Chapter 25

EMERGING INFECTIOUS DISEASES AND FUTURE THREATS

CHRIS A. WHITEHOUSE, PhD*; BRETT BEITZEL, PhD†; ZYGMUNT F. DEMBEK, PhD, MS, MPH, LHD‡; and ALAN L. SCHMALJOHN, PhD§

**Principal Investigator, Molecular and Translational Sciences Department, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702; formerly, Chief, Disease Investigation Branch, US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin*

†Principal Investigator, Center for Genomic Sciences, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702

‡Colonel (Retired), Medical Service Corps, US Army Reserve; Associate Professor, Department of Military and Emergency Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814; formerly, Chief Biodefense Epidemiology and Education & Training Programs, Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland

§Professor, Department of Microbiology and Immunology, University of Maryland School of Medicine, 655 West Baltimore Street, HSF-1, 322B, Baltimore, Maryland 21201

INTRODUCTION

What Are Emerging Infectious Diseases?

Infectious diseases have caused the deadliest pandemics in recorded human history. Some of these have included the Black Death (bubonic plague resulting in 25–40 million deaths), the 1918–1919 influenza pandemic ("Spanish Flu" resulting in an estimated 50 million deaths), and the ongoing human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) pandemic (resulting in 35 million deaths to date).[1] Emerging infectious diseases, as defined in the landmark report by the Institute of Medicine in 1992, are those diseases whose incidence has increased within the past 20 years or whose incidence threatens to increase in the near future.[2] Although some "emerging" diseases have been now recognized for more than 20 years (eg, HIV/AIDS, Lyme disease or Legionnaires' disease), their importance has not diminished, and the factors associated with their emergence are still relevant. Emerging infections include those that are recognized in their host (humans, animals, or plants) for the first time or caused by new or newly described agents. Reemerging diseases are diseases that historically have infected humans, but appear in new locations, or whose incidence had previously declined, but now are increasing. In addition, this definition includes organisms that are developing antimicrobial resistance and established chronic diseases with a recently discovered infectious origin.

Factors That Contribute to Emerging Infectious Diseases

Many factors contribute to the emergence of new diseases. In the United States, in particular, these factors include increasing population density and urbanization; immunosuppression (resulting from aging, malnutrition, cancer, or infection with HIV); changes in land use (eg, deforestation, reforestation, and fragmentation), climate, and weather; international travel and commerce; microbial or vector adaptation and change (mutations resulting in drug/pesticide resistance or increased virulence).[2] Internationally, many of these factors also hold true; however, many developing countries also have to deal with war, political instability, inadequate healthcare, and basic sanitation needs.

The numerous examples of novel infections originating from animal species (ie, zoonoses) suggest that animals are an important source of emerging diseases.[3] Although it is controversial whether HIV/AIDS should be considered a zoonotic disease,[4] it is now clear that both HIV-1 and HIV-2 had zoonotic origins.[5–7] In addition, as was observed with the 2003 outbreak of monkeypox in the United States, increasing trade in exotic animals for pets has led to increased opportunities for pathogens to "jump" from animal reservoirs to humans. The use of exotic animals (eg, Himalayan palm civets) for food in China and the close aggregation of numerous animal species in public markets may have led to the emergence of the 2002–2003 outbreak of severe acute respiratory syndrome (SARS).[8]

Many of the viruses or bacteria that cause concern as potential bioweapons are considered emerging pathogens, and most are also of zoonotic origin. In particular, some of these agents have appeared in new geographical locations where they were not previously seen (eg, the sudden occurrence of monkeypox in the midwest of the United States in 2003, and the largest recorded outbreak of Marburg hemorrhagic fever in Angola in 2005). In some cases, the specific use of a pathogen in an act of bioterrorism could classify that pathogen as an emerging or reemerging disease agent, as was the case for *Bacillus anthracis* during the 2001 anthrax attacks in the United States. Through increasingly accessible molecular biology techniques, completely new organisms—or significant modification of existing ones—can now be made in the laboratory (ie, synthetic biology). The use of these methods is beneficial and necessary for modern biomedical research to proceed. However, the same methods and techniques can be used for nefarious purposes and, along with naturally occurring emerging infections, represent significant future threats to both military and civilian populations.

More than 20 years after the Institute of Medicine Report, much progress on emerging infectious diseases has been made, including a greater awareness; use of next-generation sequencing for the characterization of pathogens, vectors, and their hosts and for enhanced diagnostics; and increased laboratory infrastructure including additional biocontainment laboratories (ie, biosafety level 3 laboratories and biosafety level 4 laboratories) to safely work with these pathogens.[9] Despite this progress, new diseases continue to emerge. This continual emergence of new infectious diseases poses a continuing challenge, requiring constant surveillance, the ability to promptly respond with new diagnostics and new vaccines and drugs, and ongoing research into the basic biology of novel pathogens.

EMERGING BACTERIAL DISEASES

Waterborne Diseases

Emerging waterborne diseases constitute a major health hazard in both developing and developed countries. From 2007 to 2008, 48 disease outbreaks associated with contaminated drinking water were reported in the United States, resulting in 4,128 ill people and 3 deaths.[10] During this same time, more than 13,966 cases of illness were associated with 134 recreational water-associated outbreaks of disease.[11] Although these numbers represent disease caused by a range of pathogenic organisms (ie, viruses, bacteria, parasites), the majority (58%) of drinking water-associated outbreaks were caused by bacterial pathogens. Bacterial pathogens associated with drinking water disease outbreaks included *Legionella* (12 outbreaks), *Campylobacter* (4 outbreaks), *Salmonella* (3 outbreaks), *Escherichia coli* O157:H7 and *Providencia* (1 outbreak each).[10] Bacterial pathogens were responsible for 21% of the outbreaks of disease associated with recreational water exposure. Diseases associated with exposure to recreational water are more diverse than those associated with drinking water and include acute gastrointestinal illness, acute respiratory illness, and dermatologic illness. Accordingly, the list of bacterial pathogens responsible is more diverse and includes *E coli* O157:H7, *Shigella sonnei*, *Legionella*, *Plesiomonas shigelloides*, and *Vibrio vulnificus*. Dermatitis outbreaks were most often attributed to *Pseudomonas*, primarily *P aeruginosa*.[12] Internationally, cholera (caused by *Vibrio cholerae*) is still a major killer, as demonstrated by recent large outbreaks in Sierra Leone, Zimbabwe, and Democratic Republic of Congo in Africa resulting in more than 120,000 cases combined and more than double that number of cases in Haiti following a massive earthquake in 2010 (see Cholera and *Vibrio cholerae*).

Cholera and Vibrio cholerae

Cholera is one of the most rapidly fatal diseases known, capable of killing within 12 to 24 hours after onset of diarrhea. The World Health Organization (WHO) estimates 3 to 5 million cholera illnesses and up to 130,000 deaths occur globally each year.[13] Cholera accounts date back to Hippocrates.[14] Seven worldwide cholera pandemics have occurred. An 1892 cholera outbreak in Hamburg, Germany, affecting 17,000 people and causing 8,605 deaths, was attributed to the inadvertent contamination of the city's water supply by bacteriologists studying the pathogen.[15] This event underscores the potential for cholera to cause widespread illness where water is not disinfected with a modern bacteriocide such as chlorine.[15]

In 1991, after almost a century without cholera, outbreaks in Latin America resulted in about 400,000 cases of cholera and more than 4,000 deaths.[16] Off the Peruvian coast, a significant correlation existed between cholera incidence and elevated sea surface temperature from 1997 to 2000, which included the 1997 to 1998 El Niño event.[17] Some people believe that the eighth worldwide pandemic began in 1992.[18] During 2011, 58 countries reported a total of 589,854 cases including 7,816 deaths from cholera.[13] Cholera cases in the United States have decreased to about 10 cases per year from 1995 through 2009; however, 42 cases were reported in 2011.[13] Most of these cases were either travel-associated or associated with consumption of undercooked seafood harvested along the Gulf Coast.

Cholera occurs through fecal-oral transmission brought about by deterioration of sanitary conditions. Epidemics are strongly linked to the consumption of unsafe water, poor hygiene, poor sanitation, and crowded living conditions (Figure 25-1). Water or food contaminated by human waste is the major vehicle for disease transmission. Cholera transmission is thought to require 10^3 organisms to exert an effect in the gut, with 10^{11} organisms as a minimum infective dose needed to survive stomach acid.[19]

Before 1992, all previous cholera pandemics were caused by the *V cholerae* serogroup O1 (classical) or El Tor biotypes. Large outbreaks in 1992 resulted from transmission of a previously unknown serogroup, *V cholerae* O139, which has since spread from India and Bangladesh to countries throughout Asia, including Pakistan, Nepal, China, Thailand, Kazakhstan, Afghanistan, and Malaysia.[20]

Enterotoxin produced by *V cholerae* O1 and O139 can cause severe fluid loss from the gut. In severe cases, profuse watery diarrhea, nausea, and vomiting can lead to rapid dehydration, acidosis, circulatory collapse, and renal failure. Successful treatment of cholera patients depends on rapid fluid and electrolyte replacement. Antimicrobial therapy can also be useful.

Mixed success has been obtained with cholera vaccines. Historically, live attenuated vaccines have been more effective than killed whole-cell vaccines.[21] No licensed cholera vaccines are available in the United States.

Figure 25-1. Typical conditions that can lead to a cholera epidemic. This photograph was taken in 1974 during a cholera research and nutrition survey amidst floodwaters in Bangladesh.
Photograph: Courtesy of Dr Jack Weissman, Centers for Disease Control and Prevention Public Health Image Library.

On January 12, 2010, a magnitude 7.0 earthquake decimated the island nation of Haiti, leaving a quarter of a million people dead, 300,000 injured, and 2 million homeless. Nine months later, in October, a cholera outbreak was confirmed in Haiti,[22] indicating the first occurrence of cholera in Haiti in at least 100 years. Some have suggested that cholera may never have been in Haiti before 2010.[23] Based on epidemiological data, the cholera outbreak began in the upstream region of the Artibonite River (Figure 25-2).[24] The presumed first case was a 28-year-old man with a history of severe untreated psychiatric disease.[25] The patient had a history of wandering nude through town throughout the day and both bathing in and drinking the water from the Latem River, one of the tributaries of the Artibonite River. On October 12, 2010, he developed acute onset of profuse watery diarrhea. In less than 24 hours after the onset of symptoms, he died at home without seeking medical attention. The first hospitalized case of cholera in Haiti occurred at the Mirebalais Government Hospital on October 17, 2010.[24]

By mid-November, cholera had spread to every part of the country and to neighboring Dominican Republic, and by mid-December a total of 121,518 cases of cholera, resulting in 63,711 hospitalizations and 2,591 deaths, had been reported from Haiti.[22] The cholera outbreak in Haiti has continued since 2010 with more than 734,983 cases and 8,761 deaths as of April 3, 2015, according to the Pan American Health Organization.[26]

The outbreak strain was identified as *V cholerae* O1, serotype Ogawa, biotype El Tor.[27] Whole genome DNA sequencing and epidemiological analysis confirmed that the outbreak strain was inadvertently introduced into Haiti by United Nations security forces from Nepal.[28–30]

A cholera outbreak was reported in Kathmandu (Nepal's capital city) on September 23, 2010, shortly before troops left for Haiti.[31] The first cholera cases in Haiti came from a village named Meye, located 150 meters downstream from the Nepalese military camp.[24,30] Taken together, evidence strongly supports the conclusion that the United Nations military camp, housing the Nepalese peacekeeping troops in Meye, was the source of the Haitian cholera epidemic. These findings led to considerable political unrest and have forever changed the global response to natural disasters. In late 2013, survivors and family members of the nearly 700,000 Haitians who contracted cholera sued the United Nations, accusing them of covering up its role in starting the cholera outbreak in Haiti. In early 2015, a US federal judge ruled that the Haitians could not sue the United Nations because the organization has legal

Figure 25-2. The Artibonite River is the longest and most important river in Haiti. It forms part of the international border between Haiti and the Dominican Republic and empties into the Gulf of Gonâve. It is believed that the 2010 cholera outbreak began in the upstream region of this river.
Photograph: Courtesy of Kendra Helmer, US Agency for International Development.

immunity against lawsuits. In August 2016, the Court upheld the United Nations' immunity from claims (http://www.ijdh.org/cholera/cholera-litigation/).

Other Vibrioses

In recent years, some noncholera vibrios have acquired increasing importance because of their association with human disease. More than 70 members are in the family *Vibrionaceae*, 12 of which have been isolated from human clinical specimens and apparently are pathogenic for humans.[32] Vibrio species are primarily aquatic and common in marine and estuarine environments and on the surface and in the intestinal tracts of marine animals. *V parahemolyticus* and *V vulnificus* are halophilic vibrios commonly associated with consumption of undercooked seafood. Diarrhea, cramping, nausea, vomiting, fever, and headache are commonly associated with *V parahemolyticus* infections.

Cases of diarrhea related to seafood consumption increased worldwide with the emergence of pandemic strain O3:K6, which was originally observed in Southeast Asia.[33] *V vulnificus* is the most common source of vibrio infections in the United States resulting in gastrointestinal symptoms similar to *V parahemolyticus*, but may also lead to ulcerative skin infections if open wounds are exposed to contaminated water. Septicemia can occur in those infected with *V vulnificus* who are immunosuppressed or have liver disease or chronic alcoholism, and septicemic patients can have a mortality rate of up to 50%. In most cases the disease begins several days after the patient has eaten raw oysters. Other human pathogenic species include *V mimicus*, *V metschnikovii*, *V cincinnatiensis*, *V hollisae*, *V damsela*, *V fluvialis*, *V furnissii*, *V alginolyticus*, and *V harveyi*; most of these have been associated with sporadic diarrhea, septicemia, and wound infections.[32]

Legionellosis

Legionnaires' disease was first recognized in 1976 after a large outbreak of severe pneumonia occurred among attendees at a convention of war veterans in Philadelphia. A total of 182 people, all members of the Pennsylvania American Legion, developed an acute respiratory illness, and 29 individuals died from the disease.[34] The cause of the outbreak remained a mystery for 6 months until the discovery by Joseph McDade, a Centers for Disease Control and Prevention (CDC) microbiologist, of a few gram-negative bacilli, subsequently named *Legionella pneumophila*,[35] in a gram stain of tissue from a guinea pig inoculated with lung tissue from a patient who died from the disease.[36] Using the indirect immunofluorescence assay, McDade showed that the sera of patients from the convention mounted an antibody response against the newly isolated bacterium,[36] marking the discovery of a whole new family of pathogenic bacteria. Retrospective analysis, however, showed that outbreaks of acute respiratory disease from as far back as 1957 have now been attributed to *L pneumophia*.[37,38] The earliest recorded isolate of a *Legionella* species was recovered by Hugh Tatlock in 1943 during an outbreak of Fort Bragg fever.[39,40]

Legionnaires' disease is normally acquired by inhalation or aspiration of *L pneumophila* or other closely related *Legionella* species. Water is the major reservoir for legionellae, and the bacteria are found in freshwater environments worldwide. Legionnaires' disease has been associated with various water sources where bacterial growth is permitted, including cooling towers,[41] whirlpool spas,[42] and grocery store mist machines.[42] The association between a portable shower and nosocomial legionellosis was demonstrated more than 30 years ago.[43] The most common source of legionellosis in hospitals is from the hot water system,[44] and sustained transmission of Legionnaires' disease in the hospital environment can be difficult to control.[42] Community-acquired legionellosis is thought to account for most infections.[45] An Italian survey of household hot water systems in 2000 found bacterial contamination, with *Legionella* species in 23% of the homes and *Pseudomonas* species in 38%. One *Legionella* species, *L longbeachae*, has been associated with disease transmission from potting soil.[16]

Legionnaires' disease is an acute bacterial illness that initially presents with anorexia, malaise, myalgia, and headache, with a rapidly rising fever and chills. Temperatures commonly reach 102°F to 105°F and are associated with nonproductive cough, abdominal pain, and diarrhea. The disease may eventually progress to respiratory failure and has a case-fatality rate as high as 39% in hospitalized cases. Nonpneumonic legionellosis, or Pontiac fever, occurs after exposure to aerosols of water colonized with *Legionella* species.[46–48] Attack rates after exposure to an aerosol-generating source, which often range from 50% to 80%, are exceptionally high. After a typical asymptomatic interval of 12 to 48 hours after exposure, patients note the abrupt onset of fever, chills, headache, malaise, and myalgias. Pneumonia is absent and those who are affected recover in 2 to 7 days without receiving specific treatment.[49]

Legionella is now recognized around the world as an important cause of community-acquired and hospital-acquired pneumonia, occurring both sporadically and in outbreaks. Although 90% of *Legionella* infections in humans are caused by *L pneumophila*, there are 50 named species of *Legionella*, with approximately 20

known to cause human infections.[50] Some unusual strains of bacteria, which infect amoebae and have been termed *Legionella*-like amoebal pathogens (LLAPs), appear to be closely related to *Legionella* species on the basis of 16S ribosomal RNA gene sequencing.[51,52] Three LLAP strains are now named *Legionella* species[53]; one of them, LLAP-3, which was first isolated from the sputum of a patient with pneumonia by coculture with amoebae, is considered a human pathogen.[54]

Foodborne Diseases

More than 200 diseases are transmitted through food, including illnesses resulting from viruses, bacteria, parasites, toxins, metals, and prions. In the United States, the burden of foodborne illness is estimated at approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year.[55] Among the bacterial pathogens estimated to cause the greatest number of US foodborne illnesses are *Campylobacter*, *Salmonella*, *Shigella*, *Clostridium*, and *Staphylococcus*.[55] Emerging bacterial illnesses include *E coli* O157:H7 and other enterhemorrhagic and enterotoxigenic *E coli*, as well as antibiotic resistant bacteria. Many of the pathogens of greatest concern today (eg, *C jejuni*, *E coli* O157:H7, *Listeria monocytogenes*, *Cyclospora cayetanensis*) were not recognized as causes of foodborne illness just 20 years ago. Other rare pathogens, such as *E coli* O104:H4, recently emerged as a cause of a foodborne outbreak of hemolytic uremic syndrome (HUS) in northern Germany resulting in more than 4,000 cases and 54 deaths (see section on Disease Caused by *Escherichia coli* O104:H4).

The majority of gastrointestinal illnesses are caused by foodborne agents not yet identified. It is estimated that 62 million foodborne-related illnesses and 3,200 deaths occur in the United States each year from unknown pathogens.[55] *Bacillus anthracis*, although rarely seen as a gastrointestinal illness in the United States, has become a concern since cases occurred in 2000 and 2009 (see next section). Even in areas of the world where gastrointestinal anthrax is more common, the oropharyngeal form is underreported because physicians are unfamiliar with it.[56] Unreported foodborne disease, deaths from unknown food agents,[57] and chronic sequelae[58] may be a huge unrecognized burden of illness.

Gastrointestinal Anthrax

Bacillus anthracis is the causative agent of anthrax, a naturally occurring zoonotic disease. The greatest bioweapons threat from anthrax is through aerosol dispersion and subsequent inhalation of concentrated spores (for more details, see Chapter 6, Anthrax). Gastrointestinal anthrax, however, is contracted through the ingestion of *B anthracis* spores in contaminated food or water. This form of the disease occurs more commonly than inhalational anthrax in the developing world, but it is rare in the United States and other developed nations.[56,59] In one large outbreak in Uganda, 155 villagers ate the meat of a zebu (bovine) that had died of an unknown disease. Within 15 to 72 hours, 143 (92%) persons developed presumed anthrax. Of these, 91% had gastrointestinal complaints and 9% oropharyngeal edema; nine of them—all children—died within 48 hours of illness onset.[59]

Although rare in the United States, gastrointestinal anthrax does occur naturally, and anthrax-contaminated meat was found to be associated with gastrointestinal illness in Minnesota in 2000.[16] Another case occurred in 2009 from exposure to animal-hide drums.[22] Purposeful contamination of food or water is possible, but it would require a high infective dose. Misdiagnosis of gastrointestinal anthrax may lead to a higher mortality than other forms of anthrax; thus awareness of this disease remains important in anthrax-endemic areas and in the setting of possible bioterrorism.

Disease Caused by **Campylobacter jejuni**

Campylobacter was first identified in 1909 (then called *Vibrio fetus*) from the placentas and aborted fetuses of cattle. The organism was not isolated from humans until nearly 40 years later when it was found in the blood of a pregnant woman who had an infectious abortion in 1947.[60] *Campylobacter jejuni* (Figure 25-3),

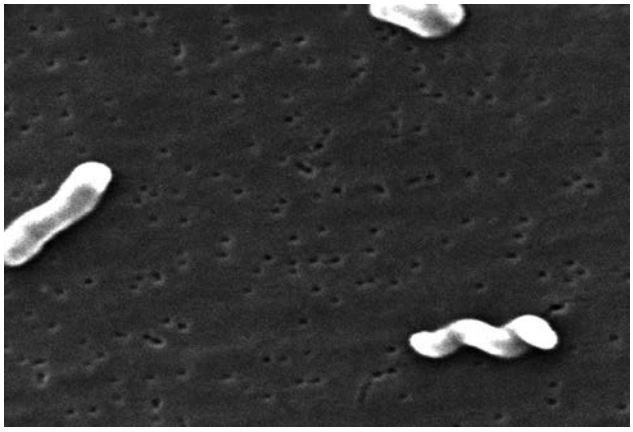

Figure 25-3. Scanning electron microscope image of *Campylobacter jejuni* illustrating its corkscrew appearance. Magnification ×11,734.
Photograph: Courtesy of Janice Carr, Centers for Disease Control and Prevention Public Health Image Library.

along with *C coli*, have been recognized as agents of gastrointestinal infection since the late 1970s. Today, *C jejuni* is considered the most commonly reported foodborne bacterial pathogen in the United States, affecting 2.4 million persons annually.[61] Campylobacteriosis is an enteric illness of variable severity including diarrhea (which may be bloody), abdominal pain, malaise, fever, nausea, and vomiting occurring 2 to 5 days after exposure. Many infections are asymptomatic; however, infection with this pathogen has also been associated with development of Guillain-Barré syndrome and arthritis.[62,63] Infants are more susceptible to *C jejuni* infections upon first exposure.[64] Persons who recover from *C jejuni* infection develop immunity. Poultry colonized with *Campylobacter* species is a major source of infections for humans.[65–67] The reported incidence of *Campylobacter* species on poultry carcasses has varied, but has been as high as 100%.[66]

Several virulence properties, including motility, adherence, invasion, and toxin production, have been recognized in *C jejuni*.[68] Along with several other enteric bacteria, *C jejuni* produces a toxin called cytolethal distending toxin that works by a completely novel mechanism; mammalian cells exposed to the toxin distend to almost 10 times their normal size from a molecular blockage in their cell cycle.[69] Although cytolethal distending toxin is the best characterized *Campylobacter* toxin, its role in the pathogenesis of human campylobacteriosis is still unclear.[70]

Because illness from *Campylobacter* infection is generally self-limited, no treatment other than rehydration and electrolyte replacement is generally recommended. However, in more severe cases (ie, high fever, bloody diarrhea, or septicemia), antibiotic therapy can be used to shorten the duration of symptoms if it is given early in the illness. Because infection with *C jejuni* in pregnant women may have deleterious effects on the fetus, infected pregnant women receive antimicrobial treatment. Erythromycin, the drug of choice for *C jejuni* infections, is safe, lacks serious toxicity, and is easy to administer. However, most clinical trials performed in adults or children have not found that erythromycin significantly alters the clinical course of *Campylobacter* infections.[71,72] Other antimicrobial agents, particularly the quinolones (eg, fluoroquinolones such as ciprofloxacin) and newer macrolides including azithromycin are also being used. Unfortunately, as the use of fluoroquinolones has expanded (especially in food animals), the rate of resistance of campylobacters to these agents has increased.[73] For example, a 1994 study found that most clinical isolates of *C jejuni* from US troops in Thailand were resistant to ciprofloxacin. Additionally, nearly one-third of isolates from US troops located in Hat Yai were resistant to azithromycin.[74] In another study conducted in 1997 in Minnesota, 13 (14%) of 91 chicken products purchased in grocery stores were contaminated with ciprofloxacin-resistant *C jejuni*,[75] illustrating the need for more prudent antimicrobial use in food–animal production.

Disease Caused by Clostridium botulinum

Clostridium botulinum produces botulinum toxin, which causes the clinical manifestations of botulism. Botulinum toxin, with a lethal dose of about 1 μg/kg, is the most potent of the natural toxins.[76] There are seven antigenic types of toxin, designated A through G with most human disease caused by types A, B, and E. Botulinum toxins A and B are most often associated with home canning and home-prepared foods, whereas botulinum toxin E is exclusively associated with ingestion of aquatic animals. Interestingly, the incidence of botulism in Alaska is among the highest in the world, and all cases of foodborne botulism in Alaska have been associated with eating traditional Alaska native foods, mostly from marine mammals; most of these cases were caused by toxin type E.[77] From 1990 to 2000, 160 foodborne botulism events affected 263 persons in the United States. Of these, 67 required intubation, and 11 deaths occurred.[77] Food items commonly associated with botulinum intoxication included homemade salsa and home-bottled garlic in oil.

Clinical illness is characterized by cranial nerve palsies, followed by symmetric descending flaccid muscle paralysis, which may involve the respiratory muscles. Full recovery may take weeks to months. Therapy includes intensive care support, mechanical ventilation as necessary, and timely administration of equine antitoxin.[78] See Chapter 14 for an in-depth discussion of the botulinum toxin.

Disease Caused by Escherichia coli O157:H7

Diarrheagenic *E coli* strains are important causes of diarrhea in humans. These strains have been divided into different pathotypes, according to their virulence attributes and mechanisms involved in the disease process. The major groups of intestinal pathogenic *E coli* strains include enteropathogenic *E coli* (EPEC), enteroaggregative *E coli* (EAEC), enterotoxigenic *E coli*, enteroinvasive *E coli* (EIEC), and enterohemorrhagic *E coli*.[79] Sometimes enterohemorrhagic *E coli* are known as Shiga toxin-producing *E coli* (STEC) and can be spread by food or water.

One STEC strain in particular, *E coli* O157:H7, has emerged as a cause of serious pediatric illness worldwide. Production of Shiga toxins depends on the presence of stx genes, located in the bacterial genome on lambdoid prophages, which are classified as mobile

genetic elements. It is these intrinsic Shiga toxins that can initiate a cascade of events that includes bloody diarrhea and HUS (exhibited by microangiopathic hemolytic anemia), acute renal failure, and thrombocytopenia.[80] HUS occurs in about 4% of all reported cases, and those younger than 5 years of age are at greatest risk for HUS subsequent to *E coli* O157:H7 infection.[55] The mortality rate for HUS is 3% to 5% and about 5% of the survivors have severe consequences, including end stage renal disease or permanent neurologic damage.[81] Antibiotic treatment of *E coli* O157:H7 is not recommended.[82] There is anecdotal evidence for an increase in the risk of HUS with the use of some antimicrobial agents. However, conclusive proof of this occurrence is lacking. Fluid replacement is the cornerstone of the treatment of diarrheal illness caused by enterohemorrhagic *E coli*.

The primary source of *E coli* O157:H7 is beef cattle. Current animal agricultural practices of grain (rather than hay) feeding of these animals decreases the pH in the colon, thereby promoting acid resistance in the bacteria and enhanced growth promotion for *E coli* pathogens.[83]

Disease Caused by Escherichia coli O104:H4

In May through June of 2011, two separate outbreaks of bloody diarrhea and HUS occurred in Europe. One was centered in Germany and comprised 3,816 cases of bloody diarrhea, 845 cases of HUS and 54 deaths; whereas, the other occurred in France and comprised 15 cases of bloody diarrhea, 9 of which progressed to HUS.[84,85] These cases, however, were not caused by *E coli* O157:H7. These outbreaks were caused by a more virulent form of Shiga toxin-producing *E coli* called *E coli* O104:H4 and represented the highest frequency of HUS and death recorded from an STEC strain. Epidemiological investigation determined that contaminated sprouts were the source of the outbreak and was a consequence of tainted fenugreek seeds from an exporter in Egypt that were obtained by a German seed distributor supplying a German sprout farm.[84] Tainted water may have led to contamination of sprout seeds that were exported from Egypt and distributed to farms in Europe. A portion of the original seed shipment was also sent to an English seed distributor, which repackaged the seeds and supplied them to French garden stores, leading to the outbreak in France.[86]

Disease Caused by Salmonella Species

Salmonella species infect an estimated 1.4 million persons annually in the United States. Severe infections are not uncommon, although most infections are self-limiting with diarrhea, vomiting, abdominal cramps, and fever. Estimates suggest that approximately 15,000 people are hospitalized and more than 500 deaths occur each year from *Salmonella* infections.[55] Food animals are the primary reservoir for human nontyphoidal *Salmonella* infections. Thousands of *Salmonella* serotypes exist, and many naturally inhabit the avian, mammalian, and reptilian gastrointestinal tracts. Poultry is the main source of the salmonellae in the food supply, but other vehicles for disease transmission include raw salads, milk, water, and shellfish.

Infection with many *Salmonella* serotypes cause gastroenteritis with associated diarrhea, vomiting, febrile illness, headache, and dehydration. Septicemia, enteric fever, and localized infections may also evolve from *Salmonella* infection. The most highly pathogenic of the salmonellae is *S typhi*, which causes typhoid fever, for which symptoms include septicemia, high fever, headache, and gastrointestinal illness. *S typhimurium* was the pathogen used in 1984 by an Oregon cult with intent to make people ill by deliberate contamination of salad bars.[87] More than 750 cases of illness resulted, but no deaths occurred, which may have not been the case had *S typhi* been chosen as the pathogenic biological weapon. A 1985 salmonellosis outbreak affecting more than 16,000 persons caused by cross-contamination of pasteurized with unpasteurized milk demonstrates the potential for large-scale illness caused by the salmonellae in the current food distribution system.[88]

Tickborne Diseases

Borreliosis

Lyme arthritis, as a distinct clinical entity, was recognized as early as 1972 in residents of three communities in eastern Connecticut.[89] Lyme disease or Lyme borreliosis is now the most commonly reported arthropod-borne illness in North America and Europe. In 1981, Dr Willy Burgdorfer and colleagues at the Rocky Mountain Laboratories in Hamilton, Montana, first observed spirochetes in adult deer ticks (*Ixodes scapularis*; then called *Ixodes dammini*) collected from vegetation on Shelter Island, New York, a known endemic focus of Lyme disease.[90] The bacteria were shown to react specifically with antibodies from Lyme disease patients,[90,91] and later, spirochetes were isolated from the blood of two patients with Lyme disease,[92] proving the infection's spirochetal etiology.[91] The spirochetes were later named, *Borrelia burgdorferi* (Figure 25-4), after Dr Burgdorfer. *I scapularis* (Figure 25-5) is now considered the primary vector of Lyme disease in the northeastern and north central United States. Other vectors are closely related ixodid ticks,

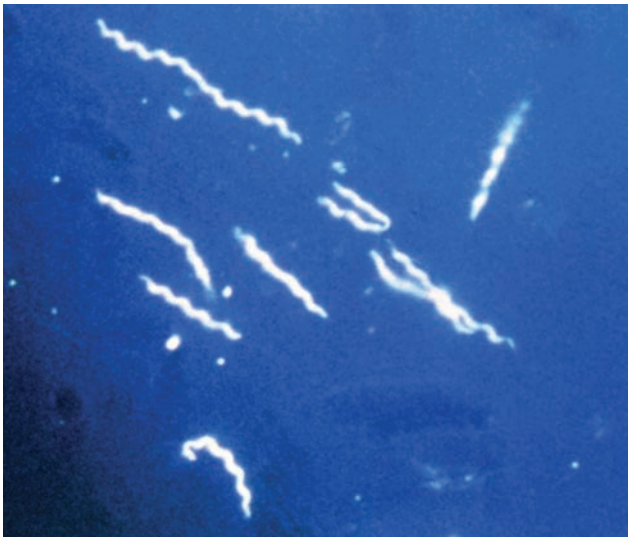

Figure 25-4. Darkfield photomicrograph of the Lyme disease spirochete, *Borrelia burgdorferi*, magnified 400x.
Photograph: Courtesy of Centers for Disease Control and Prevention Public Health Image Library.

including *I pacificus* in the western United States, *I ricinus* in Europe, and *I persulcatus* in Asia. Based on genotyping of bacterial isolates, *B burgdorferi* has now been subdivided into multiple *Borrelia* species or genospecies.[93] In North America, all strains belong to the first group, *B burgdorferi sensu stricto*. This species, along with two others, *B afzelii* and *B garinii*, are found in Europe, although most of the disease there results from the latter two species. Also, interestingly, only *B afzelii* and *B garinii* seem to be associated with the illness in Asia.[93,94] *B japonica*, which was isolated in Japan, is not known to cause human disease.[95]

Lyme disease evolves from a red macule or papule that expands annularly like a bulls-eye rash, known as erythema migrans, which may exhibit as a single lesion or as multiple lesions. However, the erythema migrans rash does not occur in all Lyme disease cases. Early systemic manifestations can include malaise, fatigue, fever, headache, stiff neck, myalgia, migratory arthralgias, and lymphadenopathy, which may last for several weeks if untreated. In weeks to months after erythema migrans onset, neurological abnormalities may develop, including facial palsy, chorea, cerebellar ataxia, motor or sensory radiculoneuritis, myelitis, and encephalitis; these symptoms fluctuate and may become chronic. Cardiac abnormalities and chronic arthritis also may result.[82]

Surveillance for Lyme disease in the United States began in 1982, and it was designated a nationally notifiable disease in 1991. Since then, the number of reported cases has increased steadily with 17,029 cases reported in 2001.[96] In 2002, 23,763 cases were reported, an increase of 40% from the previous year.[96] In 2015, approximately 300,000 people were diagnosed with Lyme disease in the United States. As with other tickborne diseases, this continued emergence of Lyme disease underscores the need for persons living in endemic areas to reduce their risk for infection through proper pest management, landscaping practices, repellent use, and prompt removal of ticks.

A newly recognized tick-transmitted disease that produces a rash (erythema migrans) similar to, and often indistinguishable from, that seen in Lyme disease has been identified in the southeastern and south central United States.[97–99] Unlike Lyme disease, however, symptoms develop following the bite of a lone star tick, *Amblyomma americanum* (Figure 25-6). The disease is named southern tick-associated rash illness,

Figure 25-5. Ixodes scapularis tick, also called the black-legged tick, is found on a wide range of hosts and is considered the main vector of the Lyme disease spirochete, *Borrelia burgdorferi*. *I scapularis* is also a vector of *Anaplasma phagocytophilum* and *Babesia microtii*, the causative agents of human granulocytic ehrlichiosis and babesiosis, respectively.
Photograph: Courtesy of James Gathany and provided by Michael L Levin, PhD, Centers for Disease Control and Prevention Public Health Image Library. Image 1669.

Figure 25-6. A female lone star tick, *Amblyomma americanum*, found throughout the southeastern United States. These ticks are considered the main vectors of *Ehrlichia chaffeensis* and *Borrelia lonestari*, the agents of human monocytotropic ehrlichiosis and southern tick-associated rash illness, respectively.
Photograph: Courtesy of James Gathany and provided by Michael L Levin, PhD, Centers for Disease Control and Prevention Public Health Image Library. Image 4407.

but it has also been referred to as Master's disease, or southern Lyme disease. *A americanum* ticks are not known to be competent vectors of *B burgdorferi*, and serological testing for Lyme disease in southern tick-associated rash illness patients are typically negative, despite microscopic evidence of spirochetes in biopsy samples. Physicians and researchers speculated that a new tick-associated spirochete may be responsible. Subsequently, molecular evidence of a novel *Borrelia* species was reported from *A americanum* ticks, from white-tailed deer, and from the skin of a patient with southern tick-associated rash illness.[100–103] The organism, named *Borrelia lonestari*, was initially described only by polymerase chain reaction (PCR) amplification of the flagellin B gene (fla B) and 16S ribosomal DNA,[104] but it has now been isolated in culture and more extensively studied.[105]

Another new tickborne *Borrelia* species has emerged to cause disease in humans. A novel *Borrelia* species was first isolated from ixodid ticks from Japan in 1995 and named *B miyamotoi*.[106] Subsequently, the bacterium was detected in ixodid ticks from North America[107,108] and Europe.[109,110] In 2011, *B miyamotoi* infection was detected in 46 patients from Russia.[111] All patients reported recent tick bite and were hospitalized with influenza-like illness with fever, headache, fatigue, myalgia, proteinuria, and elevated hepatic aminotransferase levels.[111] Cases were first described in North America in 2013.[112,113] All cases in North America have been in persons living in Lyme disease-endemic regions of the northeastern United States. Interestingly, *B miyamotoi* is genetically more similar to the tickborne relapsing-fever borreliae, which are transmitted by soft (argasid) ticks, not hard (ixodid) ticks. Some patients infected with *B miyamotoi* have even presented with clinical symptoms of relapsing fever.[111]

True relapsing fever borreliae have been known for many decades and are transmitted by ticks or lice. Relapsing fever caused by the spirochete *B recurrentis* can be transmitted by the body louse *Pediculus humanus*. *B hermsii*, which is the causative agent of tickborne relapsing fever, is transmitted by the soft tick *Ornithodoros hermsi*.[114] The disease results in fever lasting 2 to 9 days with 1 to 10 relapses. Although the total duration of louseborne disease usually averages 13 to 16 days, the tickborne disease is often longer. Gastrointestinal and respiratory involvement is common. Neuropsychiatric symptoms have also occurred.[82] Relapsing fever was first reported in the United States in 1915[115] and normally occurs in the higher elevations of the western United States and southern British Columbia (Canada). A tickborne relapsing fever outbreak occurred for the first time in Montana in 2002 among five persons visiting a cabin in the western part of the state.[114] Spirochetes were isolated from two of the patients and were identified as *B hermsii* and *O hermsi* ticks were collected from the cabin where the patients slept. This was the first report of both *B hermsii* and *O hermsi* in Montana, suggesting the risk of infection may be expanding beyond the previously recognized geographic range.

Anaplasmosis/Ehrlichiosis

Human granulocytic anaplasmosis is caused by infection with *Anaplasma phagocytophilum*, whereas the agent of human monocytotropic ehrlichiosis is *Ehrlichia chaffeensis*. Monocytotropic ehrlichiosis occurs in rural and suburban areas south of New Jersey to Kansas and in California, while granulocytic anaplasmosis occurs in areas where Lyme disease is endemic.[82] The *A americanum* tick (see Figure 25-6) transmits *E chaffeensis*, while *I scapularis* (see Figure 25-5), the Lyme disease vector, also transmits *A phagocytophilum*. A spectrum of mild-to-severe, life-threatening, or fatal disease occurs with anaplasmosis. About 20% of patients have meningoencephalitis. Infection with *A phagocytophilum*

is characterized by acute and often self-limited fever, malaise, myalgia, thrombocytopenia, leucopenia, and increased hepatic transaminases.[82] Illness ranges from mild to severe, with less than 1% case fatality.

As the *I scapularis* tick is the vector for transmission of *B burgdorferi*, *B miyamotoi*, *A phagocytophilum*, and *B microti*, coinfections of Lyme disease (and Lyme-like disease), anaplasmosis, and babesiosis (caused by the protozoan *Babesia microti*) can occur from the bite of this tick. In the United States, ticks of the *Ixodes* genus can transmit all of these diseases as well as the viral pathogens Powassan virus and the related deer–tick virus.[82,116] Coinfections with babesiosis and Lyme disease are known at times to increase the severity of both diseases.[82]

Emerging Antibiotic Resistance

Antimicrobial resistance is not a new phenomenon. Sulfonamide-resistant *Streptococcus pyogenes* emerged in military hospitals in the 1930s, and penicillin-resistant *Staphylococcus aureus* appeared in London civilian hospitals soon after the introduction of penicillin in the 1940s.[117] However, the number of resistant organisms, the geographic regions affected by drug resistance, and the number of bacterial species that are multidrug resistant (MDR) is increasing. Since the 1980s, a reemergence of tuberculosis has occurred that is often caused by MDR *Mycobacterium tuberculosis*[118] and requires the use of several—sometimes six to seven different—drugs to treat.[119] After initial reports in 2006 from South Africa of extensively drug-resistant tuberculosis (defined as tuberculosis caused by strains of *M tuberculosis* resistant to rifampicin, isoniazid, fluoroquinolones, and any of the second-line injectable drugs such as capreomycin, amikacin, and kanamycin), the number of countries reporting cases of extensively drug-resistant tuberculosis has increased to at least 84.[120] Additionally, cases of vaguely defined totally drug-resistant tuberculosis have been reported.[121,122] Other notable examples of MDR strains worldwide include *Enterococcus faecium*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *S aureus*, *Acinetobacter baumannii*, and *P aeruginosa*.[117] In developing countries, MDR enteric bacteria such as *Salmonella enteritidis*, *Shigella flexneri*, and *V cholerae* are major threats to public health.

Salmonella antibiotic resistance has emerged to become a serious concern in agriculture as well as patient management.[73,123,124] Antibiotic resistance in *E coli* O157:H7 has been shown to occur rapidly following exposure to various antibiotics, including triclosan, chloramphenicol, erythromycin, imipenem, tetracycline, and trimethoprim, as well as to a number of biocides.[125]

Few antibiotics are more potent than vancomycin. The emergence of microbial vancomycin resistance continues to be of increasing concern to clinicians and public health professionals, and surveillance systems have been instituted to monitor these pathogens.[126] *S aureus* is an important cause of illness and death and accounts for about one-fifth of bacteremia cases in the United States.[127] The discovery of vancomycin resistance in *S aureus* clinical isolates could portend the end of the antibiotic era in medicine.[42,75]

Both hospital and home healthcare patients are significantly affected by the growing emergence of antibiotic resistance.[127,128] Restrictive guidelines have therefore been developed for the use of vancomycin and other glycopeptide antimicrobials. These guidelines include a recommendation against the routine use of vancomycin as perioperative antibiotic prophylaxis for surgical site infections.[129] Vancomycin-intermediate resistance among *S aureus* has also been identified, and subsequent guidance has been developed for their identification and control of transmission.[42]

The carbapenem class of antimicrobials, which comprises imipenem, meropenem, ertapenem, and doripenem, is often the last resort for the safe and effective treatment of infections caused by MDR gram-negative bacteria, including the extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. Resistance to carbapenems occurs through several mechanisms, including the production of carbapenemases. The vast majority of acquired carbapenemases belong to one of three classes of β-lactamases, namely class B (metallo-β-lactamases) or classes A and D (serine carbapenemases). The class A group includes *Klebsiella pneumoniae carbapenemase* (KPC), which is currently the most common carbapenemase and was first detected in North Carolina in 1996 and has since spread worldwide.[130] KPC made headlines when it caused an outbreak among 18 patients at the National Institutes of Health Clinical Center in 2011.[131] Six of the patients died from their infections. The use of genomic sequencing to determine the source of this outbreak illustrates the application of this technique in epidemiological investigations (see next section).

The past few years have seen an emergence of a new type of carbapenemase, designated New Delhi metallo-β-lactamase-1 (NDM-1). It was first described in 2009 in *Klebsiella pneumoniae* (Figure 25-7) isolated from a patient receiving treatment for a urinary tract infection in a Swedish hospital, but who was of Indian origin and had previously received medical care in New Delhi, India.[132] Since this first reported case in 2009, NDM-1 producing

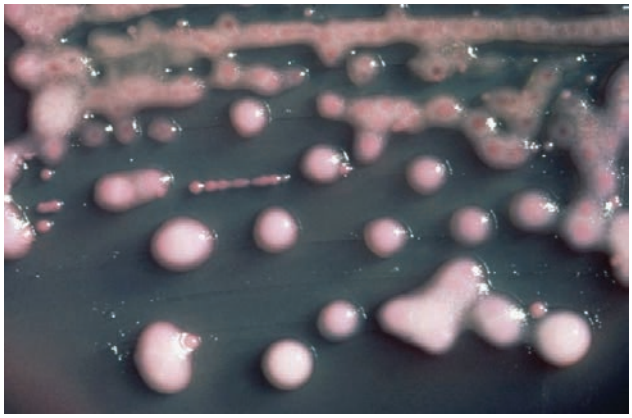

Figure 25-7. Colonies of *Klebsiella pneumoniae*, the bacterium in which New Delhi metallo-β-lactamase-1 was first identified. Magnification ×10.
Photograph: Courtesy of Centers for Disease Control and Prevention Public Health Image Library.

bacteria have rapidly spread to every continent except for Central and South America. In most of these cases, patients had been hospitalized in India, Pakistan, or Bangladesh, or had spent some time in that part of the world. It therefore suggests that the Indian subcontinent is currently the main reservoir of NDM-1 producers.[133]

A substantial number of patients have been part of the growing phenomenon of "medical tourism" resulting from delays for medical interventions such as hip and knee replacements, spinal surgery, and ophthalmologic procedures. It is estimated that in 2012 as many as 1.6 million Americans received healthcare outside of the United States.[134] Many of these medical tourists undergo such procedures in India, which may put them at risk of contracting NDM-1 strains of bacteria. NDM-1 has been identified mostly in *E coli* and *K pneumoniae*, in many cases in strains that are already MDR, making these bacterial pathogens resistant to virtually every clinically available antibiotic.

This is even more alarming considering the decreasing number of potentially new antibiotics that have come through the pharmaceutical pipeline in recent decades. The reasons for this decline are many and diverse.[135] Some of these reasons include the nature of antibiotic use, which is typically short term, compared to other drugs; the drug's uncertain future because of the constantly evolving nature of antibiotic resistance; and governmental over-regulation. Thus, appropriate antibiotic use will continue to be an important issue for clinicians and epidemiologists for the foreseeable future.[136]

Genomic Epidemiology—Use of Whole Genome Sequencing to Track Epidemics of Bacterial Pathogens

Historically, public health investigators have used techniques such as DNA–DNA hybridization, patterns of restriction endonuclease digestion of DNA on agarose gels, or pulsed-field gel electrophoresis, to determine the relatedness of bacterial pathogens isolated from different patients or different geographic regions. Although these methods can provide some information regarding strain relatedness, they vary greatly in their resolution. Bacterial genotyping techniques commonly used in outbreak investigations have limited power of resolution because they target only small parts of the genome. More recently, whole genome sequencing has emerged as a rapid and high-resolution method to investigate bacterial disease outbreaks[137–139]; this application of the technique is often referred to as "genomic epidemiology."[140] Two recent high-profile examples of using this technology to track the origin and transmission of bacterial pathogens during outbreaks include the 2010 cholera epidemic in Haiti[24] and the 2011 German *E coli* O104:H4 outbreak[84] discussed previously.

Cholera had not been previously reported from Haiti; thus, the main question was from where did the strain of *V cholerae* responsible for the outbreak come? The source of the cholera in Haiti has been controversial, with three main hypotheses being suggested. The first hypothesis was that the pathogen arrived to Haiti from the Gulf of Mexico because of tectonic shifts resulting from the earthquake. The second hypothesis was that the pathogen evolved into disease-causing strains from nonpathogenic strains naturally present in Haiti. The third hypothesis was that the pathogen was somehow inadvertently introduced into the Haitian environment, triggering the epidemic.[24] A specific form of this hypothesis, that Nepalese soldiers from a United Nations military camp were the direct source of the cholera, was a commonly held belief in Haiti. To resolve this question, Matthew Waldor of Harvard Medical School collected several samples of the *V cholerae* strain circulating in Haiti and sent them to colleagues at Pacific Biosciences, a biotech company that manufactures third-generation single-molecule real-time DNA sequencers. Scientists at Pacific Biosciences sequenced DNA from two samples from the Haitian outbreak, one strain that caused cholera in Latin America in 1991, and two *V cholerae* clinical strains isolated from Bangladesh in 2002 and 2008 and compared them to reference genomes already in the database. They analyzed single nucleotide and copy

number variations to determine the likely phylogeny of the Haitian strain and found that the Haitian isolates were more closely related to the strains from Bangladesh (South Asia) and more distantly related to isolates circulating in South America. Their conclusions were that the Haitian epidemic was probably the result of the introduction of a *V cholerae* strain from a distant geographic source.[28]

Unfortunately, this determination was as specific as these investigators could get without having a more extensive collection of strains to compare. Using a different next-generation DNA sequencer (Genome AnalyzerIIx, Illumina, San Diego, CA), Frank M Aarestrup and his team at the Technical University of Denmark sequenced 24 *V cholerae* isolates collected from August to November 2010 from five different districts in Nepal. Phylogenetic analysis showed that all 24 *V cholerae* isolates from Nepal belonged to a single well-supported clade that also contained isolates from Bangladesh and Haiti. Furthermore, direct comparison between the three Haiti outbreak strains and the three most closely related strains from Nepal show a near perfect match.[29] This finding, along with epidemiological data, strongly supports the hypothesis that the *V cholerae* strains responsible for the 2010 Haitian cholera epidemic were brought to Haiti from Nepal, most likely via Nepalese soldiers serving as United Nations peacekeepers.

A similar approach (ie, rapid whole genome sequencing) was taken to fully characterize the *E coli* from the 2011 German outbreak in near real-time.[141] This comprehensive analysis took place in the first days and weeks of the outbreak, rapidly enough to inform physicians treating infected patients and epidemiologists tracing the source of the pathogen. Only this kind of rapid whole genome sequencing allowed investigators to determine that the outbreak strain was an extremely rare form of bacterium that was a "hybrid" of enteroaggregative *E coli* and enterohemmorhagic *E coli*. Researchers also determined that this was distinct from other *E coli* O104:H4 strains because it contained a prophage encoding a Shiga toxin and a distinct set of other virulence and antibiotic-resistance factors.[141]

These are only two examples of the use of genomic epidemiology. However, it is clear that as the speed and accuracy of next-generation DNA sequencing increases (and the cost decreases), it is likely that in the near future, it will be as common a diagnostic tool as the PCR is today.

EMERGING VIRAL DISEASES

Avian Influenza and the Threat of Pandemics

Influenza is a highly contagious, acute respiratory illness, with clear evidence of human infections dating back to the Middle Ages, and probably occurring as far back as ancient Greece and Rome. The influenza viruses are members of the *Orthomyxoviridae* family and contain a segmented negative-sense RNA genome.[142] There are three genera of influenza viruses: influenza A, B, and C. Influenza A and B viruses are associated with seasonal epidemic illness in humans; whereas, influenza C infections in humans are sporadic. Because influenza A viruses are the only type of influenza viruses that have caused pandemics in the human population, this section will focus on this influenza virus type. The genome of influenza A viruses comprises eight gene segments, encoding 10 to 12 proteins.[142,143] The segmented nature of the genome allows for reassortment, or the exchange of segments (and genes) between two or more virus strains co-infecting the same cell. The major surface glycoproteins of influenza A viruses, hemagglutinin (HA) and neuraminidase (NA), are the major antigens of the virus. HA and NA are involved in the interactions between the virus and host cells, and they are the major targets of neutralizing antibodies. These proteins are seen as spikes in electron micrographs (Figure 25-8). The HA binds to sialic acid-containing moieties on the cell surface, mediating attachment and entry of the virus, and the NA is a receptor-destroying enzyme that cleaves sialic acids from the glycan backbone, thus facilitating release and spread of the virus. Subtypes of influenza A viruses are designated by their particular HA and NA types (to date, distinct subtypes of influenza B and C viruses have not been observed). Sixteen HA and 9 NA subtypes have been identified in aquatic birds, which act as the major reservoir for influenza A viruses in nature. In addition, influenza A viruses can infect many mammalian species, including pigs, horses, dogs, cats, ferrets, mink, whales, and seals. Influenza A viruses of the most recently described subtypes, H17N10 and H18N11, have not been isolated; partial genome sequences of these highly divergent influenza A viruses were identified in bats from Guatemala and Peru (see section on Influenza Viruses in Bats).[144,145] Thus far, only influenza A viruses carrying one of three HA subtypes (H1, H2, H3) have been able to achieve sustained transmission and establish themselves in the human population, causing subsequent seasonal

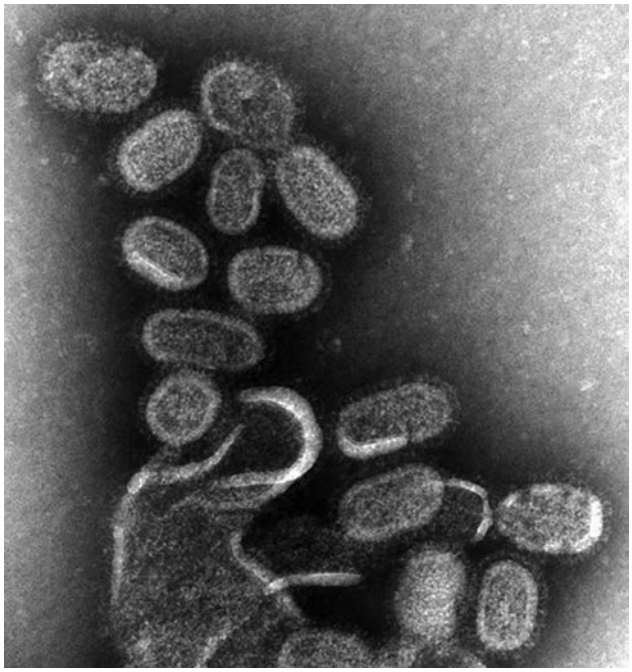

Figure 25-8. Negative-stained transmission electron micrograph showing the reconstructed 1918 influenza virons collected from the supernatants of virus-infected Madin-Darby Canine Kidney cell culture 18-hours postinfection. Surface spikes (hemagglutinin and neuraminidase) can be clearly seen extending from the surface of the virons.
Photograph: Courtesy of Cynthia Goldsmith and provided by Dr Terrence Tumpey, Centers for Disease Control and Prevention Public Health Image Library. Image 8160.

epidemics. For example, one circulating influenza virus strain is designated subtype H3N2 and has been the most commonly isolated strain during the last 4 decades.

Antigenic diversity in influenza A viruses can result from changes in the HA and NA genes. One type of variation called "antigenic drift" occurs as a result of accumulation of point mutations in the genes encoding HA and NA proteins. These point mutations occur randomly as the virus is copied in infected cells and are largely responsible for the annual epidemics of influenza seen during the winter months, and for the frequent need to reformulate the seasonal influenza vaccine.

Another type of change that can occur is called "antigenic shift," which results from the reassortment of genes that occurs when two different influenza viruses infect the same host cell, causing a shift in the HA and/or NA type of the virus. This phenomenon results in the emergence of novel influenza A strains that have the potential to cause widespread infection and disease in a susceptible population. Since 1933, when the influenza A virus was first isolated (an H1N1 subtype), major antigenic shifts (and pandemics) have occurred in 1957 ("Asian influenza," an H2N2 subtype virus) and in 1968 ("Hong Kong influenza," an H3N2 subtype virus). In 1977, the H1N1 subtype virus reappeared after a more than 20-year hiatus; however, this time it did not cause severe disease, most likely because of the immunity of persons older than 20 years of age who had been infected with the virus when it circulated earlier in the century. It is highly unlikely that this virus was maintained in an animal host for more than 20 years without changes. One possible explanation is that the virus was maintained in a laboratory freezer until it somehow was reintroduced into the human population.

In 2009, a swine-origin H1N1 influenza A virus caused the first influenza pandemic of the 21st century (see section on Swine Influenza and the H1N1 Influenza Pandemic, 2009). This H1N1 influenza A virus then replaced the circulating seasonal H1N1 influenza viruses, and continues to cause seasonal epidemic infections, with typically mild-to-moderate illness.

Of the three influenza pandemics that occurred in the 20th century, the pandemic of 1918 to 1919 was the most devastating, causing an estimated 20 to 40 million deaths worldwide. Unusually, young healthy adults between 20 and 40 years of age accounted for almost half of the influenza deaths during this pandemic. The epidemic spread rapidly, moving around the globe in less than 6 months. It is estimated that the pandemic killed 675,000 Americans, including 43,000 servicemen who were mobilized for World War I (Figures 25-9 and 25-10), and it may have played a significant role in ending the war.[146] Its impact was so profound that the average life expectancy in the United States temporarily declined by more than 10 years.[147]

Analysis of survivor antibody titers from the late 1930s suggested that the 1918 strain was an H1N1 subtype virus closely related to classical swine influenza viruses.[148] Researchers at the Armed Forces Institute of Pathology in Washington, DC—who isolated influenza viral RNA from preserved lung tissue of US servicemen who died during the 1918 pandemic, and also from a victim of the pandemic who was buried in a mass grave in Brevig Mission, Alaska—ultimately confirmed this theory. Over the next decade, all eight gene segments of the 1918 influenza virus were reconstructed, sequenced, and characterized.[149] Unfortunately, no obvious genetic changes were observed in any of these gene sequences that would account for the exceptional virulence of this pandemic virus.[150] The reconstructed virus was highly virulent in animal models, including

Figure 25-9. Emergency hospital during the 1918 influenza pandemic, Camp Fuston, Kansas. NCP 1603.
Photograph: Courtesy of the Otis Historical Archives, National Museum of Health and Medicine, Washington, DC.

mice, ferrets, and nonhuman primates.[151,152] It was determined that the HA and polymerase complex genes played important roles in virulence, although no single property of the virus has been identified to fully explain the devastating mortality seen in 1918 and 1919.[143]

Host factors undoubtedly played some role. It has been suggested that an uncontrolled cytokine response was elicited by the virus, leading to immunopathology. However, most of the mortality during the pandemic appeared to be attributable to secondary bacterial pneumonia.[9] Viral and host factors could be involved in increased susceptibility to bacterial infections, and this remains an active area of investigation. In addition, the fact that the pandemic occurred in the preantibiotic era also likely contributed to the high mortality observed.

Much has been learned from the remarkable achievement of the resurrection of the 1918 virus. Continued study of this pathogen will continue to provide valuable information for the development of vaccines and treatments for future pandemic influenza viruses.

Countermeasures do exist for the treatment and prevention of influenza. Annual vaccines include two influenza A strains (H1N1 and H3N2) and two influenza B strains (one from each of the two influenza B lineages that circulate in humans). The component strains are selected based on surveillance of the strains that are circulating in humans about 6 months prior to when vaccine will be needed for immunizations before the start of the influenza season. In some cases, the vaccine does not match the circulating strain, and low vaccine effectiveness is then observed. The strains may need to be updated annually due to antigenic drift. Influenza vaccines against potentially pandemic influenza viruses (eg, H5 and H7 subtypes) have been manufactured and evaluated in clinical trials, but these vaccines have been found to be suboptimally immunogenic, requiring higher doses or adjuvants to achieve the antibody responses needed for protection.[153] In recent years, there has been much interest in developing a universal vaccine that would provide broad cross-protection against multiple subtypes of influenza, including pandemic strains that may emerge in the future.[154] The identification of a highly conserved region of the influenza HA (the stem or stalk region) against which broadly cross-neutralizing antibodies have been detected in humans has spurred a great effort to develop ways of using the conserved HA stem region as an immunogen.[155–157] However, the availability of such a vaccine—if this strategy is successful—is still years in the future.

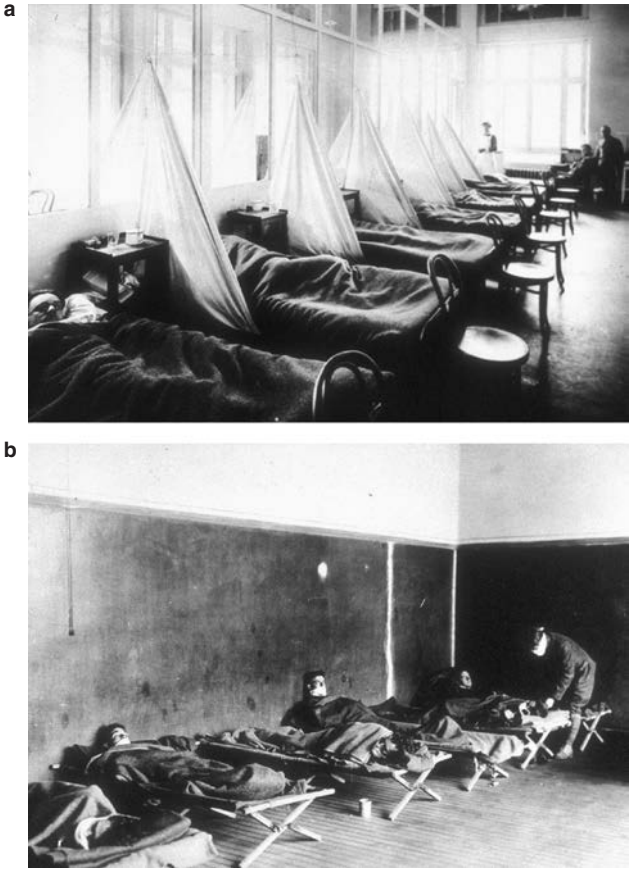

Figure 25-10. Influenza wards, US Army camp hospitals at (**a**) Aix-Les-Bains, France (Reeve 14682), and (**b**) Hollerich, Luxembourg (Reeve 15183).
Photographs: Courtesy of the Otis Historical Archives, National Museum of Health and Medicine, Washington, DC.

Two classes of drugs are available to treat influenza: (1) the NA inhibitors (oseltamivir, zanamivir) and (2) the M2 ion channel inhibitors (adamantanes). As with many antivirals, development of resistant strains is a problem that limits their use, and successful treatment with NA inhibitors must be initiated early after symptom onset to be effective.

Swine Influenza and the H1N1 Influenza Pandemic, 2009

Influenza infections in pigs were first recognized clinically during the 1918 Spanish influenza pandemic, and the first isolation of an influenza virus was from pigs in 1930.[158] Transmission of influenza virus to humans from swine has since been documented on several occasions.[159,160] Before 2009, infections of humans with swine influenza viruses were sporadic and did not result in large outbreaks of illness. In all cases, illness was indistinguishable from typical influenza virus infection in humans. Between 1958 and 2005, 37 cases of swine influenza virus infections in humans were reported, 19 of which were in the United States (reviewed in Meyers et al[159]). In 22 (61%) of these cases, recent exposure to swine was reported, and 13 of these cases resulted from occupational exposure. In the United States in 1976, there was an outbreak of swine influenza at Fort Dix in New Jersey.[161] Infection with an H1N1 swine influenza virus resulted in one soldier's death and respiratory illness in 12 additional soldiers. No exposures to pigs were reported. It was subsequently found by serological analysis that as many as 230 soldiers were infected.[161]

Influenza A viruses of the H1, H2, and H3 subtype are all present in swine. The first influenza pandemic of the 21st century occurred in 2009, and was caused by an H1N1 virus that originated in swine. The emergence of a pandemic virus from the swine reservoir was unanticipated, particularly with many influenza researchers focusing their efforts in recent years on avian influenza (AI) viruses, particularly H5N1 (see section on Human Infections with Highly Pathogenic H5N1 Avian Influenza Viruses), as possible agents for the next pandemic.

Influenza-like illness was reported in two children in southern California in March 2009, and the number of pneumonia cases increased in Mexico City around the same time. A novel H1N1 influenza A virus was isolated from individuals in the United States in April 2009. The virus was soon characterized as a quadruple reassortant virus of the H1N1 subtype, with gene segments from swine and avian influenza viruses.[162] The novel virus spread rapidly throughout the world, and the WHO declared a pandemic on June 11, 2009. Although illness caused by the nascent pandemic virus was generally mild to moderate, severe illness was observed in individuals with underlying conditions such as obesity and diabetes, in pregnant women, and—surprisingly—in older children and young adults.[163,164] This is in contrast to seasonal influenza epidemics, where the burden of disease is usually greatest in the very young and the elderly.

Antigenic characterization of the 2009 pandemic H1N1 virus and serological studies revealed that the HA was related to that of H1N1 influenza viruses that circulated in the 1930s, 1940s, and earlier, including the 1918 H1N1 influenza virus.[9,165] In addition, the 2009 pandemic H1N1 virus was antigenically similar to the H1N1 virus that caused the swine influenza at Fort Dix that triggered a national vaccination campaign in 1977.[161] Vaccination in 1977 likely afforded some protection against the pandemic virus.

The 2009 pandemic H1N1 viruses were found to be sensitive to NA inhibitors and were resistant to ion channel inhibitors. Resistance to adamantanes was conferred by a mutation in the M2 gene of the 2009 H1N1 viruses. An immediate response to the emergence of the 2009 H1N1 virus was the production of a vaccine. Vaccine manufacturers in the Northern Hemisphere had recently finished distribution of the trivalent vaccine for the 2008 to 2009 winter influenza season when the pandemic H1N1 virus emerged. Delays occurred in vaccine production, resulting from the difficulty in generating reassortant seed viruses for vaccine manufacture and the instability of the pH1N1 HA protein used for potency testing for vaccine lot release. As a result of these delays, vaccine was not distributed until the peak of infections had passed. Human clinical testing of the monovalent inactivated H1N1 vaccine revealed a high titer antibody response to vaccination in most age groups tested, providing more evidence of preexisting immunity to the pandemic H1N1 virus.[166,167]

The WHO declared the end of the 2009 influenza pandemic in August 2010. The WHO reported 18,631 laboratory-confirmed deaths caused by the H1N1 virus between April 2009 and August 2010. However, recent estimates of global mortality from this pandemic are reported to be approximately 10- to 15-fold higher.[168,169]

In 2010, the pandemic H1N1 virus replaced the circulating seasonal H1N1 influenza viruses, and it has continued to circulate concomitantly with H3N2 human influenza viruses, causing mild to moderate disease. Since the winter of 2010–2011, the pandemic H1N1 virus has been a component of the seasonal influenza vaccine. The HA of the H1N1 virus does not appear to have undergone significant antigenic drift,

and so the original vaccine strain, A/California/7/2009, has not changed (http://www.who.int/influenza/vaccines/virus/recommendations/en/).

The first influenza pandemic of the 21st century was the first influenza pandemic to occur in the molecular biology era. Much information about this virus will continue to be generated. Although delays occurred in vaccine production and deployment, the novel virus was rapidly identified and characterized. Key questions about this virus remain, including the precise point of origin of the virus and the reason for the severity of disease in pregnant women infected with this virus. These questions and other features of the virus are the subjects of intensive study.

Fortunately, the morbidity and mortality from the 2009 influenza pandemic were not on the scale of the 1918, 1957 or 1968 pandemics. The 2009 H1N1 influenza pandemic underscored several important aspects of influenza biology:

- the unpredictability of the emergence of novel influenza viruses from an animal reservoir;
- the diversity of reassortant influenza viruses in nature derived from a variety of animal hosts;
- the importance of preexisting immunity in the human population;
- the rapidity with which a human-adapted virus can spread globally; and
- the importance of surveillance of swine influenza viruses, as well as their avian counterparts.

Swine influenza viruses continue to present a pandemic threat. In 2011, and particularly in the summer of 2012, a number of cases of human infection with quadruple reassortant swine H3N2 viruses were reported. These viruses are genetically identical to the pandemic H1N1 viruses, except that the HA and NA genes are derived from circulating swine H3N2 triple reassortant viruses.[170] Importantly, the M gene segment is derived from the Eurasian swine lineage, perhaps increasing the likelihood of sustained transmission of these viruses in humans. Influenza viruses that circulate in swine are referred to as "variant" viruses when isolated in humans, so that the human cases are considered to be infections with the H3N2v virus.

From July to September 2012, 306 cases of human infection with H3N2v influenza viruses were reported.[171] H3N2v has been associated with typical influenza illness, and 16 H3N2v-associated hospitalizations and one death occurred. Almost all cases have documented histories of swine exposure, and the majority of cases were associated with attendance at state fairs. However, some cases have suggested the presence of limited person-to-person transmission.

Influenza viruses in swine do not appear to be subjected to the same immunologic pressure that leads to antigenic drift in human influenza viruses. Once introduced into swine populations, influenza viruses therefore tend to be antigenically stable. The H3N2v viruses isolated from humans are phylogenetically most closely related to human influenza viruses from the mid 1990s.[170,172] Clinical cases of H3N2v have occurred primarily in children 12 and younger, ie, individuals born after these viruses last circulated in humans.

Several studies have assessed the degree of baseline population immunity to H3N2v viruses by measuring antibody against these viruses using serum samples from different age groups. These studies have also suggested that children younger than 10 would be largely susceptible to infection based on lack of preexisting antibody.[171,172] Current seasonal inactivated influenza vaccine does not induce an antibody that recognizes H3N2v in children, although some cross-reactive antibodies are observed in adults.

These observations suggest that H3N2v viruses pose a potential pandemic risk. The viruses are prevalent in domestic swine and have a demonstrated ability to infect humans. They possess genotypes that have features that potentially enable human transmission, and some cases of human-to-human transmission have been observed. Previous swine origin viruses have already caused pandemics, and influenza viruses of the H3 subtype are clearly capable of causing widespread human disease. Although the pattern of baseline antibody possibly suggests that the impact of an H3N2v pandemic would be focused on young children, the majority of adults would also be predicted to be susceptible. Thus, development of effective vaccines for H3N2v candidate viruses is a high priority.

Human Infections With Avian Influenza Viruses

Wild aquatic birds are the major reservoirs of all subtypes of influenza A virus that have been isolated, and the viruses do not cause symptomatic infections in these species. It was generally accepted—until recently—that for an influenza pandemic to occur, AI viruses would reassort with human influenza viruses in an intermediate host, and a novel strain capable of infecting humans (with no preexisting immunity to the new virus) would emerge. Rare transmission events directly from birds or transmission of AI from other animals to humans have been reported.[173] Transmission of AI from birds to humans before 1997 occurred with AI viruses mainly of the H7 subtype.[174] Human

infections with other AI subtypes have since been reported: for example, in 1999 and 2003 with H9N2 viruses[175,176]; in 2003 with H7N7 viruses[177]; from 1997 to present with H5N1 viruses[178]; in Egypt with H10N7 viruses,[175] and in 2013 in Taiwan with H6N1.[179] These cases confirmed that AI viruses are capable of directly infecting humans without the requirement for reassortment in an intermediate mammalian host.

Human infections with AI viruses have resulted in a wide spectrum of disease, ranging from mild febrile and respiratory illness in some H5 and H9N2 influenza infections, conjunctivitis in the case of H7 influenza infections, to severe disease and death, as seen with the highly pathogenic avian influenza (HPAI) H5N1 cases that have occurred between 1997 and the present.[178] In contrast to the rarity of the isolation of AI viruses from humans, serosurveys of farmers in rural southern China suggest that many other subtypes of AI viruses have crossed the species barrier and infected humans. Specifically, seroprevalence levels of 2% to 7% for H5 viruses alone were reported,[180] and the seropositivity of human sera for H7, H10, and H11 viruses was estimated to be as high as 38%, 17%, and 15%, respectively. The dogma had been that because of receptor specificity, AI viruses were incapable of efficiently infecting humans. It has long been believed that this host restriction of AI viruses prevents the emergence of new pandemic strains via direct avian-to-human transmission. However, human cases of direct infection by AI viruses are becoming increasingly frequent; it is now known that the potential of an AI virus to infect humans is polygenic in nature, and it is widely accepted that this is not solely attributable to its HA receptor specificity. The most significant zoonotic transmissions of AI viruses in recent years have been caused by H5N1 and H7N9 AI viruses. These outbreaks are described in more detail in the following sections.

Human Infections With Highly Pathogenic H5N1 Avian Influenza Viruses

The first reported cases of H5N1 influenza infections in humans occurred in 1997 in Hong Kong. The first case was a 3-year-old boy.[181] The child died 12 days after the onset of symptoms with several complications, including respiratory failure, renal failure, and disseminated intravascular coagulopathy. An H5N1 AI virus was isolated from a tracheal aspirate specimen obtained on day 10 of illness. The nucleotide sequence of the isolate revealed a multibasic amino acid sequence at the HA cleavage site, a motif characteristic of HPAI viruses,[182] which—until this point—had only been known to cause severe disease in poultry. Phylogenetic analysis of the H5N1 Hong Kong isolate revealed no evidence of genetic reassortment with recent human influenza A viruses. The isolate was highly pathogenic for chickens and the virus displayed an AI virus-like receptor specificity. No clear epidemiological link was established between the infected child and infected poultry. However, outbreaks of influenza occurred in poultry on farms in Hong Kong between late March and early May 1997, and two viruses from one of these outbreaks were identified as H5N1 influenza viruses. It was reported that sick chickens were at the preschool attended by the child, although no evidence indicates that these chickens were infected with AI or that the child was in close contact with them.

Additional cases of H5N1 in humans in Hong Kong were confirmed in 1997.[183] In total, 18 Hong Kong residents became infected with HPAI H5N1 influenza in 1997, of whom 6 died. The cases, which were not geographically related or confined to a specific age group, occurred in children and adults with ages ranging from 1 to 60 years. In 7 of the 18 cases, histories of possible exposure to poultry existed, where the patients had either bought chickens before they became ill or had worked in proximity to chicken stalls near their homes.[184] Seven of the patients had severe complications, most prominently pneumonia, gastrointestinal manifestations, elevated liver enzyme levels, and renal failure. With one exception, patients younger than age 13 recovered from their illness whereas older patients had more severe disease that resulted in death in five cases.

An epidemiological study of the human H5N1 cases in Hong Kong in 1997 suggested that the viruses were transmitted directly from birds to humans, and serological evidence of human-to-human transmission was limited.[184] In most cases, infection was associated with recent exposure to live poultry. Sequence analysis of AI viruses circulating in China around that time resulted in the hypothesis that the H5N1 influenza viruses that infected humans in 1997 arose by reassortment between an H5N1 influenza A/goose/Guangdong/1/96-like virus and an H9N2 or H6N1 virus similar to those circulating in the live bird markets of Hong Kong in 1997. However, the actual sequence of reassortment events cannot be definitively determined from the small number of viruses available for analysis from preceding years.[173]

The human cases of HPAI H5N1 infection that occurred in 1997 coincided with further outbreaks of highly pathogenic H5N1 influenza in poultry on farms and in live bird markets in Hong Kong. Slaughter of the 1.5 million poultry in the Hong Kong Special Administrative Region was conducted between December 29 and December 31, 1997. Many experts believe that because of this action, an influenza pandemic caused

by the H5N1 virus was averted, although it has since become apparent that the H5N1 AI viruses have not adapted for efficient transmission in humans. Reintroduction of poultry to the Hong Kong Special Administrative Region began in February 1998. At this time, new practices were introduced for the live bird markets in Hong Kong. Waterfowl (eg, ducks and geese) are now sold at separate markets from chickens; ducks and geese are now slaughtered at the markets; and markets have a monthly rest day when they close for thorough cleaning, the remaining birds are culled, and restocked with fresh imported poultry. Surveillance of birds in the markets has continued since the 1997 outbreaks.

In 2003, again in Hong Kong, two cases of HPAI H5N1 infection were confirmed in a father and son of a family who had recently visited mainland China.[8] HPAI H5N1 AI infections again appeared in the human population in 2004 in Vietnam and Thailand, and they were confirmed in Cambodia, Indonesia, and China in 2005. HPAI viruses of the H5N1 subtype in Asia continued to evolve and spread in avian populations, and human cases were eventually reported in the Middle East, Europe, and Africa. Multiple genotypes and several clades of H5N1 influenza viruses have been identified.[96,185] It is believed that the highly pathogenic H5N1 viruses in Asia originated from viruses in ducks in southern China. HPAI H5N1 viruses have been isolated from dead migratory birds in Hong Kong and parts of China,[186] implicating wild birds in their spread across Asia and other parts of the world.

Human cases and fatalities have since been reported in 15 countries. As of October 2013, 641 laboratory-confirmed cases and 370 deaths have been reported to the WHO.[178] The WHO's website contains a comprehensive timeline that chronicles the panzootic spread of HPAI H5N1 viruses since 1997 and the associated incursions of the virus into the human population.[178] The HPAI H5N1 viruses of the A/goose/Guangdong lineage continue to evolve by antigenic drift, resulting in efforts to continually update stockpiled vaccine strains for use should these viruses gain the ability to transmit efficiently and cause widespread infections. Of particular concern are the HPAI H5N1 viruses circulating in Egypt since 2009, because these isolates display increased affinity for human-type sialic acid receptors.[187,188] Fortunately, HPAI H5N1 viruses have not acquired the ability to transmit efficiently from person to person, although several small family clusters of cases have been reported.[189,190]

Despite the global prevalence of HPAI H5N1 infections in birds, and the number of reported infections in humans, these viruses have not yet acquired the necessary genetic changes required for efficient, sustained transmission in the largely immunosusceptible human population. Recent controversial studies involving intentional introduction of mutations into HPAI H5N1 viruses to confer efficient transmissibility in the ferret model—the preferred animal model for the study of influenza transmission—resulted in the identification of several changes in the viral genome that achieved this state.[188,191] Changes in the HA gene and the PB2 polymerase gene were found to be necessary—but not sufficient—to confer transmissibility in ferrets.[192]

Human Infections With H7N9 Avian Influenza Viruses, 2013

Human cases of H7 AI have typically been associated with large outbreaks of H7 AI infection in birds, caused by either highly pathogenic or low pathogenicity viruses. With the exception of a fatal infection by a HPAI H7N7 in the Netherlands in 2003,[177] illness associated with H7 AI infections in humans has been relatively mild.

Recent human cases of H7N9 AI infection in China have caused great concern regarding the potential emergence of an influenza pandemic. Human infections with H7N9 AI viruses were first reported in China on March 31, 2013.[193] The first three cases reported were in two individuals from Shanghai and one individual from Anhui.[194] All three patients died. Between April and the end of May 2013, 132 laboratory confirmed cases had been reported to the WHO, 37 of them fatal. Cases occurred in eight contiguous provinces of eastern China and in the two municipalities of Beijing and Shanghai, and a single case was reported in Taiwan.[195] The infected individual from Taiwan had recently travelled to Jiangsu Province in China.

In the majority of the laboratory-confirmed cases of H7N9 in China and in the case reported in Taiwan, illness was severe.[194,195] In the initial three fatal cases reported in China, all three patients presented with fever, cough, and dyspnea. Radiologic findings were consistent with pneumonia, with diffuse opacities and consolidation.[194] The patients progressed rapidly to acute respiratory distress syndrome (ARDS) and multiorgan failure. Gao et al[194] reported the clinical features of an additional 111 laboratory confirmed cases in China. The most common early symptoms were fever and cough. Ninety-seven percent of these patients had findings consistent with pneumonia upon admission to the hospital, 77% were admitted to the intensive care unit, and 27% of patients died. The median age of patients was 61 years; 68% were male and 61% had at least one underlying medical condition—most commonly, coronary heart disease, hypertension, diabetes, or chronic obstructive pulmonary disease. Presence of an underlying medical condition

was identified in this study as the only independent risk factor for progression to ARDS. The most common complications of H7N9 AI infection in these patients were ARDS (71%), shock (26%), acute kidney injury (16%), and rhabdomyolysis (10%). Pneumonia and ARDS occurred in all fatal cases; progression to severe pneumonia, ARDS, and shock was rapid. The leading cause of death was refractory hypoxemia. In all cases, patients were treated with neuraminidase inhibitors.

It has been suggested that many mild cases may have occurred but were not reported.[179] Nevertheless, it is clear that the avian-origin H7N9 virus is capable of causing severe disease and death in humans, and age and underlying medical conditions are risk factors for severe disease.

The possible origin of the H7N9 AI viruses that emerged in China in 2013 has been extensively studied. Phylogenetic analyses determined that the viruses are the result of multiple reassortment events, and they have gene segments related to those from at least three different types of AI viruses (Figure 25-11).[194,196] The HA genes are most closely related to those from low pathogenicity AI H7N3 viruses that were isolated in parts of Asia, including China, in 2011 (A/duck/Zhejiang/12/2011-like). The NA genes are most closely related to those from low pathogenicity AI H7N9 viruses that circulated in South Korea in 2011 (A/wild bird/Korea/A14/2011-like); although notably, the human H7N9 viruses have a 15-amino acid deletion in the NA stalk region that had previously been reported to be associated with the adaptation of AI viruses to terrestrial poultry.[194,196,197] The donors of the six internal protein genes were H9N2 viruses.[194,198] It appears that ducks and chickens were probably intermediate hosts for the H7N9 reassortant viruses.

The H7N9 AI viruses isolated from humans and from birds at the time of the outbreak lack the multibasic amino acid cleavage motif in the HA gene that is seen in HPAI viruses. In addition, infection of avian species with H7N9 isolates did not result in disease, although virus was shed and the birds developed antibodies. These observations underscore the challenge for surveillance of these viruses in avian populations, since they do not cause overt disease.[193] The source of the H7N9 AI viruses appears to be live bird markets.[199,200] H7N9 AI viruses isolated from live bird markets were almost identical in sequence to the human isolates. However, H7N9 virus was isolated from only a small percentage of samples taken from birds and the environment all across China. All samples taken from swine and from slaughterhouses were negative for H7N9 AI.

In summary, AI viruses of the H7 subtype have been directly transmitted to humans on numerous occasions. The recent emergence of H7N9 AI infections in humans in China confirmed that H7 AI viruses have the potential to cause severe disease. Studies have demonstrated that the H7N9 AI viruses isolated from human cases bear some genetic markers that are associated with adaptation to mammals,[196,198] and their ability to transmit efficiently in the ferret model by direct contact and by respiratory droplets has been demonstrated.[201,202] Characterization of the crystal structure of the H7N9 AI HA glycoprotein showed preferential recognition of avian-like receptors by H7N9 AI viruses isolated from humans, suggesting that the virus may be poorly adapted for mammalian transmission at this time.[179] Although sustained, efficient human-to-human transmission has not been observed, the pandemic potential of these viruses is of great concern. It is expected that more human cases will occur in the winter months. In December 2013, two human cases of H7N9 AI infection in Hong Kong were reported. In both cases, the infections were thought to have been acquired in the neighboring city of Shenzhen (http://www.chp.gov.hk/en/view_content/32486.html). As of January 31, 2015, 677 laboratory-confirmed cases of human infection with H7N9 AI had been reported to the WHO, with at least 275 deaths.[203]

Influenza Viruses in Bats

In recent years, an increasing interest has emerged in the role of bats as reservoirs of viral pathogens.[204] The coronavirus that caused the SARS outbreak in 2003 (SARS-CoV) is closely related to CoV genomic sequences found in bats in China (see section on Diseases Caused by Emerging Coronaviruses). The paramyxoviruses Hendra and Nipah were isolated from bats. Bats are thought to be possible reservoirs of filoviruses, including Ebola virus and Lloviu virus, a novel filovirus[205,206] since viral nucleic acid sequences have been identified in a variety of bat species. Additionally, Marburg virus has been isolated from Egyptian fruit bats (*Rousettus aegyptiacus*).[207–209]

In 2012, Tong and colleagues[144] reported the identification of novel influenza virus sequences in little yellow-shouldered bats in two locations in Guatemala. Virus was not isolated, but nucleic acid sequences were derived from rectal swab samples, and from liver, intestine, and kidney tissue samples. The sequences were identified as originating from a highly divergent influenza virus. The novel virus was designated as belonging to a new subtype of influenza A viruses, H17N10. The NA gene was the most divergent gene segment, and it was found to have an older ancestral relationship to known influenza A and B viruses. The solution of the N10 crystal structure determined that, although it shares general structural features with the other influenza A

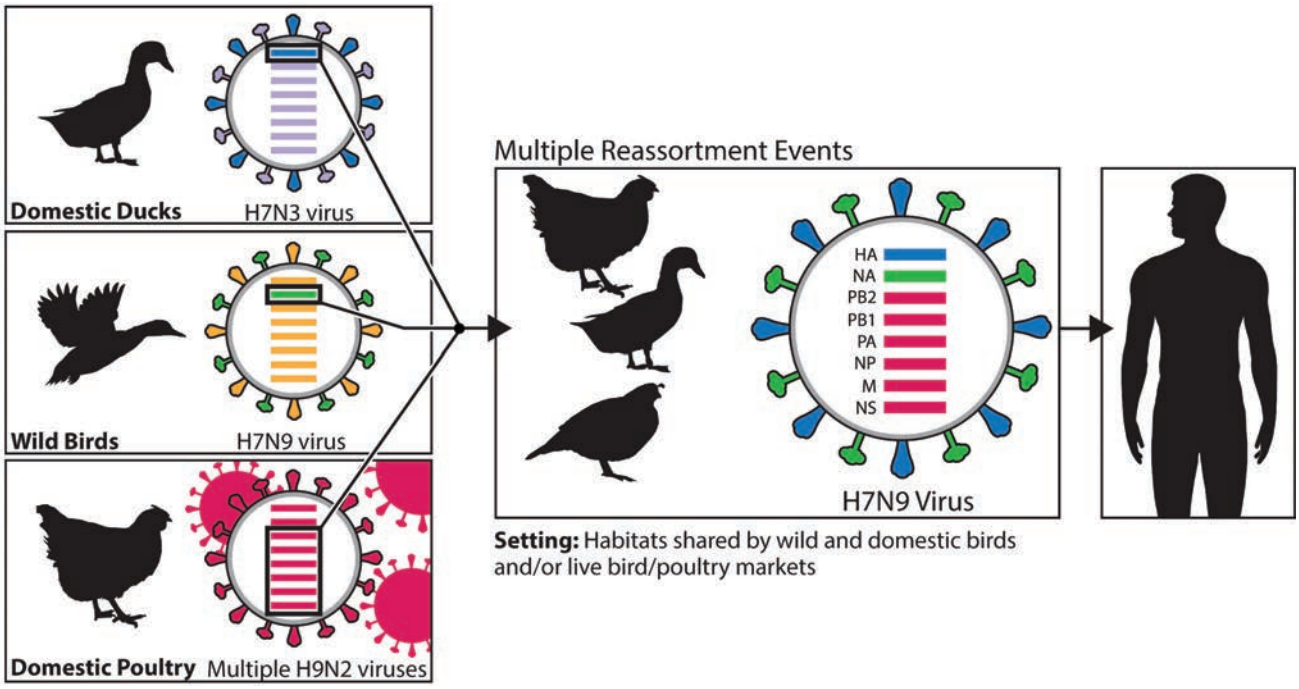

Figure 25-11. Diagram showing the likely genetic evolution of the H7N9 virus that emerged in China in 2013. The eight genes of the H7N9 virus are closely related avian influenza viruses found in domestic ducks, wild birds, and domestic poultry in Asia. The virus likely emerged from "reassortment," a process in which two or more influenza viruses coinfect a single host and exchange genes. This process can result in the creation of a new influenza virus, and it is likely that multiple reassortment events led to the creation of the H7N9 virus. These events may have occurred in habitats shared by wild and domestic birds and/or in live bird/poultry markets, where different species of birds are bought and sold for food. As the above diagram shows, the H7N9 virus likely obtained its HA (hemagglutinin) gene from domestic ducks, its NA (neuraminidase) gene from wild birds, and its six remaining genes from multiple-related H9N2 influenza viruses in domestic poultry.
M: matrix; NP: nucleoprotein; NS: nonstructural; PA: polymerase subunit A; PB1: polymerase subunit B1; PB2: polymerase subunit B2
Diagram: Courtesy of Centers for Disease Control and Prevention.

NAs whose structures have been determined, it does not have the conserved amino acids that are involved in sialic acid binding and cleavage[210,211] and the protein does not display enzymatic neuraminidase activity necessary for its function in mediating spread of the virus from infected cells. The HA structure suggests that the H17N10 virus does not use sialic acid as a receptor.[179] The polymerase complex encoded by the sequences found in the bats did function in human cells, but the sequences of the polymerase genes suggest that they may be incompatible with other influenza A subtypes.

In 2013, Tong et al[145] reported the identification of RNA encoding another distinct influenza virus, designated H18N11, in flat-faced fruit bats in Peru. Again, the sequences were highly divergent from known influenza A viruses, and they indicated a long-standing virus–host relationship. The sequences were most closely related to the H17N10 influenza sequences previously reported by this group.[144] Structural and functional studies of the HA and NA encoded by these sequences suggest that sialic acid is not a receptor for the virus and is not used for virus release from the infected cell. The H18N11 influenza virus was not isolated, but viral sequences were identified in rectal swabs and intestines of the bats. The overall H18 structure was found to be similar to that of the known influenza A trimers, but unlike the known HAs, the H18 structure infers no requirement of low pH for fusion. In addition, the receptor-binding domain of the H18 glycoprotein is dramatically different. Like the N10 NA, the general N11 structure is similar to the known influenza A NAs (ie, tetrameric), but the N11 active site is different, even from that of the N10 protein. The N11 protein does not display glycan binding or enzymatic neuraminidase activity. Seroprevalence studies found that approximately 38% of the Guatemalan bats tested had detectable antibodies to H17,[144] and 50% of bats tested had antibodies to either the recombinant H18 or N11.[145]

Some debate exists as to whether the viruses encoded by the viral sequences identified in the bats in Guatemala and Peru should even be classified as influenza A viruses. They may represent ancient ancestral viruses. The significance of these findings for the potential for the emergence of novel influenza viruses that may infect humans, or for reassortment with other influenza viruses in nature remains to be determined.

Diseases Caused by Emerging Coronaviruses

SARS, which first emerged in Guangdong province of China in November 2002, is a classic example of a newly emerging viral disease. By January 2003, the disease had spread to Guangzhou, the capital of Guangdong province, and caused major outbreaks, primarily affecting healthcare workers. In February 2003, a physician from Guangdong spent a single day in a hotel in Hong Kong, during which time he transmitted the infection to 16 other guests. These individuals quickly spread the disease to Hong Kong, Singapore, Vietnam, and Toronto.[212] Within weeks, SARS had spread to affect thousands of people in 25 countries across five continents and, by the end of the global outbreak (July 2003), more than 8,000 reported cases existed, with 744 fatalities.[213] Within 4 months of the beginning of the outbreak, a novel coronavirus (SARS-CoV) was identified as the infectious agent of the syndrome.[214–216] Cases of SARS have not been reported since 2003.

Middle Eastern Respiratory Syndrome

In June 2012, a 60-year-old man was admitted with a history of fever, cough, expectoration, and shortness of breath to a hospital in Jeddah, Saudi Arabia.[217] Despite treatment in an intensive care unit, the patient died 11 days after admission from respiratory and renal failure. Clinical isolates were initially tested and found negative for influenza, parainfluenza, enterovirus, and adenovirus. A sputum sample that was obtained upon admission was inoculated in Vero and LLC-MK2 cells, resulting in visible cytopathic effect. A viral family-wide PCR performed on nucleic acid extracted from infected cells gave a positive result for coronaviruses. Sequencing of the PCR amplicon resulted in a novel sequence that indicated the newly discovered virus was most closely related to bat coronaviruses. The first report of the novel coronavirus was made in ProMED-mail on September 20, 2012, by Dr Ali Mohamed Zaki of the Dr Soliman Fakeeh Hospital.[218]

Virus samples were sent for full genome deep sequencing to the Erasmus Medical Center in Rotterdam, Netherlands.[219] Full-genome sequencing confirmed that the novel virus was similar to BtCoV-HKU4 and BtCoV-HKU5, members of the C lineage of the betacoronavirus, but it was sufficiently different enough to warrant classification as a new species that was named HCoV-EMC/2012.

Coronaviruses have relatively large, single-stranded, positive-sense RNA genomes. The HCoV-EMC/2012 genome is approximately 30 kb in length and encodes both structural and nonstructural proteins. Before 2003, only two coronaviruses (HCoV-229E and HCoV-OC43) were known to infect humans, and those caused only mild respiratory disease.[220,221] As noted previously, SARS-CoV previously caused an epidemic in 32 countries, infecting more than 8,000 people. Since 2003, two additional human coronaviruses have been identified, HCoV-NL63[177,222] and HCoV-HKU1,[223] both of which can cause pneumonia.

On September 23, 2012, the United Kingdom Health Protection Agency reported on the case of a 49-year-old man who had become sick while in Saudi Arabia in August 2012. That illness resolved, but he subsequently presented to a physician in Qatar with a cough, myalgia, and arthralgia on September 3, 2012. Five days later he was admitted to the hospital with fever and hypoxia. His condition worsened and he was transferred to London by air ambulance. His condition deteriorated once in London, and he was placed on extracorporeal membrane oxygenation on September 20.[144] Initially, the patient was screened for common viral and bacteriological causes of respiratory illness with no positive results. After the September 20 ProMED report of a novel coronavirus identified in the Middle East, patient samples were screened by using a pan-coronavirus reverse transcriptase polymerase chain reaction (RT-PCR) assay. Sequencing of the PCR product showed it was nearly identical to the EMC/2012 virus.

Following the initial description of these two cases which came to be called Middle East Respiratory Syndrome (MERS), a retrospective investigation of an outbreak of acute respiratory disease was performed at the Zarqa hospital in Jordan.[224] In April 2012, the Zarqa hospital had 13 patients who presented with high fever and acute lower respiratory symptoms. Laboratory tests performed at the time of the outbreak were inconclusive. The cluster consisted of two phases. In the first phase, four patients had onset of symptoms between March 21 and April 2. The patient with the earliest onset (a 25-year-old student) and a 40-year-old nurse who worked at the hospital died within 2 to 4 weeks of symptom onset. MERS-CoV infection was confirmed in both of these cases by specific RT-PCR. A second wave of disease followed with onset of symptoms between April 11 and April 26. This second

wave consisted of seven healthcare workers from the hospital and two family members of patients from the first wave of disease. Three of the healthcare workers and the two family members had close contact with individuals in the first wave, raising the possibility of limited person-to-person transmission of the virus.

A published report in June 2013 confirmed person-to-person transmission in a cluster of 23 confirmed and 11 probable MERS-CoV infections in hospitals in the Al-Hasa governorate of Saudi Arabia.[200] The first patient was admitted to the hospital on April 5, 2013, with dizziness and diaphoresis. He was not tested for MERS-CoV, but infection was subsequently confirmed in his son. The first patient is thought to have transmitted the virus to a patient in an adjacent room (in addition to his son), who then transmitted the virus to an additional seven patients (six in the dialysis unit and one in the intensive care unit). Further transmission of the virus was documented to an additional 10 patients, two healthcare workers, and three family members.

In May 2015, Republic of Korea health officials reported a case of MERS-CoV infection in a 68-year-old man who had been traveling in the Middle East for several weeks. He was asymptomatic while traveling,

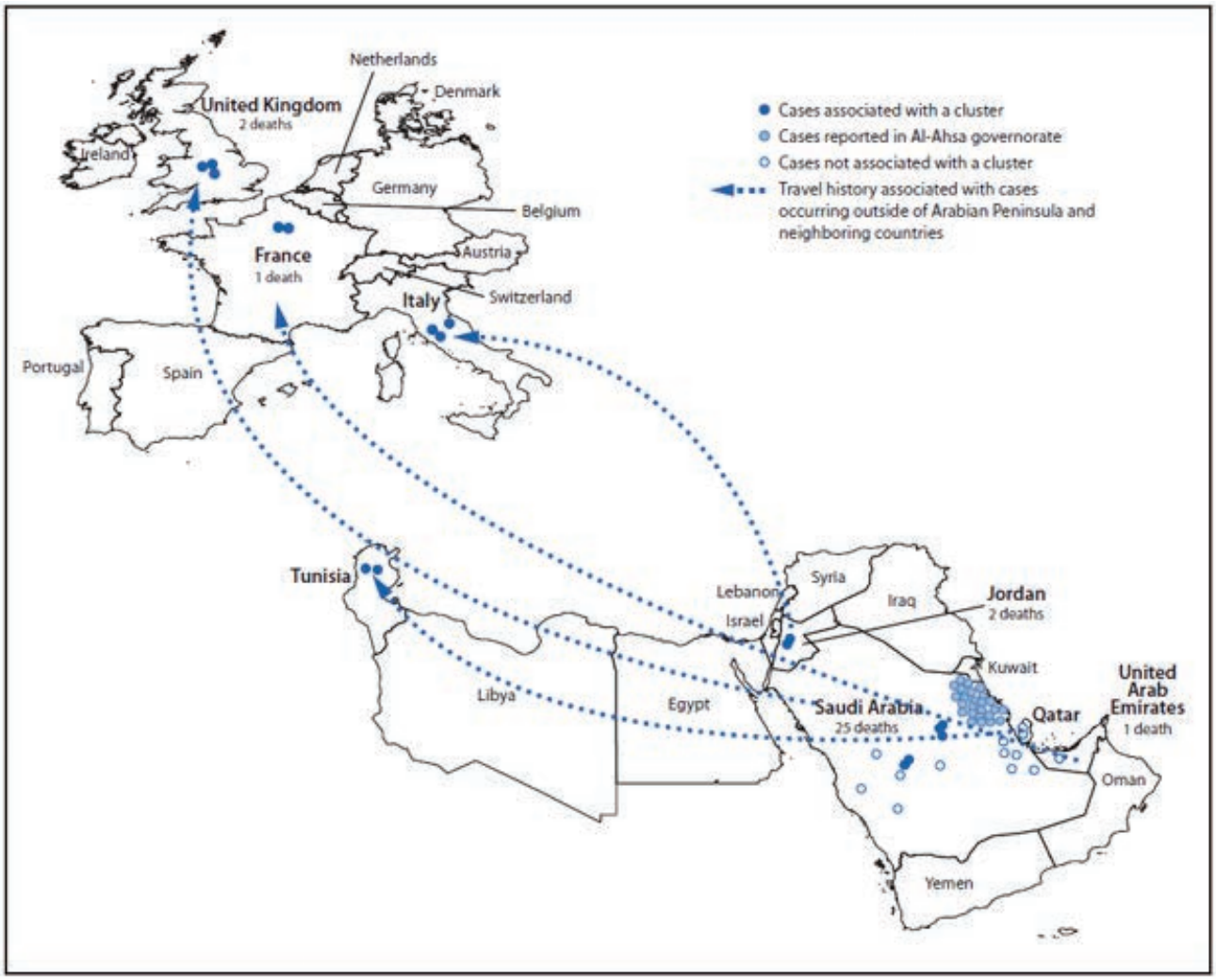

Figure 25-12. History of travel from in or near the Arabian Peninsula within 14 days of illness onset for confirmed cases (N = 130) of Middle East respiratory syndrome coronavirus infection reported to the World Health Organization from 2012 to 2013. All cases have been directly or indirectly linked through travel to or residence in Saudi Arabia, Qatar, Jordan, and the United Arab Emirates. Figure does not include recent cases in South Korea.
Data source: Centers for Disease Control and Prevention. Updated information on the epidemiology of Middle East respiratory syndrome coronavirus (MERS-CoV) infection and guidance for the public, clinicians, and public health authorities, 2012–2013. *MMWR Morb Mortal Wkly Rep*. 2013;62:793–796.

but developed symptoms within a week of returning to Korea. He was seen at four separate hospitals and was admitted to the fourth one in Seoul on May 18, where it was confirmed that he was infected with MERS-CoV.[225] During his medical visits, before being diagnosed, he infected around 30 other individuals who were present in the hospital at the same time. A secondary case from the second hospital went on to infect more than 80 additional people. In total, 186 cases of MERS (185 in the Republic of Korea and 1 in China[226]) were confirmed during the Korean outbreak, more than half of which had been infected by one of the two "superspreaders."

As of September 2, 2015, 1,493 confirmed cases of MERS-CoV infection resulted in 527 deaths (35% case fatality rate).[226] The majority of these cases were from Saudi Arabia, but Korea, France, Italy, Jordan, Qatar, Tunisia, the United Kingdom, and the United Arab Emirates also have reported cases. During the recent outbreak in South Korea, secondary and tertiary transmission occurred from the index case who traveled to the Middle East. All other cases outside of the Middle East had recorded recent travel to the Middle East (Figure 25-12).[227]

A meta-analysis of 47 laboratory-confirmed cases of MERS-CoV infection from Saudi Arabia found that most patients presented with fever, cough, shortness of breath, and myalgia.[200] Almost all of the identified patients had underlying comorbidities, including diabetes (68%), chronic kidney disease (49%), hypertension (34%), chronic heart disease (28%), and chronic lung disease (26%). Twenty-eight (60%) of the patients died, and the fatality rate increased with increasing age.

The receptor for MERS-CoV was identified within 6 months of the original characterization of the virus. Dipeptidyl peptidase 4 (DPP4) from extracts of cells susceptible to virus infection co-purified with the receptor-binding domain of the EMC/2012 spike protein.[228] Transient expression of DPP4 in nonsusceptible cells also rendered them susceptible to infection and preincubation of cells with anti-DPP4 polyclonal antibodies made them resistant to MERS-CoV infection. The rapid identification of the receptor opens several avenues for generating antiviral therapeutics. Manipulation of DPP4 levels or the development of small molecules or monoclonal antibodies that can block the interaction of MERS-CoV with DPP4 could potentially alter the course of disease in infected individuals.

Other therapeutic interventions may prove useful for treatment of MERS-CoV infection. The virus has been shown to be sensitive to type I interferon in vitro, with its replication limited by two to four orders of magnitude when cells are pretreated with interferon.[229,230] In addition, in a rhesus macaque model of MERS-CoV infection,[231] treatment with interferon α2b and ribavirin was shown to ameliorate some of the disease symptoms.[231] Additionally, a monoclonal antibody isolated from an infected patient has shown prophylactic and postexposure protection against MERS-CoV infection in a mouse model of infection.[232] Vaccination of mice, camels, and rhesus macaques with a DNA vaccine expressing the MERS-CoV spike protein was shown to elicit neutralizing antibody responses in all three species, and it could also protect macaques from challenge with MERS-CoV.[233]

Parallels can be drawn between the current MERS-CoV epidemic and the SARS epidemic of 2002 to 2003. Although the reservoir for MERS-CoV has not been identified, both viruses are thought to circulate in bats. SARS-like coronaviruses were identified in three species of bats from the genus *Rhinolophus*.[186] The virus may have spilled over into palm civets (*Paguma larvata*) (Figure 25-13), which served as an amplifying host. The virus could then be transmitted to humans when they came into contact with infected civets in wild animal markets. The genome sequence of MERS-CoV indicates that it is closely related to two bat coronaviruses, BtCoV-HKU4 and BtCoV-HKU5. A bat survey conducted in Saudi Arabia identified a fecal sample from a *Taphozous perforatus* bat that yielded a PCR product with 100% identity to the sequence of MERS-

Figure 25-13. The masked palm civet was originally implicated as the possible animal source for the SARS coronavirus after SARS-like coronaviruses were isolated from animals found in a live animal market in Guangdong, China. These animals are trapped and butchered for food in southern China. This photograph was taken at a wet market in Guangzhou in May 2003.
SARS: severe acute respiratory syndrome
Photograph: Courtesy of Dr Meirion Evans, Cardiff University, United Kingdom.

CoV EMC/2012.[234] In another survey, a fecal sample from a South African *Neoromicia zuluensis* bat yielded a PCR product whose nucleotide sequence indicated that it was closely related to the MERS-CoV.[235]

Although MERS-CoV-like viruses have been identified in bats, nothing indicates that MERS-CoV is jumping directly from bats into humans. One possibility is that MERS-CoV may move from bats through an intermediate host that has a closer association with humans, as was the case of the SARS-CoV. Serum surveys of livestock in Egypt, Oman, and Spain identified high levels of MERS-CoV reactive antibodies in dromedary camels.[236,237] Subsequently, MERS-CoV RNA was detected in three camels that had close association with two human cases.[218] In November and December 2013, a large, nationwide serosurvey of livestock in the Kingdom of Saudi Arabia found that 74% of the sampled dromedary camels had antibodies reactive to MERS-CoV.[238] Testing of archived serum samples found MERS-CoV reactivity dating back to 1992, indicating that the virus has been circulating in the Kingdom of Saudi Arabia since at least that time. In addition to the serological data, two groups isolated replication-competent MERS-CoV from dromedary camels in late 2013 through early 2014.[239,240]

Public health measures were an important aspect of halting the spread of the SARS epidemic, and public health officials worldwide have been proactive with measures intended to reduce the possibility that MERS could become another pandemic. One of the main concerns has centered on the Hajj, the annual event in which millions of Muslim pilgrims from around the world travel to Mecca in Saudi Arabia. The Saudi Arabian Ministry of Health recommended that persons older than 65, pregnant women, children younger than 12, or those with chronic diseases should postpone performing the Hajj in 2013. Many may have heeded those warnings, as participation in the 2013 Hajj was estimated at just less than 2 million pilgrims, down from 3.2 million pilgrims in 2012.

As of this writing, cases of MERS are still occurring on the Arabian Peninsula. Unfortunately, unlike SARS-CoV, MERS-CoV shows no sign of abating and continued efforts to understand this virus will be important to control this emerging disease.

Diseases Caused by Emerging Paramyxoviruses

Hendra Virus

In 1994, a new member of the paramyxoviruses emerged for the first time in Brisbane, Australia, killing 14 race horses and a horse trainer.[241] Another worker at the stable survived with an influenza-like illness. One year later, a farmer from Mackay (800 km north of Brisbane) died as a result of encephalitis caused by this novel virus.[242] Two of his horses were subsequently shown to have died from the same virus 13 months earlier. Genetic analysis of the virus showed it was distantly related to the morbilliviruses, which contain other members such as rinderpest, measles, and canine distemper viruses, and so the virus was initially named equine morbillivirus,[241] but was later renamed Hendra virus after the Brisbane suburb where the outbreak occurred. Serologic evidence[243] and later evidence of infection was found in several species of Australian flying foxes (ie, fruit bats of the genus *Pteropus*) (Figure 25-14), supporting epidemiological evidence that fruit bats are the natural reservoir for Hendra virus. Field, experimental, and molecular investigations indicate that Hendra virus is an endemic fruit bat virus that has probably co-evolved with its pteropid hosts.[53,244,245]

Although additional occurrences of Hendra virus have been relatively rare and sporadic, as of June 2014, 50 outbreaks of Hendra virus occurred in Australia, all involving infection of horses. Four of these outbreaks have spread to humans as a result of direct contact with infected horses. The case fatality rate in humans is 60% and in horses 75%.[246]

Nipah Virus

Nearly 5 years after the discovery of the Hendra virus, a massive outbreak of porcine respiratory disease occurred in Malaysia and subsequently caused the deaths of 105 pig farm or abattoir workers, the eventual culling of more than 1 million pigs, and the discovery of a new virus closely related to Hendra called Nipah virus.[247] The predominant clinical syndrome in humans was encephalitic (not respiratory as was seen in the infected pigs) with clinical signs including fever, headache, myalgia, drowsiness, and disorientation sometimes leading to coma within 48 hours.[248,249] The majority of human cases had a history of direct contact with infected pigs, most of whom were pig farmers. Preliminary research on the new virus revealed that it had ultrastructural, antigenic, serologic, and molecular characteristics similar to Hendra virus.[247] Follow-up molecular studies showed the genome of Nipah virus to be highly homologous to that of Hendra virus, with specific genes sharing from 70% to 88% nucleotide homologies and amino acid homologies ranging from 67% to 92%.[247] Given the degree of similarity and other unique features of these viruses, they both were placed in a new genus, *Henipavirus*, within the family *Paramyxoviridae*.[250] With the knowledge of the similarities between Nipah and Hendra viruses, it was natural that attention focused on Malaysian bats as the source of the

infection in pigs.[53] Initial surveillance efforts identified the presence of neutralizing antibodies to Nipah virus in the sera of 21 bats from five species (four species of fruit bat, including two flying fox species, and one insectivorous bat species).[251] Although no virus was isolated or viral RNA amplified from these seropositive bats, later attempts proved successful and virus was isolated from pooled urine samples collected from a colony of seropositive flying foxes from Tioman Island off the coast of Malaysia.[252]

The virus reemerged in Bangladesh in 2001, each resulting in a cluster of febrile neurologic illnesses with nine reported deaths.[253] Since 2001, outbreaks of Nipah have occurred nearly every year in Bangladesh. More than 70% of those infected have died and one-third of the survivors have permanent neurological deficits.[254] Outbreak investigations in Bangladesh have identified consumption of raw date palm sap as the primary route of transmission of Nipah virus from *Pteropus* bats to people. Date palm sap is harvested in the winter in Bangladesh by shaving the bark from the sugar date palm tree and collecting the sap into open clay pots. *Pteropus* bats (see Figure 25-14) that shed Nipah virus in their saliva frequently visit the trees during sap collection and lick the sap as it is running into the pot, thereby contaminating the sap.[255] However, similar to other viruses, such as Ebola virus, transmission can also occur by direct contact with infected individuals, particularly during patient care (ie, nosocomial transmission) or exposure to infected patients' bodily secretions during traditional burial practices.[256] Thus, patients from regions where Nipah virus is known to occur who present with meningoencephalitis should be placed in an isolation room or ward and healthcare workers caring for these patients should wear gloves and masks.

Emerging Mosquitoborne Viruses: Dengue, West Nile, and Chikungunya

Mosquitoborne viruses are members of the more general category of arthropodborne viruses or arboviruses. Human infection with arboviruses can be asymptomatic or can cause diseases ranging from a mild febrile illness to encephalitis or even severe hemorrhagic fever in some cases. Others cause rash and epidemic arthralgia. Most arboviruses require a reservoir host such as a bird or small mammal while using a vector—usually a mosquito or tick—for transmission to another host.[257] From this complex life cycle, many arboviruses are restricted to specific geographical regions. For example, Ross River and Murray Valley encephalitis viruses are restricted to Australia and surrounding islands, whereas o'nyong-nyong virus

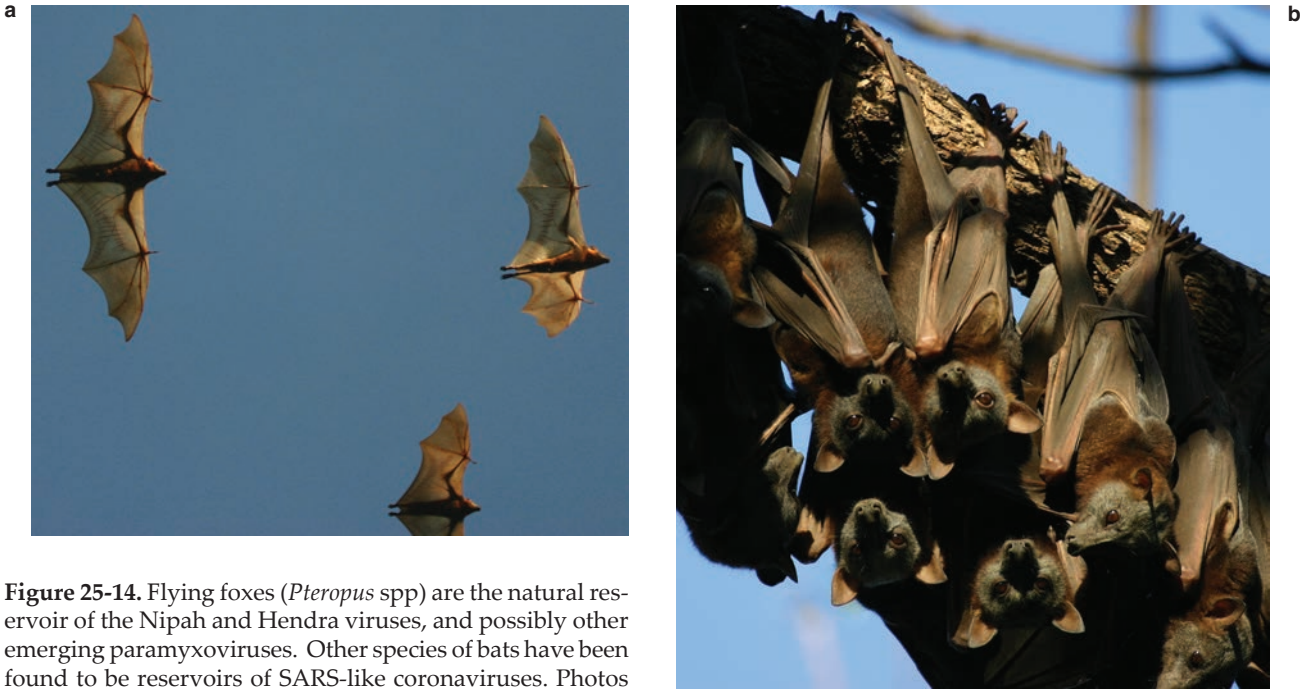

Figure 25-14. Flying foxes (*Pteropus* spp) are the natural reservoir of the Nipah and Hendra viruses, and possibly other emerging paramyxoviruses. Other species of bats have been found to be reservoirs of SARS-like coronaviruses. Photos show the little red flying fox (*Pteropus scapulatus*) in flight (**a**) and roosting (**b**).
Photographs: Courtesy of Raina Plowright, Department of Veterinary Medicine and Epidemiology, University of California, Davis, California.

occurs only in Africa. However, because of various ecological or environmental changes (whether natural or manmade) that lead to changes in the mosquito vector distribution or genetic changes in the viruses themselves, some arboviruses may not always remain restricted to their previously known geographical regions.

Dengue Virus

Dengue fever, which is caused by one of four viral subtypes (designated DENV-1 to DENV-4), is one of the most common mosquitoborne viral infections of humans, with up to 100 million cases reported annually and some 2.5 billion people living at risk of infection in tropical and subtropical regions of Africa, Asia, and the Americas.[258] Infection with dengue virus (DENV) can present in several clinical manifestations. Classical dengue fever is an acute febrile illness that often occurs in children and is characterized by fever, severe headache and muscle aches, nausea, vomiting, and rash. This acute illness, which usually lasts for 8 to 10 days, is rarely fatal. A more severe form of dengue infection is dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). DHF usually begins during the first week of the acute illness and can lead to hemorrhagic manifestations, including petechiae, ecchymoses, epistaxis, bleeding gums, and gastrointestinal tract bleeding.[259] DSS occurs if the patient goes on to develop hypotension and shock due to plasma leakage and circulatory failure, which happens in about one-third of severe dengue cases (especially children) and is often associated with higher mortality. Convalescence for patients with DHF is usually short and uneventful, and if shock is overcome, patients usually recover within 2 to 3 days.[259]

The pathogenesis of DHF/DSS is complicated and not well understood. Two theories are frequently cited to explain the pathogenetic changes that occur in DHF/DSS. The most commonly accepted theory is known as immune enhancement.[260,261] This idea suggests that patients experiencing a second infection with a heterologous DENV serotype have a significantly higher risk of developing DHF and DSS. Preexisting heterologous dengue antibody recognizes the infecting virus and forms an antigen-antibody complex, which is then bound to and internalized by immunoglobulin Fc receptors on macrophages. Thus, it is hypothesized that prior infection, through a process known as antibody-dependent enhancement, enhances the infection and replication of DENV in mononuclear cells.[259] The other theory assumes that DENV changes genetically as a result of selective pressures as it replicates in humans and/or mosquitoes and that the phenotypic expression of these genetic changes may include increased virus replication and virulence. All the data suggest that a combination of one's viral, immunopathogenic, age, and genetic background plays a role in disease severity.[258]

Although first identified in southeast Asia in the 1940s and 1950s, evidence suggest that DENVs were derived from a primitive progenitor introduced to Asia from Africa about 1,000 years ago.[262] Studies of DENV ecology in sylvatic habitats of west Africa and Malaysia have identified transmission cycles involving nonhuman primates as reservoir hosts and arboreal, tree-hole dwelling *Aedes* species mosquitoes as vectors.[263,264] Efficient interhuman DENV transmission probably requires a human population of 10,000 to 1 million people, a feature of urban civilization that did not exist until about 4,000 years ago, suggesting the sylvatic cycle is probably ancestral.[265] Further support for this idea comes from studies suggesting that a zoonotic transfer of DENV from sylvatic to sustained human transmission occurred between 125 and 320 years ago.[262] In the past 300 years, these viruses have become established in the urban centers of the tropics. The principal urban vector, *Aedes aegypti*, is highly domesticated and is adapted to humans, preferring to feed on them and lay their eggs in artificial containers in and around houses. *Ae. albopictus* (the Asian tiger mosquito) (Figure 25-15) is a secondary vector of DENVs.

In the past 25 years, a marked global emergence of epidemic dengue has occurred, with more frequent and larger epidemics associated with more severe disease.[259,266,267] The reasons are not fully understood, but are thought to stem from major demographic and

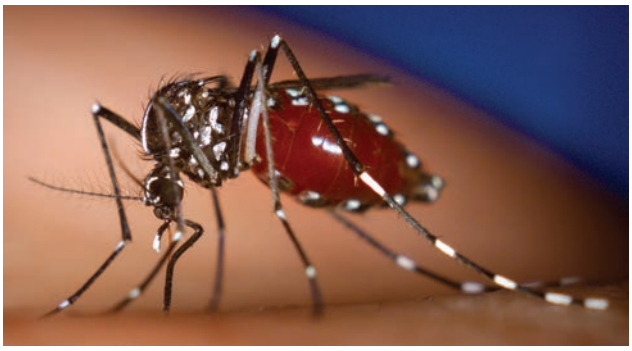

Figure 25-15. A female *Aedes albopictus* mosquito feeding on a human host. This mosquito, along with *Aedes aegypti*, are competent vectors of dengue virus.
Photograph: Courtesy of James Gathany, Centers for Disease Control and Prevention Public Health Image Library. Image 4490.

societal changes that have occurred over the past 50 years. In particular, unprecedented global population growth and associated unplanned and uncontrolled urbanization occurred, especially in the tropical developing countries.[259] Other potential factors associated with the global emergence of dengue include the lack of effective mosquito control in many tropical areas where dengue is endemic, increased international air travel, and a general decay in public health infrastructure in most countries over the past 30 years.[259] Dengue does occur—albeit rarely—in the United States, primarily in southern Texas and Florida. Because the mosquito vectors that transmit DENVs are distributed throughout much of the southeastern United States, there is likely a greater potential for emergence of dengue. This situation may be unfolding in southern Florida.[267a] Florida has a history of epidemic DENV transmission, but more recent cases were most likely imported by tourism or triggered by infected individuals traveling into the area. However, in the late summer of 2009, DENV-1 infection was confirmed in a person who acquired the virus while traveling to Key West in Monroe County, Florida. DENV-1 infections were subsequently confirmed in two Monroe County residents without history of recent travel.[268] In 2010, additional dengue cases from Monroe County were reported, and DENV-1 was isolated from a mosquito pool[269] and a blood donor from Key West.[270] Phylogenetic analyses of these viral isolates indicated that endemic DENV-1 was transmitted in Key West over at least a 2-year period.[268] In 2013, Martin County in east-central Florida reported 29 cases of locally transmitted DENV-1, and occasional locally transmitted cases have continued to occur since then.[267a]

West Nile Virus

West Nile virus (WNV) was first isolated in 1937 from the blood of a febrile patient in the West Nile district of northern Uganda. It is now one of the most widely distributed of all mosquitoborne arboviruses, and it is found in areas throughout Africa, Europe, Asia, and the Americas. Yet, until 1999, it was completely exotic to the Western Hemisphere. In the late summer of 1999, WNV emerged in the New York City area as the cause of an outbreak of meningoencephalitis resulting in 7 deaths among 62 confirmed cases.[75] A concurrent outbreak occurred among the horse population on Long Island, resulting in 25 equine cases, including 9 fatalities.[271] The principal mosquito vectors were *Culex pipiens* or other related *Culex* species; however, the virus has been isolated from other mosquito species and even from ticks in some cases.[266,272] The virus has been shown to be capable of infecting more than 50 species of mosquitoes and ticks.[272,273] Since the introduction of WNV into New York in 1999, the virus

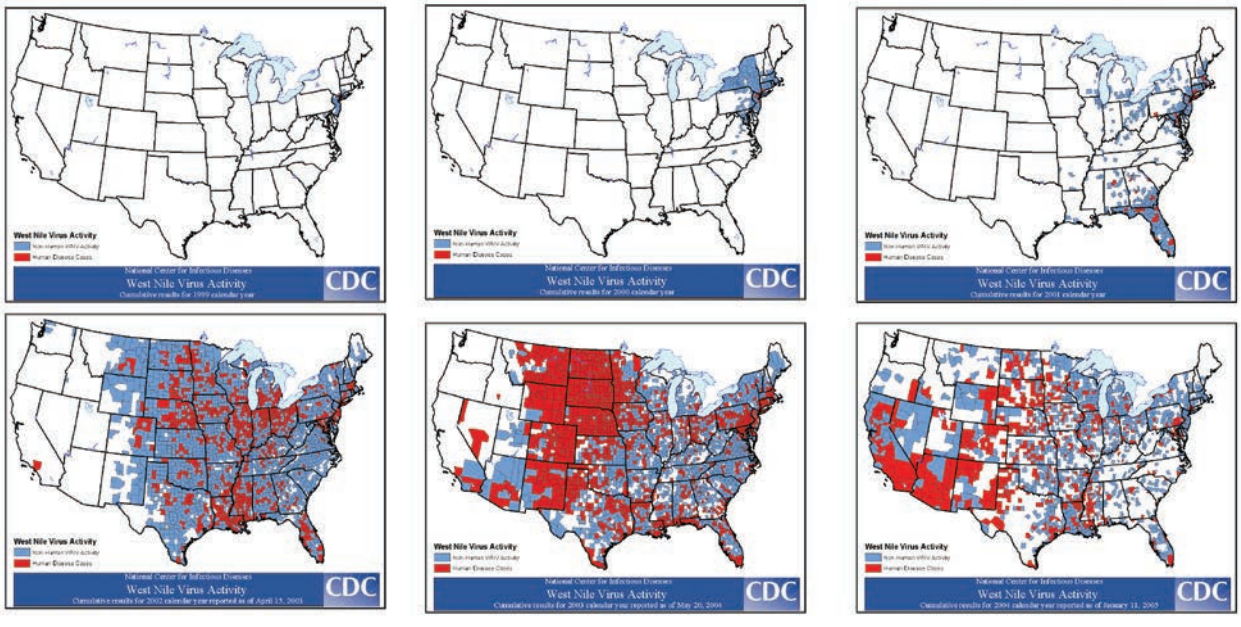

Figure 25-16. Yearly spread of West Nile virus activity across the United States, 1999 to 2004. Data represent counties reporting West Nile virus activity in humans (*red*) and nonhuman (eg, birds, mosquitoes, equines, and other mammals) (*blue*) in the United States.
Data source: National Center for Infectious Diseases, Centers for Disease Control and Prevention.

has spread across the United States (Figure 25-16), north into Canada, and south into Mexico, Central America, and the Caribbean Islands.

Recent years have seen a high incidence of human infection with WNV through blood transfusion, mother-to-fetus transmission, and transmission in breast milk, and also by organ transplantation, causing even greater public health concerns.[96,212,274,275] After several years of low WNV activity in the United States, a multistate outbreak was seen in 2012, with more than 5,600 cases and 286 deaths recorded.[276]

Chikungunya

The first recorded outbreak of chikungunya (CHIK) occurred in the Newala District of Tanzania (formerly Tanganyika) in 1952 to 1953.[277,278] The outbreak was initially thought to have been caused by DENV because it shared many clinical features with dengue infection and was thought to be transmitted by *Ae aegypti* mosquitoes. The infection manifested with a sudden onset of incapacitating joint pain and high fever, leading locals to call it chikungunya, meaning "that which bends up" in the local Makonde language. The disease also often led to development of a maculopapular rash, anorexia, and constipation. Most symptoms usually resolved within 7 to 10 days, but the arthralgia could last for months following the infection. In some patients, the joint pain was so severe months after infection that they were unable to change position without help.

A viral agent was recovered from the serum of acutely ill patients by intracerebral inoculation into mice.[279] Hyperimmune serum raised against the virus could cross neutralize Semliki Forest virus (an alphavirus) but not DENV, indicating that the virus was more closely related to the alphaviruses than flaviviruses.

The virus isolated from the outbreak in the Newala District, chikungunya virus (CHIKV), is an Old World

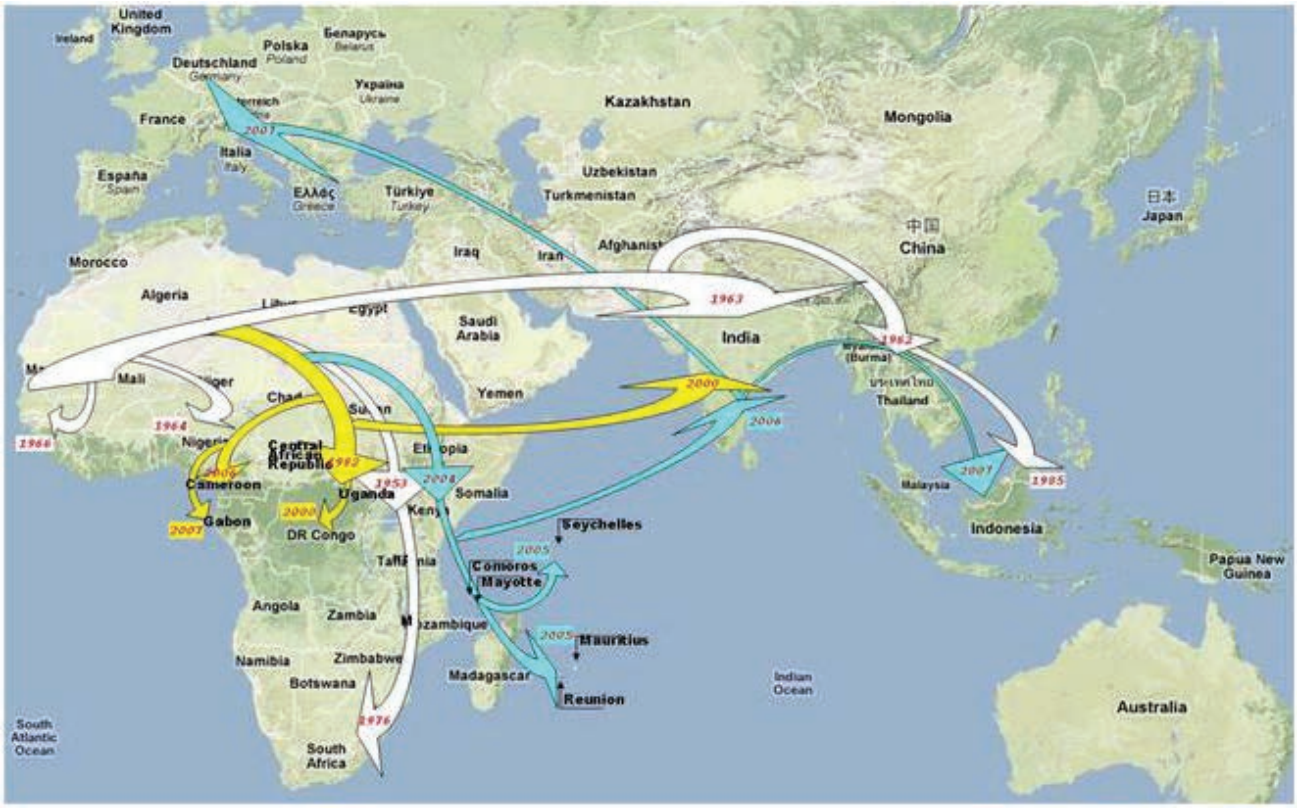

Figure 25-17. Predicted dispersal pattern of chikungunya virus from Africa to the Indian Ocean and Europe during the past 20 to 50 years.
DR Congo: Democratic Republic of the Congo
Photograph: Courtesy of Creative Commons, licensed under CC BY 2.0. https://virologyj.biomedcentral.com/articles/10.1186/1743-422X-5-33.
Data source: de Lamballerie X, Leroy E, Charrel RN, Ttsetsarkin K, Higgs S, Gould EA. Chikungunya virus adapts to tiger mosquito via evolutionary convergence: a sign of things to come? *Virol J*. 2008;5:33.

alphavirus in the Semliki Forest antigenic complex that is found mainly in Africa and Southeast Asia. African transmission follows a sylvatic cycle between nonhuman primates, small mammals, and *Aedes* species mosquitoes, with occasional spillover into human populations when vector populations are high.[263] Asian transmission follows an urban cycle, with the virus transmitted between humans via the urban dwelling *Ae aegypti* and *Ae albopictus* mosquito vectors.

Between 1960 and 1980 numerous documented outbreaks of CHIK occurred throughout Africa and Asia, followed by relative quiet between 1980 and 2000.[280] In 2000, the virus reemerged when an estimated 50,000 people were infected in Kinshasa, Democratic Republic of the Congo, the first reappearance of the virus there in 39 years.[281,282] From May until July 2004, an outbreak occurred on Lamu Island off the coast of Kenya. There were 1,300 reported cases of CHIKV infection of a total population of 18,000 on the island. A seroprevalence study conducted after the epidemic found that 75% of the population had detectable IgG and/or IgM antibodies to the virus, indicating that approximately 13,500 people had been infected.[283] The virus spread to Mombasa, Kenya, and then to the Comoros Islands, where an estimated 215,000 people contracted the disease on Grand Comore Island between February and May of 2005.[284]

Additional outbreaks occurred on the Indian Ocean islands of Mauritius, the Seychelles, Madagascar, and Mayotte, culminating in a large outbreak on Reunion Island between March 1, 2005 and April 30, 2006. During the Reunion Island outbreak, an estimated 255,000 people were infected.[285] The outbreak on Reunion Island was unusual because the main mosquito vector, *Ae aegypti*, was not abundant on the island. It appeared that the main vector responsible for transmission during the Reunion outbreak was *Ae albopictus*. Genetic characterization of the virus from Reunion Island identified a key single amino acid change (A226V, ie, the alanine at position 226 was changed to valine) in the envelope glycoprotein that enabled the virus to infect *Ae albopictus* more efficiently.[286,287]

A large outbreak of CHIKV occurred in India in 2006, marking a return of the virus that had been absent for 33 years (Figure 25-17; note: the *yellow arrow* indicating the presence of CHIKV in India in 2000 is derived from a virus isolated from mosquitoes in Yawat, Maharashtra, not a human case). It is estimated that 1.4 million people were infected. Genetic analysis of the virus showed that it was related to the East African and Indian Ocean strains from the previous couple of years, but it lacked the A226V mutation.[288] During a second wave of infection in 2007 in Kerala, India, the virus had obtained the A226V mutation (likely independently from the Reunion strains of the virus), indicating that it had adapted to the high population densities of *Ae albopictus* in the area at the time.[288,289]

The rapid adaptation of CHIKV to *Ae albopictus* mosquitoes may represent a threat to Europe and North America. *Ae aegypti* mosquitoes have only been detected in a small swath of the southern United States. *Ae albopictus*, however, has been detected as far north as Pennsylvania, New Jersey, and southern New York in the United States, and as far north as Germany and the Netherlands in Europe. An outbreak in Ravenna, Italy during the summer of 2007 may foreshadow potential future outbreaks in the United States and Europe. A visitor from the active outbreak area of Kerala, India became ill with CHIKV 2 days after arriving in Ravenna on June 21. Virus was transmitted locally by *Ae albopictus* mosquitoes, resulting in 205 autochthonous cases identified between July 4 and September 27, peaking during the third week of August.[290] This was the first observation of sustained CHIKV transmission in a temperate climate.

In December 2013, the WHO reported confirmed cases of CHIKV infection on the Caribbean Island of Saint Martin including two confirmed cases, four probable cases, and another 20 suspected cases.[291] None of the cases reported recent travel outside of Saint Martin, indicating that these were the first reported cases of local transmission of CHIKV in the Western Hemisphere.

After the initial detection in Saint Martin, the virus spread rapidly throughout the Caribbean, and South and Central America. The cumulative case number for 2014 throughout the Americas reached nearly 25,000 confirmed and more than 1.1 million suspected cases, with the highest incidence rate of 56% occurring on the island of Martinique.[292]

In the summer of 2014, the Florida Department of Health reported detection of the first autochthonous transmission of CHIKV in the United States.[293] Eleven cases of transmission were detected in Miami-Dade, Palm Beach, Saint Lucie, and Broward counties.

No treatment is available for CHIKV infection. The sole remedy available consists of treating to alleviate the symptoms. An attenuated CHIKV vaccine developed by the US Army Medical Research Institute for Infectious Disease and the University of Maryland progressed through phase II clinical trials in 2000 before it was discontinued because of a change in funding priorities.[294] The recent explosion in the size of CHIK outbreaks and the demonstration that the virus can cause outbreaks in Europe and potentially the United States may call for a reinvestment in the development of CHIKV vaccines.

Emerging Tickborne Phleboviruses

Until recently, most bunyaviruses (family *Bunyaviridae*) within the genus *Phlebovirus* that were of concern to human health were transmitted by either mosquitoes or sandflies, including viruses such as Rift Valley fever virus or sandfly fever virus, respectively. Recently, new tickborne diseases caused by novel phleboviruses have emerged in China (and later seen in Japan and South Korea) and in the midwestern United States.

Severe Fever With Thrombocytopenia Syndrome Virus

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tickborne disease first described from rural areas of central China.[295,296] The major clinical symptoms include fever, thrombocytopenia (low platelet count), gastrointestinal symptoms, and leukocytopenia (low white blood cell count). Initial cases (79 cases with 10 deaths, case fatality rate of 12.7%) were found in 2007 from the Henan Province.[295] Interestingly, because of the similarity in clinical symptoms, investigators first suspected human granulocytic anaplasmosis, another tickborne disease caused by the bacterium *Anaplasma phagocytophilum*.[295,296] However, when no bacterial DNA or antibodies against this bacterium could be detected in the blood samples from the majority of the patients, a viral etiology was suspected. In 2009, a novel virus was isolated from the blood of a patient from Xinyang City in Henan Province.[296] Independently, another group identified the virus from the same region of China using high-throughput sequencing of acute-phase sera from 10 patients who had fever, thrombocytopenia, leukopenia, and a history of tick bite.[295] The group also isolated a virus that reacted with patients' sera in immunoflorescence assays and had characteristic virion morphology consistent with that of a bunyavirus (Figure 25-18).[295] These investigators named the disease thrombocytopenia and leukopenia syndrome and the new virus, Henan fever virus, after the location of the index patient.[295] However, SFTS and SFTS virus (SFTSV) are generally accepted.

As the name implies, prominent manifestations of the disease include thrombocytopenia and leukopenia. Other major symptoms include sudden onset of fever and gastrointestinal symptoms (vomiting, diarrhea, and upper abdominal pain).[295,296] Multiorgan failure developed rapidly in most patients as shown by elevated levels of serum alanine aminotransferase, aspartate aminotransferase, creatine kinase, and lactate dehydro-

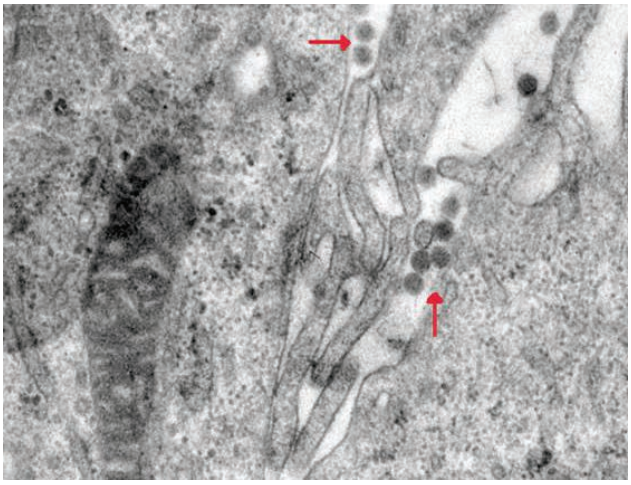

Figure 25-18. Thin-section electron microscopy of the novel bunyavirus (*red arrows*) associated with fever, thrombocytopenia, and leukopenia syndrome (now called severe fever with thrombocytopenia syndrome) in China. Original magnification ×50,000.
Photograph: Courtesy of Creative Commons, licensed under CC BY 2.0. http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1002369.
Data source: Xu B, Liu L, Huang X, et al. Metagenomic analysis of fever, thrombocytopenia and leukopenia syndrome (FTLS) in Henan Province, China: discovery of a new bunyavirus. *PLoS Pathog*. 2011;7:e1002369.

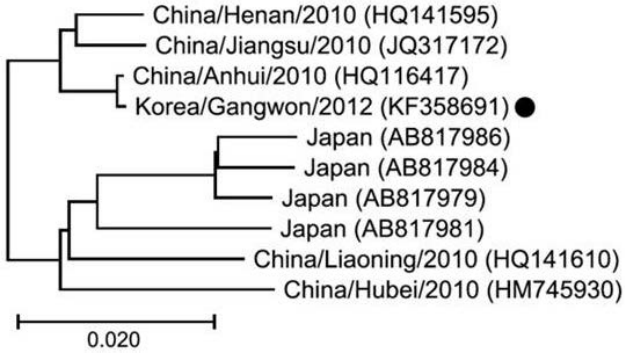

Figure 25-19. Phylogenetic tree for the RNA-dependent RNA polymerase (RdRP) gene sequences of the large segment of an isolate obtained from a fatal case of severe fever with thrombocytopenia syndrome (SFTS) in South Korea (*black dot*) compared with representative SFTS virus strains from China and Japan. The tree was constructed on the basis of the nucleic acid sequences of the RdRP genes by using the neighbor-joining method. Location, year of isolation, and GenBank accession numbers are indicated. Branch length of the tree shows the evolutionary distance. *Scale bar* indicates 2.0% sequence distance.
Data source: Kim KH, Yi J, Kim G, et al. Severe fever with thrombocytopenia syndrome, South Korea, 2012. *Emerg Infect Dis*. 2013;19:1892–1894.

genase. In one study of 285 patients from the Henan Province, investigators reported that a small number of patients experienced mental status alterations, ecchymosis, gastrointestinal hemorrhage, pulmonary hemorrhage, and disseminated intravascular coagulation.[295] Interestingly, many of these symptoms are similar to those of hemorrhagic fevers caused by other bunyaviruses such as Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndrome, and Rift Valley fever. Although no indications of person-to-person transmission exist in the initial clusters of cases, several recent reports demonstrate person-to-person transmission of SFTSV among healthcare workers, family members, and mortuary workers.[211,297,298]

Epidemiological investigations during the initial cluster of cases showed that the majority of patients were farmers living in wooded and hilly areas and were working in the fields before the onset of disease.[296] Also, mosquitoes and ticks were commonly found in the patients' home environment. Thus, the role of arthropod vectors was highly suspected. Although no viral RNA was found in any of 5,900 mosquitoes tested, more than 5% of *Haemaphysalis longicornis* ticks collected from animals in the areas were the patients lived contained SFTSV RNA.[296] *H longicornis* is widely distributed in the Asia-Pacific region, including China, Korea, Japan, Australia, the Pacific Islands, and New Zealand.[299] SFTSV-specific antibodies and viral RNA have also been found in several domesticated animals in China.[300] In a sampling of more than 3,000 domesticated animals in Shandong Province, specific antibodies were detected in 69.5% of sheep, 60.5% of cattle, 37.9% of dogs, 3.1% of pigs, and 47.4% of chickens. SFTSV RNA was detected in all these animal hosts as well, albeit at a somewhat lower prevalence, ranging from 1.7% to 5.3%.[300] These findings demonstrate that natural infections of SFTSV occur in several domesticated animals in disease-endemic areas and that the virus has a wide host range. However, the role of domesticated animals in the circulation and transmission of SFTSV remains unclear.[300]

The disease has also been detected in Japan and South Korea, killing at least eight people in each country thus far.[301] This occurrence is perhaps not too surprising given that the range of the tick vector includes these countries and SFTSV was detected in *H longicornis* ticks collected during 2011 to 2012 in South Korea.[301] The strains isolated from South Korea were closely related to those from China, but were somewhat more distantly related to those from Japan (Figure 25-19),[301] which is consistent with the geographic distance between these countries and presumably reflects the greater evolutionary history between these viruses.

Heartland Virus

At about the same time that SFTSV was discovered in rural China (ie, 2009), a similar but distinct virus infected two men in rural Missouri in the United States. The men, one in his late 50s and the other in his late 60s who both lived on large farms in northwestern Missouri, independently presented to Heartland Regional Medical Center in Saint Joseph, Missouri, in early June 2009.[302] Both individuals had elevated temperatures exceeding 39°C, thrombocytopenia, and leukopenia. Other symptoms included elevation of the liver enzymes alanine aminotransferase and aspartate aminotransferase, nonbloody diarrhea, fatigue, and anorexia. The two men were hospitalized for 10 and 12 days, respectively, and both had short-term memory difficulty, which slowly improved over 4 to 6 weeks.[302] These symptoms sound remarkably similar to those of the Chinese patients suffering from SFTS.

Because all the specimens collected from these two individuals were negative for all the known pathogens, blood was sent to the CDC in Atlanta, Georgia, for further testing. Electron microscopy revealed viruses consistent with members of the family *Bunyaviridae*. Next-generation sequencing and phylogenetic analysis identified the viruses as novel members of the *Phlebovirus* genus. The authors named it the Heartland virus. The Heartland virus is most closely related to the SFTSV, but is clearly distinct because it shows amino acid differences in the viral polymerase and nucleoprotein of 27% and 38%, respectively.[302] This novel virus was also distinct from an uncharacterized bunyavirus called Lone Star virus, which was isolated in 1967 from an *A americanum* tick found on a woodchuck in western Kentucky. Comparison of the polymerase amino acid sequence showed that the Lone Star virus shared only 34% identity with the Heartland virus.[302]

Both patients infected with the Heartland virus in Missouri had reported being bitten by ticks 5 to 7 days before the onset of their illness. Given the similarity to SFTS, both in terms of the disease symptoms and the high percent identity of the virus to SFTSV, it was highly likely that Heartland virus was also tickborne. In 2012, investigators from the CDC collected and tested arthropods in areas of northwestern Missouri, including the farms of the two patients, to identify potential arthropod vectors for this new pathogen.[303] These investigators collected 56,428 ticks at 12 sites including both patients' farms. *A americanum* was the most frequently encountered tick and represented 97.5% of the collected ticks.[303] They grouped the ticks into pools by site, collection date, species, sex, and life stage. Ten pools composed of nymphs of *A americanum* were RT-PCR positive for the Heartland virus, and

eight pools yielded viable virus in cell culture. None of the 758 specimens (representing 12 species) of mosquitoes collected was positive for the virus.[303] Although more epidemiological and laboratory work is needed, these data strongly incriminate the *A americanum* tick as the vector of Heartland virus, at least in Missouri.

Ebola Epidemic in West Africa

On December 26, 2013, an 18-month-old boy from the village of Meliandou in the Guéckédou District of Guinea had experienced an illness characterized by fever, vomiting, and black stools. He died 2 days later.[304] Within a few weeks, his 3-year-old sister, mother, grandmother, and a nurse who had treated him all developed similar symptoms, and all died within a week.[305] Others in the surrounding area continued to get sick and die for the next several months. By the end of March 2014, the disease that had circulated in the southeastern corner of Guinea was identified as Ebola (for more detailed information on Ebola virus, see Chapter 23, Filoviruses).[306,307] By then, 111 clinically suspected cases and 79 deaths had occurred.

Full-genome sequencing of viral isolates from Guinea identified the virus as Zaire ebolavirus, but it represented a new genetic clade of virus, different from those circulating in the Democratic Republic of the Congo and Gabon. These data suggest that the virus may have been indigenous to Guinea and had not just "jumped" from another area where Ebola outbreaks have occurred. Ebola had not been seen in West Africa before, although serological evidence from patients with febrile illnesses had detected Ebola virus-specific antibodies in a subset of the population in Sierra Leone.[308] An epidemiological investigation found that the likely source of the virus that had infected the young boy was a colony of insectivorous free-tailed bats (*Mops condylurus*) that lived in a hollow tree in the village. Villagers reported that children played regularly in the tree and that a colony of bats lived in the tree.[309] Subsequent sequencing of a large number of Ebola virus isolates from the outbreak also indicated that the outbreak had likely started with a single transmission from the animal reservoir to human, followed by human-to-human transmission to sustain the outbreak.[310]

By March 30, 2014, cases had begun to be reported from the Foya district across the border in Liberia, and on May 24, Sierra Leone reported its first laboratory confirmed case.[311] The West African outbreak quickly became the largest outbreak of Ebola in recorded history, surpassing the previous record of 425 cases from the 2000 to 2001 outbreak in Uganda.[312] Previous outbreaks had occurred in relatively remote areas, limiting the ability of the virus to spread to large numbers of people. With the West African outbreak, however, cases were reported from the large capital cities of Monrovia, Liberia (~1 million inhabitants), Conakry, Guinea (~1.6 million inhabitants), and Freetown, Sierra Leone (~1.2 million inhabitants). Some models predicted that—if left unchecked—the total number of Ebola cases could exceed 1 million cases.[313] Spurred in part by worst-case scenario predictions such as these, international partners tried to bring the outbreak under control. US assistance focused on Liberia, and included the deployment of 3,000 soldiers to Liberia under Operation United Assistance to support logistics, train health workers, and build 17 Ebola treatment units.[314] British assistance efforts focused on Sierra Leone, and the French assisted the Guinean government.

The number of new cases per week peaked in late 2014 (September in Liberia, November in Sierra Leone, and December in Guinea).[315] During the height of the outbreak, Ebola virus-infected individuals were exported to Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom, and the United States. Nigeria, Mali, and the United States also had limited local transmission of the virus originating from the imported cases. In the United States, a traveler from Liberia presented at a hospital in Dallas County, Texas, on September 25, 2014.[316] He presented with fever, abdominal pain, and headache, but was misdiagnosed with sinusitis and discharged. He returned to the hospital on September 28 with worsening symptoms, was admitted, and tested positive for Ebola virus infection on September 30. The patient died on October 8, but two nurses became infected while caring for him. The nurses both survived.

As of April 13, 2016, there were a total of 28,616 cases of Ebola (confirmed, probable, and suspected) with 11,310 deaths (40% case fatality rate).[317] Sierra Leone was the last country to have active cases of Ebola virus disease and was declared Ebola-free on March 17, 2016. An investigational vaccine for Ebola was tested in Guinea in 2015 and was shown to be 100% effective in preventing disease in contacts of confirmed Ebola cases.[318] The trial used a "ring vaccination" design in which contacts and contacts of contacts of confirmed Ebola patients were either immediately vaccinated or vaccinated after a delay of 21 days. After interim analysis of the data from the trial showed the high effectiveness of immediate vaccination, the data safety and monitoring board recommended that the delayed vaccination arm be dropped and all participants be offered immediate vaccination. An additional Ebola vaccine trial, the Sierra Leone Trial to Introduce a Vaccine Against Ebola (STRIVE) was performed in Sierra Leone between April and August 2015. Nearly 9,000 volunteers consisting of healthcare and frontline workers were vaccinated in a phase II/III trial. Due

to the decrease in incidence of Ebola virus disease during the duration of the STRIVE trial, efficacy data could not be obtained for the vaccine, but a subset of the participants was enrolled in safety and immunogenicity substudies. No adverse events were reported in the safety study, and the immunogenicity study is ongoing as of October 2016.

Viral Pathogen Discovery by High-Throughput DNA Sequencing

Traditional diagnosis of viral infections has required some foreknowledge of the viral pathogen that is suspected to be causing disease. For example, enzyme-linked immunosorbent assays require either recombinant antigens or antigens isolated from whole organisms. Real-time PCR assays require sequence information from the genomes of the organisms that are suspected to be present. Any novel agent that has a genome that is sufficiently divergent enough to alter the structure of an antigen or change a binding site for a real-time PCR probe may not be detected in these assays. The advent of microarrays that contain millions of short nucleic acid probes increases the likelihood that a pathogen can be detected. These arrays can screen for hundreds to thousands of pathogens in a single sample,[319,320] but still require the genome sequences of each pathogen to design probes. Novel or divergent pathogens may also escape identification by arrays.

When high-throughput massively parallel sequencing became available in 2005,[321] it was quickly realized that the hundreds of thousands to millions of sequencing reads obtained by this new form of sequencing could be used as a diagnostic tool. The first demonstration of this occurred in 2007 with the detection of Israeli acute paralysis virus in colonies of bees that suffered from colony collapse disorder (CCD).[319] RNA was isolated from bees from CCD and non-CCD bee colonies, amplified by random RT-PCR, and then sequenced on a 454 sequencer. Examination of the sequence reads identified large numbers of bacteria, fungi, and viruses, but only reads from Israeli acute paralysis virus seemed to correlate with the presence of CCD in a colony.

The first demonstrated use of massively parallel sequencing for the detection of a novel human pathogen occurred in 2008, when a novel arenavirus was detected in patients that had received visceral organ donations from a single donor.[322] The donor had died of cerebral hemorrhage 10 days after returning from a 3-month trip to the former Yugoslavia. His liver and kidneys were transplanted into three recipients, whose initial recoveries were unremarkable. Within 4 to 6 weeks of receiving the transplants, however, all three recipients died displaying various levels of encephalopathy. Tissues were collected from each recipient, and RNA was extracted for sequencing. After sequencing, 14 sequence reads out of more than 100,000 reads obtained showed amino-acid level similarity to lymphocytic choriomeningitis virus. The reads obtained by massively parallel sequencing were used to design PCR primers, and standard PCR and sequencing enabled the recovery of the full genome sequence of the novel virus. The virus was 72% to 87% identical to lymphocytic choriomeningitis virus at the nucleotide level, and 79% to 97% identical at the amino acid level. Despite these relatively high levels of identity to lymphocytic choriomeningitis virus, initial tests with diagnostic microarrays had failed to identify any candidate viruses as a cause of the infection.

Another example of the use of massively parallel sequencing to identify a novel pathogen was published in 2009.[323] In September 2008, a patient in critical condition with hemorrhagic fever was airlifted from Lusaka, Zambia to Standton, South Africa (a suburb of Johannesburg). Although the index patient was under care in the hospital, the disease spread to a paramedic who had been on the air ambulance flight from Lusaka, a nurse who attended to the index patient, and a hospital worker who had cleaned the index patient's room. A tertiary case of disease also spread to a nurse who attended to the sick paramedic. The first four patients died, and the fifth patient, who was treated with ribavirin, survived. Liver and skin sections were submitted to the CDC, where immunohistochemical staining with a monoclonal antibody broadly cross-reactive for Old World arenaviruses gave a positive result.[323] Subsequent RT-PCR with conserved arenavirus primers yielded partial sequences of the glycoprotein and nucleoprotein genes, indicating the presence of a novel arenavirus. Serum and tissue samples from some of the cases were submitted for massively parallel sequencing, yielding 5.6 kb of sequence from the novel arenavirus. The partial genome was used to design PCR amplicons, and those were used to recover the full genome of the virus. Analysis of the full genome indicated that the novel virus was phylogenetically distinct from previously known arenaviruses, and it was named Lujo virus after Lusaka and Johannesburg.

Both of the cases described previously used relatively low numbers of sequencing reads from a 454 sequencer (around 100,000 per sample) to identify genome fragments of the novel viruses. The fragments were then joined by PCR and conventional Sanger sequencing to obtain the full genome sequences. A third example demonstrated a different approach using the extremely high capacity of the Illumina HiSeq sequencer. In 2009, three people from a remote village in the Bas-Congo province of the Democratic

Republic of the Congo were stricken with a hemorrhagic fever of unknown origin.[324] The first case was a 15-year-old boy who presented to the hospital with malaise, epistaxis, conjunctival injection, gingival bleeding, hematemesis, and bloody diarrhea. The hemorrhagic symptoms had only started the previous day, and the patient died 2 days later from sudden circulatory collapse. The second case was a 13-year-old girl who presented with similar symptoms and died 3 days after onset of her disease. The final case was a 32-year-old male nurse who worked in the clinic where the original two patients had been seen. He was transferred to a regional hospital 2 days after the onset of disease, where he was treated with fluid resuscitation, blood transfusion, and antibiotics. He recovered spontaneously a few days later.

A serum sample that was taken from the nurse before his recovery tested negative by PCR for all viruses known to cause acute hemorrhagic fever in Africa. RNA was extracted from the serum and sequenced by 454, yielding a single read (of approximately 4,500) that had 41% identity to known rhabdoviruses. Attempts to recover more of the virus genome sequence by PCR were stymied by limited sample, so the sample was subjected to sequencing on the HiSeq. The HiSeq run yielded more than 140 million reads, with 30,000 of them mapping to the novel rhabdovirus. The large number of reads obtained allowed reconstruction of 98.2% of the genome and showed that the new virus, named Bas-Congo virus, was only distantly related to other rhabdoviruses.

Several other examples of the utility of next generation sequencing are applied to viral pathogen discovery, including Heartland virus and SFTSV mentioned previously in this chapter. However, several bottlenecks still remain to the widespread adoption of this technology in diagnostic settings. One of the most difficult aspects is simply analyzing the extremely large datasets that can be generated. In many cases, the overwhelming majority of sequence reads will be from the host (human) and trying to identify a pathogen is like looking for a needle in a haystack. Many groups are working on computer algorithms that can sort through the datasets to rapidly and correctly identify pathogen reads. No one algorithm has been successful, prompting the US Defense Threat Reduction Agency in 2013 to offer a $1 million prize through Innocentive.com to the team that can develop the most reliable and efficient algorithm. The prize ultimately went to a bioinformatics team at the University of Tübingen in Germany.

The unprecedented ability to detect all of the nucleic acid present in a diagnostic sample is a powerful tool for pathogen discovery, but it does have some pitfalls. An example of this pitfall was published in September 2013 when the genome of a highly divergent single stranded DNA virus was detected in samples from patients with chronic seronegative hepatitis and diarrhea of unknown etiology.[325] Deep sequencing of serum samples from patients with chronic hepatitis identified a virus that was related to both circoviruses and parvoviruses, and it was provisionally named parvovirus-like hybrid virus (PHV-1). Deep sequencing of diarrheal stool samples in a separate laboratory independently identified a virus that had 99% identity to PHV-1, which were named PHV-2. Both PHV-1 and PHV-2 were 99% identical to a virus named National Institutes of Health-Chonqing virus that had been identified in samples from Chinese seronegative hepatitis patients.[179] Suspicions about the frequency at which these viruses were being detected led to a reanalysis of the samples using different nucleic acid extraction reagents, and eventually led to the conclusion that all of the detections of these viruses were likely linked to commercial nucleic acid isolation spin-columns that had been used in all of the studies. PHV sequences were identified in metagenomic sequencing datasets from the coastal marine waters of North America, suggesting that PHV was linked to diatoms present in the marine waters that generate the silica matrix used in the commercial spin-columns.

As next-generation sequencing continues to increase in throughput and decreases in price, its utility for identifying novel viral pathogens will continue to increase. One can imagine a scenario in the not so distant future in which a clinician will be able to test for every pathogen present in a patient's sample without needing to pre-select tests for specific pathogens based on the patient's symptoms.

FUTURE THREATS

Genetically Engineered Organisms

Without human intervention, the natural world has produced innumerable microbial agents that continue to emerge as new or newly observed causes of disease. Human activity has also played a huge role in the emergence of many diseases, but this effect has—for the most part—been inadvertent, rather than deliberate. The spread of HIV, for example, can be attributed almost entirely to human behavior, and the same was true of the spread of smallpox. Historically, both microbial agents and the affected populations have tended toward change during the disease outbreaks. Examples from the human experience include the way in which diseases

such as smallpox and measles favored the survival of several generations of Europeans who were most resistant to these diseases, followed later by unchecked contagion and decimation of new populations when the same diseases were introduced to isolated islands and the New World.[326,327] A classic example of agent–host adaptation in animals was the intentional introduction of myxoma virus (an orthopoxvirus, reminiscent of smallpox in rabbits) into Australia to control or eliminate a scourge of rabbits. At first, mortalities were high in the Australian rabbits, but in time the rabbits acquired a degree of genetic resistance. In parallel, the circulating virus became diminished in its virulence, persisting and being shed over a longer period of time in infected rabbits.[328] For both rabbit and virus, natural selection blindly favored survival of the species. This natural order has been intentionally perturbed by humans, from the lifesaving selection of relatively benign forms of disease to use as vaccines against the most virulent forms (eg, variolation, or the classical adaptation of measles, mumps, and rubella vaccines) to the intentional selection of the most virulent disease agents in biological weapons programs (the latter finally stigmatized and outlawed as such in the Biological Weapons Convention Treaty). Other microbial perturbations have been unintended, such as the treatment-based selection of antibiotic resistant bacteria now widespread in hospitals.[329]

More recently, humankind has acquired the technical capacity to create microbial threats far more deadly than natural evolution could create or sustain. Genetic engineering, the intentional molecular manipulation of genes, has proven to have capacity for both good and ill. A few examples from open scientific literature will follow to illustrate the seriousness of the threat of genetically engineered microorganisms.

Antibiotic resistant strains of *B anthracis*, the causative agent of anthrax, have been derived not only by biological selection, but also more directly by genetic engineering.[330–332] Scientifically, the capacity to do so with any bacterial threat is unsurprising, but the implications are ominous. Similarly, for anyone moderately skilled in microbiology, it is obvious that otherwise harmless bacteria may be engineered to synthesize toxins made by unrelated lethal strains of bacteria. Buffering the threat, unauthorized conduct of most such experimentation has become not only difficult but also illegal—subject to fines and incarceration—in many countries including the United States. In the United States, federally funded research that may result in knowledge that could be used for nefarious purposes, so called dual use research of concern, is subject to review before initiation of research and also at the stage when the findings from such research are ready for submission for publication.

Viral genomes can now be easily manipulated in the laboratory and infectious viruses can be generated from plasmid DNA. The progression of this technology with human pathogens began some 20 years ago with the simpler viruses (positive sense, single-strand, small genomes) such as poliovirus,[333] alphaviruses,[205] and flaviviruses.[334] It has grown to include negative-strand viruses (eg, vesicular stomatitis virus, respiratory syncytial virus, Ebola virus) and segmented viruses (eg, influenza virus, Crimean-Congo hemorrhagic fever virus). Even the relatively large genome of vaccinia virus can be derived from DNA cloned into bacteria.[335] In a parenthetical observation that was alarming to some in its simplicity, the capacity to derive a human pathogenic virus (poliovirus) by chemical synthesis was demonstrated.[336] Even more controversial were the efforts to genetically resurrect the 1918 influenza virus that killed some 20 million persons before disappearing[152,337–339] and the proposals to genetically manipulate smallpox virus.[340] Experiments designed to create or improve vaccines, to understand interactions between virus and host, or to unveil some arcane mysteries of the viruses themselves have simultaneously proven the ease with which bioactive and sometimes harmful molecules may be inserted into viruses. Symbolizing this, a large body of work with recombinant poxviruses was widely considered to be entirely benign until it was reported that a mouse poxvirus (ectromelia virus) was rendered more virulent by its modification to co-express a molecule of the immune system (ie, interleukin-4).[341] This result was merely part of a progression of studies of similar design and outcome,[342] but its timing (2001) crystallized the potential problem.

Perhaps the most prominent example of dual use research of concern in recent years occurred in late 2011, when two independent research groups prepared to publish research studies in which mutations were introduced into highly pathogenic influenza H5N1 viruses that facilitated efficient transmission of the viruses in the ferret model.[188,191] The ensuing debate resulted in a self-imposed moratorium on such research by influenza scientists in the United States and internationally,[343] while a regulatory framework for the review of proposals for such gain-of-function studies was constructed.[344] As a result, research proposals for this type of study submitted for US federal funding are subject to additional layers of review. It is expected that other countries will follow suit, if they do not already have such a framework. For more detailed information, the reader is directed to a special issue of *Science* specifically devoted to the H5N1 gain-of-function research debate.[345]

Ultimately, the capacity to create deadly pathogens through genetic engineering is restrained in large part by technical knowledge and opportunity, and in the final analysis, by intent. That is, what is straightforward for skilled scientists is impossibly difficult for the untrained and unequipped. However, a determined person with the appropriate set of knowledge and skills may succeed in creating genetically engineered microorganisms. Unfortunately, such organisms could also be created by well-intentioned scientists who underestimate the unexpected consequences of their work.

What countermeasures and solutions exist? Laws and regulations to emphatically restrict accidental or intentional creation of new deadly organisms, or possession of the deadly agents already existing in nature, have been implemented in the United States (eg, 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73), but these bounds are difficult—if not impossible—to enforce internationally. Also helpful are the myriad coordination meetings and rehearsals for public health responses to pandemic natural threats such as smallpox or a pandemic influenza virus; in the case of the outbreak of a contagious genetically engineered microorganism, classical methods of epidemiology and quarantine would likely be exceedingly helpful. Also encouraging, the likely period of ignorance as to the nature and design of a newly emerged causative agent has been compressed as the newest technologies have been applied to both diagnostics and bioforensics. Less encouraging, development of specific medical countermeasures (vaccines, therapeutic drugs) for a previously unknown organism usually takes years. Some regard this as impetus to redirect greater funding toward discovery of generic methods of boosting innate immunity in persons to provide increased resistance to most or all infectious agents. A related approach is to target common cellular pathways used and shared by many unrelated agents, especially viruses. As with conventional agents, great localized harm could be done and widespread panic produced by genetically engineered microorganisms, even if medical countermeasures were nominally available.

Synthetic Biology

Genome synthesis is no longer limited to the realm of viral genomes. In 2008, Gibson et al published a paper describing the complete chemical synthesis of all 582,970 bases of the *Mycoplasma genitalium* genome.[346] The starting material for the synthesis was short oligonucleotides of the kind that can be purchased for $0.10 per base or less. These were gradually assembled into larger and larger pieces of DNA until the researchers cloned and maintained the complete genome in the form of a yeast artificial chromosome in *Saccharomyces cerevisiae*.

Following closely on the heels of this achievement, the same group published a second paper in 2010 detailing the complete chemical synthesis of the 1.08 megabase-pair genome of *M mycoides*.[347] This genome was synthesized in a manner similar to that described above, but the group went one step farther. The group transplanted the synthetic genome into the husk of a *M capricolum* cell from which the normal genome had been removed. The cellular materials left behind after removing the normal genome accepted the new, synthetic genome and kick-started replication of the novel bacterium called *M mycoides* JCVI-syn1.0 after the J. Craig Venter Institute where the work was performed. To prove that the new bacterium had the synthetic genome, the group had included watermarks encoded in the genome during synthesis. These watermarks used a cipher made of short nucleotide sequences to encode e-mail and web addresses, the names of the authors, and the following famous quotes:

- "To live, to err, to fall, to triumph, to recreate life out of life" (by James Joyce),
- "See things not as they are, but as they might be" (by Robert Oppenheimer), and
- "What I cannot create, I cannot understand" (by Richard Feynman).

SUMMARY

Emerging infectious diseases are among some of the most important future threats facing both military and civilian populations. These diseases are caused by a variety of infectious agents (ie, bacteria, viruses, fungi, and parasites), some of which are new to mankind, whereas others have been around for millennia, but are only newly recognized. Still others may be common commensals that have acquired virulence factors (eg, toxins) or antimicrobial resistance genes though natural or unnatural (ie, genetic engineering) means.

Despite many successes in disease control and prevention, infectious diseases remain the leading cause of death worldwide and the third leading cause of death in the United States. HIV/AIDS, which was first recognized in 1981, is the most dramatic example of an infectious disease that has rapidly emerged during the last 35 years. Despite the significant advances in treatment of HIV/AIDS, the pandemic will continue to put large numbers of people at risk for new and reemerging opportunistic infections. The rapid spread

of the West Nile virus across the United States after its introduction in 1999 and the increasing problem of antimicrobial resistance are other examples of the ability of microbes to emerge, adapt, and spread.

Future threats are difficult to predict but will likely include many of the topics covered in this chapter, including the following:

- increasingly complex challenges of foodborne and waterborne diseases,
- the threat of another influenza pandemic,
- emerging antibacterial and antiviral resistance, and
- the increasing incidence of zoonotic diseases.

Meeting these challenges will require a multidisciplinary approach using the expertise of physicians and veterinarians trained in public health, microbiologists, pathologists, ecologists, vector biologists, and military and civilian public health officials.

Emerging infectious diseases have been defined as those diseases which have been newly recognized or whose incidence has increased within the past 20 years. What new diseases will be encountered in the next 20 years? What role will the increasingly advanced fields of molecular biology, genomics, and synthetic biology play? Will infectious agents from the past be resurrected, as has been done with the 1918 influenza virus? Or will increasingly advanced bioterrorists or rogue nations create weapons though genetic engineering or synthetic biology? Only through increased knowledge gained from continued research in infectious diseases will we be able to meet the challenges of these future threats.

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