough to completely eradicate the prevalent mosquito-borne illnesses completely. Therefore, mosquito netting is suggested along with the insecticides by the health control agencies to control the various mosquito-borne illnesses effectively. Mosquito nets keep out mosquitoes, flies, and other insects and defend people against various mosquito-borne diseases. An ideal mosquito net will allow air to circulate inside and outside while keeping the mosquitoes out. Insecticide-impregnated bed nets act as insect repellents by increasing the individual protection and decreasing their effect on a population level.

Types of Mosquito Nets

Many types of mosquito bed nets and screens are available for effective mosquito control, including bed netting (a mesh cover draped over the bed), barrier screens for doorways and windows, net barrier clothing, and pop-up screen rooms for the outdoors.

- **Insecticide-treated mosquito nets (ITNs)**: Mosquito bed nets are soaked in a suitable insecticide (pyrethroid), which spreads throughout the net and then dries by the treatment called *impregnation*. These insecticide-saturated bed nets are referred as impregnated mosquito nets. For impregnation purposes, the insecticides to be used should be safe for

human body and also potent enough to kill mosquitoes effectively. After every 6 washes, these nets must be replaced or further retreated with the insect repellent.

- **Long-lasting insecticide-treated nets (LLINs)**: Mosquito netting and pest control companies provide LLINs, which bind or incorporate the insecticide such that it is released slowly over a few years and can be washed at least 20 times still retain its potency.
- **Mosquito Nets for Households**:
  1. Newborn babies and infants are quite susceptible to most of the infectious diseases because of poorly or undeveloped immune system. To protect them, baby mosquito nets can be used for covering cots, playpens, prams, and pushchairs.
  2. Many mosquito nets are designed with various attractive features in the bedroom along with the required protection. One of the popular types is canopy mosquito netting, which does not require tying or using pole or rope; it just needs to be strapped to the mattress. Elastic trap is attached to support the mosquito net.
  3. Mosquito tents simply spring open, pop into shape spontaneously when unpacked, and are then fixed at place.
  4. Travel mosquito nets are also available, which are useful for people moving from one place to another. These nets are particularly suitable for camping or for using outside in the garden, where there is minimal hanging or fixing to do.
  5. Mosquito nets can be applied to windows and doors, which effectively blocks the entry of mosquitoes. These nets have holes that are big enough to let air and light in, but small enough to keep mosquitoes and other insects out.
  6. Mosquito roller screens are also an excellent option because these are an elegant window-and-door system that not only block out disease causing mosquitoes but also add aesthetic appeal.

References

Becker N, Margalit J. 1993. Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies. In: Entwistle PF, Cory JS, Bailey MJ, Higgs S, eds., Bacillus thuringiensis, *An Environmental Biopesticide: Theory and Practice*, pp. 147–170. New York: Wiley.

Brown L. 1993. *The New Shorter Oxford English Dictionary on Historical Principles*. Oxford: Clarendon Press.

Brown M, Hebert AA. 1997. Insect repellents: An overview. *J Am Acad Dermatol* 36:243–249.

Cao Jun-li, Varnell A, Cooper, DC. 2011. Gulf War Syndrome: A role for organophosphate induced plasticity of locus coeruleus neurons. *Nature Precedings*. Accessed February 24, 2013, at http://precedings.nature.com/documents/6057/version/1/html.

Cappiello D. 2009. Bedbugs on the rebound, feds take note. Accessed February 24, 2013, at http://www.pestproductsonline.com/bedbugcontrol.

Casida JE, Quistad GB. 1995. *Pyrethrum Flowers: Production, Chemistry, Toxicology and Uses*. Oxford: Oxford University Press.

Communicable Diseases Module: 9. Malaria Prevention: Environmental Management and Larviciding for Vector Control. Accessed March 25, 2013, at http://labspace.open.ac.uk/mod/oucontent/view.php?id=439267&section=1.4.2.

Corbel V, Stankiewicz M, Pennetier C, Fournier D, Stojan J, et al. 2009. Evidence for inhibition of cholinesterases in insect and mammalian nervous systems by the insect repellent deet. *BMC Biology* 10:86.

Fox M, Wiessler D. 2008. For mosquitoes, DEET just plain stinks. *Reuters*. Accessed February 24, 2013, at http://www.reuters.com/article/2008/08/18/us-mosquitoes-deet-id USN1849399820080818.

Garrett H. 2012. Safe and Natural Mosquito Control. *Dallas Metroplex edition*. Accessed February 24, 2013, at http://www.nadallas.com/DAL/June-2012/Safe-and-Natural-Mosquito-Control/.

Lines JD, Myamba J, Curtis CF. 1987. Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Med Vet Entomol* 1:37–51.

Merritt RW. 1989. A broad evaluation of B.t.i for black fly (*Diptera: Simuliidae*) control in a Michigan river: Efficacy, carry and nontarget effects on invertebrates and fish. *J Am Mos Contr Ass* 5:397–415.

Molavi A. 2003. Africa's malaria death toll still outrageously high. *National Geographic* Accessed February 24, 2013, at http://news.nationalgeographic.com/news/2003/06/0612_030612_malaria.html.

Schreck CE. 1991. Permethrin and dimethyl phthalate as tent fabric treatments against *Aedes aegypti*. *J Am Mosq Control Assoc* 7:533–535.

Schreck CE, Posey K, Smith D. 1978. Durability of permethrin as a potential clothing treatment to protect against blood-feeding arthropods. *J Econ Entomol* 71:397–400.

Sukumar K, Perich MJ, Boobar LR. 1991. Botanical derivatives in mosquito control: A review. *J Am Mosq Control Assoc* 7:210–237.

Syed Z, Leal WS. 2008. Mosquitoes smell and avoid the insect repellent DEET. *Proc Natl Acad. Sci USA* 105:13598–13603.

Wigglesworth VB. 1932. *The Adaptation of Mosquito Larvae to Salt Water*. Department of Entomology, London: London School of Hygiene and Tropical Medicine.

Wilcox BA, Ellis B. 2006. Forests and emerging infectious diseases of humans. *Unasylva*, a publication of Food and Agriculture Organization of the United Nations 57:2.

Chapter 23
New and Potential Techniques: Mosquito Control

Myco-Insecticides

With an increasing global risk of mosquito-borne diseases, as well as, continuous and increasing levels of insecticide resistance, health authorities reviving an interest in entomopathogenic fungi. New and existing entomopathogenic fungi can contribute to an expansion of the limited available mosquito control tools, which contribute in a significant and sustainable manner to control various vector-borne diseases, including malaria, dengue, and filariasis. Many fungal agents, such as *Coelomomyces*, *Lagenidium*, *Metarrhizium*, *Culinomyces*, and *Tolypocladium*, have been isolated and tested (Roberts and Strand, 1977) with this respect. Fungal agents, such as *Lagenidium*, *Coelomomyces*, and *Culicinomyces*, have also been extensively studied and known to affect mosquito populations (Chandrahas and Rajagopalan, 1979). *Lagenidium giganteum* was highly pathogenic to immature forms of *Culex quinquefasciatus*, *Culex tritaeniorhychnus*, *Anopheles culicifacies, Anopheles stephensi*, and *Anopheles subpictus*. However, some other fungi infect and kill mosquitoes at the larval or adult stage. The fungal mosquito pathogen *Leptolegnia chapmanii* (ARSEF 5499) had been tested against larva of 12 mosquito species and found to be effective against *Anopheline* and *Culicine* mosquitoes (Lopez et al., 2004). Similarly a soil fungus *Trichophyton ajelloi*, caused high larval mortality in *A. stephensi* and *C. quinquefasciatus* (Mohanty and Prakash, 2003). Residual sprays of these fungal bio-pesticides might replace chemical insecticides for malaria control, particularly in areas of high insecticide resistance (Blanford et al., 2005).

Oomycota (Watermolds)

Some aquatic organisms designated as water molds are facultative parasites of mosquito larvae. *Leptolegnia*, *Pythium*, and *Cryptiloca* are pathogenic to mosquitoes and one

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

organism *L. giganteum* (Couch) is commercially available as a mosquito control agent. *Leptolegnia caudata* was isolated from the malaria vector *Anopheles culicifacies Giles* (Bisht et al., 1996) and is used in larval control campaigns to reduce malaria transmission. A *Pythium* species proved to be pathogenic to early instars of *Aedes aegypti*, *Aedes africanus*, *Aedes simpsoni*, *C. quinquefasciatus*, *Culex tigripes*, and *Anopheles gambiae* in a laboratory with mortalities between 50 and 100 percent (Nnakumusana, 1985). This is an opportunistic fungal pathogen rather than a strictly entomopathogenic one and infects mechanically injured larvae rather than healthy larvae. Another organism *L. giganteum* is a facultative parasite of mosquito larvae and has high mortalities in mosquito populations in laboratories in small- and large-scale field studies (California and North Carolina), especially in *Culex* (Merriam and Axtell, 1982; Jaronski and Axtell, 1983), *Mansonia* (Florida) (Cuda et al., 1997), and *Anopheles* species (Kerwin and Washino, 1987). McCray et al. (1973) also showed that this fungus could infect and kill larvae of many mosquitoes, including *A. aegypti*, *Ochlerotatus triseriatus*, *Aedes mediovittatus* (Coquillett), *Ochlerotatus taeniorhynchus Wiedemann, Ochlerotatus sollicitans* (Walker), *Ochlerotatus taeniorhynchus Theobald*, *C. quinquefasciatus*, and *Culex restuans* except anophelines.

Chytridiomycota

Coelomomyces is the only genus of this phylum that has an anti-mosquito property. This genus consists of more than 70 species of obligatory parasitic aquatic fungi undergoing a complex life cycle involving alternating sexual (gametophytic) and asexual (sporophytic) generations. Coelomomyces is usually restricted to aquatic *Diptera* insects, including *Culicidae*, *Psychodidae*, *Chironomidae*, *Simuliidae*, and *Tabanidae* families (Arêa Leão and Carlota Pedroso 1964; Chapman, 1974; Roberts, 1970). This fungus prefers the mosquito host *Anopheles* followed by *Aedes* and *Culex* (Chapman, 1974) and requires an intermediate microcrustacean host (e.g., cyclopoid copepods, harpacticoid copepods, or ostracods) and two mosquito generations. The zygotic stage of this fungus encyst on the intersegmental membranes of young or recently molted mosquito larva (Lacey and Undeen, 1986) and enter the epithelial cells through a penetration tube and then the hemocoel where it uses the larval fat body reserves to develop into irregularly shaped hyphae without cell walls (Roberts, 1974). Sporangia are then produced within the hyphae at their tips, and the larva then dies in most cases and forms resting spores to complete the life cycle.

Zygomycota

The order *Entomophthorales* of the phylum Zygomycota contains approximately 200 known species classified in six genera, and some of them predominantly infect mosquitoes. An example is *Conidiobolus coronatus*, which has been found in several orders of insects and in two other classes of invertebrates. It is considered to have the widest host range among the entomophthorales, but it is a weak pathogen. Genus *Entomophthora* primarily infects adults as compared to larval mosquitoes. Apart from these, *Erynia aquatica* is found to be pathogenic to adult mosquitoes, where infection levels, particularly in overwintering populations of adults, frequently approaches 100 percent.

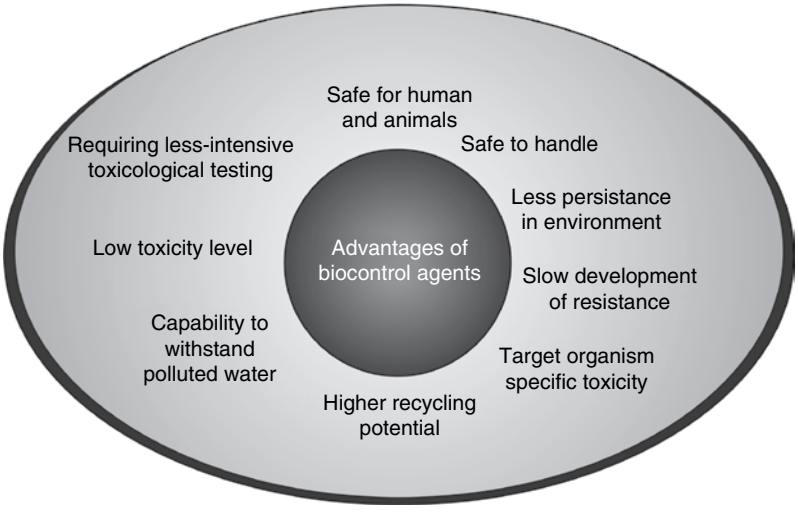

Figure 23.1. Advantages of biocontrol agents.

Deuteromyces

Many genera of entomopathogenic fungi belong to this group of fungi and it shows the widest host ranges among entomopathogens, including several mosquito species. The most common route of host invasion is through the external integument, although digestive tract infection is also possible (Goettel and Inglis, 1997). Conidia attach to the cuticle, germinate, and then start penetrating the cuticle. Once in the hemocoel, the mycelium grows throughout the host body, forming hyphal bodies called *blastospores*. Insects may die probably as a result of a combination of the action of fungal toxins, physical obstruction of blood circulation, nutrient depletion, and invasion of organs. After the death of the host, hyphae usually emerge from the cadaver and produce conidia under suitable abiotical conditions on the exterior of the host, which are then dispersed by wind or water. As an example, *Culicinomyces clavisporus* fungus is pathogenic to mosquito larvae and culicines shows more susceptibility than anophelines. To control mosquito populations, conidial suspensions have been effective but require large numbers of conidia. Another genera, *Beauveria*, also has a cosmopolitan distribution, infecting *Culex tarsalis*, *Culex pipiens*, and *Anopheles albimanus*.

Entomopathogenic Bacteria and Viruses

In the past few decades, there is growing realization that bacterial and viral agents may be useful in controlling mosquito larva in different breeding habitats. These biocontrol agents have many advantages over pre-existing chemical agents (Figure 23.1).

Bacterial Agents

From the last three decades, bacilli-based mosquito larvicides (biocides or biolarvicides) are becoming popular in controlling vector population. Many commercial formulations

Table 23.1. Bacterial strains and their mosquito host.

Entomopathogenic Bacterial Strain	Susceptible Mosquito Host
Bacillus thuringiensis subsp. *Israelensis* (Bti)	*Aedes aegypti*, *Culex*, and *Anopheles*
Bacillus thuringiensis subsp. *Kurstaki* (Btk)	*Aedes aegypti*
Bacillus thuringiensis subsp. *Jegathesan* (Btj)	*Anopheles stephensi*, *Culex pipiens*, and *Aedes aegypti*
Bacillus sphaericus (Bs)	*Culex*, *Anopheles*, and *Aedes aegypti*
Clostridium bifermentans ser. *Malaysia*	*Anopheles*, *Aedes detritus*, *Aedes caspius*, and *Aedes aegypti*

of these organisms are available for this purpose, which can be used in large-scale mosquito control operations. These biocides have reduced application costs and are quite safe to the environment, humans, animals, and other nontarget organisms. The two commonly used bacterial biocidal agents are *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*). Both of them are spore-forming entomopathogenic bacteria, which produces toxin to some invertebrates, mostly insects and nematodes upon sporulation (Feitelson et al., 1992). They produce protein depending on the quantity and type of strain and each type of crystal protein is characterized by a specific host range. Insecticidal endotoxins produced by *Bacillus thuringiensis* subsp. *israelensis* have been classified into several groups on the basis of the difference in their sequence and specificity and designated as *Cry* (Crickmore et al., 1995). Whereas the toxic strains of *Bacillus sphaericus* have been divided into two groups, one is low toxicity strains and other is high toxicity strains. *Bacillus sphaericus* SS11 is a low toxicity strain (Singer, 1973), which generates toxicity initially in the vegetative growth phase before the onset of sporulation and is markedly unstable. In contrast, the high toxicity strains of *Bacillus sphaericus* (strains 1593 and 2362) are relatively stable (Broadwell and Baumann 1986). These high toxicity strains produce binary toxins with protein components of 41.9 kDa and 51.4 kDa, and both are required to kill mosquito larvae (Broadwell et al., 1990). Both of these biocidal agents are useful in controlling mosquito larva, but their low persistence in nature requires frequent application, which is considered a limiting factor for the use of these organisms as potent biolarvicide. Nevertheless, they are by far the best choice for controlling mosquitoes (Riehle and Jacobs-Lorena 2005). Recently two other bacterial species *Peanibacillus macerans* and *Bacillus subtilis* were isolated showing mosquito larvicidal activity against Chikungunya vector *A. aegypti* (Ramathilaga et al., 2012). Table 23.1 details some of bacterial strains and their mosquito host.

Bti actually produces a proteinaceous parasporal crystalline inclusion during sporulation. When insects ingest it, this is solubilized in the midgut, releasing proteins called *delta-endotoxins* that are activated by midgut proteases. These activated toxins then interact with the larval midgut's epithelium causing a disruption in membrane integrity and ultimately leads to insect death (Poopathi et al., 1999). Similarly, on ingestion *Bs* protein destroys the gut epithelium of mosquito larva, resulting in the death of the insect. Generally, the genus *Culex* larvae are especially susceptible to this toxin, *Anopheles* spp. are moderately susceptible, whereas the larvae of *Aedes* spp. are quite resistant. The larvicidal properties of these crystals have made *Bs* a useful and preferable agent for the biological control of mosquitoes.

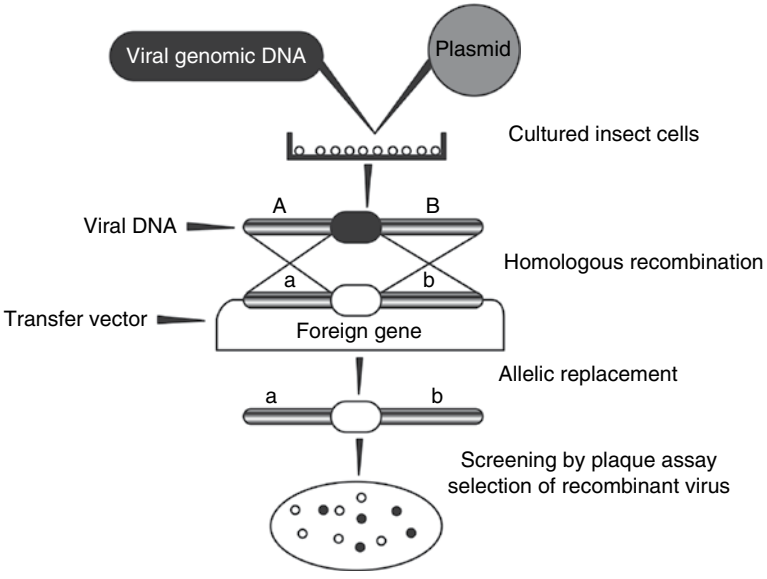

Figure 23.2. Method for the generation of recombinant viruses for vector control.

Viral Agents

Some noninfectious viruses, such as Iridescent virus, densonucleosis virus, cytoplasmic polyhedrosis virus, and Baculoviruses as nuclear polyhedrosis virus (NPV), granulosis virus (GV), and oryctes granulosis virus, have been studied in the past for controlling mosquitoes (Jenkins, 1964; Roberts and Strand, 1977). These viruses can attack a wide range of hosts, are highly lethal, and are not infectious for the human population. The best known example is baculoviruses, which are largely restricted to insects and are host specific. GV and NPV usually cause death of larvae within 5 to 12 days after infection. Commercially these viruses are produced on the host insects and are formulated both as liquid and dust formulation. The problems of insufficient infectivity or virulence handicap the development of viruses as biocontrol agents. Because the viruses require host cell to cultivate, the most serious obstacle to their development and use is the unavailability of an efficient method for their mass production because they are highly specific obligate pathogens. However, they can be produced at mass scale using homologous recombination method (Figure 23.2).

Hormonomimetic and Plant-Derived Substances

Some plant-derived substances have an insect hormonelike activity; an external supply of the hormonelike substances, from the plant without respect to the physiological control mechanisms, disturbs the precise synchronization of insect development and leads to the appearance of malformed creatures unable to survive and reproduce. Plants that contain analogues of insect hormones show resistance, especially against susceptible insects. These substances fall into two categories:

1. The first category has an activity similar to insect juvenile hormone; these are mainly aliphatic or monocyclic sesquiterpenes of farnesane or bisabolane types.
2. The second category has an activity similar to molting hormone; these are polyhydroxy-steroid compounds related to ecdysone.

Actually the normal progressive development of an insect from its immature stage to an adult stage occurs in the presence of juvenile hormone (JH) in its tissues. JH is synthesized in paired glands, the corpora allata, situated on either side of the oesophagus. This hormone suppresses the development of an adult mosquito; hence its concentration must be reduced to a specific level for the metamorphosis of insect to form into a sexually mature adult mosquito. If this JH is artificially applied to the insect at a concentration higher than that required and triggers the metamorphosis activities, it can completely disturb the insect's programmed life processes. Hence JH or its analogue have similar modes of action and provides a novel and efficient way to control medically important arthropods.

Apart from these hormomimetic compounds, some other naturally occurring plant-derived oils could also be used as potential bioactive agents against mosquitoes; for example, basil, cinnamon, citronella, and thymus are considered promising mosquito larvicides.

Larvivorous Fish and Crustaceans

A large number of organisms help to regulate mosquito populations naturally through predation, parasitism, and competition. Larvivorous fishes were the first biocontrol agents employed to control mosquito vectors, and since the early 1900s larvivorous fish have been used throughout the world as biological mosquito control agents (Raghavendra and Subbarao, 2002). Larvivorous fishes feed on immature stages of mosquitoes. The major advantages of these larvivorous fish as biocontrol agents are that these fishes are environment friendly and are self-perpetuating after its establishment and continuous to reduce mosquito larva for longer time period with lower application cost than that of chemical control. Most of these fishes prefer residing in shallow water, the breeding area of mosquito larvae, *Gambusia* and *Poecilia*.

Required Characteristics of Larvivorous Fish for Use in Mosquito Control

1. Should be small in size and easily reside in shallow waters among thick weeds where mosquitoes find suitable breeding grounds.
2. Should be drought resistant and capable of flourishing in both deep and shallow waters as well as in drinking water tanks and pools without any contamination.
3. Should be surface feeders and carnivorous.
4. Should be capable of surviving in the absence of mosquito larvae.
5. Should be easy to rear and be prolific breeders with shorter life span.
6. Should be capable of withstanding a wide range of temperature and light intensity.
7. Should be hard enough and able to withstand rough handling and transportation for long distances.
8. Should not be attractive or bright colored.
9. Should not be significant or used as food for other predators.

Table 23.2. Categories of larvivorous fishes.

Categories	Features
Typical surface feeders	They fulfill the characteristic features of larvivorous fish such as *Aplocheilus* and *Gambusia*
Some surface feeders	They are less efficient owing to their mode of life, for example, *Oryzias*, *Lebistes (Poecilia)*, *Aphanius*, etc.
Subsurface feeders	Prefers to grow under surface, for example, *Amblypharyngodon mola*, *Danio*, *Rasbora*, etc.
Column feeders	They feed on mosquito larvae when chance permits like *Puntius* spp., *Colisa*, *Chanda*, *Anabas*, etc.
Fry of carps and mullets	Helpful in controlling mosquito larvae
Predatory fishes	Their fry may destroy mosquito larvae but their adults may be predatory on other fishes including larvicidal fish species like *Wallago*, *Channa*, *Notopterus*, and *Mystus*

10. Should have a special predilection for mosquito larvae even in the presence of other food materials.
11. Should be compatible with the existing fish life in the environment.
12. Should not have any food value, so that fish-eating people used to discard them.

Although it is difficult to find a fish species that satisfies all these parameters, the choice usually depends on those, which satisfy as many of the qualities.

Categories of Larvivorous Fishes

In 1938, Hora and Mukherjee classified larvivorous fishes into six categories based on their efficiency of mosquito control (Table 23.2).

Aplocheilus

It is approximately 9 cm (3.6 inches) long and suitable for ponds and impounded water bodies. Three species of this genus are useful in mosquito control: *Aplocheilus blockii* (Dwarf panchax), *Aplocheilus lineatus* (Malabar killie), and *Aplocheilus panchax* (Panchax minnow). *A. blockii* is a potential larvivorous fish that can control the spread of chikungunya fever by controlling *Aedes albopictus* mosquito, whereas *A. lineatus* controls dengue fever by controlling *A. Aegypti* and *A. panchax* controls several vector species existing in different types of natural and man-made habitats. It controls *A. culicifacies* mosquitoes in breeding habitats such as rain water pools, irrigation channels, sluggish streams with sandy margins and little vegetation, river bed pools, borrow pits, cemented tanks, swimming pools, and freshly laid rice fields.. It also helps in controlling a *Culex* species, *C. quinquefasciatus* in various sites including cesspools, drains, chocked severs, storm water drains, ponds, polluted waterways, septic tanks, disused wells, well, and manure pits.

Gambusia Affinis (Common Name: Top Minnow)

G. affinis is an exotic species that has been distributed throughout the tropical and some temperate parts of the world. It is a hardy fish that can adapt to wide variations in

temperature as well as to chemical and organic content of the water but is not capable of tolerating a high organic pollution. Although the optimum temperature for reproduction ranges from 75.2 to 93.2° F (2–34° C) and the optimum pH is 6.5–9.9, but the fish can also survive at freezing temperatures. *Gambusia* are frequently found in areas especially suitable for the mosquito larvae. Its life span is approximately 4 + 1 years. The maximum size for male fish is 4.5 cm, and for female it is 5.2 cm to 6.8 cm. The female *Gambusia* requires about 3 to 6 months for complete maturation. Its ovary contains approximately 120 eggs and after maturity, breeds throughout the year, especially in tropical conditions. A single full grown fish eats about 100 to 300 mosquito larvae per day, and it is a surface feeder and is suitable for feeding on both anophelines and culicines.

Poecilia (Lebistes) Reticulata (Common Name: Guppy)

It lives on artificial food and prefers mosquito larva. The male is of around 2 cm (0.75 inch), and the female is 4 cm (1.5 inch) long. *Poecilia reticulata* has effectively been used to suppress the larval and adult population of *A. gambiae* in washbasins and cisterns.

Aphanius Dispar (Common Name: Dispar Top Minnow)

It is a larvivorous fish approximately 7.5 cm (3 inches) long, which thrives both in fresh and brackish waters. It is a delicate species and is not suitable for transporting. It is particularly suited for drains and polluted water bodies and also useful for stagnant water bodies, disused wells, and cesspools. It is mainly useful for the control of *C. quinquefasciatus* a vector of Bancroftian filariasis. *A. dispar* effectively suppresses the growth of *Anopheles arabiensis* and *A. gambiae* breeding in wells, cisterns, and barrels.

Danio Rerio (Common Name: Zebra Danio)

It is approximately 5 cm (2 inch) long, useful in moving streams and ponds, is common in rivulets at foothills, and is also beneficial in clear water. This fish is mainly use to control malaria vector.

Colisa

Three species of this genus are useful as larvicide: *Colisa fasciatus* (as Giant gourami), *Colisa lalia* (Dwarf gourami), and *Colisa sota* (Sunset gourami). *C. fasciatus* is effective in controlling vectors of both brugian filariasis and malaria, whereas *C. lalia and C. sota* only control malaria vector.

Chanda Nama (Common Name: Elongate Glass Perchlet)

It is widely distributed in fresh water bodies with thick vegetation and breeds freely in confined waters and rivers. The species is useful as a larvicidal fish in forest pools, streams, tanks, and ditches overgrown with vegetation for control of malarial vector.

Mixed Culture of Larvivorous Fishes as Biocontrol Agents

To obtain high productivity per hectare of the water body, fast-growing compatible species of fish either of different feeding habits or different weight classes of the same species are stocked together in the same pond so that all its ecological niches are occupied

by fish. This system of pond management is called *mixed fish farming*, *composite fish culture*, or *polyculture*, which has proven to be useful in controlling various mosquito population.

The Mosquito Crustacean (Macrocyclops Albicus)

This is a small, shrimplike member of the *Crustacea* family, which can be found in ditches, ponds, and clogged gutters of a house. This can eat up to 27 mosquito larvae per day and can easily survive in a small amount of water, the place where mosquitoes prefer to breed. Unlike mosquito fish, these crustaceans never pose a risk to the balance of the ecosystem and are thus more helpful for use in biological control of mosquitoes.

Dragonfly Nymphs

Dragonfly larvae, designated as nymphs, feed on mosquito larvae, whereas adult dragonflies feed on adult mosquitoes. The nymphs will eat mosquito larvae in the water, and adults will confine themselves on the rocks, waiting for the adults to leave the water. Singh et al. (2003) conducted an experiment to check the biocontrol potential of dragonfly nymph, *Brachythemis contaminata* against the larvae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* and observed that they had very good predatory potential and, therefore can be used as a biological control agent for controlling of mosquito. Dragonflies are also considered one of the best mosquito control solutions because one adult dragonfly can eat its own body weight in mosquitoes each night. Both stages the nymph and adult dragonflies are the ultimate opportunist of nature because they eat whatever is plentiful. In the presence of mosquitoes, they will eat them and can reduce their population, but once the mosquito population start to gets scarce, they will eat every last one and then simply switch to some other insect prey. Hence, dragonflies could be used to control the population of mosquitoes but not eradicate them.

Protozoa

The mosquito population can be kept in check by the activities of various parasites and predators. Lamborn (1921) first reported *Lambornella stegomyiae*, an endoparasitic ciliated protozoan infection in the larvae of *A. albopictus* in a sample collected in Kuala Lumpur. After almost 50 years after this study, a second species of *Lambornella* (*L. clarki*) was isolated from tree hole-breeding mosquito larvae, *Aedes sirensis* in California (Corliss and Coats, 1976). Another known protozoan group, designated as *Microsporidia*, contains many species useful in biological control. Although insect infections with *Microsporidia* are common and responsible for naturally occurring insect mortality, these are slow-acting organisms, taking days or weeks to debilitate their host. Microsporidians such as *Nosema*, *Thelohania*, *Parathelohania*, *Amblyospora*, and *Vavraia* have already been studied for mosquito control efficacy (Poopathi and Tyagi, 2006). Microsporidia can infect a wide range of insects and enters the insect body via the gut wall, spreads to various tissues and organs, and finally starts multiplication that causes tissue breakdown and septicemia.

Another important protozoan is the genus *Helicosporidium*, which is found to be associated with the diseased larvae of *C. pipiens* L and showing larvicidal potential.

Helicosporidium was also infectious to *A. caspius* (Pallas), *Culex antennatus* (Becker), *Culex perexiguus* (Theobald), and *Culiseta longiareolata* (Macquart). *Chilodonella uncinata* is also a ciliated protozoan found associated with chronic and fatal infection in mosquitoes in and around Delhi, North India (Das, 2003). Das reported that anopheline larvae were less susceptible to *Chilodonella* infection(14.13 percent) than culicine larvae (75.21 percent). *C. uncinata* is a free-living facultative parasite of mosquito larvae exhibits virulence against highly susceptible host including *C. tritaeniorhychnus* and *C. pseudovishnui*. It affects host tissues, such as gut epithelium, fat bodies, muscles, and malpighian tubules and causes chronic infection in susceptible host mosquito larvae with high mortality rate. They preferentially attack young mosquito larvae and invade the host hemocoel by dissolving the host cuticle and forming cuticular cysts. On reaching the host hemocoel, the ciliate allows the host larva to grow and acquires the fourth stage. Simultaneously, the ciliate also multiplies and increases its number immensely at the expense of host tissues. Finally, the ciliate kills the host and the larva turns discolored and opaque. After the death of the host larva, *C. uncinata* continues to grow and fulfill almost the entire body cavity of the susceptible host. At this stage, the infected larva turns transparent, with thousands of visible motile microscopic endoparasitic stage in the host's body cavity.

Mermithid Nematodes

Mermithid nematodes are roundworms, which are free-living adults and parasitic in their developing stages. They are also named rainworms because they were once thought to fall from the sky with the rain. At least 25 species of Mermithid nematodes are known to parasitize mosquito larvae, which makes them of substantial potential in biological vector control (Platzer, 1981). The best known genus for this purpose is *Romanomermis* particularly the species, *Romanomermis culicivorax*. The life cycle of *R. culicivorax* involves only larval stages of mosquitoes. Preparasitic juveniles of the nematode attack the mosquito larva and penetrate into the hemocoel via the cuticle (Figure 23.3). Mosquito larvae die within 7 to 10 days post-infection; at that time the postparasitic juveniles penetrate the host's body and complete their development. Here the mermithids molt, mate, and the females ovipositin the bottom sediments. *R. culicivorax* has a broader host range comprising more than 90 species of mosquitoes in 13 genera (Peterson, 1985). The susceptibility of any host mosquito is related to its larval stage, the first instars are more susceptible, and the fourth instars are least. In mixed mosquito populations, anophelines are more susceptible than culicines (Peterson, 1985).

Other mermithids are *Romanomermis iyengari* and *Octomyomermis muspratti*, both with high specificity to mosquito larvae. These mermithids also undergo part of their development cycle within mosquito larvae, and they further recycle and infect mosquito larvae season after the season in nature. However, they cannot tolerate extreme pH and pollution; hence they have yet to be developed for use in a polluted environment. *R. iyengari* and *R. culicivorax* have a broad host range and are promising biocontrol agents for different species of mosquitoes (Petersen, 1985). The nematode *Strelkovimermis spiculatus* was also reported as a promising biocontrol agent against the mosquito *C. quinquefasciatus* in Cuba (Rodriguez et al., 2003). Although these mermithids do not prevent female mosquitoes from blood feeding, they can cause biological castration, preventing the mosquitoes from reproducing (Trips et al., 1968).

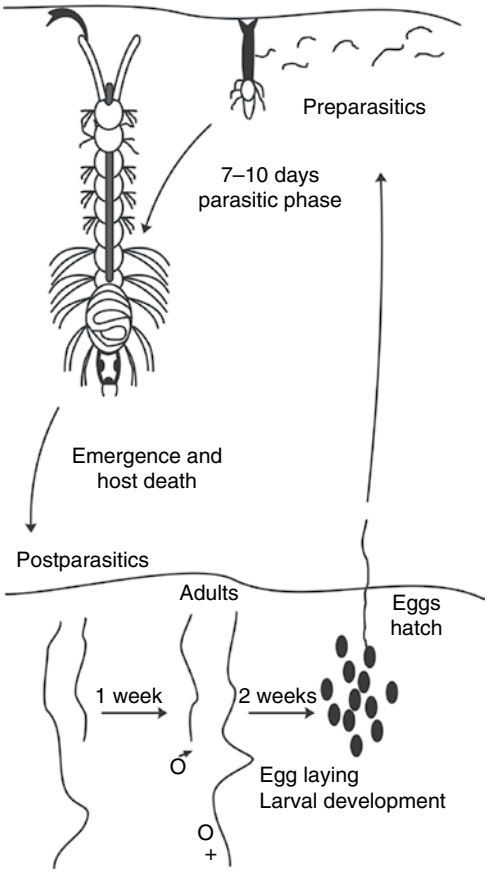

Figure 23.3. Life cycle of *Romanomermis culicivorax* in *Culex pipiens* mosquito.

Predator Larvae

Some mosquito species do not require a blood meal because they suck plant sap and nectar, but their larvae are a predator of other mosquito larvae. These predatory mosquitoes can be used in biological control, which results in complete control of the targeted population. One of the genera in this category of mosquito is *Toxorhynchites*, which is the most common arthropod that has been used for control of container-breeding mosquitoes and thereby are attractive in biological control. Its species *Toxorhyncites splendens* is a nonbloodsucking predatory mosquito whose larvae were found to be effective in controlling *Anopheline* and *Culicine* larvae (Amalraj and Das, 1998). The predatory effect of this mosquito species was also proven through field evaluation studies against 13 mosquito species especially *Culex* and *Aedes* conducted in Japan (Miyagi et al., 1992). *T. splendens* larvae also found to suppress *A. aegypti* populations in household water storage containers in Jakarta (Annis et al., 1989). They can eat as many as 400 larval mosquitoes during larval development. One of the major advantages of a *Toxorhynchites* release program is that the adult mosquito can disperse and lay eggs in

areas most likely to escape treatment with insecticides. Another predatory mosquito is *Rhantus sikkimensis*, whose prey-killing capability has been expected more as compared to the larvae of *T. splendens* (Gautam et al., 2006).

Genetic Engineering

Vector-borne diseases impose enormous health and economical burdens throughout the world; this burden will further increase with the excessive use of insecticide and spread of drug resistance unless new control measures are developed. The design, development, and testing of new and improved vector control tools and techniques are a critical research issue. Along with the conventional vector control strategies based on the chemical, biological, and environmental interventions, new methods based on biotechnological advances and the success of mosquito control can be developed for use in addressing human health issues. One possible control strategy is genetic modification of vectors so that they are no longer able to transmit parasites. Along with the use of several biocontrol agents, several effector molecules have been identified that interfere with the parasite development in its insect vectors and vectors can then be transformed in a manner such that genes start encoding these effector molecules. These antiparasitic effector molecules could be transferred to wild vector populations by bacteria. The mosquitocidal toxin genes from *Bti* and different *Bs* strains can be cloned that allow the re-expression of a combination of toxins from both species in one recombinant cell in an attempt to broaden the host range of toxins and obtain beneficial synergistic effects (Bourgouin et al., 1990; Bar et al., 1991). Homologous recombination technique can be applied to *Bti* or *Bs* (or other bacteria) to integrate novel combinations of mosquitocidal toxin genes into the bacterial chromosome and then study their expression during the vegetative or sporulation phase of cell growth. A novel approach to controlling pests uses heat-killed, encapsulated vegetative cells of *Pseudomonas fluorescens*, which are genetically engineered to express high levels of *Bt* toxins. Efforts have been made to evaluate genetically engineered vegetative bacteria, such as cyanobacteria, *Caulobacter*, and *Ancylobacter aquaticus*, as delivery vehicles form mosquitocidal toxins (Thanabalu, 1992a, 1992b a,b). Being surface dwellers, cyanobacteria, caulobacters, and *A. aquaticus* can resist rapid inactivation by ultraviolet light. These bacteria are not pathogenic and are easily grow in a simple, inexpensive media. Moreover, the reproduction of these bacteria occurs naturally in environments with low nutrient levels, unlike the spores of bacilli, which depend on protein-rich larval cadavers for their germination. Various cyanobacterial species have been investigated as vehicles for prolonged delivery of insecticidal toxins to the larval feeding zone. (de Marsac et al., 1987; Angsuthanasombat and Panyim, 1989; Chungjatopornachai, 1990; Murphy and Stephensen, 1992). Various toxin genes from *Bti* and *Bs* have been cloned into different cyanobacteria, *Caulobacter crescentus*, or *A. aquaticus*, and as a result, recombinant cyanobacterial cells have been shown to be significantly toxic to *Culex* and *Aedes* larvae. Although toxin expression levels were low, the characteristic buoyancy of the cells may prolong their larvicidal action. Before releasing engineered bacteria, many issues must be raised, and experiments performed to determine that the modified organism would kill only mosquito larvae and not disturb the ecosystem. There are some powerful arguments both for and against the release of genetically manipulated microorganisms. Protagonists point out that there is an urgent need for the development of a new approach to control these biological vectors of

parasitic and viral diseases. The decision regarding the release of a genetically modified organism must be taken on a case-by-case basis and should be made after careful consideration.

One variation of genetic technique employs killing of female mosquitoes only. Genetically modified mosquitoes (GMM) can be raised that are not infected with any diseases. For this purpose, the mosquito eggs are injected with a gene and then put a new chemical into the DNA, which kills only the female mosquitoes while allowing the male mosquitoes to survive. The males carry the female-killer, but they are not sterile so they can mate with females in the wild, but all of their surviving offspring will be male because the female offspring was killed by the female-killer DNA, which they have inherited. Now the resulting offspring of the modified mosquitoes have only one copy of the introduced DNA as compared to the two copies of their fathers. Approximately half of their male offspring will carry the female-killer gene and half will not so this gradually gets filtered out of the population over time. New batches of modified males would need to be released quite often, about once a week, to keep the lower number of wild mosquitoes. The advantage of this female-killing process over pesticide sprays is that the pesticides kill a broad range of species, including insects that are liked, but this technique only kills the specific type of mosquitoes.

The mosquito host range of the toxins is narrow and difficult to predict. The spores do not significantly multiply outside the larval cadaver and the spore-crystal complex is sensitive to ultraviolet light, which limits the duration of biological control. Insecticidal bacteria are generally costlier to produce than chemical insecticides. However, a combination of novel genetic manipulation approaches, coupled with existing formulation technology or the use of engineered vegetative bacteria that can exist in the upper layer of water, may become helpful in these problems. Biological control of mosquito larvae mainly relies on the use of two important entomopathogenic bacteria, *Bs* and *Bti*. During sporulation both bacteria synthesize proteins that assemble into crystals, which are toxic for the larvae upon ingestion. *Bti* crystals are composed of four major polypeptides with molecular weights of 125, 135, 68, and 28 kDa, respectively, and now referred to as Cry IVA, Cry IVB, Cry IVD, and Cyt A, respectively (Priest et al., 1997). Genes encoding all these polypeptides are located on a 72 kDa resident plasmid (Delecluse et al., 1995) and have all been cloned and expressed in various hosts. The initial detection and field surveillance for resistance will likely continue to be based upon simple bioassays, biochemical and molecular tools, and the deeper understanding of how resistance arises. Resistance detection should be always made an integral part of all control programs. The resources for vector control, even under emergency situations, are limited and, therefore, must be used as effectively as possible. One of the major weapons for countering resistance has been the use of alternative chemicals with structures that are unaffected by cross-resistance. As resistance has been developed against many chemicals, there is a need to maximize the effect of new chemicals through their application under conditions that delay or prevent the development of resistance. Hence, it is essential to understand the parameters influencing the selection process. The development of new field of metabolic engineering involves the improvement of cellular activities by manipulating enzymatic, transport, and regulatory functioning of the cell using recombinant DNA technology. In the last decade metabolic engineering has emerged as an interdisciplinary field aiming to improve cellular properties by using modern genetic tools to modify pathways (Nielsen, 2001). With the rapid developments in new analytical techniques and cloning procedures, it is now possible to introduce genetic changes directly in microbes and then subsequently

analyze the consequences of those introduced changes at the cellular level. Advancements in the field of genetic engineering, whole genome sequencing, and developments in bioinformatics have sped up the process of gene cloning and transformation. Metabolic engineering is, therefore, concerned with modifying pathways and then assessing the physiological outcome of such genetic alterations in an effort to improve the degradative abilities of microorganisms (Jain et al., 2005).

References

Amalraj D, Das PK.1998. Estimation of predation by the larvae of *Toxorhynchites splendens* on the aquatic stages of *Aedes aegypti*. *Southeast Asian J Trop Med Public Health* 29: 177–183.

Angsuthanasombat C, Panyim S. 1989. Bioasynthesis of 130-kd mosquito larvicide in the cyanobacterium *Agmenellum quadruplicatum* PR-6. *Appl Environ Microbiol* 55:2428–2430.

Annis B, Krisnowardojo S, Atmosoedjono S, Supardi P. 1989. Suppression of larval *Aedes aegypti* populations in household water storage containers in Jakarta, Indonesia, through releases of firstinstar *Toxorhynchites splendens* larvae. *Amer Mosq Control Assoc* 5:235–238.

Arêa Leão AE, Carlota Pedroso M. 1964. Nova especie do genero Coelomomyces parsito de ovos de Phlebotomus. *Mycopathologia Et Mycologia Applicata* 26:305–307.

Bar E, Lieman-Hurwitz J, Rahamim E, Keynan A, Sandler N. 1991. Cloning and expression of *Bacillus thuringiensis* d– endotoxin DNA in *B. sphaericus*. *J Invertebr Pathol* 57:149–158.

Bisht GS, Joshi C, Khulbe RD. 1996. Watermolds: Potential biological control agents of malaria vector *Anopheles culicifacies*. *Current Science* 70:393–395.

Blanford S, Chan BH, Jenkins N, Sim D, Turner R J, et al. 2005. Fungal pathogen reduces potential for malaria transmission. *Science* 308:1531–1533.

Bourgouin C, Delecluse A, de la Rorre F, Szulmajster J. 1990. Transfer of the toxin protein genes of *Bacillus sphaericus* into *Bacillus thuringiensis* subsp. *israelensis* and their expression. *Appl Environ Microbiol* 56:340–344.

Broadwell AH, Baumann P. 1986. Sporulation associated activation of *Bacillus sphaericus* larvicide. *Appl Environ Microbiol* 52:758–764.

Broadwell AH, Baumann L, Baumann P. 1990. Larvicidal properties of the 42 and 51 kilodalton *Bacillus sphaericus* proteins expressed in different bacterial hosts: evidence for a binary toxin. *Curr Microbiol* 21:361–366.

Chandrahas RK, Rajagopalan PK. 1979. Observations on mosquito breeding and the natural parasitism of larvae by a fungus *Coelomomyces* and a mermithid nematode *Romanomermis* in paddy fields in Pondicherry. *Indian J Med Res* 69:63–67.

Chapman HC. 1974. Biological control of mosquito larvae. *Annu Rev Entom* 19:33–59.

Chungjiatupornchai W. 1990. Expression of the mosquitocidal protein of *Bacillus thuringiensis* subsp *israelensis* and the herbicide resistance gene bar in *Synechocystis* PCC6803. *Curr Microbiol* 21:283–288.

Corliss JO, Coats DW. 1976. A new cuticular cyst producing tetrahymenid ciliate, *Lambornella clarki* n. sp. and the current status of ciliatosis in culicine mosquitoes. *Trans Am Microsc Soc* 95:725–739.

Crickmore N, Bone EJ, Williams JA, Ellar DJ. 1995. Contribution of the individual components of the -endotoxin crystal to the mosquitocidal activity of *Bacillus thuringiensis* subsp. *israelensis*. *FEMS Microbiol Lett* 131:249–254.

Cuda JP, Hornby JA, Cotterilli B, Cattell M. 1997. Evaluation of *Coelomomyces* for biocontrol of Mansonia mosquitoes in Florida (*Diptera: Culicidae*) *Biological Control* 8:124–130.

Das BP. 2003. *Chilodonella uncinata*–a protozoa pathogenic to mosquito larvae. *Current Science* 85:483–489.

de Marsac NT, de la Torre F, Szulmajster J. 1987. Expression of the larvicidal gene of *Bacillus sphaericus* 1593 Min the cyanobacterium *Anacystis nidulans* R2. *Mol Gene Genet* 209:396–398.
Delecluse A, Rosso ML, Ragni A. 1995. Cloning and expression of a novel toxin gene from *Bacillus thuringiensis* susp. *Jegathesan* encoding a highly mosquitocidal protein. *Appl Environ Microbiol* 61:4230–4235.
Feitelson JS, Payne J, Kim L. 1992. *Bacillus thuringiensis*: insects and beyond. *BioTechnol* 10:271–275.
Gautam A, Anirban A, Goutam S K. 2006. Predatory activity of *Rhantus sikkimensis* and larvae of *Toxorhynchites splendens* on mosquito larvae in Darjeeling, India. *J Vect Borne Dis* 43:66–72.
Goettel MS, Inglis GD. 1997. Fungi: Hyphomycetes. In: Lacey LA, ed., *Manual of Techniques in Insect Pathology*, pp. 213–249. San Diego: Academic Press.
Hora SL, Mukherjee DD. 1938. The absence of millions, *Lebistes reticulates* (Peters) in India. Malaria Bureau No.4, *Health Bulletin Delhi* 12:1–49.
Jain RK, Kapur M, Labana S, Lal B, Sharma P M, et al. 2005. Microbial diversity: application of microorganism for the biodegradation of xenobiotics. *Current Science* 89:101–112.
Jaronski S, Axtell RC. 1983. Persistence of the mosquito fungal pathogen *Coelomomyces giganteum* (*Oomycetes; Lagenidiales*) after introduction into natural habitats. *Mosquito News* 43:332–337.
Jenkins DW. 1964. Pathogens, parasites and predators of medically important arthropods. *Bull WHO* 30 suppl:28–37.
Kerwin JL, Washino RK. 1987. Ground and aerial application of the asexual stage of *Coelomomyces giganteum* for control of mosquitoes associated with rice culture in the central valley of California. *Jour Am Mosq Contl Assoc* 3:59–64.
Lacey LA, Undeen AH. 1986. Microbial control of black flies and mosquitoes. *Annu Rev Entom* 31:265–296.
Lamborn WA. 1921. A protozoan pathogenic to mosquito larvae. *Parasitology* 13:213–215.
Lopez CC, Scorsetti AC, Marti GA, Garcia JJ. 2004. Host range and specificity of an Argentinean isolate of the aquatic fungus *Leptolegnia chapmanii* (*Oomycetes: Saprolegniales*), a pathogen of mosquito larvae (Diptera: Culicidae). *Mycopathologia* 158:311–315.
McCray EM Jr, Umphlett CJ, Fay RW. 1973. Laboratory studies on a new fungal pathogen of mosquitoes. *Mosquito News* 33:54–60.
Merrriam TL, Axtell RC. 1982. Evaluation of the entomogenous fungi *Culicinomyces clavosporus* and *Coelomomyces giganteum* for control of the salt marsh mosquito, *Aedes taeniorhynchus*. *Mosquito News* 42:594–602.
Miyagi I, Toma T, Mogi M. 1992. Biological control of container breeding mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus*, in a Japanese island by release of *Toxorhynchites splendens* adults. *Med Vet Entomol* 6:290–300.
Mohanty SS, Prakash S. 2003. Laboratory evaluation of Trichophyton ajelloi, a fungal pathogen of *Anopheles stephensi* and *Culex quinquefasciatus*. *Jour Am Mosq Contl Assoc* 16:254–257.
Murphy RC, Stephensen Jr S E. 1992. Cloning and expression of the cryIV gene of *Bacillus thuringiensis* subsp. *israelensis* in the cyanobacterium *Agmenellum quadruplicatum* PR-6 and its resulting larvicidal activity. *Appl Environ Microbiol* 58:1650–1655.
Nielsen J. 2001. Metabolic engineering. *Appl Microbiol Biotechnol* 55:263–283.
Nnakumusana ES. 1985. Laboratory infection of mosquito larvae by entomopathogenic fungi with particular reference to *Aspergillus parasiticus* and its effects on fecundity and longevity of mosquitoes exposed to sporal infections in larval stages. *Current Science* 54:1221–1228.
Petersen JJ. 1985. Nematodes as biological control agents: Pt I, Mermithidae. *Adv Parasitol* 14:307–334.
Platzer EG. 1981. Biological control of mosquitoes with mermithids. *Jour Nematol* 13:257–262.
Poopathi S, Tyagi BK. 2006. The challenge of mosquito control strategies: From primordial to molecular approaches. *Biotech Mol Biol Rev* 1:51–65.

Poopathi S, Rao DR, Mani TR, Baskaran G, Kabilan L. 1999. Effect of *Bacillus sphaericus* and *Bacillus thuringiensis* var *israelensis* on the ultrastructural changes in the midgut of *Culex quinquefasciatus* Say. (Diptera: Culicidae). *Jour Entmol Res* 23:347–357.

Priest F, Ebdrup L, Zahner V, Carter, P. 1997. Distribution and characterization of mosquitocidal toxin genes in some strains of *Bacillus sphaericus*. *App Environ Microbiol* 63:1195–1198.

Raghavendra K, Subbarao SK. 2002.Chemical insecticides in malaria vector control in India. *ICMR Bull* 32:1–7.

Ramathilaga A, Murugesan AG, Prabu CS. 2012. Biolarvicidal activity of *Peanibacillus macerans* and *Bacillus subtilis* isolated from the dead larvae against *Aedes aegypti*—Vector for Chikungunya. *Proceed International Acad Ecol Environ Sc* 2:90–95.

Riehle MA, Jacobs-Lorena M. 2005. Using bacteria to express and display anti-parasite molecules in mosquitoes: current and future strategies. *Insect Biochem Mol Biol* 35:699–707.

Roberts DW, Strand A. 1977. Pathogens of medically important arthropods. *Bull WHO* 55 (Suppl I):419.

Roberts DW. 1970. *Coelomomyces*, *Entomophtora*, *Beauveria*, and *Metarrhizium* as parasites of mosquitoes. *Miscellaneous Publications of the Entomological Society of America* 7:140–155.

Roberts DW. 1974. Fungal infections of mosquitoes. In: Aubin A, Belloncik S, Bourassa JP, LaCoursière E, Péllissier M, eds., *Le contrôle des moustiques/Mosquito control*, pp 143–193. Québec: Presses de l'Université du Québec.

Rodriguez J, Garcia IG, Diaz M, Avila IG, Sanchez JE. 2003. Pathogenic effect of the parasite nematode *Strelkovimermmis spiculatus* (*Nematoda Mermithidae*) in larvae of mosquito *Culex quinquefasciatus* (Diptera: Culicidae) under laboratory conditions in Cuba. *J Med Ent* 55:124–125.

Singer S. 1973. Insecticidal activity of recent bacterial isolates and their toxins against mosquito larvae. *Nature (London)* 244:110–111.

Singh RK, Dhiman RC, Singh SP. 2003. Laboratory studies on the predatory potential of dragonfly nymphs on mosquito larvae. *Commun Dis* 35:96–101.

Thanabalu T, Hindley J, Berry C.1992a. Proteolytic processing of the mosquitocidal toxin from *Bacillus sphaericus* SSII-1. *J Bacterial* 174:5051–5056.

Thanabalu T, Hindley J, Brenner S, Oei C, Berry C.1992b. Expression of the mosquitocidal toxins of *Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis* by recombinant *Caulobacter crescentus*, a vehicle for biological control of aquatic insect larvae. *Appl Environ Microbiol* 58:905–910.

Trips M, Haufe WO, Shemanchuk JA. 1968. Mermithid parasites of the mosquito *Aedes vexans* Meigen in British Columbia. *Canad Jour Zool* 46:1077–1079.

Chapter 24

Other Disease Vectors and Their Control

Housefly

The housefly (*Musca domestica*) is the most common of all the domestic flies and is one of the most widely distributed insects. They are considered common household pests, which visit dumps, sewers, and garbage heaps, feed on fecal matter, discharges from wounds and sores, sputum, and all sorts of moist, decaying matter, such as spoiled fish, eggs, and meat. House flies mechanically transmit various disease organisms because they regurgitate and excrete wherever they come to rest. House flies are suspected of transmitting around 65 diseases to humans. They are gray in color, approximately 1/4-inch (6-mm) long, and have four dark longitudinal stripes on top of the thorax or middle body region. Their mouth parts are well adapted for sponging up liquids. They cannot bite only ingest liquid or semisolid food, although also feed on solid food by regurgitating saliva onto it. The solid material liquefies by the saliva, which is then sponged up with the proboscis. They require water to salivate continuously. Because they ingest large amount of food they constantly deposit feces; this factor makes them a dangerous carrier of pathogens. A house fly is a domestic fly, which usually confined to human habitations, but it can also fly up to several miles from its breeding place. They are active only in daytime, and rest at night in places, such as at the corners of rooms, ceiling hangings, cellars, and barns. Here they can survive the coldest winters by hibernation, and when spring arrives, adult flies are seen only a few days after the first thaw. They are capable of carrying more than 100 pathogenic agents causing typhoid, cholera, salmonellosis, bacillary dysentery, tuberculosis, anthrax, ophthalmia, and parasitic worms. Table 24.1 lists the various pathogens transmitted by house fly.

Each female house fly deposits about 100 to 150 eggs on dead and decaying organic matter, such as garbage and human and animal excreta. The preferred breeding medium is horse manure. These eggs hatch to form wormlike creatures called *maggots* in 1 to

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

Table 24.1. Pathogenic agents transmitted by the house fly.

Type of Agent	Pathogen and Disease
Parasite	For example protozoan cysts as *Entamoeba histolytica*, *Giardia lamblia*, and helminthic eggs (e.g., *Ascaris lumbricoides*, *Hymenolepis nana*, *Enterobius vermicularis*, and *Trichuris trichiura*)
Bacteria	For example *Salmonella typhi*, *Vibrio cholerae*, *Shigella dysenteriae*, pyogenic cocci, etc.
Virus	For example enteroviruses, poliomyelitis, viral hepatitis, etc.

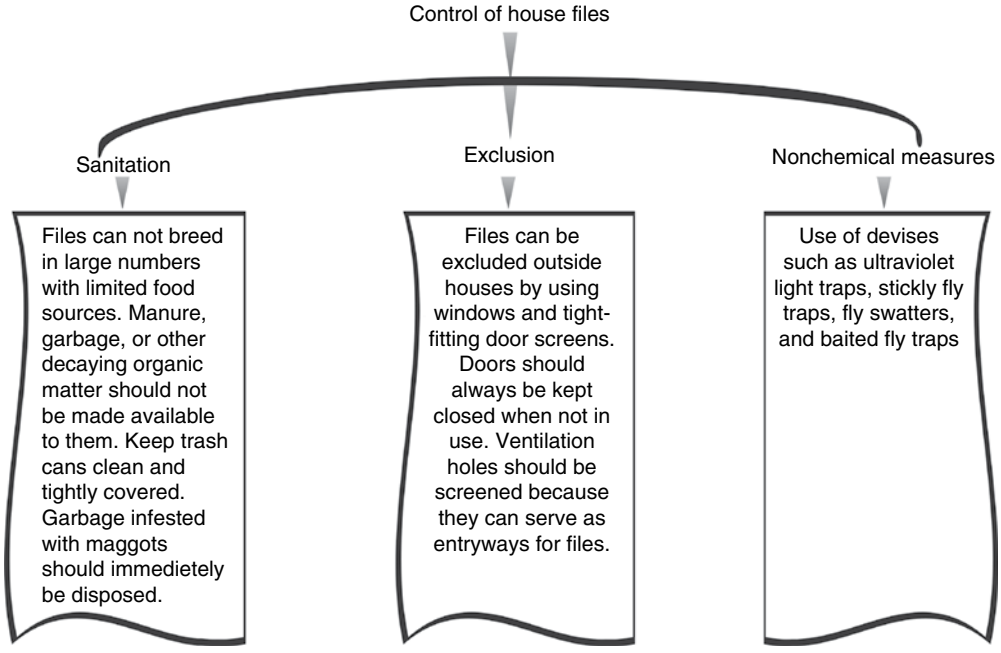

Figure 24.1. Control of house flies.

2 days. After three larval molts, mature larvae stop feeding and then burrow into drier surrounding areas, where they pupate. Their pupa is a chestnut-brown, oval object within which the larva converts into an adult house fly. Adults start mating within 1 to 2 days after emerging from their pupal cases. The life cycle from egg to adult can also be completed in 1 week, but it typically takes 3 weeks. House fly adults normally live about 2 1/2 weeks during the summer season, but they can usually survive up to 3 months at lower temperatures.

To control the population of house flies, three basic principles should be considered, including sanitation, exclusion, and some nonchemical measures (Figure 24.1). Sanitation will provide the best long-term control, whereas exclusion and nonchemical measures provide shorter term management.

Sand Fly

Sand flies are the most widespread and one of the most irritating of all the insects that bite human beings. The sand fly is a colloquial name given to the genus of biting, blood-sucking flies belong to the order *Diptera*, which are especially encountered in sandy areas. In the United States, sand fly are categorized with certain horse flies (also called as *greenheads*) classified under family *Tabanidae*, whereas outside the United States, it is classified under subfamily *Phlebotominae* within *Psychodidae*. Only the female counterpart of sand fly bites and sucks the blood of mammals, reptiles, and birds because she requires a protein present in the blood to complete the reproductive cycle and to make eggs. Sand fly bites a host and injects its saliva, which contains an anticoagulant nd helps the flea in drawing blood from the host. Its saliva also contains allergens, which trigger the immune system of the host body and to develop specific inflammatory lesions.

Sand flies generally breed in fast-flowing streams or rivers and lay their eggs on rocks or plants around or below water level. Larvae hatch and then pupate on collecting the food and spend around 12 days there, before emerging as flies at the surface of the water. After mating, the female starts searching for a blood meal, but the male is a vegetarian. Sand flies cannot see at night, so they seldom bite in the dark and generally remain outdoors. Peaks in biting occur when light intensity increases in the morning and decreases at dusk. They are most active in dull, overcast, and humid conditions, where they can bite at a similar rate throughout the day and carry the parasite of visceral leishmaniasis and the Chandipura virus encephalitis. It is better to prevent the sand fly bite because there is no known efficient method of controlling sand fly bites. Severe reactions may require medical treatment with antihistamines. The preferred areas of attack by the sand flies are exposed legs and ankles followed by other extremities, such as the hands, arms, neck, and face of humans, therefore, biting could be reduced by covering these areas.

There are many remedies, both pharmaceutical and home, for sand fly bites (Figure 24.2). Some of the mosquito repellents as DEET and lemon eucalyptus oil are also reported being effective against sand flies (Centers for Disease Control and Prevention [CDC], 2009).

Deer Fly

Deer flies are pests of cattle, horses, and humans and belong to the genus *Chrysops* of the family *Tabanidae* distributed worldwide. They are commonly called horse flies because their distinguishing characteristic feature is patterned gold or green eyes (Milne and Milne, 1980). They are usually found in damp environments, such as wetlands or forests, and are considered as the potential vectors for the mechanical transmission of tularemia, anthrax, and loa loa filariasis diseases. Their predators include nest-building wasps and hornets, dragonflies, and some birds including the killdeer. Similar to mosquitoes, the female deer fly is actually responsible for inflicting a bite, whereas males are mainly pollen and nectar feeders. They are usually active during daylight hours.

Female deer fly laid around 100 to 1,000 eggs in layers on a vertical surface such as overhanging foliage, projecting rocks, sticks, and preferably aquatic vegetation.

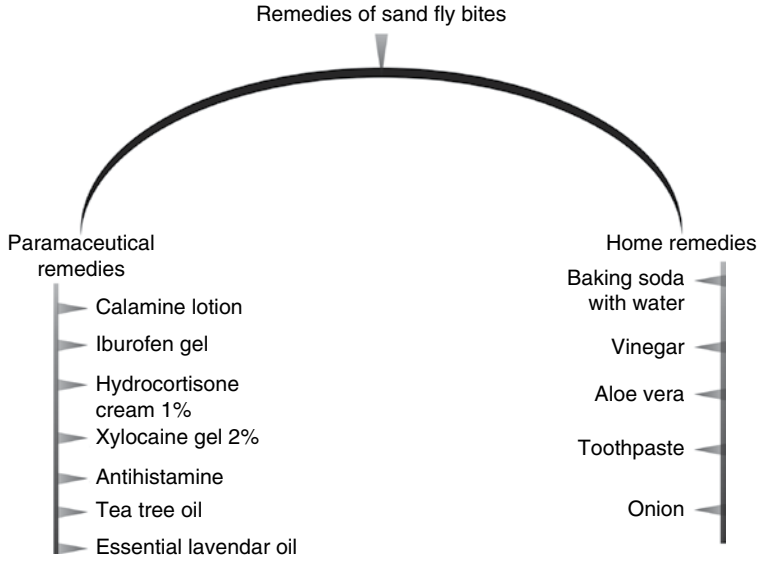

Figure 24.2. Pharmaceutical- and home-based medications for sand fly bite.

Chrysops spp. are "hydrobionts" because they are usually found in areas with high water content, whereas *Tabanus* spp. prefers dryer substrates and "hemi-hydrobionts." Eggs finally hatch into larvae and the larval stage can last from a few months to a year. The larvae of *Chrysops* feed on organic matter in the soil, whereas *Tabanus* spp. feed on insect larvae, crustaceans, and earthworms. The larva moves into the upper 2.5 to 5.0 cm of the soil, where it gets dry and then readies to pupate. Within 2 days after moving to the surface it turns to a brown-colored pupa, which are rounded anteriorly, tapering posteriorly, and have leg and wing cases attached to the body. This pupal stage generally lasts from 2 to 3 weeks and then the adult fly emerges from the pupae via a slit located along the thorax. In most of the species, the males emerge before the females and then mate. Mating is initiated in the air but completed on the ground. The female then deposits the egg mass and is ready to seek a host.

Deer flies penetrate the host skin in a scissorlike action and pump the anticoagulants present in the saliva into the wound then ingest the blood through the sponging labella. A deer fly attack in livestock animals results in lowered gains and low milk production. A deer fly bite can be extremely painful, and the victim can develop an allergic reaction, resulting in discomfort with pain and itching; more significant allergic reactions are also possible (Bartlett, 1999).

Currently there are no effective biological control programs for controlling tabanids. There are native beneficial insects that target tabanids and their eggs are parasitized by *Hymenoptera* families as *Trichogrammatidae*, *Scelionidae*, and *Chalcididae*, whereas larvae and pupa are parasitized by *Diapriidae* and *Pteromalidae* (*Hymenoptera*) and *Bombyliidae* and *Trachinidae* (*Diptera*). Personal protection can be accomplished by wearing a long sleeve shirts and pants in combination with a repellent containing DEET, citronella, or geraniol.

Black Fly

Black flies are small, dark flies belonging to the family *Simuliidae* of the order *Diptera*, and have a painful bite. Unlike a mosquito, black flies slash the skin and lap up the pooled blood. Their larvae and pupae attach themselves to rocks and vegetation in flowing streams. It is also referred to as buffalo gnat, turkey gnat, or white socks. There are around 1,800 known species of black flies and all females gain nourishment by feeding on the blood of mammals, including humans, although the males feed mainly on nectar. They are usually small, black or gray with short legs and antennae.

Black flies are the vectors of various diseases, including the filarial disease river blindness in Africa (*Simulium damnosum* and *Simulium neavei*) and the America (*Simulium callidum* and *Simulium metallicum* in Central America, *Simulium ochraceum* in Central and South America). River blindness is also called onchocerciasis because it is caused by a nematode parasite *Onchocerca volvulus*. The parasite resides on human skin and is transmitted to the black fly during feeding (Service, 2008). When this infected black fly carrying the larvae of *O. volvulus* bites a victim, it transmits the infection. In the host body, the larvae grow into groups of stringlike worms that live wrapped up in a bump under the skin. Adult female worms produce thousands of tiny microfilariae for approximately 8 to 12 years, which migrate throughout the victim's skin and cause skin disfigurement and intense itching. The worms can also cause permanent damage if they reach the eyes, impairing sight and finally causing complete blindness. When another black fly bites the victim and then bites an uninfected person, it begins the infectious cycle.

Intense feeding by the black flies can cause "Black Fly Fever," which is associated with headache, nausea, fever, swollen lymph nodes, and aching joints. Severe allergic reactions may require patient hospitalization (Mullen and Durden, 2009). Black flies can also reduce the milk production from dairy cows.

Tsetse Fly

Tsetse flies belong to the genus *Glossina* of the family *Glossinidae*. These are the large biting flies inhabiting mid-continental Africa between the Sahara and the Kalahari deserts (Rogers et al., 1996). They feed on the blood of vertebrates and are the primary biological vectors of the parasite *Wuchereria bancrofti*, which causes elephantitis and trypanosomes, which cause human sleeping sickness and animal trypanosomiasis (nagana). It transmits the parasite both through mechanical and biological transmission. Tsetse can be distinguished from other flies as having four characteristic features:

1. A distinct proboscis, a long, thin structure attached to the bottom of the head and pointing forward.
2. At resting, tsetse fold their wings completely one on top of the other.
3. The discal medial cell of the wing has a characteristic hatchet shape resembling a meat cleaver or a hatchet.
4. The antennae have arista with hairs which are branched.

Tsetse has been extensively studied among flies because of their medical, veterinary, and economic importance. They are relatively large in size thus can be raised in a laboratory.

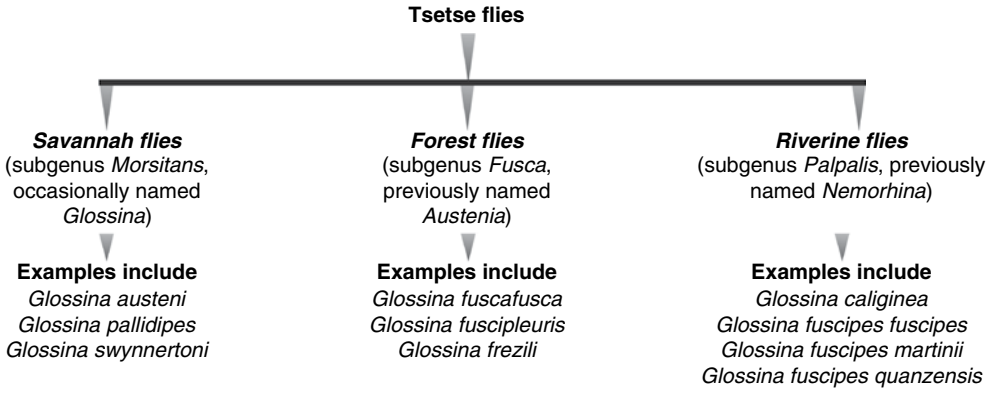

Figure 24.3. Categories of Tsetse flies.

The tsetse genus is further categorized into three groups based on a combination of distributional, behavioral, molecular, and morphological characteristics (Jordan, 1993) (Figure 24.3).

Tsetse flies have an unusual life cycle pattern because of the richness of their food source. The female fertilize only one egg at a time and retain each egg within their uterus to allow the internal development of offspring during the first larval stages through the process of adrenotrophic viviparity. In this duration, the female feeds the developing offspring with a milky substance secreted by a modified gland in the uterus. The tsetse larvae then leave the uterus and begin their independent life in the third larval stage. However, the newly independent tsetse larva simply crawls into the ground and forms a hard outer shell called the puparial case, in which it completes its morphological transformation to an adult fly. This life cycle shows a variable duration, ranging from 20 to 30 days, and during this time the larva must rely on stored resources. The female must get enough energy for the needs of her developing offspring and also to store the resources required by her offspring until it emerges as an adult.

Diseases caused by tsetse flies can be reduced and then controlled either by prophylaxis, treatment, and surveillance or through an entomological process of reducing flies population. Control techniques should also involve slaughter of wild animals on which tsetse feed, land clearing involving complete removal of brush and woody vegetation from an area thereby making it inhospitable for the flies, pesticide campaigns, and trapping tsetse using an electric blue colored cloth as the blue color attracts the flies.

Water Flea (Cyclops)

Water fleas are small crustaceans belongs to the order *Cladocera*. They are designated as water flea because of their saltatory swimming style. *Daphnia* is the best known example of these water fleas; it is a small, planktonic crustacean, around 0.2 and 5 mm in length, and resides in various aquatic environments ranging from acidic swamps to freshwater lakes, ponds, streams, and rivers. *Daphnia* reproduce parthenogenetically and the newly hatched *Daphnia* then molt several times before they are fully grown into adults, usually around 2 weeks. The younger ones are small copies of the adult because there are no true nymph

or instar stages. Under ideal conditions, the fully mature females of *Daphnia* are able to produce young generation about every 10 days. The reproduction process continues while the environmental conditions support their growth. Under unfavorable environmental conditions (e.g., drought), the mature female switches to the production of parthenogenic males rather than females. Males are smaller than females and possess a specialized abdominal appendage. After fertilization, the eggs acquire an extra shell layer called ephippium, which preserves and protects them from harsh environmental conditions until the more favorable times, such as the spring, when the reproductive cycle starts once again.

The life span of water fleas is usually less than 1 year, which is further dependent on the temperature. Thereby *Daphnia* may be used as an indicator species because of this shorter life span and reproductive capabilities in certain environments, surveyors can check the effects of toxins on an ecosystem. Water fleas are responsible for the transmission of a nonfatal parasitic disease called as *dracunculiasis* or *Guinea worm*. The disease is transmitted through the drinking water containing water fleas already infected with the larva of the parasite *Dracunculiasis medinensis*. One year after the ingestion of infected water by the human being, a female adult worm emerges, typically from a lower extremity and produces painful ulcers that can impair mobility for up to several weeks. The cycle repeats again when the worm comes in contact with water and releases its larvae. This disease occurs annually, especially when agricultural activities are at their peak, and has plagued Africans for decades. Guinea worm disease has also been referred to as forgotten disease for forgotten people because of its debilitating effect on people in remote rural communities. It is also one of the next diseases targeted for eradication by the World Health Organization (WHO). Although the disease has a low mortality rate, it causes an enormous amount of morbidity and is often economically devastating for affected villages (Cairncross et al., 2002).

Sand Flea (Jigger or Chigoe Flea)

The chigoe flea or jigger (*Tunga penetrans*) is a parasitic arthropod found in most tropical and subtropical climates and causes a skin disorder called as *Tungiasis*. The parasitic flea resides in soil and sand and feeds intermittently on several warm-blooded hosts such as humans, cattle, sheep, dogs, mice, and other animals. During reproduction, the female flea burrows its head-first into the hosts' skin, often leaving the caudal tip of its abdomen visible through an orifice in a skin lesion. This orifice allows the chigoe flea to breathe and defecate while feeding on blood vessels located in the cutaneous and subcutaneous dermal layer of the host body. After 2 weeks, its abdomen swells up with several dozen eggs, which it releases through the caudal orifice to fall to the ground when ready to hatch. The flea then dies and is shed off with the host's skin. The released eggs start hatching and mature into adult fleas within 3 to 4 weeks. It causes an infection on the foot of the host, which usually starts with an itching or irritation. With the swelling of the flea's abdomen with eggs later in the cycle, the pressure from the swelling may press nearby nerves or blood vessels. Depending on the particular site, this can cause sensations ranging from mild irritation to serious discomfort.

Most of the victims of sand fleas are poor and walk barefoot hence the lesions of tungiasis usually appear on the toes, between them and on the soles. Children's hands will show more lesions because they play in the sand or dusty ground. Apart from these the elbows, buttocks, and the genital area (gluteal area) are the other common ectopic sites of infestation.

Rat Flea

They are blood-sucking external parasites that primarily infect rats like Norway rats or roof rats. They are considered to have originated in Europe but can now be found in temperate environments all over the world. Although these rat flea principally hosts on rats, they can also feed on the blood of other mammals, including humans. Rats are already known to carry diseases, and people assume that rats are the main vector for transmitting various diseases to people. However, fleas are actually the problematic vectors for transmitting diseases. A rat flea will host on a rat carrying a disease, become carriers for the pathogen, and when this hungry flea bites a human, the flea regurgitates the bacteria into this new host. Two genus of rat flea are considered as a potential vector of human diseases, the oriental rat flea and the northern rat flea.

The Oriental Rat Flea (Xenopsylla Cheopis)

It is also called as the tropical rat flea, which is rodent parasite, of the genus *Rattus*, and is a primary vector for bubonic plague and murine typhus. It transmits the disease while feeding on an infected rodent and then bites a human body. The flea's body consists of three regions: head, thorax, and abdomen. The head and the thorax consist of rows of bristles (called combs), and the abdomen consists of eight visible segments. Its mouth serves two functions: it squirts the saliva or partly digests blood into the bite and then sucks up the blood from the host. This process mechanically transmits pathogenic organisms. The oriental flea is wingless and cannot fly, but it can jump long distances with the help of small powerful legs.

The oriental rat flea is particularly famous for transmitting the pathogen causing plague (Black Death), and it is also a host for tapeworms *Hymenolepis diminuta* and *Hymenolepis nana*. The eggs of the flea transmit those diseases from their one generation to the next (Farhang-Azad et al., 1985).

The Northern Rat Flea (Nosopsyllus Fasciatus)

These are found on domestic rats and house mice. They are external parasites, living by hematophagy off the blood of rodents; they originated in Europe and have been transported to temperate regions all over the world (Wall and Shearer, 1997). The northern rat flea is principally a parasite of the Norway rat *Rattus norvegicus* and is occasionally observed feeding on humans and wild rodents. It is also considered a minor vector for plague and a host of the rat tapeworm *Hymenolepis diminuta* in South America, Europe, and Australia (Wall and Shearer, 1997).

Reduviid Bug

Reduviid bug belongs to the family *Reduviidae*, a large cosmopolitan family of predatory insects of the order *Hemiptera*. It is the insect that carries and transmits the parasite *Trypanosoma cruzi*, a cause of Chagas disease (American trypanosomiasis). It is also called the kissing bug (*Triatoma* sp.) because it usually appears to bite soft tissues such as lips and eyes of people, especially babies when they are sleeping. The bug resides in cracks

and holes of substandard housing in South and Central America and is also found living in the roofs especially thatched roofs of poor housing. When the bug bites an animal or patient of Chagas disease, it becomes infected and then spreads the infection to other people by depositing feces on a skin of a healthy person, usually at night when the person is sleeping. The person acquires the parasite *T. cruzi* accidently through rubbing the feces into the bite wound, an open cut, the eyes, or the mouth. Infected persons, especially children, often develop swelling as the first sign at the site of the bite called Romaña's sign and later develop an ulcer, which may eventually heal, but the parasite is still present in the body and finally moves to the heart. Hence Chagas disease is a major cause of heart failure and heart arrhythmias, especially in Latin America. Animals usually also become infected by the same way, but additionally they can also contract the disease by eating an infected bug. *T. cruzi* parasite can also be transmitted by blood transfusion and can cross the placenta during pregnancy to infect the fetus in the uterus. The bug mostly comes out at night, so prevention should be aimed at both spraying for the fly, improving housing, and sleeping in bed nets.

Some species of Reduviid bug are considered beneficial in many locations and have been known to feed on cockroaches or bedbugs. The saliva of reduviid *Rhynocoris marginatus* showed some insecticidal activity in vitro against the lepidopteron pest, and it also reduced food consumption, assimilation, and utilization efficiency of the pest; additionally the anti-aggregation factors of saliva alters prey hemocytes aggregation and spreading (Kitherin and Muthukumar, 2011). Thereby some people breed them as pets and for insect control.

Ticks (Hard and Soft)

Ticks are obligate hematophagous ectoparasites (external parasites) of amphibians, reptiles, birds, and mammals. They are small arachnids belonging to the order *Ixodida*, which are widely distributed around the world (Magnarelli, 2009). However, they usually survive in countries with warm, humid climates because they need some moisture in the air to undergo metamorphosis, and the low temperatures of temperate countries also inhibit the development of their eggs to larva (Nuttall, 1904). Two tick families are important medically, *Ixodidae* (hard ticks) and *Argasidae* (soft ticks) because they transmit many human diseases. Ticks have eight legs, which are similar to all arachnids. Each leg is composed of six segments: the coxa, trochanter, femur, patella, tibia, and tarsus; each of these segments are connected by muscles, which allow for flexion and extension. The legs remain tightly folded against the body in the resting stage (Nicholson et al., 2009). Ticks can infect humans with bacteria, viruses, and parasites that can cause serious illness and are the vectors of various diseases, such as anaplasmosis, babesiosis, ehrlichiosis, Lyme disease, Q fever, Colorado tick fever, Rocky mountain spotted fever, African tick bite fever, tularemia, tick-borne relapsing fever, tick paralysis, and tick-borne meningoencephalitis (CDC, 2012).

Hard Ticks (Family Ixodidae)

They are called hard ticks because of the presence of a toughish back plate or scutum. They have three distinct stages in their life cycle: larva, nymph, and adult. Larvae have six legs (Figure 24.4), emerge from the egg, obtain a blood meal from a vertebrate host and

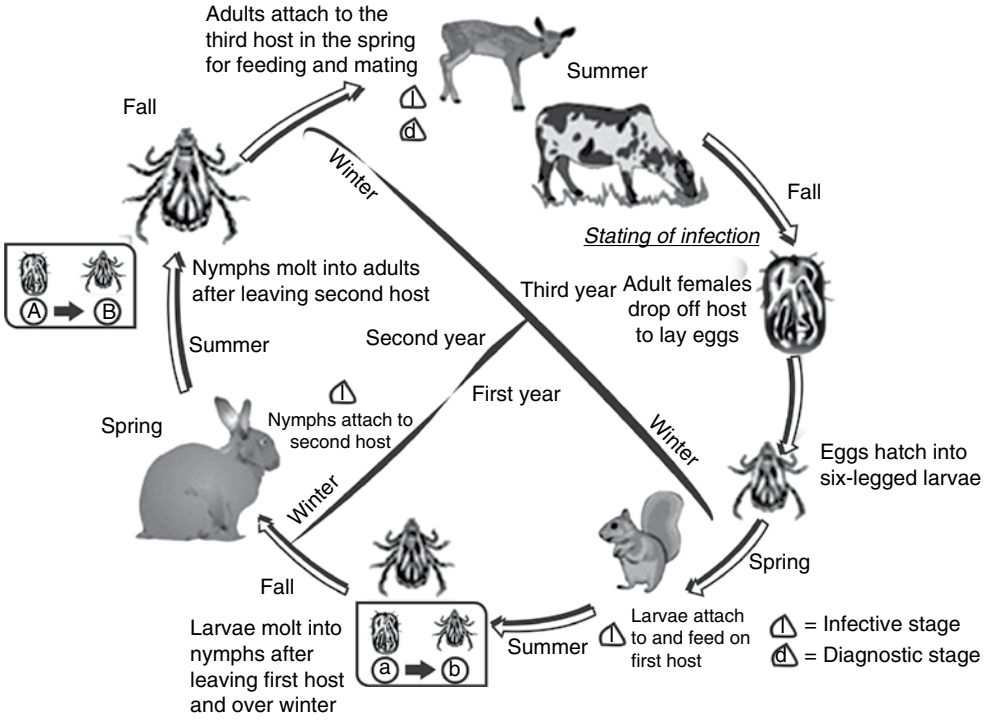

Figure 24.4. The life cycle of ticks with humans as alternate hosts.

molt to the nymphal stage. Nymphs feed and further molt to the final stage, the adult with eight legs. After feeding, the adult female hard ticks lay a batch of thousands of eggs and then die. They take only one blood meal during each of the three life stages. The time duration for the completion of the entire life cycle may vary from less than a year in tropical regions to more than 3 years in cold climates, where certain stages may pause until hosts are again available. Many hard ticks can survive for up to several months without feeding, depending on the environmental conditions. Hard ticks seek hosts by unique behavior called "questing." Questing ticks crawl up the stems of grass or perch on the edges of tree leaves on the ground in a unique posture with the front legs extended, especially in response to a passing by host. Carbon dioxide released by the host as well as heat and movement serve as stimuli for this questing behavior. Subsequently, these ticks climb on to a potential host that brushes against their extended front legs then attach and feed for hours to days. Disease transmission usually occurs at the end of a meal when the tick becomes full of blood.

Figure 24.4 shows the life cycle of ticks in general, and Figure 24.5 shows several hard ticks and the various stages in their life cycle. The smallest stages, larva and nymph, are sometimes referred to as "seed ticks" because of their resemblance with a small plant seeds.

Hard ticks show a variety of life cycles based on their contact with an appropriate host for survival. Some ticks feed on only one host throughout all three life stages, and these are called as *one-host ticks*. One-host ticks remain on one host during the larval and nymphal stages until they become adults, and females drop off the host after feeding to

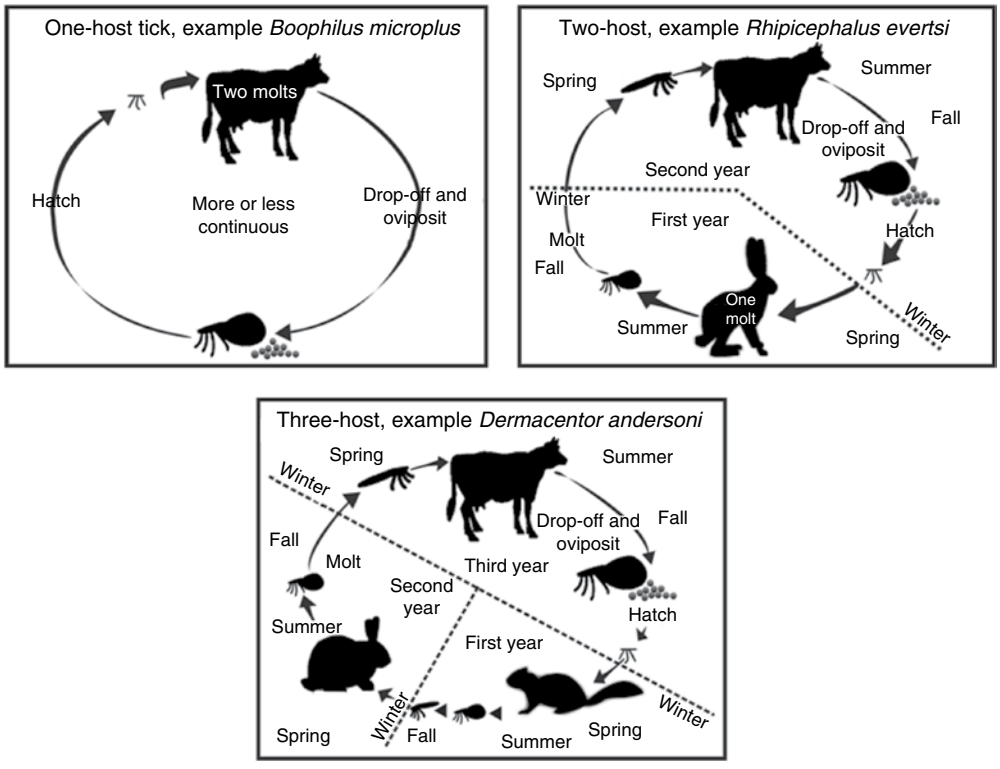

Figure 24.5. Life cycle of hard ticks.

lay their batch of eggs. The second category of hard ticks feed on two hosts during their lives and are referred to as two-host ticks. Two-host ticks feed and remain on the first host during the larval and nymphal life stages and then drop off and attaches to a different host as an adult for its final blood meal. The third category ticks feed on three hosts, one during each life stage. These ticks drop off and reattach to a new host during each life stage until finally the adult females lay their batch of eggs. In each individual case, the fed adult stage is terminal, in which after laying one batch of eggs the female dies, and the male also dies after reproduction.

Generally tick bites are painless; most people may not even notice the bite and may not find the tick if it falls off. Some small ticks (e.g., the deer tick that transmits Lyme disease) are so tiny that they may be nearly undetectable. However, after a tick bite, some symptoms may occur that can be directly related to the tick itself. Occasionally, a neurotoxin is secreted at the time of attachment, which makes the bite unnoticeable to humans and other hosts and can cause muscle weakness or paralysis. Simply removing the tick stops any further neurotoxin production, and the person recovers quickly and completely. The actual bite usually causes symptoms only after the tick drops off. However, some people may report local redness, itching, burning, and rarely, localized intense pain (especially in case of soft ticks) before or after the tick drops off. The illnesses transmitted by ticks often begin days to weeks after the removal of the tick from the body. This is probably the reason that doctors may not suspect a tick-related illness because many people ignore or

forget about barely noticeable "bites." The most important clue about any tick-related illness is to inform the physician about a tick bite. The individual also needs to tell their physician about outdoor activity (camping, hiking, etc.) in tick-infested areas, if the person does not remember a tick bite. Individuals develop flulike symptoms, fever, numbness, rashes, confusion, weakness, pain and swelling in joints, palpitations, shortness of breath, and nausea and vomiting after a tick bite, which is probably as a result of the transmission of pathogen through the corresponding tick.

Soft Tick (Family Argasidae)

Soft ticks are round or oval, lacking a shield on their back. Their skin is leathery, wrinkled, and tough. Their development is quite similar to hard ticks, but they may have two to several larval and nymphal stages and require a meal of blood each time (Aeschlimann and Freyvogel, 1995). Their life cycle ranges from months to years. Soft ticks are secretive in their habits, usually feeding at night and concealing themselves during the day in crevices or cracks near the nest or roost of the host. The female tick feeds and lays eggs alternately over a relatively long period; thereby a single soft tick may feed on several different hosts during its life time, which increases its disease-carrying potential. Many soft ticks feed on birds and reptiles, although others prefer mammals as their host. The main genera of soft ticks are *Antricola*, *Argas*, *Nothaspis*, *Ornithodoros*, and *Otobius* (Guglielmone et al., 2010). They primarily feed on birds and rarely are found to parasitize land animals or humans (Allan, 2001).

Control of Ticks

Mechanical removal is the best way to remove the adult hard tick, followed by punch biopsy to remove any remaining parts of tick body (Zuber and Mayeaux, 2003). The parasite ichneumon wasp (*Ixodiphagus hookeri*) has also been used to control tick populations, which lays its eggs into ticks and then the hatching wasps kill their host. A bird Guinea fowl consumes mass quantities of ticks (Duffy et al., 1992); just two birds can clear 2 acres (8,100 m^2) in a single year. A chemical phenothrin (85.7%) in combination with methoprene can be used for the topical treatment of felines. Phenothrin directly kills adult fleas and ticks, whereas methoprene is an insect growth regulator that interrupts the life cycle of insect by killing eggs.

Lice

Lice are the wingless insects belonging to the order *Phthiraptera*. They are the obligate ectoparasites of most of the avian as well as mammalian hosts. Most of them are scavengers, thereby feeding on skin and other debris present on the host's body, but some species prefer to feed on sebaceous gland secretions and blood. They exhibit type specificity and are only found on specific animals, especially in a particular part of their body; for example, most louse species inhabit the scalp and pubic hair in humans. They are not capable of surviving a longer time period after being removed from their host body (Hoell et al., 1998). The order *Phthiraptera* has been divided into two suborders, *Anoplura* (Sucking lice) and *Mallophaga* (Chewing lice). Humans may carry three types of lice: head lice, pubic lice, and body lice. Lice are usually born as miniature versions of the

adults, nymphs (exopterygotism), and the young louse has to molt three times before reaching the final adult form, usually within a month of hatching (Hoell et al., 1998). Generally its color varies from pale beige to dark gray, however, on feeding blood it will become darker. Female lice are more common than males, with some species being parthenogenic. A louse egg, also called a *nit*, is attached to the host's hairs by means of saliva. Following lice infection, the symptoms include intense itching, small, red bumps on the scalp, neck, and shoulders and tiny white specks on the bottom of each hair that are hard to get off. On the other side, lice are helpful in the study of human evolution because they are the subject of significant DNA research. David et al. (2007) suggested that pubic lice spread to humans approximately 2 million years ago from gorillas. Similarly, the DNA differences of head lice and body lice provide corroborating evidence that humans started losing body hair, about 2 million years ago (Travis, 2003).

Lice infections can simply be controlled using lice combs and medicated shampoo. Shampoos containing 1% permethrin (Nix) proved to be effective for lice treatment. Another way of lice treatment is removing its eggs (nits), which can be removed using a nit comb (metal combs with very fine teeth) after applying olive oil in the hair. Maintenance of proper hygiene by washing of clothes and bed linens in hot water with detergent also helps prevent head lice from spreading to others during the short period when head lice can survive outside the human body. The US Food and Drug Administration (FDA) has also approved the use of the formulation, 0.5% malathion in isopropanol for treating head lice, but it should be applied to dry hair. However, this treatment can cause significant side effects in children younger than 6 months old, the elderly, and anyone weighing less than 110 pounds (50 kg), especially when it is used repeatedly within a short period of time. Hence prevention is always better, and it is suggested that people not share hair brushes, combs, hair pieces, hats, bedding, towels, or clothing with someone who has head lice. Children infected with lice should not be allowed to be at school until they have been completely treated.

Cockroach

Cockroaches are well-known pests belonging to the order *Blattaria* or *Blattodea* of insects. Their widely known species include, American cockroach (*Periplaneta Americana*), German cockroach (*Blattella germanica*), Asian cockroach (*Blattella asahinai*), and Oriental cockroach (*Blatta orientalis*). The name *cockroach* originated from the Spanish word *cucaracha* (chafer, "beetle,") from *cuca*, a kind of caterpillar. Cockroaches inhabit a wide range of environments throughout the world and can readily adapt to a variety of environments but usually prefer tropical and subtropical climates. They have nocturnal habits and will usually run away when exposed to light; the exception is the Asian cockroach, which is always attracted toward light. Most of them are omnivorous with the exception of the wood-eating species, *Cryptocercus*. Cockroaches have a broad, flattened body with a relatively small head. The mouthparts are on the underside of the head, which include generalized chewing mandibles. They have large compound eyes, two ocelli and long, flexible antennae. The first pair of wings, called *tegmina*, are tough and protective, lying as a shield on top of the membranous hind wings. All four wings have branching longitudinal veins, as well as multiple cross-veins. The abdomen of a cockroach has 10 segments and several cerci (Hoell et al., 1998). Quite similar to other insects, cockroaches breathe through tracheae, which are attached to the spiracles, excluding the

head. Thus, they are not dependent on the mouth and windpipe for breathing. They do not have lungs and thus do not actively breathe like vertebrates.

Cockroaches are one of the most commonly observed household pest insects (Hatch, 2008). They feed on human and pet food and usually leave an offensive odor. They can also passively transport microbes on their body surfaces, including some which are potentially dangerous to humans, particularly in hospital environments (Elgderi et al., 2006). They have also been found to be associated with some allergic reactions in humans (Kutrup, 2003) and one of the proteins that trigger allergic reactions has been identified as tropomyosin (Santos et al., 1999). These allergens have also been found associated with asthma (Kang et al., 1979).

Cockroaches can be controlled by general preventive measures employed against other household pests, such as keeping all food properly stored in sealed containers, using garbage cans with a tight lid, frequent cleaning of the kitchen, and regular vacuuming. Any water leakage, such as dripping taps, should also be repaired timely. All the entry points, such as holes around baseboards, in between kitchen cabinets, pipes, doors, and windows should be sealed with some steel wool or copper mesh and some cement, putty or silicone caulk. They can also be eliminated by using diatomaceous earth, which is harmless to humans, feels like talcum powder and, therefore, can be applied as a fine powder. Some cockroaches have been known to survive up to 3 months without food and a month without water. They frequently live outdoors, although they prefer warm climates and are considered cold intolerant; they are resilient enough to survive occasional freezing temperatures, which makes them difficult to eradicate once they have infested an area. As far as biological control is concerned, many parasites and predators are there for cockroaches, but only a few of them have proven to be highly effective for the biological control of these pest species. Wasps are one of the most effective insect predators because they attack the eggs, adult, and nymphal stages of cockroaches. Bait stations, gels containing hydramethylnon or fipronil, and boric acid powder are also proved to be toxic to cockroaches. In addition, some pest control products containing deltamethrin or pyrethrin are also found to be effective.

Mites (Chiggers)

Mite is a group of insectlike organisms, which bite or cause irritation to humans, parasitize animals, scavengers, and prey on some insects and other arthropods. Quite similar to ticks, they also pass through four developmental stages from egg to larva to nymph to adult. All the stages have eight legs except the larva stage which has six. Mites are freely living in the soil or water, but there are a large number of species that live as parasites on plants, animals, and on molds. Some mites may also parasitize some insect species, for example, *Varroa destructor* is a parasite of honeybee.

An important member of mites is the red bug whose larvae, a chigger, are extremely small (0.5 mm) in size and are usually encountered in low, damp places where grass and weeds are overgrown. Chigger develops as an adult in soil and becomes active in the spring and lays eggs in soil. On hatching, the larvae crawl and then attach to a suitable host injecting its salivary fluid and producing a hardened, raised area around them. It withdraws the body fluids from the host by means of a feeding tube and feeds for about 4 days and then molt to nonparasitic nymphs and adults. Chiggers usually feed on a variety of wild and domestic animals as well as humans and complete their life cycle in

about 50 days. Common biting sites are around the ankles, waistline, armpits, or other areas where clothing fits tightly against the skin. People develop redness within 24 hours of biting by a chigger, which is further accompanied by intense itching persisting for a week or longer, and if untreated, followed by fever.

Another important mite member is the house dust mite, which is cosmopolitan in human habitation. It feeds on organic matter, such as flakes of shedded human skin. These mites are generally responsible for the asthmatic and allergic symptoms occurring worldwide because their genitourinary tract contains some proteolytic enzymes that persist in their feces and are, therefore, the major inducers of allergic reactions, such as wheezing. The exoskeleton of mites can also contribute to these allergic reactions. Allergens associated with mites can be further subdivided into various groups. Among them, group 1 and group 2 allergens are the most potent one. Group 1 consists of some catalytic proteins, for example, Dermatophagoides pteronyssinus group 1 (Der p 1) allergen is a cysteine protease. Group 2 comprises proteins important for the mite.

The first step in the mite management is to determine the true cause of irritation, which may involve thorough inspection of the premises and then identifying organisms. All mites are tiny creatures and can only be seen with magnification, which often makes their inspection and identification difficult. Similarly, mites are a diverse group, each having different habits; investigators should also be aware of all these habits. Appropriate control measures can be employed only after the proper identification of mite species. A vacuum sweeper has proven to be useful in controlling mites because infestations of clover mites and rodent and bird mites in and around structures can sometimes be eliminated by vacuuming alone. Vacuuming might be less effective, but still important, in controlling various food mites, straw itch mites, and dust mites. A high-efficiency particulate air (HEPA) filter can also be installed to prevent air-borne allergens, including dust mite particles. Moisture should also be controlled because mites transfer air and water through their body walls and are subject to desiccation at low humidity. The best known example is dust mite populations, which suffer at relative humidity of 50 percent or fewer. On the other hand, high humidity increases mite populations exponentially. Daily activities, such as air-conditioning and showering, will cause fluctuation in humidity levels, especially in homes. Thus, along with humidity control, other means should be employed to control the mite population. To control dust mites, chemical products containing benzyl benzoate, and possibly abrasive dust formulations, may be useful if applied on to flooring and floor coverings. Pillows, mattresses, and upholstered furniture can be discarded or sealed in plastic covers to help prevent dust mite infestation and also to reduce associated allergens.

Exclusion methods also can be used for certain mites, including clover mites. Some structural entry points, such as gaps in and around foundations, doors, windows, vents, and utility lines, should be properly sealed, which will help to keep clover mites outdoors along with rats, mice, birds. If nests are found, they should be removed completely, and the area around them vacuumed. Other nonchemical methods include constructing a plant-free border around foundations and reducing the use of fertilizer in lawns, both of which help to keep away clover mites. Pesticides formulated against mites, known as miticides or acaricides, can be effective against clover mites and chiggers in grassy areas. To control chiggers, however, insect repellents containing 7to 30% DEET should be used as the first line of defense.

General Principles of Vector Control

Vector-borne diseases (VBD) significantly contribute to morbidity and mortality, especially in developing and underdeveloping countries. Vector control measures have been employed throughout the world, are quite successful, and are the easiest way of preventing VBD. Vector control essentially requires a good understanding of the life cycle of the vector so as to eradicate them before they transmit disease. An extrinsic cycle always offers a good opportunity to control the disease without directly interfering with the behavior of human beings. The other required thing for vector control is a good knowledge of vector behavior. Therefore, before embarking on any vector control exercise, a good understanding of the vector's breeding places, nesting places, feeding patterns, and flying distances is needed. Different vectors show different behaviors; for example, some bite during the day and others attack during the night. Therefore, in certain areas, more than one vector control measures has to be employed at one particular time. There are some general principles that should be followed while controlling disease vectors.

1. **Targeting the adult using insecticides**. Insecticides normally kill the adult vectors; they are either residual or non-residual. Residual insecticides are mainly organic chemicals, which remain effective for several months when applied on surfaces. These include DDT, Dieldrin, Lidane, HCH, and organophosphates. DDT is not recommended in most countries because it does not degraded easily and also contaminates the environment.
2. **Targeting the larvae using larvicides**. Larvicides mainly target the larvae and are mainly used in the breeding sites; however, most of the larvicides are easily diluted by water. For greater effectiveness larvicides are generally sprayed on the water surfaces already covered with oil.
3. **Preventing the breeding of the vector through environmental interventions**. Prevention of vectors breeding is an economic and effective way of disease control. Some of the environmental control measures include:
   - Clearing bushes and grass along water bodies.
   - Draining and collecting all containers that may hold water.
   - Covering water containers and tanks with lids.
   - Draining waterholes, ditches, and any unwanted water bodies around villages.
   - Disturbing snail habitat by changing the water levels, filling or draining the water bodies, and use of molluscides.
   - Protecting susceptible individuals,

These interventions are basically to prevent the vector from coming into contact with a susceptible individual. The most common ways this can be achieved are:

- Use of insecticide-treated bed nets, such as the long-lasting insecticide treated nets (LLITNS).
- Use of house screens.
- Use of insect repellents.
- Use of protective clothing.
- Avoid staying outdoors at night.

Integrated Vector Management

Integrated vector management (IVM) is a rational decision-making process to optimize the use of various resources for vector control (WHO, 2008). It requires a management approach that improves the efficacy, cost effectiveness, ecological soundness, and sustainability of vector control interventions with the available tools and resources. The IVM approach is vital to achieve the national and global targets for controlling vector-borne diseases. IVM can be defined as "a process of evidence based decision making procedures particularly aimed to plan, deliver, thoroughly monitor and properly evaluate targeted, cost-effective and sustainable combinations of regulatory and operational vector control measures, along with a measurable impact on transmission risks, adhering to the principles of subsidiarity, intersectorality and partnership" (WHO, 2007, p. 25).

A wide variety of vector-borne diseases sometimes coexist in the same environments and impose a heavy burden on human populations, particularly in developing countries of tropical and subtropical zones. They cause the direct human suffering and are a significant obstacle to the socioeconomic development in a particular country.

Characteristic Features of Integrated Vector Management

Characteristic features of an effective IVM approach include:

- Selection of appropriate vector control methods based on knowledge of local vector biology and ecology, disease transmission, and morbidity.
- Use of a range of interventions, separately or in combination. and more often synergistically.
- Collaboration within the health sector and with other public and private sectors that have an impact on vector breeding.
- Engagement with local communities and other stakeholders.
- A public health regulatory and legislative framework.
- Rational use of insecticides.
- Good and healthy management practices.

Basic Elements of an Integrated Vector Management Program

Vector control is an important component of the prevention and management of all the vector-borne diseases, because for some diseases, the vector is the only feasible target for control. If it is well organized and well targeted, vector control can reduce or interrupt the transmission of many diseases. Table 24.2 shows the basic elements for an IVM approach.

Reasons for Employing Integrated Vector Management

The main reason for employing an IVM strategy is to strengthen the impact of vector management through complementary methods of vector control with operational flexibility, without disrupting the integrity of the ecosystem. Ideally IVM should reduce the vector capacity at the level of preventing transmission risks. IVM programs usually involve:

Table 24.2. Basic elements of an integrated vector management approach.

Element	Description
Advocacy, social mobilization, and legislation	Promotion and embedding of IVM principles in designing policies in all relevant agencies, organizations and civil society; establishment or strengthening of regulatory and legislative controls for public health; empowerment of communities
Collaboration within the health sector and with other sectors	Consideration of all options for collaboration within and between public and private sectors; application of the principles of subsidiary in planning and decision making; strengthening channels of communication among policy makers, vector-borne disease program managers, and other IVM partners
Integrated approach	Ensure rational use of available resources by addressing several diseases, integrating nonchemical and chemical vector control methods and integrating with other disease control methods
Evidence-based decision making	Adaptation of strategies and interventions to local ecology, epidemiology, and resources, guided by operational research and subject to routine monitoring and evaluation
Capacity building	Provision of the essential material infrastructure, financial resources, and human resources at national and local level to manage IVM strategies on the basis of a situational analysis

IVM, integrated vector management.
Adapted from World Health Organization (WHO). 2004. *Global Strategic Framework for Integrated Vector Management*. WHO/CDS/CPE/PVC/2004.10. Geneva: WHO.

- Implementation of a sound ecosystem and eco-epidemiological analysis to reveal the critical points in disease transmission, which can be further targeted for maximizing the impact on disease interruption.
- Designing a series of incremental interventions, whereby each additional intervention yields maximum health gains at its least additional cost.
- Coordinating and refocusing pre-existing resources for IVM against multiple vectors, through both intersectoral and intrasectoral cooperation to maximize benefits and cost- efficient use of available resources.
- Using an alternate or multiple interventions to reduce dependency on the use of pesticides.
- Achieving sustainable long-term prevention of vector-borne diseases at minimal cost.

In the long term, IVM should also help to prevent and overcome costly setbacks, especially those that may arise as a result of changes in vector behavior or development of insecticide resistance.

Guiding Principles for the Development and Implementation of Integrated Vector Management Interventions

- IVM is an essential element in controlling vector-borne diseases. It should be economically feasible, cost effective, sustainable, environmentally sound, and socially acceptable.
- Vector control interventions are components of integrated vector-borne disease control programs.

- Wherever and whenever possible, vector control interventions should be planned for prevention and control of multiple vector-borne diseases.
- Incentives and regulatory or institutional arrangements need to be designed and employed to ensure effective intersectoral collaboration.
- Program management can be optimized by enabling decision making at local levels. This should be considered in the context of the health sector decentralization and the need for active community participation.

Priority Actions for Implementation of Integrated Vector Management

- Incorporating IVM principles into national health policies.
- Strengthening vector control capability within the national health system.
- Establishing or strengthening national capacity to implement IVM by providing training, promoting career opportunities, enhancing collaboration, guiding reorientation of vector control activities, and ensuring availability of skilled staff.
- Engaging in advocacy to ensure political commitment for IVM as an important component of communicable disease control, and developing policies and legislation to increase community participation, empowerment, and mobilization of human and financial resources.
- Promoting intersectoral and intrasectoral cooperation to optimize the allocation of resources within the health sector (e.g., environmental health and different vector-borne disease programs) and intersectoral collaboration between different government sectors, (especially agriculture, environment, and local government/municipalities) supported by appropriate policies, legislation, and impact assessment.
- Establishing partnerships to mobilize public and private sectors, together with civil society, nongovernmental organizations (NGOs), and donors to optimize allocation of resources and ensure effective implementation of IVM.
- Monitoring and evaluating ongoing vector control activities by employing entomological surveillance and conducting operational research, including post-registration monitoring of pesticide use.

Role of Member States

Depending on the conditions in each country, effective implementation of IVM will require the establishment, strengthening, or reorganization of vector control services to facilitate multidisciplinary and intersectoral collaboration. The basic step for all countries is implementing a comprehensive needs assessment, which is to be used as a basis for fulfilling the following targets:

- Identification of the technical, human, and financial resources for the effective implementation of IVM activities.
- Development of a proposal for the establishment of IVM services within the existing framework of national health policies and health systems and reaching an agreement with relevant authorities.
- Establishment and strengthening of a structure for the planning, implementation, monitoring, and evaluation of an IVM program. A core group can guide, support, and whenever necessary, participate in IVM activities.

- Development of national guidelines for the planning, implementation, monitoring, and evaluation of effective IVM activities.
- Establishment of various mechanisms to ensure intersectoral and intrasectoral collaboration, public–private partnership, cross-border coordination, and community participation.
- Planning and implementation of the operational research for evidence-based IVM interventions.

Role of World Health Organization

Finally, the WHO can assist countries in fulfilling the targets of IVM by finalizing and disseminating the draft regional strategic framework among member states. During intervals, the WHO can prepare and disseminate the technical guidelines for planning, implementation, monitoring, and evaluation of vector control interventions based on the IVM for communicable disease control. It should also provide the necessary technical support for the member states to conduct situation analyses, needs assessment, planning, implementation, monitoring, and evaluation of vector control interventions based on an effective IVM approach.

Rodents

Rodents are mammals placed in the order *Rodentia* and are characterized by two continuously growing incisors in the upper and lower jaws, which must be kept short by gnawing. Actually 40 percent of mammal species are rodents (Myers, 2000), and most of them are present in large numbers on all continents other than Antarctica. Mice, rats, porcupines, squirrels, guinea pigs, beavers, and hamsters are the common rodents having sharp incisors and are used to gnaw wood, break into food, and bite predators. All of these rodents share a characteristic dentition, which is highly specialized for gnawing. This is the basis of their nomenclature from the Latin, *rodere*, to gnaw and, *dentis*, of tooth (Pearsall, 2002). All of them have a pair of upper and a pair of lower incisors, followed by a gap, called *diastema*, and one or more molars or premolars. Rodent incisors grow continuously, which must be kept worn down by gnawing. Their anterior and lateral surfaces are covered with enamel, but the posterior surface is exposed dentine. During gnawing, the incisors grind against each other, wearing away the softer dentine, leaving the enamel edge like the blade of a chisel (Hurst, 1999). This self-sharpening system is effective and is probably one of the keys to the enormous success of rodents.

Rodents lack canine teeth and use their teeth for cutting wood, biting through the skin of fruit, or for defense. Nearly all rodents feed on plants particularly plant seeds, but a few exceptions eat insects (grasshopper mouse [*Onychomys leucogaster*]) or fish (beavers, *Castor* spp.), although some have more varied diets. Some species have historically been pests, eating seeds stored by people (Meerburg et al., 2009a) and spreading disease (Meerburg et al., 2009b). Rodents make up the largest order of mammals in terms of the number of species; there are about 2,277 species of rodents (Wilson and Reeder, 2005). Their success is particularly because of their small size, short breeding cycle, and ability to gnaw and eat a variety of foods.

Rodents can function as food sources for predators, participates in mechanisms of seed dispersal and as disease vectors. Therefore, they are an important part of many ecosystems.

Humans use rodents as a source of fur, as pets, as an experimental model organism, for food, and even for detecting land mines (Wines, 2004). Rodents show wide diversity in their characteristic features, some of which are considered unique among mammals; hence they are widely used in research (Sherwin, 2010). The best known example is the naked mole rat, *Heterocephalus glaber*, which is used in studies on thermoregulation and pain because it is the only known mammal that does not produce the neurotransmitter substance P and is poikilothermic.

Methods for Rodent Control

Rodents (especially rats and mice) sometimes get indoors and create a nuisance. They damage property, contaminate food, and transmit many diseases. Therefore, effective control methods are essential.

Cleaning

There should be proper hygiene inside, and food should be stored in packed glass and metal containers. Floors should be swept properly to deny rats and mice food. Garbage should be placed in trash cans covered with proper lids. Human, animal, or pet food should not be left out overnight unprotected. The weeds around the outside entryways should also be controlled.

Trapping

Traps should be placed along walls and in paths where rodents usually travel. People should be aware of trap-shy rats and mice and leave traps unset until bait has been taken at least once. Traps do not have to be washed after a catch, some recommended baits for traps are peanut butter, pineapple, nuts, doughnuts, cake, fried bacon, raisin, chocolate, and gum drops.

Poisoning

Poison bait can be used along with trapping to increase rodent capture and killing. Some useful available poisons include warfarin, pival, fumarin, chlorophacinone, bromadiolone, and cholecalciferol. Mice tend to nibble; thereby a longer period of time is needed to control them with poison. Poison may also cause rodents to die in inaccessible areas, leaving unpleasant odors of decaying carcasses. Children and pet animals should be kept away from poison baits.

Rodent Proofing

Rodent proofing is an essential part of a complete rodent control program. The objective of rodent proofing is to close all present and potential openings that may serve as entries for rats and mice. The important thing is that not to seal rodents indoors until baiting, trapping, and removal is completed. The materials used for this purpose should be rodent resistant, such as metal sheet, heavy wire mesh, concrete, brick, and mortar. In cases of the unavailability of materials, steel wool is a good substitute for plugging holes, but it

should be used only as a temporary measure. Appropriate metal guards should be employed to prevent rats and mice from using wires and pipes to enter, such as a flat funnel 12 inches wide for a single vertical line next to the building, and an 18- inch radius disk guard for horizontal lines.

Rodent Control

Finally, after controlling the problem, both inside and outside the residence should be monitored to make sure that property is rodent free. Reinfestation can occur inside rodent-proofed buildings when doors and windows are left open. Reinspection is necessary to ensure effective rodent proofing.

References

Aeschlimann A, Freyvogel TA. 1995. Biology and distribution of ticks of medical importance. In: Meier J, White J, eds., *Handbook of Clinical toxicology of Animal Venoms and Poisons*, pp. 177–189. Boca Raton: CRC Press.

Allan SA. 2001. Ticks (Class *Arachnida*: Order *Acarina*). In: Samuel WM, Pybus MJ, Kocan AA, eds., *Parasitic Diseases of Wild Mammals*, pp. 72–106. New York: Wiley-Blackwell.

Bartlett K. 1999. Deer and horse flies. Accessed October 9, 2008, at http://www.uri.edu/ce/factsheets/sheets/ deerhorseflies.html.

Cairncross S, Muller R, Zagaria N. 2002. Dracunculiasis (guinea worm disease) and the eradication initiative. *Clin Microbiol Rev* 15:223–246.

Centers for Disease Control and Prevention. 2009. West Nile virus-updated insect repellent. Retrieved June 15, 2011, at http://www.cdc.gov/ncidod/dvbid/westnile/mosquitorepellent.htm.

Centers for Disease Control and Prevention. 2012. DEET, Showers, and Tick Checks Can Stop Ticks. Retrieved March 10, 2013, at http://www.cdc.gov/features/stopticks/.

David LR, Light JE, Allen JM, Kirchman JJ. 2007. Pair of lice lost or parasites regained: The evolutionary history of anthropoid primate lice. *BMC Biology* 5:7.

Duffy DC, Downer R, Brinkley C. 1992. The effectiveness of Helmeted Guineafowl in the control of the control of the deer tick, the vector of Lyme disease (PDF). *Wilson Bulletin* 104:342–345.

Elgderi RM, Ghenghesh KS, Berbash N. 2006. Carriage by the German cockroach (*Blattella germanica*) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospitals and households in Tripoli, Libya. *Ann Trop Med Parasitol* 100:55–62.

Farhang-Azad A, Traub R, Baqar S. 1985. Transovarial transmission of murine typhus rickettsiae in *Xenopsylla cheopis* fleas. *Science* 227:543–545.

Guglielmone AA, Robbing RG, Apanaskevich DA, Petney TN, Estrada-Peña A, et al. 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari:Ixodida) of the world: a list of valid species names" (PDF). *Zootaxa* 2528:1–28.

Hatch E. 2008. How to get rid of cockroaches. Accessed March 25, 2013, at http://www.getridofthings.com/pests/cockroaches/.

Hoell HV, Doyen JT, Purcell AH. 1998. *Introduction to Insect Biology and Diversity* 2nd ed., Oxford: Oxford University Press.

Hurst JL. 1999. Introduction to rodents. In: Poole, T., ed., *The UFAW Handbook on the Care and Management of Laboratory Animals 1, Terrestrial Vertebrates*, 7th ed., pp. 262–273. Oxford: Blackwell Publishing.

Jordan AM. 1993. Tsetse-flies (*Glossinidae*). In: Lane RP, Crosskey RW, eds. *Medical Insects and Arachnids*, pp. 333–382. New York: Chapman and Hall.

Kang B, Vellody D, Homburger H, Yunginger JW. 1979. Cockroach cause of allergic asthma. Its specificity and immunologic profile. *J Allergy Clin Immunol* 63:80–86.

Kitherin S, Muthukumar S. 2011. Zootoxic effects of reduviid *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) venomous saliva on Spodoptera litura (Fab.). *Toxicon* 58:415–425.
Kutrup B. 2003. Cockroach Infestation in Some Hospitals in Trabzon, Turkey. *Turk J Zool* 27:73–77.
Larsen B. 2010. How to get rid of cockroaches. http://summitpestmanagement.com/how to get rid of cockroaches.php.
Magnarelli LA. 2009. Global importance of ticks and associated infectious disease agents. *Clinical Microbiology Newsletter* 31:33–37.
Meerburg BG, Singleton GR, Kijlstra A. 2009a. Rodent-borne diseases and their risks for public health. *Crit Rev Microbiol* 35:221–270.
Meerburg B G, Singleton G R, Leirs H. 2009b. The Year of the Rat ends: Time to fight hunger! *Pest Manag Sci* 65:351–352.
Milne LJ, Milne M. 1980. *The Audubon Society Field Guide to North American Insects and Spiders*. New York: Alfred A. Knopf, Inc.
Mullen G, Durden L. 2009. *Medical and Veterinary Entomology*. New York: Academic Press.
Myers P. 2000. Rodentia. *Animal Diversity Web*. University of Michigan Museum of Zoology. Retrieved May 25, 2006, at http://animaldiversity.ummz.umich.edu/site/accounts/information/Rodentia html.
Nicholson WL, Sonenshine DE, Lane RS, Uilenberg G. 2009. Ticks (*Ixodida*). In: Mullen G, Durden L, eds., *Medical and Veterinary Enomology*, pp. 483–532. New York: Academic Press.
Nuttall GHF. 1904. Ticks and tick-transmitted diseases. *Trans Epidemiol Soc London* 24:12–26.
Pearsall J. 2002. *The Concise Oxford English Dictionary*, 10th ed. rev. Oxford: Oxford University Press.
Rogers DJ, Hay SI, Packer MJ. 1996. Predicting the distribution of tsetse flies in West Africa using temporal Fourier processed meteorological satellite data. *Annal Trop Med Parasi* 90:225–241.
Santos AB, Chapman MD, Aalberse RC, Vailes LD, Ferriani VP, et al. 1999. Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. *J Allergy Clin Immunol* 104:329–337.
Service MW. 2008. *Medical Entomology for Students*. Cambridge: Cambridge University Press.
Sherwin CM. 2010. The husbandry and welfare of non-traditional laboratory rodents. In: Hubrecht R, Kirkwood J, eds., *UFAW Handbook on the Care and Management of Laboratory Animals*, pp. 359–369. New York: Wiley-Blackwell.
Travis J. 2003. *The naked truth? Lice hint at a recent origin of clothing. Science News* 164:118.
Wall R, Shearer D.1997. *Veterinary Entomology: Arthropod Ectoparasites of Veterinary Importance*. Dordrecht: Springer-Verlag.
World Health Organization (WHO). 2004. *Global Strategic Framework for Integrated Vector Management*. WHO/CDS/CPE/PVC/2004.10. Geneva: WHO.
Wilson DE, Reeder DAM, eds. 2005. *Mammal Species of the World. A Taxonomic and Geographic Reference*, 3rd ed. Baltimore: Johns Hopkins University Press.
Wines M. 2004. Gambian rodents risk death for bananas. *The Age* (The Age Company Ltd.). Retrieved May 25, 2006, at http://www.theage.com.au/articles/2004/05/18/1084783512636.html.
World Health Organization (WHO). 2007. Strategic plan for malaria control and elimination in the WHO Eastern Mediterranean Region, 2006–2010. Accessed March 25, 2013, at http://applications.emro.who.int/dsaf/dsa741.pdf.
World Health Organization (WHO). 2008. *WHO Position Statement on Integrated Vector Management*. WHO/HTM/NTD/VEM/2008.2. Geneva: WHO.
Zuber TJ, Mayeaux EJ. 2003. Tick removal. In: *Atlas of Primary Care Procedures*, pp. 63–67. Baltimore: Lippincott Williams & Wilkins.

Glossary

aerobic Growing or thriving only in the presence of oxygen

aerosol transmission A cloud or mist of solid or liquid particles containing pathogenic microorganisms, released by sneezing or coughing

AIDS (Acquired Immune Deficiency Syndrome) A virus that weakens the immune system and subjects the patient to opportunistic diseases, such as pneumonia and tuberculosis

amebiasis Disease caused by the protozoan *Entamoeba histolytica*, a type of amoeba

analgesics Drugs that relieve pain

antibiotic A substance made either from a mold or bacterium or synthetically that inhibits or kills certain microorganisms, specifically bacteria, and that treats infections

antibodies Any of a large number of proteins of high molecular weight that are produced as part of the immune response in response to an antigen, which it then neutralizes, tags, or destroys

antigen Any foreign substance that when introduced into the body stimulates an immune response

arthropod An invertebrate having jointed limbs and a segmented body with an exoskeleton

asexual reproduction A type of reproduction in which an organism replicates itself, by budding or dividing, without the involvement of other organisms

attenuated Reduced in strength

autoimmune disease Any of a large group of diseases characterized by abnormal functioning of the immune system that causes it to produce antibodies against the body's own tissues.

autotrophs Microorganisms that use inorganic materials as sources of nutrients

B cells One of the two major classes of lymphocytes; during infections, B cells mature into plasma cells, which produce antibodies directed at specific antigens

babesiosis A rare, often severe(and sometimes fatal illness that is caused by a pathogen transmitted by ticks

bacterium A single celled microscopic organism, whose genetic material is not enclosed by a membrane

binary fission A form of asexual reproduction in which a cell divides into two daughter cells after DNA replication

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

biological warfare (or **biowarfare**) The use of bacteria or viruses or their toxins as weapons

Bubonic plague A bacterial infection transmitted from the flea bite of an infected rat to humans

budding A form of asexual reproduction in which a bud or outgrowth from the end or side of the parent cell emerges and develops into a new organism

capsid The outer protein shell surrounding the nucleic acid of a virus

capsomeres Repetitive protein subunits that form the capsid; often arranged in a symmetric pattern

carbohydrate Chemical substances containing carbon, oxygen, and hydrogen atoms that are an essential structural component of living cells and a source of energy for animals (i.e., sugars and starches)

Carotid arteries Paired large caliber vessels that pass on either side of the neck, supplying oxygenated blood to the brain

cell-mediated The branch of the immune system in which specific defense cells, rather than antibodies, respond and act against a foreign antigen

cellulose A complex carbohydrate that is the chief element of all plant tissues and fibers

Centers for Disease Control and Prevention (CDC) US governmental agency whose mission is to promote health and quality of life by preventing and controlling disease, injury, and disability.

Chagas' disease A parasitic infection that is transmitted by biting insects; it can be prevented by sleeping with bed netting and using insecticide to kill insects

Chandipura virus (CHPV) A virus belonging to the *Vesiculovirus* genus and *Rhabdoviridae* family, has been associated with a number of encephalitis epidemics, with high mortality in children, in different parts of India

chemolithotrophs Organisms that obtain their energy from the oxidation of inorganic compounds

chemostat An apparatus designed to grow bacteria indefinitely, while keeping the conditions and the colony size constant by having a continuous flow of liquid nutrient wash the colony and steadily remove bacteria

chicken pox A viral infection spread through direct contact or by coughing, sneezing, and touching contaminated clothing; it causes a blisterlike rash on the surface of the skin and mucous membranes

Chikungunya fever A viral disease transmitted to humans by the bite of infected *Aedes Aegypti* mosquitoes. Chikungunya virus (CHIKV) is a member of the genus *Alphavirus*, in the family *Togaviridae*. Chikungunya fever typically lasts from 5 to 7 days and frequently causes severe and often incapacitating joint pain that sometimes persists for much longer periods

Chlorophyll A group of green pigments found in green plants, algae, and some bacteria necessary for energy production

cholera A disease transmitted by fecal-contaminated food and water and by ingesting raw or undercooked seafood.

chromosome A threadlike body in the cell nucleus that carries the genes in a linear order

cilia Short hair-like appendages found on the surfaces of some types of cells and organisms; used for either propelling trapped material out of the body or for locomotion

coadaptation Mutual adaptation in two or more interactive species

computerized axial tomography (CAT) or **computed tomography (CT)** A special radiographic technique that uses a computer to assimilate multiple X-ray images into a two-dimensional cross-sectional image

conjugation A mating process in which the temporary union of 2 one-celled organisms results in the exchange of genetic material

cryptosporidiosis An infection caused by an intestinal parasite, transmitted through the ingestion of food or water contaminated with animal feces

cytoplasm The living substance of a cell excluding the nucleus

deforestation The state of being clear of trees

dengue fever A virus transmitted from the bite of the *Aedes* mosquito. Dengue is transmitted by the bite of an *Aedes* mosquito infected with any one of the four dengue viruses and occurs in tropical and subtropical areas of the world

diagnosis The act of identifying a disease and its cause

dialysis A medical procedure that uses a machine to filter waste products from the bloodstream and restore the bloods normal constituents

diphtheria An acute bacterial infectious disease that is spread by droplets sprays from an infected person; children can be immunized against this disease

DNA (deoxyribonucleic acid) The primary genetic material of a cell

Ebola A deadly virus that is transmitted through direct contact with the blood or bodily fluids of an infected person, unsterilized needles, or an infected animal

ecosystem A community of organisms and their physical environment interacting as an ecological unit

electron microscopy A form of microscopy in which a beam of electrons deflected by electromagnets can magnify a specimen up to 400,000 times its original size

encephalitis A virus caused by the bite of an infected mosquito

endemic A disease that is constantly present to a greater or lesser degree in people of a certain class or in people living in a particular location

endoplasmic reticulum An extensive network of internal membranes within an eukaryotic cell that is necessary for protein synthesis

enzyme Any of several complex proteins that are produced by cells and act as catalysts in specific biochemical reactions

epidemic A widespread outbreak of an infectious disease in which many people are infected at the same time

epidemiology The branch of medical science dealing with the incidence, distribution, and control of disease in a population

eukaryote A cell that possesses a defined nucleus surrounded by a membrane; protists, fungi, plants, and animals are eukaryotes.

exposure The act of coming into contact with a disease-causing microorganism; exposure may or may not lead to infection

extremophiles Organisms (typically bacteria) that are adapted to living in extreme conditions, such as high salt, in ice, or in thermal springs

fermentation The oxidation of compounds by the enzyme action of microorganisms

flagellum A thin filamentous appendage on cells, such as bacteria and protists, responsible for locomotion

flora In microbiology, the microorganisms present in a given environment; normal flora are those microorganisms that reside harmlessly within the human body

fluke A parasitic trematode worm, which has a flat, leaf-shaped body and two suckers

fungi Molds, mushrooms, and yeasts that comprise the group of flowerless and seedless plants that reproduce by means of asexual spores showing no differentiation into stem, root, and leaf and are deprived of chlorophyll

genotype The particular set of genes found within an organism

genus A category in biological classification comprising one or more phylogenetically related species

germ theory A theory in medicine that infectious diseases result from the action of microorganisms

Golgi apparatus A membrane-bound structure found within the cytoplasm of eukaryotic cells, which function in protein synthesis

habitat The type of environment in which an organism or group normally lives or occurs

Hantavirus A virus that is carried by rodents, especially deer mice and is found in their urine and feces but does not make the animal sick; believed that humans can get sick with this virus if they come in contact with contaminated dust from mice nests or droppings

Hantavirus pulmonary syndrome (HPS) Transmitted by exposure to rodent excrement via aerosol distribution, especially in moist areas, and rodent saliva from bites

helix Something spiral in form

helminth A worm that is a multicellular animal and can be either free-living or parasitic (i.e., roundworms, tapeworms, flukes)

hemagglutinin (H) protein One of the two main proteins found on the surface of the virus that causes the flu; it is necessary for attaching the virus to the host cell

hemorrhagic Showing evidence of bleeding; certain infections (hemorrhagic fevers) result in the loss of blood and body fluids

Hepatitis A, B, C Three types of this viral disease are transmitted in different ways: A, through ingestion of contaminated food or water; B, sexually transmitted and the use of unsterilized needles; and C, transfusion of tainted blood or transplant of infected tissue affecting the liver

hermaphroditic Having both male and female reproductive organs

herpes A recurrent viral infection caused by *Herpesvirus hominis* (HVH); consists of the following five viruses: Herpes simplex virus types 1 and 2, human cytomegalovirus, Varicella-Zoster virus, and Epstein-Barr virus

heterotrophs Microorganisms that require carbon dioxide and other organic compounds for their nutrition and energy needs

HIV (human immunodeficiency virus) A type of retrovirus that is responsible for the fatal illness acquired immunodeficiency syndrome (AIDS)

hookworm An intestinal parasitic infection caused by larval hookworms that penetrate the host's skin

host An organism that provides food or shelter for another organism

humoral immunity The branch of the immune system in which antibodies are produced in response to a foreign antigen

hypodermic Administered by injection beneath the skin

icosahedral Having 20 equal sides or faces

immune system The parts of the body that prevent and fight disease

inflammatory disease Disease with inflamed tissue, characterized by pain, swelling, redness and heat

influenza More commonly known as the "flu," is transmitted from the sneeze or cough of an infected person, person-to-person contact, or contact with objects that an

infected person has contaminated with nose and throat secretions; an infectious disease of birds and mammals caused by RNA viruses of the family *Orthomyxoviridae*, the influenza viruses. The most common symptoms are chills, fever, sore throat, muscle pains, headache (often severe), coughing, weakness/fatigue, and general discomfort

inhibitor A molecule that represses or prevents another molecule from engaging in a reaction

intravenous Occurring within or entering by way of a vein

Japanese encephalitis (JE) A disease caused by a flavivirus that affects the membranes around the brain; transmitted by mosquitoes

Junin virus South American arenavirus is known as the Argentine hemorrhagic fever, inflicting several hundred people annually; a rodent-borne virus whose origin or cause of spread remain unknown

Kuru A slow-virus disease rarely seen today because of the discontinuance of cannibalism and ritualistic butchering

Kyasanur Forest disease (KFD) A tick-borne encephalitis complex; a flavivirus transmitted between infected ticks and monkeys that can cause severe hemorrhagic fever. KFD is caused by Kyasanur Forest disease virus (KFDV), a member of the virus family *Flaviviridae*, isolated from a sick monkey from the Kyasanur forest in the Karnataka (formerly Mysore) State of India. The main hosts of KFDV are small rodents, but shrews, bats, and monkeys may also carry the virus. KFD is transmitted from the bite of an infected tick (*Haemaphysalis spinigera* is the major vector). Humans can get these diseases from a tick bite or by contact with an infected animal, such as sick or recently dead monkey

leishmaniasis A parasitic infection transmitted through the bite of a female sandfly

leptospirosis A bacterial infection that is transmitted through direct contact with water, food, or soil, containing urine from an infected animal; a rare and severe infection that occurs when a human comes in contact with *Leptospira* bacteria

limbic system A system of functionally related neural structures in the brain that are concerned with emotion and motivation

lipid A fat or fatlike substance that is insoluble in water but soluble in organic solvents and is an essential structural component of living cells (along with proteins and carbohydrates)

Lyme disease Transmitted from the bite of a deer tick, this bacterium subjects the victim to a circular rash with a clear center area

lymphocyte A white blood cell present in the blood, lymph, and lymphoid tissue; two major types are T cells and B cells

lysosomes Structures found within the cytoplasm of certain eukaryotic cells that contain digestive enzymes; responsible for ridding the cell of debris

macrophage A large, immune system cell that devours foreign antigens and stimulates the action of other immune system cells

macroscopic Large enough to be visible to the naked eye

magnetic resonance imaging (MRI) A special imaging technique used to image internal structures of the body, particularly the soft tissues; creating an image superior to a normal X-ray

malaria A tropical parasitic disease that kills more people than any other communicable disease except tuberculosis; reemerging in areas that had controlled or eradicated the disease

measles A viral infection that is spread through contact with the saliva from an infected person

metabolic reaction Chemical changes in living cells by which energy is provided for vital processes

melioidosis An infectious disease caused by a gram-negative bacterium, *Burkholderia pseudomallei*, found in soil and water

meningitis An infection of the fluid of a person's spinal cord and the fluid that surrounds the brain sometimes referred as *spinal meningitis*

miasmatic One who has made a special study of infectious particles or germs floating in the air

microbe A microscopic organism, such as a bacterium, a virus, or a protozoan

microorganism Any organism that can only be seen with a microscope; protozoan, bacteria, fungi, and viruses are examples of microorganisms

mitochondria Small intracellular organelles, found in eukaryotic cells, which are responsible for energy production and cellular respiration

multicelluar Consisting of, or having, more than one cell or many cells

multiple fission Splitting multiple times

mumps A virus that lives in the mouth, nose, and throat, can be transmitted when an infected person cough, sneezes, talks, or touches someone else

mutagen An agent that can cause an increase in the rate of mutation, includes X-rays, ultraviolet irradiation, and various chemicals

natural selection A natural process that directs the evolution of organisms best adapted to the environment

nematode An unsegmented worm with an elongated, round body pointed at both ends; mostly free-living but some are parasitic

Nipah virus (NiV) A paramyxovirus whose reservoir host is fruit bats of the genus *Pteropus;* occasionally the virus is introduced into human populations and causes severe illness characterized by encephalitis or respiratory disease

nucleotide The basic structural unit of nucleic acids (DNA or RNA)

nucleus The membrane-bound structure found in eukaryotic cells that contains DNA and RNA and is responsible for growth and reproduction

organelles Subcellular, membrane-bound structures found within eukaryotic cells, which perform discrete functions necessary for the life of the cell

organism Any individual living thing, whether animal, plant or microorganism

outbreak The occurrence of a large number of cases of a disease in a short period of time

pandemic An epidemic that affects multiple geographic areas at the same time.

paragonimiasis A food-borne parasitic infection caused by the lung fluke, most commonly *Paragonimus westermani*

paramecia Ciliate protozoa of the genus *Paramecium* that have an elongated body, rounded at the anterior end and a funnel-shaped mouth at the extremity

parasite An animal or plant that lives in or on another and from which it obtains nourishment

pasteurization Partial sterilization of food at a temperature that destroys harmful microorganisms without major changes in the chemistry of the food

pathogen Any disease-producing agent (i.e., virus, bacteria, or other microorganisms)

pertussis A disease of the respiratory mucous membrane, also known as *whooping cough*

phagocytosis The intake of material into a cell by the formation of a membrane-bound sac

photosynthesis The process by which green plants, algae, and some bacteria absorb light energy and use it to synthesize organic compounds (initially carbohydrates)
phototrophs Microorganisms (bacteria) capable of using light energy for metabolism
phylogeny The evolutionary history of a particular taxonomic group
phylum The second highest taxonomic classification for the kingdom *Animalia* (animals), between kingdom level and class level
pili Threadlike structures present on some bacteria; pili are shorter than flagella and are used to adhere bacteria to one another during mating and to adhere to animal cells
placebo A medicinal preparation having no specific pharmacological activity against a patient's illness or complaint; given solely for the psychophysiological effects of the treatment
plague A severe and potentially deadly bacterial infection caused by the enterobacteria *Yersinia pestis*
plankton Small (often microscopic) plants and animals floating, drifting, or weakly swimming in bodies of fresh or salt water
plasmid A small, independently replicating circle of DNA found in bacteria that can be transferred from one organism to another during certain types of mating
polio A disease caused by a virus that causes paralysis, which is irreversible, and in more severe cases this paralysis can lead to death by asphyxiation; gains entry to the body by fecal-oral contact or person-to-person contact
polygyny The mating of a single male with several females
polymerase chain reaction (PCR) The first practical system for in vitro amplification of DNA, and as such, one of the most important recent developments in molecular biology
polymorphonuclear leukocytes Also called *neutrophils;* white blood cells that respond quickly, phagocytose and destroy foreign antigens, such as pathogenic microorganisms
polypeptides Two or more amino acids bound together that upon a chemical reaction with water yields multiple amino acids
polysaccharide Any of a class of carbohydrates whose molecules contain chains of monosaccharide (simple sugar) molecules
prions An infective group of complex organic compounds (proteins) suggested as the causative agents of several infectious diseases
proglottids A segment of a tapeworm containing both male and female reproductive organs; capable of a brief independent existence
prokaryotes Organisms, namely bacteria and blue green algae, characterized by the lack of a distinct nucleus
prophylactic Preventive measure or medication
protease Any enzyme that catalyses the splitting of interior peptide bonds in a protein
protein Any of a group of complex organic compounds that contain carbon, hydrogen, oxygen, nitrogen, and usually sulfur, the characteristic element being nitrogen; widely distributed in plants and animals
protist Unicellular, colonial, or multicellular organisms including protozoa and most algae
protozoa Simple, unicellular animals comprising some 50,000 organisms
pseudopods Temporary blunt-ended projections of the cytoplasm of a cell that is used for locomotion or food collecting (in amoeba)
Q Fever A bacterial infection that is contracted by contact with materials contaminated with animal feces, blood, inhaling contaminated dust or droplets, or ingesting

contaminated food or liquids receptor, a molecule on the surface of a cell that serves as a recognition or binding site.

recombination Formation by the process of crossing over an independent assortment of new genes in the offspring that did not occur in the parents

reservoir host A host that carries a pathogen without injury to itself and serves as a source of infection for other host organisms

ribosomes A structure found within the cytoplasm of cells, made up of protein and RNA, that serves as the site of protein synthesis

Rift Valley fever (RVF) An acute, fever-causing viral disease that affects domestic animals (such as cattle, buffalo, sheep, goats, and camels) and humans; RVF is most commonly associated with mosquito-borne epidemics during years of heavy rainfall

rinderpest virus (RPV) A highly infectious viral disease that can destroy entire populations of cattle and buffalo; the only way to prevent this disease is to vaccinate all animals and livestock

river blindness A parasitic worm disease that is spread by the bite of a blackfly; the best way to prevent the disease is insect repellent

RNA (ribonucleic acid) A nucleic acid that governs protein synthesis in a cell

serum The clear, thin fluid portion of the blood that remains after coagulation; antibodies and other proteins are found in the serum

severe acute respiratory syndrome (SARS) A serious form of pneumonia, caused by a viral infection with the SARS virus and causes acute respiratory distress (severe breathing difficulty) and sometimes death

sexually transmitted disease (STD) A communicable disease transmitted by sexual intercourse or genital contact

spore formation Formation of unicellular, often environmentally resistant, dormant, or reproductive bodies produced by some microbes

symbiotic The relationship between two interacting organisms or populations

T cells Thymus-derived white blood cells (lymphocytes) that participate in a variety of cell-mediated immune responses

T-cytotoxic cells A subset of T lymphocytes that are able to directly kill foreign cells, especially virally infected host cells

tegument The covering of a living body or of some part or organ of such a body

T-helper cells A subset of T lymphocytes that normally orchestrate the immune response by signaling other cells in the immune system to perform their special functions

thymus The lymphoid organ in which T lymphocytes are educated, mature, and multiply

toxigenic *E. coli* A bacterial infection transmitted through the ingestion of undercooked ground beef, unpasteurized milk, or water that has been contaminated by sewage

trypanosomiasis (African sleeping sickness) A parasitic infection that is spread through the bite of the Tsetse fly

tuberculosis, MTB, or TB (short for *tubercle bacillus*) A common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*

unicellular When an organism has only one cell

vaccine A substance that contains antigenic components , either weakened, dead, or synthetic, from an infectious organism that is used to produce active immunity against that organism

vector An organism that transmits a pathogen

vertebrate One of the grand divisions of the animal kingdom, comprising all animals that have a backbone composed of bony or cartilaginous vertebrae

virions A single virus particle, complete with coat

virulence The degree or ability of a pathogenic organism to cause disease

virus Ultramicroscopic infectious agent that replicates itself only within cells of living hosts

West Nile virus (WNV) A mosquito-borne zoonotic arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*, an infectious disease; found in temperate and tropical regions of the world

white blood cell A white corpuscle in the blood; spherical, colorless mass involved with host defenses; blood cell that engulf and digest bacteria and fungi; an important part of the body's defense system

World Health Organization (WHO) An agency of the United Nations founded in 1948 to promote technical cooperation for health among nations, carry out programs to control and eradicate disease, and strives to improve the quality of human life

Index

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.

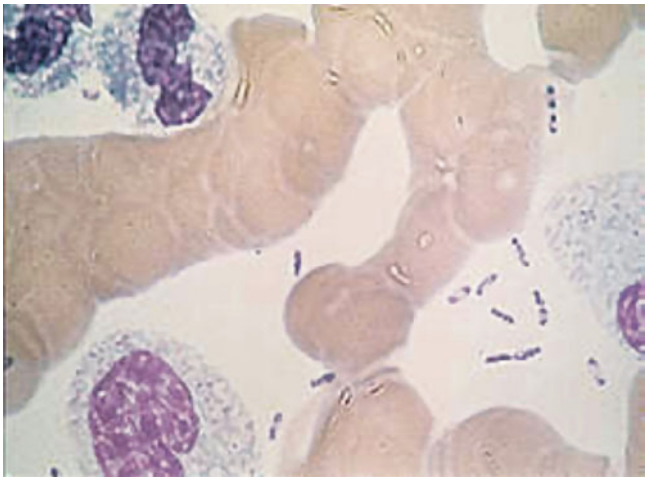

Figure 6.6 Wayson's stain of *Yersinia pestis* with the characteristic safety pin appearance of the bacteria. http://www.cdc.gov/ncidod/dvbid/plague/wayson.htm.

Emerging Epidemics: Management and Control, First Edition. Prakash S. Bisen and Ruchika Raghuvanshi.