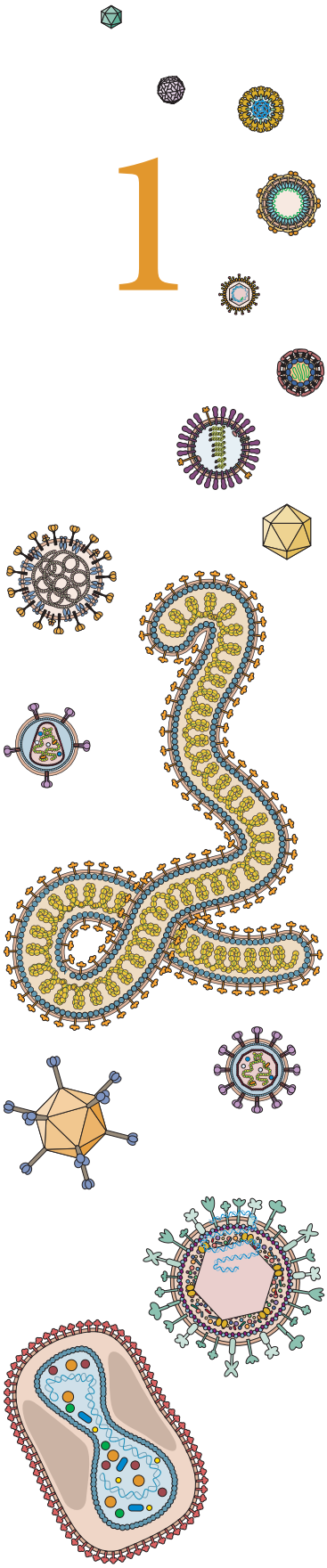


1 Infections of Populations: History and Epidemiology

LINKS FOR CHAPTER 1

- ⏭ *Video: Interview with Dr. W. Thomas London*
  http://bit.ly/Virology_London
- ⏭ *Epidemiology causes conclusions (p less than 0.01)*
  http://bit.ly/Virology_Twiv169
- ⏭ *Slow motion sneezing*
  http://bit.ly/Virology_1-23-13
- ⏭ *CD4 Hunter*
  http://bit.ly/Virology_Twiv489

Swords, lances, arrows, machine guns, and even high explosives have had far less power over the fates of nations than the typhus louse, the plague flea, and the yellow-fever mosquito.

HANS ZINSSER
Rats, Lice and History, 1934

Introduction to Viral Pathogenesis

While the title of Zinsser's classic volume *Rats, Lice and History* may trigger a wry smile (for how, after all, could lice be part of history?), the ideas proposed in this famous history of pathogens and the diseases they cause remain as relevant today as when they were published in 1934. As Zinsser argued, the global impact of pathogens, including viruses, has shaped human history as much as any war, natural disaster, or invention. This view may seem an exaggeration to today's student of virology, who may perceive most viral infections as annoyances that result in a few missed classes or days of work. But in the context of history, **epidemics** of smallpox, yellow fever, human immunodeficiency virus, and influenza have caused an incalculable loss of life and have changed entire societies. Smallpox alone killed well over 300 million people in the 20th century, more than twice the number of deaths from all the wars that occurred in the same time period. Huge empires fell to a relatively small number of invaders, in part because the conquerors inadvertently introduced viruses that crippled the empires' defense forces. Not all such victories were accidental, however: in the 1760s, British traders offered blankets and handkerchiefs from the smallpox hospital at Fort Pitt to American Indians who had never been exposed to this pathogen. As William Trent, one of the traders, noted, "I hope it will have the desired effect." Smallpox, used in this way, was the first bioweapon.

Although vaccines and antivirals have reduced, and even eliminated, some of these scourges, we are reminded of the challenges we still face by the looming threat of an influenza **pandemic**, the devastation caused by Ebolaviruses in Africa, the lack of success in developing a human immunodeficiency virus vaccine, and the resurgence of vaccine-preventable infections. We also face the emergence of "new" human viral pathogens, such as the coronavirus that is the cause of the worldwide pandemic in 2019–2020, and Zika virus, which causes gross defects in brain formation. Of equal importance, viruses that cause disease in crops and animals have crippling consequences for the infected species, the farmers who depend on them for their livelihoods, and human populations that may face starvation.

The ways that viruses cause diseases in their hosts, the tug-of-war among viruses and the host's defenses, and the impact that viral epidemics have had on human, animal, and plant populations are therefore not just interesting academic pursuits, but rather life-and-death issues for all organisms. That said, it is important to bear in mind this critical principle: **pathogenesis** (the processes that lead to disease) is often a collateral outcome of the parasitic nature of viruses. As is true for humans, selective pressures that control evolution of viruses act only on their abilities to survive and reproduce. From this perspective, one could argue that the most successful viruses are those that cause no apparent disease in their natural host.

In the first chapter of Volume I, we recounted an abbreviated history of virology and described milestones that established the foundation for our current understanding of viral reproduction. In this chapter, we return to history, focusing on watershed events that catalyzed the fields of viral **epidemiology** and pathogenesis. Subsequent chapters in this volume will consider the impact of viral infections on individual hosts, tissues, and cells. Our goal in Volume II is to build on the principles of viral reproduction that were established in Volume I, providing an integrated view of how viruses cause disease in single cells, discrete hosts, and large populations, as well as the host responses that mitigate or prevent such diseases.

PRINCIPLES *Introduction to viral pathogenesis*

- Diseases associated with viral infections are a collateral outcome of the parasitic nature of these pathogens.
- Koch's postulates helped to identify causal relationships between a microbe and the disease it causes in the host, although these postulates may not be fulfilled when associating some viruses with a particular disease.
- Major insights in viral pathogenesis have come from exploitation of technical advances in the fields of molecular biology and immunology.
- The increased mobility of human and animal populations on the planet has accelerated the emergence of epidemics.
- Many viruses that can infect multiple species establish a reservoir in an animal host in which the virus causes negligible disease. Spread into new human hosts, called a zoonosis, is usually a dead-end infection.
- Epidemiology, the study of infections in populations, is the cornerstone of public health research and response.
- Individual differences among prospective hosts, group dynamics and behaviors, geography, and climate all influence how efficiently a virus can establish infection within a population.
- The regional occurrence of viral infections may be due to the restriction of a vector or animal reservoir to a limited geographical area.
- Seasonal differences in the appearances of some viruses may be due to variations in viral particle stability at various temperatures or humidity, changes in the integrity of host barriers (such as the skin or mucosa), or seasonal changes in the life cycles of viral vectors, such as mosquitos.
- Susceptibility to infection does not necessarily signify susceptibility to disease.

A Brief History of Viral Pathogenesis

The Relationships among Microbes and the Diseases They Cause

Long before any disease-causing microbes were identified, poisonous air (miasma) was generally presumed to cause epidemics of contagious diseases. The causative association of particular microorganisms, initially bacteria, with specific diseases can be attributed to the work of the German physician Robert Koch. With his colleague Friedrich Loeffler, Koch developed four criteria that, if met, would prove a causal relationship between a given microbe and a particular disease. These criteria, **Koch's postulates**, were first published in 1884 and are still used today as a standard by which pathogens are identified. The postulates are as follows:

- the microorganism must be associated regularly with the disease and its characteristic lesions but should not be found in healthy individuals;
- the microorganism must be isolated from the diseased host and grown in culture;
- the disease should be reproduced when a pure preparation of the microorganism is introduced into a healthy, susceptible host; and
- the same microorganism must be reisolated from the experimentally infected host.

Guided by these postulates and the methods developed by Pasteur for the sterile culture and isolation of purified preparations of microorganisms, researchers identified and classified many pathogenic bacteria (as well as yeasts and fungi) during the latter part of the 19th century. Identifying a cause-and-effect relationship between a microbe and a pathogenic outcome set the stage for transformative therapeutic advances, including the development of antibiotics to treat bacterial infections.

However, during the last decade of the 19th century, it became clear that not all diseases could be attributed to bacterial

BOX 1.1

DISCUSSION

Why viruses may not fulfill Koch's postulates

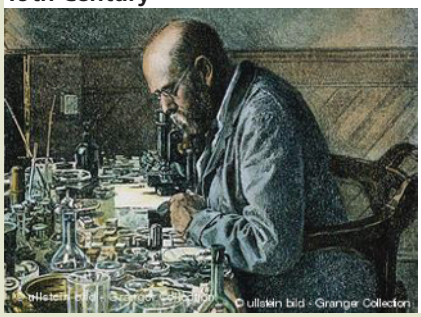
Although Koch's postulates provided a framework to identify a pathogen as an agent of a particular disease unambiguously, not all postulates can be applied to some infectious agents of disease. Koch himself became aware of the limitations of his postulates upon discovery that the bacterium *Vibrio cholerae*, which causes cholera, could be isolated from both sick and healthy individuals. In fact, it has been argued that the rigid application of these criteria to viral agents may have impeded early progress in the field of virology.

Application of Koch's postulates to viruses can be problematic for several reasons. For example, the first postulate, which states that the microorganism must be "regularly associated" with the disease, does not hold true for many animal reservoirs, such as bats, in which the virus actively reproduces but causes no disease. Similarly, arthropod vectors, such as mosquitos, support reproduction of a variety of hemorrhagic viruses but do not die as a result. Moreover, the second postulate states that the microorganism must be grown in culture. However, some viruses, including papillomaviruses that cause warts and cervical cancer, could be propagated in culture only recently, requiring complex conditions that must mimic the tissue complexity found in the infected host. Finally, recent studies addressing polymicrobial infections (that is, infections with more than one pathogen) have shown that, for some diseases, two or more organisms must work in synergy to cause a disease.

Assiduously applying the postulates has been particularly problematic for identifying viruses that cause human tumors. As noted in a review, Koch's postulates "are a brilliant example of precision in scientific thinking, but they hold little practical value for 21st-century tumor virology, as they cannot prove nor disprove most candidate tumor viruses to cause cancers." Consequently, the postulates are a guide, not an invariant set of requirements to fulfill.

More recently, detection methods based on nucleic acid sequence have rendered Koch's original postulates even less relevant. Such approaches are sufficiently sensitive to detect the presence of small quantities of viral nucleic acid in an apparently healthy individual. As such, a revised set of Koch's postulates that takes into consideration new technical capabilities has been proposed (Volume I, Box 1.4).

19th Century



21st Century

Highly sensitive molecular technologies, including quantitative polymerase chain reaction and DNA sequencing, have triggered a revision of Koch's postulates for the modern era. Left panel courtesy of Granger Historical Picture Archive, image no. 0008494.

Fredricks DN, Relman DA. 1996. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clin Microbiol Rev* **9:**18–33.

Moore PS, Chang Y. 2014. The conundrum of causality in tumor virology: the cases of KSHV and MCV. *Semin Cancer Biol* **26:**4–12.

or fungal agents. This apparent breakdown of the paradigm led to the identification of a new class of infectious agents: submicroscopic particles that came to be called viruses. Koch's postulates can often be applied to viruses, but not all virus-disease relationships meet these criteria. While compliance with Koch's principles **will** establish that a particular virus is the causative agent of a specific disease, failure to comply does not rule out a possible cause-and-effect relationship (Box 1.1).

The First Human Viruses Identified and the Role of Serendipity

The first human virus that was identified was the agent that causes yellow fever. The story of its identification in 1901 is instructive, as it highlights the contributions of creative thinking, collaboration, serendipitous timing, and even heroism in identifying new pathogens.

Although not recognized, yellow fever was widespread in tropical countries since the 15th century, and was responsible for devastating epidemics associated with extraordinary rates of mortality (for example, over a quarter of infected individuals died in the New Orleans epidemic of 1853). While the disease can be relatively mild, with transient symptoms that include fever and nausea, more-severe cases result in major organ failure. Destruction of the liver causes yellowing of the skin (jaundice), the symptom from which the disease name is derived. Despite its impact, little was known about how yellow fever was spread, although it was clear that the disease was not transferred directly from person to person. This property prompted speculation that the source of the infection was present in the atmosphere, and led to desperate efforts to "purify" the air, including burning barrels of tar and firing cannons. Some believed that the pathogen was carried on **fomites**, such as bedding or clothing, although this hypothesis was disproved when volunteers remained healthy after sleeping in the nightwear of yellow fever victims.

The first real advance in establishing the origin, or **etiology**, of yellow fever came in 1880, when the Cuban physician Carlos Juan Finlay proposed that a bloodsucking insect, most likely a mosquito, played a part in the transmission of the disease. A commission to study the basis of yellow fever was established in 1899 in Cuba by the U.S. Army under Colonel Walter Reed. This commission was formed in part because of the high **incidence** of the disease among soldiers who at that time were stationed in Cuba. Jesse Lazear, a member of Reed's commission, confirmed Finlay's hypothesis when he allowed himself to be bitten by a yellow fever virus-infected mosquito. "I rather think I am on the track of the real germ," wrote Lazear to his wife, sadly just days before he died of yellow fever himself. The results of the Reed Commission's study proved conclusively that mosquitos are the **vectors** for this disease. In retrospect, a mosquito-borne mode of transmission made sense, as the disease was predominantly found in warm and humid regions (e.g., Cuba, New Orleans) where mosquitos were, and remain, abundant. The members of this courageous team, perhaps the first true epidemiologists, are depicted in a dramatic 1939 painting (Fig. 1.1).

The nature of the pathogen was established in 1901, when Reed and James Carroll injected diluted, filtered serum from the blood of a yellow fever patient into three healthy individuals. Two of the volunteers developed yellow fever, leading Reed and Carroll to conclude that a "filterable agent," which we now know as yellow fever virus, was the cause of the disease. In the same year, a professor at the University of Havana attempted to produce immunity by exposing volunteers to mosquitos that were allowed to take a blood meal from an individual who showed signs of yellow fever. Of 19 volunteers, 8 contracted the disease, and 3 died. One of the deceased was Clara Louise Maass, a U.S. Army nurse. Maass's story is of interest, as she had volunteered to be inoculated by infected mosquitos some time before, developed only mild symptoms, and survived. Her agreement to be infected a second time was to test if her earlier exposure provided protection from a subsequent challenge. This was a prescient idea, because at

Figure 1.1 Conquerors of yellow fever. This painting by Dean Cornwell (1939) depicts the experimental exposure of James Carroll to infected mosquitos. Walter Reed, in white, stands at the head of the table, while Jesse Lazear applies the infected mosquitos to Carroll's arm. Also depicted in this painting is Carlos Finlay, in a dark suit. Despite the care that Cornwell took to ensure accuracy of his portrayal of the participants and their uniforms, the event documented in this painting never took place; rather, artistic license was used to place all the major players in one depiction of a watershed moment in medical history. Courtesy of the Cornwall Historical Society, accessed April 1, 2020, http://www.cornwallhistoricalsociety.org/omeka/items/show/241.

that time, virtually nothing was known about immune memory, which is the underlying principle of vaccines. Maass's death prompted a public outcry and helped to end yellow fever experiments in human volunteers.

Yellow fever had been **endemic** in Havana for many years, but the conclusions of Reed and his colleagues about the nature of the pathogen, and the vector that transmitted it, led to rapid implementation of effective mosquito control measures that dramatically reduced the incidence of disease within a year. To this day, mosquito control remains an important method for preventing yellow fever, as well as other viral diseases transmitted by arthropod vectors (Box 1.2).

Other human viruses were identified during the early decades of the 20th century (Fig. 1.2). However, the pace of discovery was slow, in great part because of the dangers and difficulties associated with experimental manipulation of human viruses so vividly illustrated by the experience with yellow fever virus. Consequently, agents of some important human diseases were not identified for many years, and then only with some good luck.

A classic example is the identification of the virus responsible for influenza, a name derived in the mid-1700s from the Italian language because of the belief that the disease resulted from the "influence" of contaminated air and adverse astrological signs. Worldwide epidemics (**pandemics**) of influenza had been documented in humans for well over 100 years. Such pandemics were typically associated with mortality among the very young and the very old, but the 1918–19 pandemic following the end of World War I was especially devastating. It is estimated that one-fifth of the world's population was infected, resulting in more than 50 million deaths, far more than were killed in the preceding war. Un-

BOX 1.2

BACKGROUND

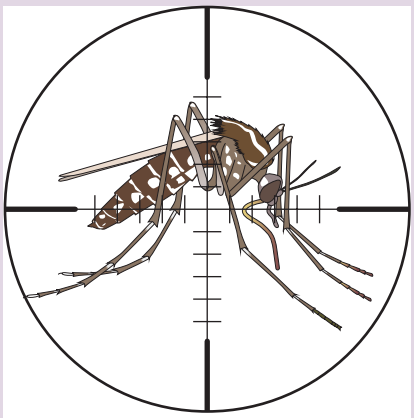
Mosquito control measures

In the 1930s, a vaccine was developed for yellow fever virus that dramatically reduced the mortality associated with infection by this virus. Nevertheless, mosquitos remain a primary vector for transmission to humans of viruses for which vaccines do not exist, including Zika and chikungunya viruses, as well as the parasite that causes malaria. Consequently, mosquito control remains a major public health initiative worldwide, but these ubiquitous flying syringes pose a formidable challenge to such efforts.

As mosquitos breed in standing water, reducing the prevalence of seemingly innocuous water traps such as old tires, inflatable pools, birdbaths, clogged gutters, and discarded soda bottle caps can have a substantial impact on mosquito populations. However, such strategies are likely to be only moderately effective in humid, rainy, or swampy environments. Mosquito netting, with a maximum effective mesh size of 1.2 millimeters, has proven effective when hung over beds or incorporated into tents, and variants of this physical barrier were in effect even in the time of Cleopatra. Similarly, the widespread use of insecticides and repellents has reduced spread of mosquito-borne infections.

Recently, more-creative strategies for mosquito control have been added to the anti-mosquito arsenal, including **biocontrol**, the use of natural enemies to manage mosquito populations. For example, certain fish, lizards, and other insects, such as dragonflies, feed on mosquito larvae. Their presence may thus help to limit populations naturally, although careless introduction of these species into mosquito-rich environments could destabilize fragile ecosystems. Genetic manipulation of the mosquitos themselves is an active area of research: studies are ongoing to breed and then release large numbers of sterile male mosquitos; females that mate with a sterile male produce no offspring, thus reducing the next generation's population size. An even more sophisticated control has been the development of genetically modified strains that require an antibiotic to develop beyond the larval stage. Modified males develop normally when provided with the antibiotic in nurseries. However, when the males are released into the wild and mate with normal females, the genetic vulnerability is transferred to future generations in an environment where the antibiotic is not available. As a result, progeny maturation cannot occur. In April 2014, Brazil's National Technical Commission for Biosecurity approved the commercial release of a genetically modified mosquito, and the U.S. Food and Drug Administration is considering such measures in the United States.

Other successful, and creative, mosquito control campaigns have been waged. For example, to reduce transmission of dengue virus, a community in Australia released millions of mosquitos infected with a bacterial species, *Wolbachia*, which prevents transmission of viruses such as dengue. When *Wolbachia*-infected mosquitos were released, they bred with others, infecting them with the bacteria and, in turn, preventing the infected mosquitos from transmitting viruses.



Aedes aegypti

Dengue fever virus
Yellow fever virus
Chikungunya virus
Zika virus
Venezuelan Equine Encephalitis virus
West Nile virus (hypothesized)

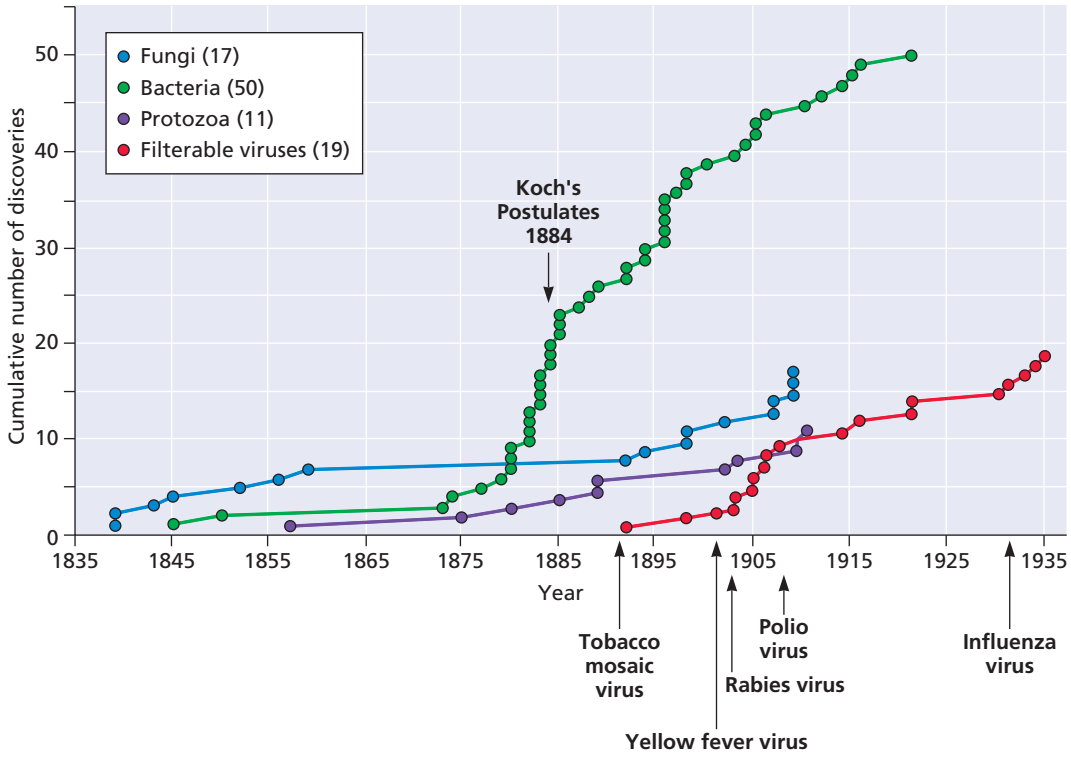


Figure 1.2 The pace of discovery of new infectious agents in the dawn of virology. Koch's introduction of efficient bacteriological techniques spawned an explosion of new discoveries of bacterial agents in the early 1880s. Similarly, the discovery of filterable agents launched the field of virology in the early 1900s. Despite an early surge of virus discovery, only 19 distinct human viruses had been reported by 1935. Data from Burdon KL. 1939. *Medical Microbiology* (MacMillan Co., New York, NY), with permission.

like in previous epidemics, healthy young adults were often victims (Fig. 1.3).

Despite many efforts, a human influenza virus was not isolated until 1933, when Wilson Smith, Christopher Andrewes, and Patrick Laidlaw serendipitously found that the virus could be propagated in an unusual host. Laidlaw and his colleagues at Mill Hill in England were using ferrets in studies of canine distemper virus, a paramyxovirus unrelated to influenza. These ferrets were secluded from the environment and other pathogens (for example, all ferrets were housed separately, and all laboratory personnel had to disinfect themselves before and after entering a room). Despite such precautions, it is thought that an infected lab worker transmitted the influenza virus to a ferret. When this ferret developed a disease very similar to influenza in humans, Laidlaw and colleagues realized its implications. These researchers then infected naïve ferrets with throat washings from sick individuals and isolated the virus now known as influenza A virus. (Note the effective use of Koch's postulates in this study!) Subsequently, influenza A virus was shown to also infect adult mice and chicken embryos. The latter proved to be an especially valuable host system, as vast quantities of the virus are produced in the allantoic sac. Chicken eggs are still used today to produce most influenza virus vaccines.

New Methods Facilitate the Study of Viruses as Causes of Disease

Technological developments propelled advances in our understanding of how viruses are reproduced (Volume I, Chapter 1) and also paved the way for early insights into viral pathogenesis. The period from approximately 1950 to 1975 was marked by remarkable creativity and productivity, and many experimental procedures developed then are still in use today. With these techniques in hand, scientists performed pioneering studies that revealed how viruses, including mousepox virus, rabies virus, poliovirus, and lymphocytic choriomeningitis virus, caused illness in susceptible hosts.

Revolutionary developments in molecular biology from the mid-1970s to the end of the 20th century and beyond further accelerated the study of viral pathogenesis. Recombinant DNA technology enabled the cloning, sequencing, and manipulation of host and viral genomes. Among other benefits,

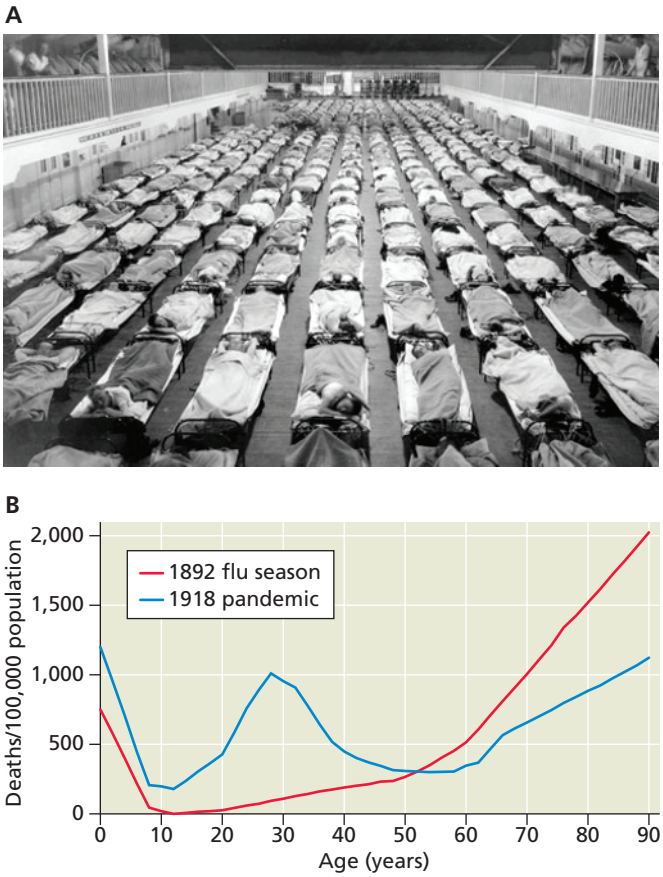


Figure 1.3 Consequences of the 1918 influenza pandemic. (A) The 1918–19 influenza pandemic infected a staggering number of people, resulting in the hasty establishment of cavernous quarantines in college gymnasia and large halls, filled with rows and rows of infected patients. Photo courtesy of the Naval History and Heritage Command (Catalog # NH 2654). **(B)** Of particular concern, this epidemic had a high death rate among young, otherwise healthy individuals compared to deaths in previous flu seasons, in which deaths occurred mostly among the very young and very old (representative data from Massachusetts shown). Based on data from Dauer CC, Serfling RE. 1961. *Am Rev Respir Dis* 83:15–28.

these techniques allowed investigators to mutate particular viral genes and to determine how specific viral proteins influence cell pathology. The polymerase chain reaction (PCR) was first among the many new offshoots of recombinant DNA technology that transformed the field of virology. PCR can be used to amplify extremely small quantities of viral nucleic acid from infected samples. Once sufficient viral DNA has been obtained and the sequence determined, the virus can be more easily identified, studied, and manipulated experimentally. The ability to sequence and manipulate DNA also led to major advances in the related field of immunology and greatly improved our understanding of the infected host's immune response to viral infection. The Nobel Prizes since the 1980s often acknowledge the importance of new technologies and concepts, and include awards for the establishment of transgenic animals, gene targeting methods, and immune cell recognition of virus-infected cells.

The emergence of molecular biology and cell biology as distinct fields marked a transition from a descriptive era to one that focused on the mechanisms underlying viral reproduction, transmission, and disease. Genomes were isolated, proteins were identified, functions were deduced by application of genetic and biochemical methods, and new animal models of disease were developed. These approaches also ushered in practical applications, including the development of diagnostic tests, antiviral drugs, and vaccines. As the 20th century came to a close, another paradigm shift was occurring in virology, as many scientists realized the power of large-scale, unbiased screens to study virus-host relationships. These scientists embraced the notion that all the molecules or reactions that govern a biological process could be identified and monitored during an infection, allowing discovery of new molecules and mechanisms that would be overlooked by more reductionist, gene-specific approaches. Large data sets were acquired, initially using microarray technology, which enabled a global and unbiased snapshot of both host and viral RNAs under defined conditions. Today, next-generation strategies, including high-throughput RNA sequencing (RNA-Seq) and nanopore sequencing, are used to reveal the type and quantity of nucleic acid in a biological sample at a given moment (Box 1.3).

New tools continue to expand our capabilities, and methods once considered cutting-edge are eclipsed by more-powerful, faster, or cheaper alternatives. Parallel developments in information technology and computer analyses (often called "data mining") have been critical to draw conclusions from the massive data sets, requiring in-depth expertise in bioinformatics and biostatistics. Computer-aided approaches have enabled scientists to define cellular pathways that are triggered during viral infection, to identify common features among seemingly diverse viruses, and to make structural predictions about small-molecule inhibitors that could prevent infection. While these new tools are exciting and powerful, it is likely that traditional approaches will still be required to validate and advance the hypotheses that are emerging from these more global analyses.

Although the methods that virologists employ may be ever-changing, one fundamental question asked by early pioneers remains with us: how do viruses cause disease? The remainder of this chapter focuses on how outbreaks and epidemics begin, and the impact of viral infections in large populations.

Viral Epidemics in History

In the apocalyptic movies *I Am Legend* (2007), *Contagion* (2011), and *World War Z* (2013), fictional epidemics are depicted following introduction of a virus into a naïve human population.

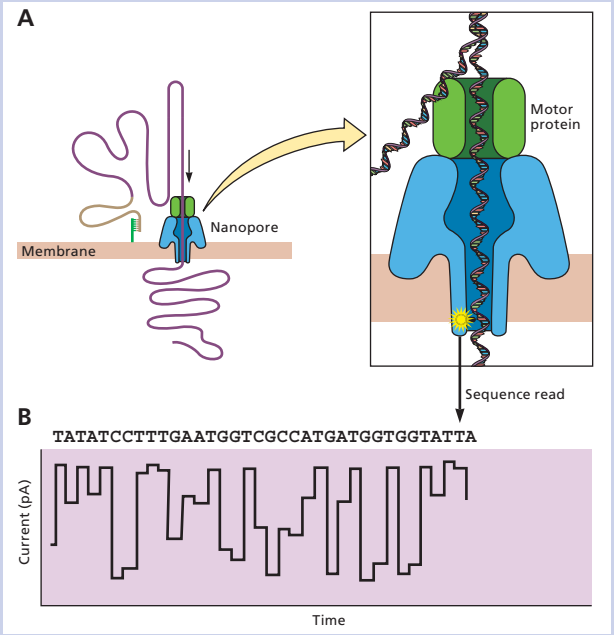
BOX 1.3

METHODS

Nanopore sequencing

A new approach for determining the sequence of a nucleic acid has been developed, referred to as "nanopore sequencing." This method relies on the use of biological nanopores, such as the bacterial hemolysin, which forms extremely small holes, or pores, in a membrane. These pores have a diameter wide enough to allow only a single strand of RNA or DNA to pass through. When an ionic current is applied to the membrane, each of the four nucleotides passing through the pore alters the current in a characteristic manner, which can be interpreted by a sensor (yellow starburst in panel A in the figure) and decoded to provide the sequence. This approach obviates the need for PCR amplification, greatly reducing experimental error that can accompany other sequencing techniques that rely on such amplification. Moreover, this approach is portable to remote locations, accelerating pathogen identification at sites of outbreaks.

Kafetzopoulou LE, et al. 2019. Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak. *Science* **363:**74–77.



Simplified overview of the process of nanopore sequencing.

(In some of these films, the virus turned the infected victims into zombies; although viruses cause many diverse outcomes, zombification is not among them.) Some of these doomsday films include a scene in which an epidemiologist ominously describes the devastating consequences of uncontrolled, exponential viral spread through a population. These movies were certainly frightening, but ultimately comforting, as humans, with improbable speed, developed strategies to limit viral spread. But how realistic is this Hollywood vision? One could argue that proof of our triumph over viral pathogens can be found in the eradication of smallpox and the development of vaccines to prevent infection by many viruses that historically resulted in much sickness and loss of life. However, there is a risk in becoming self-congratulatory. Doing so makes us ignorant of how quickly a virus can spread in a susceptible population, as the recent SARS-CoV-2 pandemic has taught us. When epidemics and pandemics occur in real life, there is a pervasive feeling of helplessness, and often interventions are not developed in time to mitigate substantial clinical impact. The stories that follow highlight the financial toll, loss of life, and historical ramifications of viral outbreaks, and underscore a new reality: the increased mobility of human and animal populations on the planet has almost certainly accelerated the emergence of epidemics.

Epidemics Shaped History: the 1793 Yellow Fever Epidemic in Philadelphia

One powerful example of a deadly viral epidemic that influenced American history and changed how cities are managed is the yellow fever outbreak that occurred in Philadelphia, Pennsylvania. In 1793, when this epidemic occurred (and a full century before Walter Reed's commission), nothing was known about yellow fever virus, the disease, or how it was spread. Worse, no one at the time knew that viruses existed, so the seemingly random way that individuals became sick compounded the confusion and sense of helplessness. Furthermore, this epidemic struck at a pivotal time for the fledgling Union. At that time, Philadelphia was the new nation's temporary capital and a city of active commerce and trade. One can easily imagine the panic in Philadelphia when scores of individuals became ill and died of this mysterious disease within a very short time. In the 101 days between August 1 and November 9, some 5,000 people perished in a city of about 45,000, making this one of the most severe epidemics in the history of the United States (Fig. 1.4). There were few families that did not lose a relative to this disease, and many entire families were lost. Those who could flee the city did so, including the new president, George Washington, and his cabinet.

Figure 1.4 Deaths caused by the yellow fever epidemic in Philadelphia, 1793. This map records the locations of deaths due to yellow fever, with red and orange streets marking those with highest mortality. Yellow fever was most deadly near the northern wharves, where poorer people lived, and where Hell Town was located (just blocks away from Independence Hall and the current home of the Liberty Bell). These areas furnished breeding places for *Aedes aegypti*, the species of mosquito that transmits the disease. Adapted from Paul Sivitz and Billy G. Smith, with permission.

Others stayed behind to aid the sick, including men of the Free African Society, who volunteered on the basis of the incorrect notion of Benjamin Rush, a prominent Philadelphia physician, that black people were immune to infection.

Because Philadelphia was a major port city, it is likely that the agent, which we now know was the yellow fever virus, was transported by infected individuals on cargo ships, and that standing water in the city provided a hospitable breeding ground for local mosquitos and rapid expansion of the disease along the wharves. Credit goes to Rush, who noticed identical symptoms in many victims and who recommended that individuals either leave the city or quarantine themselves, practices that helped to curtail the epidemic. Rush's belief that the scourge arose from a pile of rotting coffee beans left on a dock, and his treatment regimen of purging and bloodletting, are less worthy of praise.

The city of Philadelphia was transformed after the epidemic. The outbreak, believed by many to be due to contaminated water (which was, in part, true), spurred the local government to establish a municipal water system, the first major city in the world to do so. Infirmaries to tend to the sick and isolate them from the healthy were developed. Finally, the epidemic promoted a city-supported effort to keep streets free of trash, leading to the development of a sanitation program that would be a model for similar programs elsewhere. Although an effective vaccine now exists, yellow fever still kills 30,000 people every year, about 90% of them in Africa.

Tracking Epidemics by Sequencing: West Nile Virus Spread to the Western Hemisphere

It took a full century to determine the cause of the Philadelphia epidemic, but technological advances have greatly accelerated our ability to understand the natural histories of some modern-day outbreaks. While the sudden appearance of West Nile virus in the Western Hemisphere in 1999 fortunately did not result in massive loss of life, this epidemic is notable for the role that viral genome sequencing played in defining its origin in the Middle East.

Prior to the summer of 1999, West Nile virus infections were restricted to Africa and the Mediterranean basin. Upon introduction to the United States, West Nile virus spread with remarkable speed; in 3 years, the incidence of infection expanded from eight cases in Queens, New York City, to virtually all of the United States and much of Canada, where it is now endemic (Fig. 1.5).

The eight cases first identified in Queens held the key for major epidemiologic efforts to identify the source of this new infection. All victims had been healthy, and many had engaged in outdoor activities soon before showing signs of sickness. At about the same time, a high proportion of dead birds was found in and around New York City, including exotic birds within the Bronx Zoo, prompting epidemiologists to consider the possibility that the same virus had infected both hosts. PCR and genome sequencing were used to confirm that West Nile virus was the cause of both the bird deaths and the human illnesses. Subsequently, it was discovered that the virus was rapidly disseminated among avian and mammalian populations, and that mosquitos (again) were the vector that transmitted the virus to mammals, including humans. Fortunately, the consequences of infection are far less severe than for yellow fever: in 2009, 720 cases were diagnosed, but epidemiologists believe the true number to be >54,000; the discrepancy is likely due to the mild symptoms that the infection causes in most healthy individuals. Most deaths occur in the immunocompromised and the elderly.

How West Nile virus arrived in North America will never be known conclusively, but many think that the culprit was an infected mosquito (the natural **reservoir**) that arrived as a stowaway on a flight from Israel to New York. This scenario was deduced from the remarkable identity between genome sequences of the virus isolated in New York and an isolate

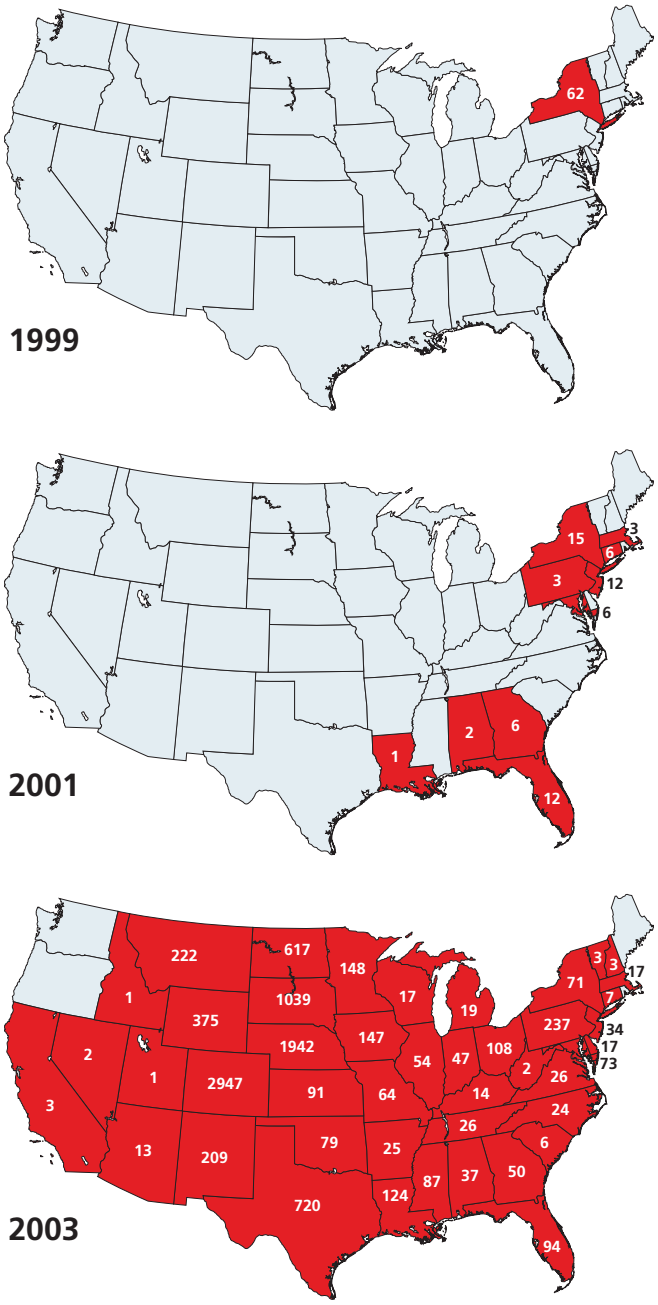


Figure 1.5 Spread of West Nile virus in the United States. The maps show the spread of West Nile virus from Queens, New York, throughout the country in four years (1999 to 2003). States highlighted in red indicate confirmed human infections (with numbers of confirmed cases). Data from Centers for Disease Control and Prevention.

obtained from a dead goose in Israel. It is sobering to contemplate that a virus that can now be found in virtually all states and provinces of North America may have begun with a single infected human, or perhaps a mosquito trapped in a suitcase or purse, an invisible passenger on a trans-Atlantic flight.

Zoonotic Infections and Epidemics Caused by "New" Viruses

Viral epidemics often appear unexpectedly, raising questions about their origins. Some viral epidemics begin with a zoonotic infection, discussed in detail in Chapters 10 and 11. **Zoonoses** are infections transmitted from other animals to humans. Many viruses that can infect multiple species establish a reservoir in a host in which the virus causes no disease or only nonlethal disease. When a new host is in proximity to an infected reservoir animal, a species jump may occur. While zoonotic transmission may cause disease in the new host, trans-species infection is usually a dead end for the virus. Consequently, zoonotic infections rarely spread from human to human, as is the case for rabies virus, West Nile virus, and avian influenza. Although relatively rare, zoonotic infections are a concern to epidemiologists, because the human host will not have immunity, and the disease that occurs in the new host may be different (often more severe) than that in the reservoir host. The trans-species spread of a human immunodeficiency virus-like ancestor from monkeys to humans is a prime example of zoonotic transmission (Chapter 12), as is the likely zoonosis of SARS-CoV-2 from bats to humans.

Over the past few decades, new, or at least newly discovered, zoonotic and vector-borne viral diseases have emerged, many originating in Southeast Asia and the Western Pacific. These include viruses with which most people have little familiarity, including Japanese encephalitis, Ross River, chikungunya, Nipah, and Hendra viruses. While the first three of these are transmitted by mosquitos, the natural reservoir of both Nipah and Hendra viruses is fruit bats, prevalent in Southeast Asia (Fig. 1.6). As increased contact between animals is the predominant risk factor for trans-species infection, one can envision how changes in the environment or ecosystems of some animals may increase the risk for contact among different species. These changes are of particular concern when humans invade wilderness areas. For example, it is thought that Nipah virus, a paramyxovirus, underwent species-to-species transmission in 1999 in Malaysia, when pig farming began in the habitat occupied by infected fruit bats. The infection spread from bats to pigs and ultimately to the farmers themselves. Hendra and Nipah pose a significant human health threat: in a 2004 outbreak in Bangladesh, Nipah virus killed 60% of the people it infected, and additional outbreaks have occurred in almost every year since. Symptoms of infection vary widely: some people experience a transient fever and cough, although complications can include life-threatening inflammation of the brain, respiratory failure, and, following recovery, seizures. Because infection by the virus depends on direct contact with fruit bats or infected hosts, the number of cases is typically low. Nevertheless, as the geographic range of the fruit bat is large, including all of India and much of Southeast Asia, the number of people who

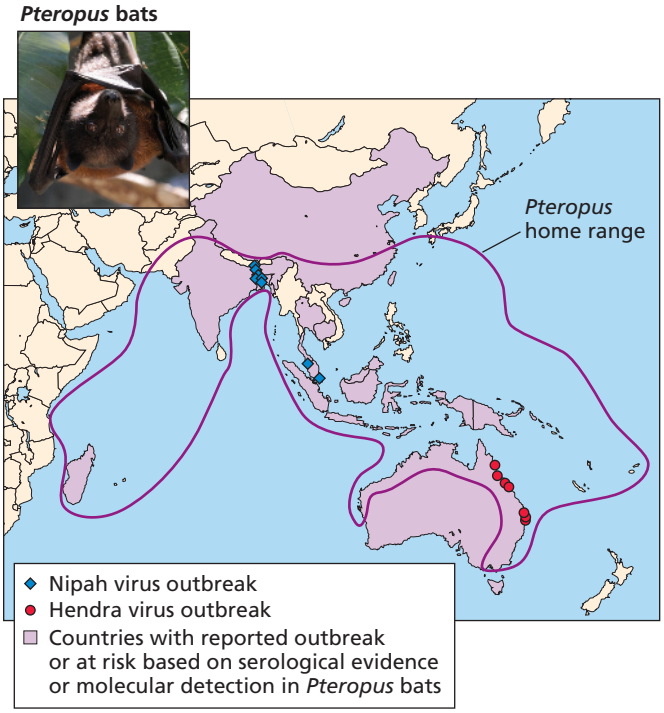


Figure 1.6 Fruit bat geographic range in Southeast Asia, and prevalence of Nipah and Hendra viruses. Outbreaks of Hendra and Nipah virus infections from the late 1990s through 2014 are indicated, as is the geographic range of the *Pteropus* fruit bat.

could be exposed is enormous. Moreover, it is not necessary to invoke an exotic locale or complex combination of animals for zoonosis to occur; petting zoos, open markets, and state fairs provide sufficient human-animal contact to allow a virus to jump species.

The Economic Toll of Viral Epidemics in Livestock

Epidemics affect animals other than humans as well, especially those in dense farming populations. The outbreak of foot-and-mouth disease in the United Kingdom in 2001 caused an agricultural crisis of historical proportions; over 10 million sheep and cattle were killed, an average of 10,000 to 13,000 a day, in an attempt to stop the infection from spreading. As there was no easy way to distinguish infected from uninfected, all of the farm animals in affected areas were destroyed, independent of signs of disease in the livestock. In the 2001 outbreak, the infection could be traced to one pig (the "**index case**") on a specific farm in Northumberland. Unfortunately, the farmer did not inform the authorities of the appearance of foot-and-mouth disease, which is relatively easy to identify by characteristic lesions on the snout, mouth, and feet. The epidemic spread rapidly, accelerated by the use of the same trucks to transport animals from both contaminated and uncontaminated farms to slaughterhouses. While this outbreak did not affect humans directly, the indirect financial impact on farming and tourism was enormous; it is estimated that this crisis cost the United Kingdom over $16 billion, and created great stress within the government. A vaccine for foot-and-mouth disease virus exists, and was available at the time. However, vaccine use had been rejected because farmers feared that they would then not be able to ship their meat to other countries, as vaccinated animals cannot be distinguished serologically from infected ones. A positive outcome of this epidemic is that all farm animals in the United Kingdom are now vaccinated for foot-and-mouth disease virus. Nevertheless, agricultural threats remain: while one virus of livestock has been eradicated (rinderpest virus, a relative of measles virus), and some are controlled by vaccines, others, including bluetongue virus, continue to pose a significant threat to cattle, sheep, antelopes, and deer.

Population Density and World Travel Are Accelerators of Viral Transmission

While the thought of an ocean cruise may evoke images of endless buffets and poolside piña coladas, to viral epidemiologists, such pleasure ships appear as prime breeding grounds for viral epidemics. Norwalk virus, a member of the norovirus family, is often associated with cruise ship outbreaks of gastroenteritis resulting in vomiting and diarrhea, although other viruses can also cause these nautical nightmares. Moreover, Norwalk virus is not restricted to ships; hot spots include any place in which many people from various locations are in close proximity for an extended period. Other high-density environments include prisons, airplane cabins, day care facilities, dormitories, and elderly care communities. The risk of transmission is enhanced by the fact that noroviruses are quite hardy, and can be transmitted either person to person or via contaminated food or surfaces, resulting in the need to decontaminate all shared surfaces with chlorine-containing solutions following an outbreak. While the gastrointestinal effects of a noroviral infection are unpleasant, the disease is short-lived, and patients usually recover quickly. However, the frequency with which these outbreaks strike is a chilling reminder that, despite improved tools to characterize viral epidemics and reduce their spread, the ease and prevalence of world travel greatly facilitate the encounter between viruses and new hosts.

Focus on Frontline Health Care: Ebolavirus in Africa

In December 2013, a one-year-old boy in Guinea died from complications of Ebola virus: he was the first victim of what would become an epidemic that claimed over 11,000 lives and lasted more than two years. During this period, the virus spread to the neighboring countries of Liberia and Sierra Le-

one, and it is conservatively estimated that more than 28,000 individuals became infected from December 2013 to late 2016. The epidemic was fueled, in part, by poverty, social unrest, armed conflict, and inadequate or absent health care systems. Furthermore, local burial customs, including ritual washing of the corpse, facilitated person-to-person transmission. Air transportation of infected persons out of these areas caused infections in health care workers, including in hospitals in Spain and the United States. While these latter cases did not spread further, the entry of this highly lethal virus into these countries created widespread public anxiety in these countries. Such anxiety likely contributed to greater awareness of the devastation that was in progress on the west coast of Africa.

Ebola virus is probably transmitted by bats, and, indeed, the index patient's village was located near a large bat colony. Ebola virus is spread by direct contact with body fluids: mucus, saliva, blood, and, as determined later, semen. Ebola hemorrhagic fever, which typically starts with high fever, headache, and muscle pain, often progresses to vomiting, diarrhea, and rash, and eventually kidney and liver impairment. In some infected individuals, rupture of infected blood vessels leads to internal and external bleeding (hence the name hemorrhagic fever), which can cause death from low blood pressure and fluid loss. The disease carries an extremely high risk of death, killing between 25 and 90% of those infected, although the odds of survival are directly dependent on the efficiency and quality of health care: providing fluids (saline, blood transfusions) greatly increases an infected individual's chances of survival.

More than any epidemic in recent memory, media attention was particularly focused on the health care workers on site, who provided care for the victims and potential victims (Fig. 1.7). The Médecins Sans Frontières (Doctors Without Borders), which received the 1999 Nobel Peace Prize for its work throughout the developing world, provided much of this frontline care. In late 2014, at the peak of the epidemic, physicians and support personnel were exhausted, hospitals had little room for new patients, and lack of adequate resources forced heartbreaking choices on those doctors: provide optimal care to a few or substandard care to many. To care for the victims, medical personnel put themselves in extreme danger: despite protective gear, approximately 10% of Ebola virus fatalities occurred in health care workers. Lack of running water, oppressive temperatures, and outdated supplies were all likely contributors.

Eventually, border closings, mandatory quarantines, and public education that led to changes in burial practices slowed the spread of the epidemic. In December 2016, the WHO announced, after a two-year trial, that a recombinant vaccine appeared to offer protection from the Zaire strain of Ebola responsible for the West Africa outbreak (Chapters 7 and 9).

Figure 1.7 Ebola outbreak. Health care workers in areas of the Ebola virus outbreak are completely protected from any contact with body fluids from a potentially infected individual. Standard safety protection includes a suit, apron, boots, gowns, gloves, masks, and goggles. One physician working in Sierra Leone stated: "After about 30 or 40 minutes, your goggles have fogged up; your socks are completely drenched in sweat. You're just walking in water in your boots. And at that point, you have to exit for your own safety ... Here it takes 20–25 minutes to take off a protective suit and must be done with two trained supervisors who watch every step in a military manner to ensure no mistakes are made, because a slip up can easily occur and of course can be fatal." AP Photo/Jerome Delay, File 288676002851.

Though the impact of the virus abated, epidemics have long-lasting economic ramifications: it has been estimated that the financial toll of this epidemic exceeded $1.6 billion, accelerating poverty, which, as estimated by one news organization, likely caused as many deaths as the outbreak itself.

Emergence of a Birth Defect Associated with Infection: Zika Virus in Brazil

As the Ebola outbreak was resolving in Africa in 2015, a new viral epidemic was beginning in South America. The first confirmed case of Zika virus infection in the Americas was reported in northeast Brazil in May 2015, although phylogenetic studies indicated that the virus had been introduced as early as 2013.

Zika virus is transmitted by mosquitos in temperate climates and causes a relatively mild disease in most cases: many adults seroconvert without ever knowing they were infected. However, during the 2015 outbreak it was rapidly appreciated that Zika virus infection of pregnant women can be associated with a terrifying new symptom in their newborns: small, misshapen heads (microcephaly) and severe developmental defects. As the virus spread throughout Brazil and beyond (Fig. 1.8), people in Mexico and North America quickly realized that the geographic range for the mosquito vector, *Aedes aegypti*, extended well into these areas. The rest of the Americas awaited the summer months braced for disaster, as mosquitos were predicted to carry Zika virus inexorably north. The outbreak coincided with the Summer Olympics in Rio de

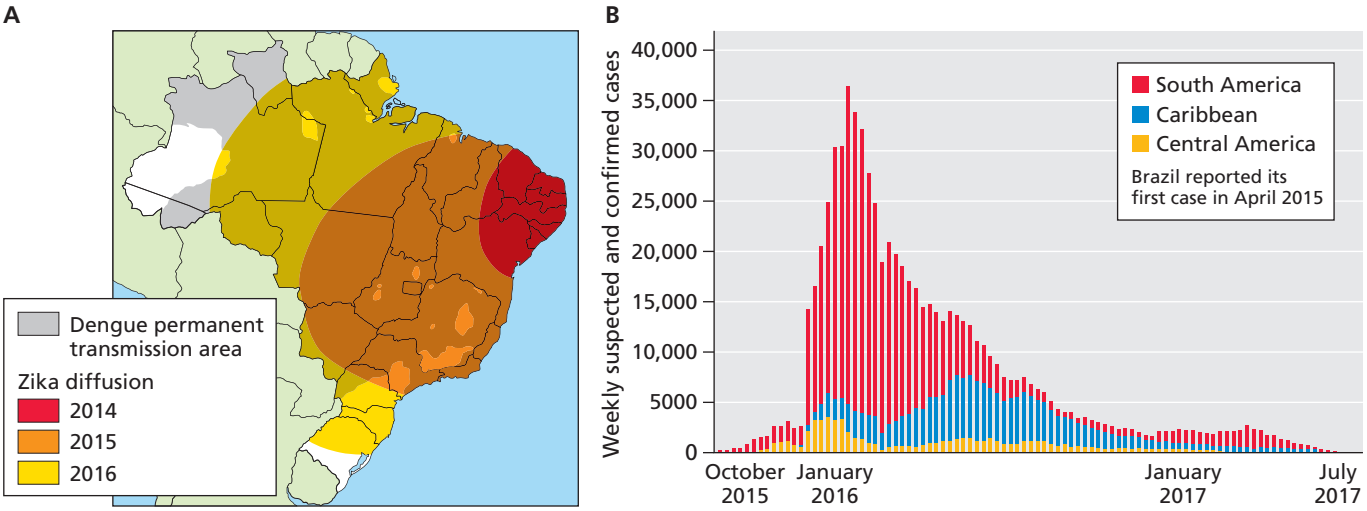


Figure 1.8 Zika spread in Brazil. (A) In three short years, from 2014 to 2016, Zika virus moved progressively north and westward, spreading from the coastal region of Brazil to other countries in South America. **(B)** Decline of Zika virus cases since 2016. Adapted from Lowe R et al. 2018. *Int J Environ Res Public Health* 15:E96, under license CC BY 4.0.

Janeiro, and prompted many enthusiastic supporters and athletes to stay home. For reasons still unclear, these fears did not materialize. By 2017, most of Latin America and the Caribbean had a massive decline in Zika virus infections. It has been suggested that the sharp reduction in cases is due, at least in part, to a phenomenon known as **herd immunity** (Chapter 7). Virus transmission between humans and mosquitos is greatly reduced in a population when enough people become immune to a virus, through vaccination or, as in the case of the Zika virus, natural immunity following infection.

Epidemiology

The stories above highlight some of the unique challenges, uncertainty, and urgency that face epidemiologists during an outbreak. The study of viruses can be likened to a set of concentric circles. The center comprises detailed analyses at the molecular level of the genome and the structures of viral particles and proteins that are crucial to understanding viral reproduction, and the biochemical consequences of interactions of viral and host cell proteins. How infection of individual cells affects the tissue in which the infected cells reside, and how that impacted tissue disturbs the biology of the host, define the landscape of the field of viral pathogenesis, in the next level (discussed in the following four chapters). But if a viral population is to survive, transmission must occur from an infected host to susceptible, uninfected ones. The study of infections of populations is the discipline of epidemiology, the cornerstone of public health research and response. Within this broad, outer circle, major areas of epidemiological research include outbreak investigation, disease transmission, surveillance, screening, biomonitoring, and public education.

An epidemiologist investigates outbreaks by undertaking careful data collection in the field (that is, where the infections occur) and performing statistical analyses. Questions often asked include "How might the symptoms observed in an infected individual implicate one mode of viral transmission over another?" or "Can a timeline be established to trace back the origins of an epidemic to a single event?" The goal is to learn more about the pathogen and how it caused the epidemic. Individual differences among prospective hosts, group dynamics and behaviors, geography, and climate all influence how efficiently a virus can establish infection within a population. Epidemiologists lack the luxury of performing controlled experiments, in which only one variable is manipulated. Instead, they must consider many parameters simultaneously to identify the source and transmission potential of a viral pathogen within a host community. Many of these variables are captured in various video games and mobile phone apps that simulate outbreaks (Box 1.4). In the next section, we identify some crucial terms and concepts used in this field. (For commentary and a personal account related to the topic, see the interview with Dr. Thomas London: http://bit.ly/Virology_London.)

Fundamental Concepts

Incidence versus Prevalence

Determining the number of infected individuals in a population is a primary goal of epidemiological studies. This information is required to establish both the **incidence** and the **prevalence** of infection. Incidence is defined as the number of new cases within a population in a specified period. Some epidemiologists use this term to determine the number of

BOX 1.4

DISCUSSION

Video games model infectious-disease epidemics

The hugely popular online video game *World of Warcraft* became a model for the transmission of viral infections. In 2005, a dungeon was added to the fantasy world in which players could confront a powerful creature called Hakkar. In his death throes, Hakkar hit foes with "corrupted blood," infected with a virus that killed the virtual player. The infection was meant to affect only those in the immediate vicinity of Hakkar's corpse, but the virus spread as players and their virtual pets traveled to other cities in the game. Within hours after the software update, a full-blown virtual epidemic ensued as millions of characters became infected.

Although such games are meant only for entertainment, they do model disease spread in a mostly realistic manner. For example, as in real life, the spread of the virus in Hakkar's blood depended on the ease of travel within the game, zoonotic transmission by pets, and transmission via asymptomatic carriers. Moreover, such games have a large number of participants, at one point more than 10 million for *World of Warcraft*, creating an excellent community for experimental study of infectious diseases. The players' responses to dangerous situations approximated real-world reactions. For example, during the "corrupted-blood" epidemic, players with healing ability were the first to rush to the aid of infected players. This action probably affected the dynamics of the epidemic because infected players survived longer and were able to travel and spread the infection. A more reality-based smartphone app called *Plague Inc.*, downloaded more than 85 million times, asks: "Can you infect the world?" and gives players the opportunity to choose a pathogen and influence its evolution. Players compete against the clock, trying to destroy humanity before the world can develop a cure.

Scientists themselves recognize the educational value of such games. A professor at Drexel University developed *CD4 Hunter*, in which players enter the bloodstream as a human immunodeficiency virus type 1 particle. The goal is to find and infect $CD4^+$ T cells, white blood cells of the adaptive immune system that are the main targets in this infection. The game mimics virus binding and entry, and was created as a supplementary teaching tool for graduate students and undergraduates in advanced-level courses (http://bit.ly/Virology_Twiv489).

With all of these games, successful players learn to integrate multiple variables simultaneously, including environment, time, and population density. These applications also demonstrate how the reproductive cycle of a virus may change over the course of an epidemic. However, the parallels to real-world epidemiology end there; a defeated player can begin again with the click of a button or the flick of a finger. Alas, real life does not come with "do-overs."

Lofgren ET, Fefferman NH. 2007. The untapped potential of virtual game worlds to shed light on real world epidemics. *Lancet Infect Dis* **7:**625–629.

new cases in a community during a particular period, while others use incidence to indicate the number of new disease cases per unit of population per period. For example, the incidence of influenza can be stated as the number of reported cases in New York City per year or the number of new cases/1,000 people/year. Disease prevalence, on the other hand, is a measure of the number of infected individuals at one moment in time divided by an appropriate measure of the population. A highly infectious and lethal disease (such as the 1793 epidemic of yellow fever in Philadelphia) may have a high incidence but a low prevalence, because many of the infected individuals either died or cleared the infection. In contrast, a virus that can persist in a host for decades is likely to have high prevalence. An example of high prevalence is provided by hepatitis B virus; of the 300 million to 400 million people infected globally, one-third live in China, with 130 million carriers. For this reason, incidence is an informative measure for acute or highly lethal infections, whereas prevalence is often used to describe long-lasting or persistent infections.

Prospective and Retrospective Studies

Although infections of natural populations differ from those under controlled conditions in the laboratory, it is possible to determine if one or more variables affect disease incidence and viral transmission in nature. Two general experimental approaches are used: **prospective** (also called cohort or longitudinal) and **retrospective** (or case-controlled) studies. In prospective studies, a population is randomly divided into two groups (cohorts). One group then gets the "treatment of interest," such as a vaccine or a drug, and the other does not. The negative-control population often receives a placebo. Whether a person belongs to the treatment or placebo cohort is not known to either the recipient or the investigator until the data are collected and the code is broken ("**double blind**"). This strategy removes potential investigator bias and patient expectations that may otherwise influence data collection. Prospective studies require a large number of subjects, who often are followed for months or years. The number of subjects and time required depend on the incidence of the disease or side effect under consideration

BOX 1.5

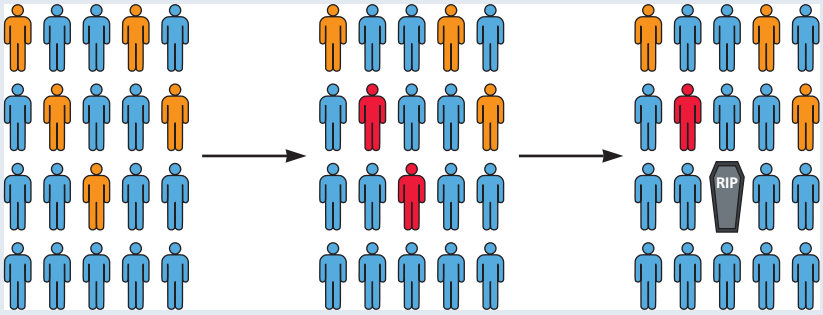
TERMINOLOGY

Morbidity, mortality, incidence, and case fatality

The terminology used to calculate the number of people who are infected and/or who become ill following a viral outbreak can be confusing. The following fictional example will be used to clarify these definitions.

Imagine that, in a city of 200,000 residents, a virus causes infection of 50,000 persons (as determined by serology). Of these, 20,000 develop signs of illness and 10,000 die of the infection.

- The **incidence** of this infection is the number of people infected divided by the population (50,000/200,000, or 25%).
- **Morbidity rate** is the number of individuals who became ill divided by the number of individuals at risk (20,000/200,000, or 10%).
- **Mortality rate** is the number of deaths divided by the number of individuals who are at risk (10,000/200,000, or 5%).
- The **case fatality ratio** is the proportion of deaths within a population of infected individuals. This value is typically expressed as a percentage. Case fatality ratios are most often used for diseases with discrete, limited time courses, such as outbreaks of acute infections. In the above example, the case fatality ratio is the number of deaths divided by the number of individuals with illness (10,000/20,000, or 50%).



Representation of incidence, morbidity, and mortality rates in a population. Each person represents 10,000 members of a community, as in the example above. Orange individuals are those who are infected; red are those who show symptoms of infection; the coffin indicates those who have died of the infection.

As a real-world example, Nipah virus infection and resulting encephalitis in Southeast Asia in 2011–12 resulted in 280 cases and 211 deaths, a staggering case fatality ratio of 75%.

and the **statistical power**, the probability of detecting a difference that is sufficiently significant to draw conclusions.

In contrast, retrospective studies are not encumbered by the need for large numbers of subjects and long study times. Instead, some number of subjects with the disease or side effect under investigation is selected, as is an equal number of subjects who do not have the disease. The presence of the variable under study is then determined for each group. For example, in one retrospective study of measles virus vaccine safety and childhood autism, a cohort of vaccinated children and an equivalent cohort of age-matched, unvaccinated children were chosen randomly. The proportion of children with autism was then calculated for each group to determine if the rate of occurrence of autism in the vaccinated group was higher, lower, or the same as in the unvaccinated group. The incidence of the side effect in each group is then calculated; the ratio of these values between groups is the relative risk associated with vaccination. In this example, the rate of autism was not found to be different in the two groups, showing that vaccination is not a risk factor for the development of this disorder (Chapter 7).

Mortality, Morbidity, and Case Fatality Ratios

Three other measures used in epidemiology can cause confusion because of the similarity of their definitions: **mortality**, **morbidity**, and **case fatality ratios** (Box 1.5). The mortality rate is expressed as a percentage of deaths in a known population of infected individuals normalized to the whole population in a period of time. The morbidity rate is similar but refers to the number of infected individuals in a given population who show symptoms of infection. The morbidity percentage will always be higher than the mortality percentage, of course, because not all sick individuals will die of the infection.

In contrast, a case fatality ratio is a measure of the number of deaths among clinical cases of the disease, expressed as a percentage. As an example, if 200 people are diagnosed with a respiratory tract infection and 16 of them die, the case fatality ratio would be 16/200, or 8%. In a technical sense, the use of the word "ratio" is incorrect; a case fatality ratio is more a measure of relative risk than a comparison between two numbers.

R-naught (R_0)

Virus particles must spread from host to host to maintain a viable population. Spreading will occur if, on average, each infected host passes the agent to more than one new host before the original host dies or clears the infection. The probability of such transmission is related to the size of the host population: infections can spread only if population density exceeds a minimal value. These concepts have been incorpo-

rated into a comprehensive theory of host-parasite interactions that is well known in ecological circles, but not always appreciated. This theory describes the parameters of viral infection in quantitative terms. The basic reproductive number for a virus population, R_0 (pronounced "R-naught"), is defined as the number of secondary infections that can arise in a large population of susceptible hosts from a single infected individual during its life span. If R_0 is <1, it is impossible to sustain an epidemic; in fact, it may be possible to eradicate the pathogen, especially if the species of hosts that it infects is limited. If R_0 is >1, an epidemic is possible, but random fluctuations in the number of transmissions in the early stages of infection in a susceptible population can lead to either extinction or explosion of the infection. If R_0 is **much** greater than 1, an epidemic (or perhaps a pandemic) is almost certain (Table 1.1). The proportion of the susceptible population that must be vaccinated to prevent virus spread is calculated as $1—1/R_0$. In the simplest model, $R_0 = \text{tau} \times c \times d$, where tau is the probability of infection, given contact between an infected and uninfected host; c is the average duration of contact between them; and d is the duration of infectivity. Consequently, the longer the exposure among individuals and the length of the infectious period, the higher R_0 will be.

The original host-parasite theory assumed well-mixed, homogeneous host populations in which each individual host has the same probability of becoming infected. Although the general concepts remain valid, additional parameters and constraints have been added to the mathematical models as more has been learned about population diversity and the dynamics of viral infections (Chapter 10). For example, immune-resistant viral mutants with differences in virulence and transmissibility can be selected, and some individuals (called super transmitters) can pass infection to others much more readily than the majority. We also now know that virus populations are more diverse than first imagined, and the constellation of possible host populations affects their evolution in ways not easily captured by mathematical equations. Consequently, although the calculations are useful indications of the thresholds that govern the spread of a virus in a population (that is, they help to determine if a disease is likely to die out [R_0 is <1] or become endemic [R_0 is >1]), they cannot be used to compare possible outcomes in particular cases or for different diseases.

While mathematical formulas and statistics are crucial to all studies in virology, they are of particular value in viral epidemiology. An understanding of some essential principles concerning the use of statistics in virology is provided in Box 1.6.

Table 1.1 Reproductive numbers for selected viruses

Virus	R_0[a]
Measles	12–18
Smallpox	5–7
Polio	~7
SARS-CoV-2	2–3
Influenza	
2009 (H1N1)	1.47
1957, 1968 pandemics	1.8
1918 pandemic	2.4–5.4
Ebola	1.3–1.8[b]

[a]Values from Centers for Disease Control and Prevention website.
[b]Source: Chowell G et al. 2004. *J Theoret Biol* 229:199–126.

Methods Used by Epidemiologists

We have considered some of the terms that epidemiologists use, but how do these scientists monitor and develop strategies to control the spread of viruses in populations? An investigation begins at the site of an outbreak, where as much descriptive data as possible about the infected individuals and the environment are gathered. In cases of viral infections in humans, information on recent travel, lifestyle, and preexisting health conditions is considered, along with the medical records of infected individuals to generate a testable hypothesis about the origin of the outbreak. The word "descriptive" can have a negative connotation in virology, often used to imply the opposite of "mechanistic." However, in epidemiology, descriptive studies are essential to establish or exclude particular hypotheses about the origins of an outbreak. Indeed, descriptive epidemiology was the cornerstone for the discovery of human immunodeficiency virus during the AIDS epidemic in the 1980s (Box 1.7).

Following the descriptive phase, analytical epidemiological methods are used to test hypotheses using control populations in either retrospectively or prospectively focused studies. Clinical epidemiology focuses on the collection of biospecimens, such as blood, sputum, urine, and feces, to search for viral agents or other pathogens and to help determine the potential route of transmission. Once specimens are collected, nucleic acid sequencing is often performed on the samples to determine the nature of the infectious agent, or to define how genetic variants may have spread within a population. Studies may also include serological analyses, in which antibodies in the blood that implicate previous infection are identified.

Surveillance

The establishment of vigilant surveillance procedures that can shorten the period between the beginning of an epidemic and its detection is crucial to mitigating the impact of an outbreak. One could argue that the development of worldwide surveillance programs and information sharing have had as profound an impact on limiting viral infections as antiviral medications and vaccines. The U.S. Centers for Disease Control and Prevention (CDC) was established in 1946 after World War II, with a primary mission to prevent malaria from spreading across the country. The scope of the CDC quickly expanded, and this institution is now a central repository for information and

BOX 1.6

METHODS

The use of statistics in virology

When studying viral infections in hosts, scientists do not always obtain results that are so clear and obvious that everyone agrees with the conclusions. Often the effects are subtle, or the data are highly variable from sample to sample or from study to study (sometimes referred to as "noise"). This ambiguity is particularly true in epidemiological studies, given the large number of parameters and potential outcomes. How do you know if the data that you generated or are reading about in a paper are significant?

Statistical methods, properly employed, provide the common language of critical analysis to determine whether differences observed between or among groups are significant. Unfortunately, surveys of articles published in scientific journals indicate that statistical errors are common, making it even more difficult for the reader to interpret results. In fact, the term "significant difference" may be one of the most misused phrases in scientific papers, because the actual statistical support for the statement is often absent or incorrectly obtained. While a detailed presentation of basic statistical considerations is beyond the scope of this text, some guiding principles are offered.

It is essential to consider experimental design carefully before going to the bench or to the field. A fundamental challenge in study design is to predict correctly the number of observations required to obtain a reliable significant difference. The significance level is defined as the probability of mistakenly reporting that a difference is meaningful; by convention, this probability is minimally set at 0.05 (5%; see the table for hypothetical data). Scientists do not usually refer to experimental outcomes as "true" or "false" but rather use quantitative approaches to provide a sense of the significance between two data sets (e.g., experimental versus control). An important concept is statistical power, the probability of detecting a difference that truly is significant. In the simplest case, power can be increased by having a larger sample size (see table). Even when results seem black and white, having too few animals (or replicates) will be insufficient for drawing a statistically meaningful conclusion.

It is essential to include a detailed description of how statistical analyses were performed in all communications linked with the data. Benjamin Disraeli, a 19th-century British prime minister, once said, "There are three kinds of lies: lies, damned lies, and statistics." Indeed, a gullible reader may be persuaded that a certain set of data is significant, but this conclusion depends on the stringency and appropriateness of the tests that were applied, as well as the data points included in the analysis.

While this text cannot define what tests are applicable for which assays, we can make a couple of strong suggestions. First, statistics should not be considered an afterthought or a painful process that one does after putting data together for a publication. Reliable studies that stand the test of time have considered statistics throughout the scientific process, and good statistics are essential for good study design. Second, be wary of reports in which an investigator inappropriately influences the statistical analyses to produce a "statistically significant" result. This process, sometimes referred to as "*p*-hacking," can result in false positives or data that, while sufficiently different to qualify as "statistical," in fact have no bearing on biological significance. Finally, while it is true that the field can be complex, most of the tests used by virologists are reasonably straightforward. Computer programs such as Excel and GraphPad have made the calculations easy, but you need to know which tests to apply. Fortunately, there are excellent books available that make statistics logical and accessible (e.g., *Intuitive Biostatistics*, by Harvey Motulsky). For more complex data, study design issues, and analyses, one may require consultation with a statistician.

***P* values for the differences in infection rates between experimental and control groups[a]**

No. of animals per group	*P* value for indicated group[b]: All control animals infected and no experimental animals infected	All control animals and one experimental animal infected or one control animal uninfected and no experimental animal infected	One control animal uninfected and one experimental animal infected
3	0.1	0.4	1.0
4	0.03	0.1	0.5
5	0.008	0.05	0.2
6	0.002	0.02	0.08
7	<0.001	0.005	0.03
8	<0.001	0.001	0.01

[a]Data from Richardson BA, Overbaugh J. 2005. *J. Virol* 79:669–676.
[b]Determined by Fisher's exact test, using a two-sided hypothesis test with the significance level fixed at 0.05. Fisher's exact test is used because it is appropriate for experiments with small numbers of observations.

Motulsky H. 2013. *Intuitive Biostatistics: a Nonmathematical Guide to Statistical Thinking*, 3rd ed. Oxford University Press, Oxford, United Kingdom.

biospecimens available to epidemiologists; it also offers educational tools to foster awareness and ensure public safety. The World Health Organization (WHO), founded in 1948 as an international agency of the United Nations, is charged with establishing priorities and guidelines for the worldwide eradication of viral agents. The WHO provides support to countries that may not have the resources to combat infectious diseases, and coordinates results from a global network of participating laboratories. While the WHO provides coordination, the experimental work is performed in hundreds of laboratories throughout the world, often in remote locations, which process samples and relay information back to the WHO. These WHO-

BOX 1.7

BACKGROUND

Descriptive epidemiology and the discovery of human immunodeficiency virus

Acquired immunodeficiency syndrome (AIDS) was first recognized as a new disease in the United States by physicians in New York, Los Angeles, and San Francisco, who independently noticed that some of the young homosexual male patients in their practices had developed unusual diseases, such as *Pneumocystis carinii* pneumonia and Kaposi's sarcoma, which were typically associated with immunosuppressed patients. The first report in the medical literature that described this apparently new syndrome appeared in June 1981, and described five homosexual men in Los Angeles with *P. carinii* pneumonia. Other reports of a similar syndrome in individuals who injected drugs intravenously soon followed. While these "descriptive" observations raised many questions and incited much anxiety, they also laid the foundation for the subsequent, mechanistically focused work that identified the human immunodeficiency virus as a new human pathogen.

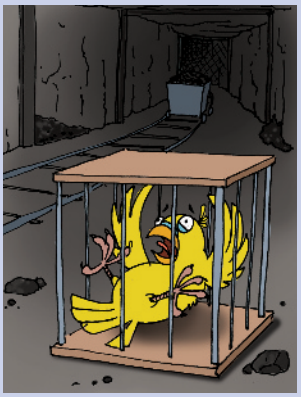
Centers for Disease Control (CDC). 1981. Pneumocystis pneumonia—Los Angeles. *MMWR Morb Mortal Wkly Rep* **30:**250–252.

BOX 1.8

METHODS

Sentinel animals

Underground coal mines are dangerous places to work, in part because toxic, even fatal, levels of carbon monoxide can build up in caves with poor ventilation. Because carbon monoxide is odorless, miners would often keep a bright yellow canary in the mines with them; if the canary remained alive, no carbon monoxide was present, but if the canary died, the miners were forewarned. The "canary in a coal mine" is perhaps the best-known case of using animals as sentinels or harbingers, though this approach has been used to identify viral infections in the wild as well. For example, sentinel species, such as monkeys, are placed inside cages near the entrance to caves where bats reside. Epidemiologists periodically check on the health of the monkey; if the animal became sick, not only would this indicate a health concern, but virus could be isolated from the affected host to determine the nature of the pathogen, and perhaps to learn something about how it was spread. Methods that do not require the incarceration of the sentinel organism are also in use, including collection of feces and urine in the wild for subsequent laboratory analysis. In fact, in 1947, scientists conducting routine surveillance for yellow fever virus in the Zika forest of Uganda recovered a novel virus, later named Zika virus.



certified laboratories adhere to stringent standards to ensure consistency of methods and interpretations. The laboratories conduct field surveillance using wild and sentinel animals, and perform periodic blood screening for signs of infection or immunity (Box 1.8). The chief successes of such global-surveillance efforts to date include the eradications of smallpox virus and rinderpest virus, the latter of which causes disease in agricultural animals, such as cattle and sheep.

Publications and websites help to distribute consistent and timely information to health care workers across the globe. The *Morbidity and Mortality Weekly Report*, published by the CDC, provides a central clearinghouse for health care providers in the United States to communicate individual cases of infectious diseases or to report unusual observations. ProMED (Program for Monitoring Emerging Diseases), sponsored by the International Society for Infectious Diseases, is a worldwide effort to promote communication among members of the international infectious disease community. Reporting of individual cases, when considered by epidemiologists in the aggregate, may catch an epidemic in its earliest days, when intervention is most effective.

More-informal "crowdsourced" approaches have recently gained attention for their power to share data, educate the public, and rapidly identify a potential outbreak. Real-time data-gathering tools, such as Google Flu Trends and Google Dengue Trends, are Web-based applications that survey search queries from more than 25 countries to predict epidemics. The predictions made from these applications have been generally consistent with more traditional surveillance data-gathering approaches. The innovative use of keyword collection to monitor viral outbreaks underscores how collaboration between distinct fields (e.g., epidemiology and

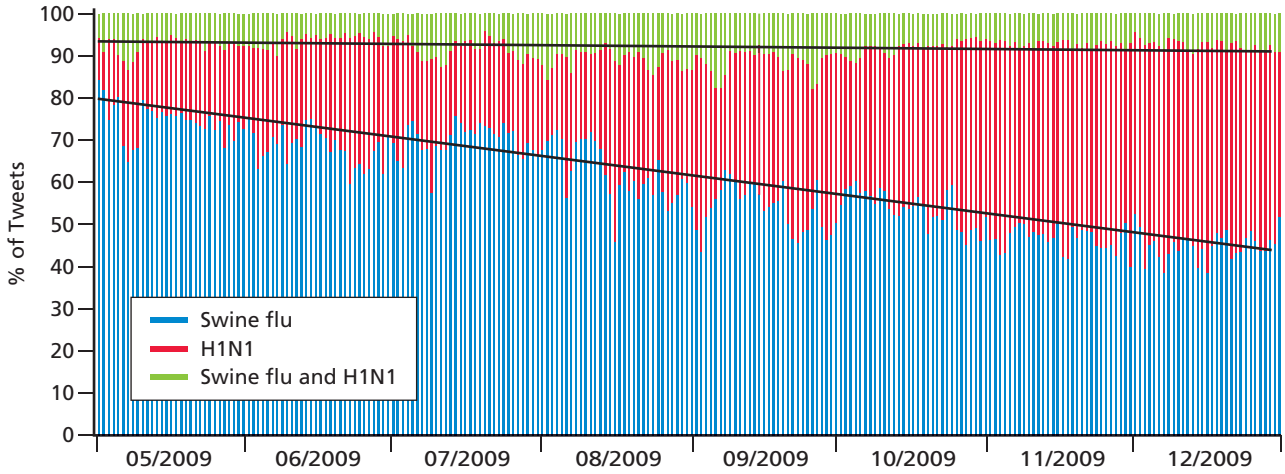


Figure 1.9 Twitter as a tool in viral epidemiology. Between May 1 and December 31, 2009, the relative proportion of tweets using "H1N1" increased in an almost linear fashion, indicating a gradual adoption of the WHO-recommended H1N1 terminology as opposed to "swine flu." Blue = use of the term "swine flu"; red = use of the term "H1N1"; green = combined use of "swine flu" and "H1N1." Adapted from Chew C, Eysenbach G. 2010. *PLoS One* 5:e14118, under license CC BY 4.0. © 2010 Chew et al.

search engine design) can lead to creative ways to detect incipient epidemics. Social media monitoring also is an excellent way to gauge the impact of public education efforts in understanding viral infections (Fig. 1.9).

Network Theory and Practical Applications

How many people do you encounter each day in conversation, in the classroom, or passing on the street? Dozens to hundreds of interactions—some long-lasting, others fleeting—can occur in a day, and each of these individuals has a personal "network" as well. The science of social networks as a tool to understanding spread of pathogens within communities has revolutionized epidemiology. Such networks define potential transmission routes; for example, contact tracing identifies likely transmission network connections from known infected cases and then applies this information to treat or contain their contacts, thereby reducing the spread of infection. Contact tracing is a highly effective public health tool, as it uses the underlying transmission dynamics to target control efforts and does not rely on a detailed understanding of the etiology of the infection.

Network analysis has been used most effectively when considering viruses that are spread via sexual activity. In contrast to airborne infections, sexually transmitted viruses, such as human immunodeficiency virus type 1 and some herpesviruses, have transmission routes that should be easily identified, provided one can recall recent sexual partners. In these cases, an individual identifies their sexual partners over a given period, these partners are then contacted and asked for their partners, and so on; this process is known as **snowball sampling**, and is used by many public health officials to contact individuals who may be at risk for infection.

Developing methods to trace viruses' spread via aerosols is far more challenging. One successful strategy took advantage of the fact that most people carry mobile phones; in this study, data were collected using Bluetooth to sense other mobile phones in the vicinity. These data gave a highly detailed account of an individual's behavior and contact patterns, and allowed highly detailed interaction maps to be developed.

Parameters That Govern the Ability of a Virus to Infect a Population

One often hears that a virus is "going around," and such comments usually correlate with particular times of year (flu season). The seasonal appearance of some viruses, especially those that cause respiratory and gastrointestinal disease, raises the question of what parameters facilitate seasonal or temporal spread in a population. This question is relevant both to viruses that cause widespread epidemics and to more mundane infections, such as the common cold. Identifying the variables associated with increased risk in a population has obvious value in clinical and educational efforts to prevent or limit outbreaks. As discussed below, multiple aspects of both the host and the environment contribute to maintaining a virus in a community.

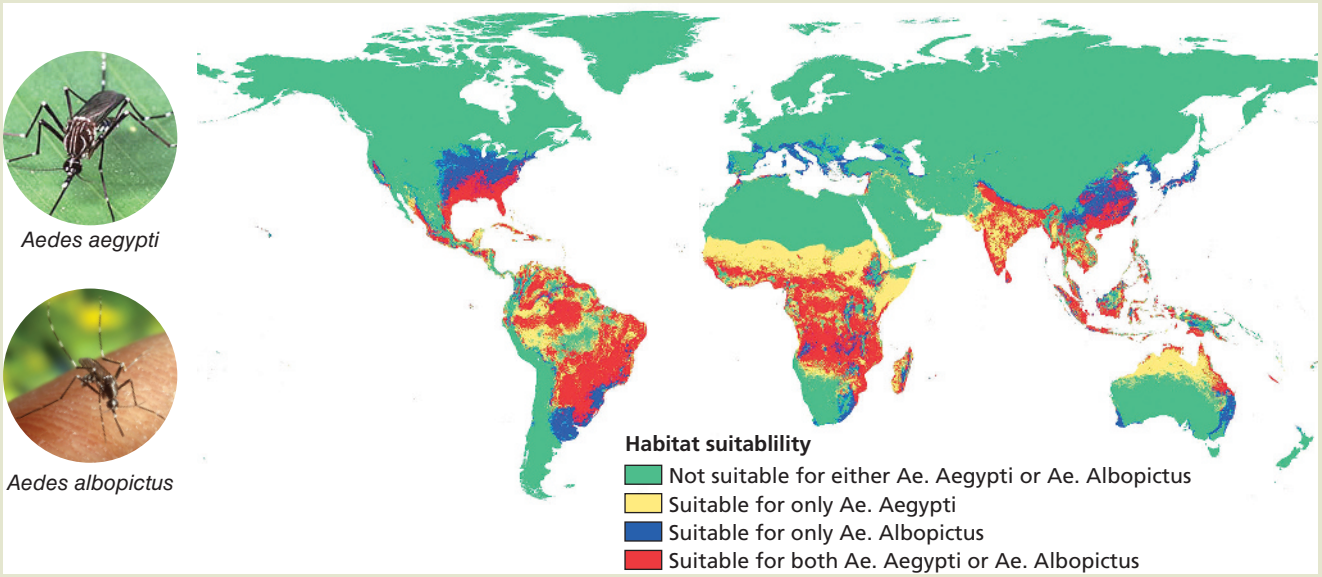
Geography and Population Density

Some viruses are found only in specific geographical locations. The regional occurrence of viral infections may be due to the restriction of a vector or animal reservoir to a limited area. For example, most insect vectors are restricted to a specific region or ecosystem; unless this vector "escapes" its natural habitat, the viruses that it harbors will also be geographically constrained. Changes in migration routes or territory of a reservoir species may therefore influence the distribution of a virus and lead to new interactions with other

BOX 1.9

DISCUSSION

A virus on the move



Habitat suitability for both *Aedes aegypti* and *Aedes albopictus*. *A. albopictus* image courtesy of CDC/James Gathany (CDC-PHIL ID 1863). Right panel reprinted from Leta S et al. 2018. *Int J Infect Dis* **67:**25–35, with permission.

Chikungunya virus is an alphavirus in the family *Togaviridae*. The virus is spread by mosquitos (primarily the notorious *Aedes aegypti*). The viral disease has been known for more than 50 years in the tropics and savannahs of Asia and Africa, but had never been a problem in the developed countries of Europe or the United States. The disease causes unpleasant rashes and joint pain, but infections are not fatal. In the last decade, however, something changed dramatically and brought this once exotic disease into the forefront of public concern.

In 2004, outbreaks of chikungunya disease spread rapidly from Kenya to islands in the Indian Ocean and then to India, where it had not been reported in over 30 years. In some of the Indian Ocean islands, more than 40% of the population fell ill. In 2007, there was an outbreak in Italy, the first ever in Europe. What had happened to change the pattern of infection?

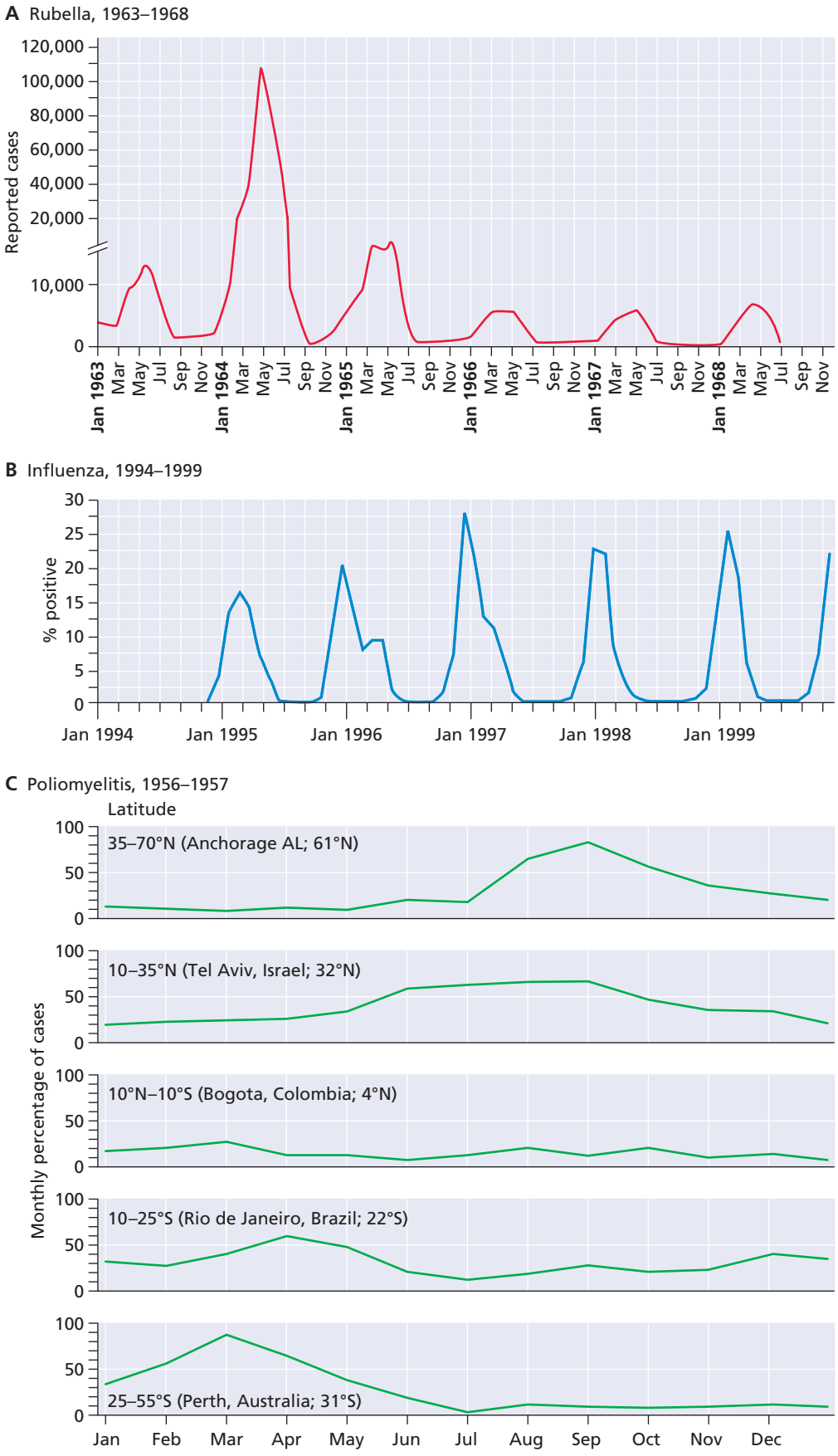
An alarming finding was that the Asian tiger mosquito (*Aedes albopictus*) became an efficient new vector for the virus. A point mutation in the viral genome appears to be the cause of the vector expansion and, perhaps, for the epidemic spread of the disease in areas in which it had been unknown. *A. albopictus*, which has a greater geographical range than *A. aegypti*, is spreading across the globe from eastern Asia and is now found in mainland Europe and the United States. This mosquito is a maintenance (occasionally epidemic) vector of dengue viruses in parts of Asia, and is a competent vector for several other viral diseases. Since its discovery in the United States, five arboviruses (Eastern equine encephalitis, Keystone, Tensaw, Cache Valley, and Potosi viruses) have been isolated from *A. albopictus*.

Enserink M. 2007. Infectious diseases. Chikungunya: no longer a third world disease. *Science* **318:**1860–1861.

species, increasing the risk of zoonotic transmission. A striking example of how a vector can change the location in which a virus is found is provided by the global spread of chikungunya virus (Box 1.9).

Host population density is a critical parameter for some virus populations to be sustained. Person-to-person transmission of some acute viral infections occurs only if the host population is large and interactive. For example, measles virus can be maintained only in human populations that exceed ~200,000, most likely because there is no animal reservoir, and infected individuals develop complete and long-lasting immunity. These infections are rarely found in isolated groups that might populate small islands or areas with extreme climates. Before global travel was possible, isolated host populations were the norm, and the distribution of viruses was far more limited. Now, however, as illustrated by the rapid colonization of the Western Hemisphere by West Nile virus, viruses are transported routinely and efficiently around the globe. In fact, epidemiologists have begun to think about the potential for epidemics in terms of the "effective distances" between airports, arguing that London is actually closer to New York than to other British towns, based upon air traffic densities. The larger the number of people that travel between airports and the cities that they serve, the smaller the effective distance. Data derived from consideration of population density might also influence public health measures. It was recently shown that

Figure 1.10 Seasonal variation in disease caused by three human pathogens in the United States. **(A)** Annual cycles of rubella between larger epidemics, which occurred every 6 to 9 years (1963 to 1968). **(B)** Annual cycles of influenza virus infection (1994 to 1999). Note the strong seasonal prevalence. **(C)** Monthly incidence of poliomyelitis at different latitudes, with representative cities denoted. Data from Dowell SF. 2001. *Emerg Infect Dis* 7:369–374.



influenza epidemics are governed by both population size and humidity, which led to the proposal that large metropolitan areas should focus their public health efforts on reducing influenza spread, whereas smaller cities and towns should focus on minimizing harm from such infections.

Climate

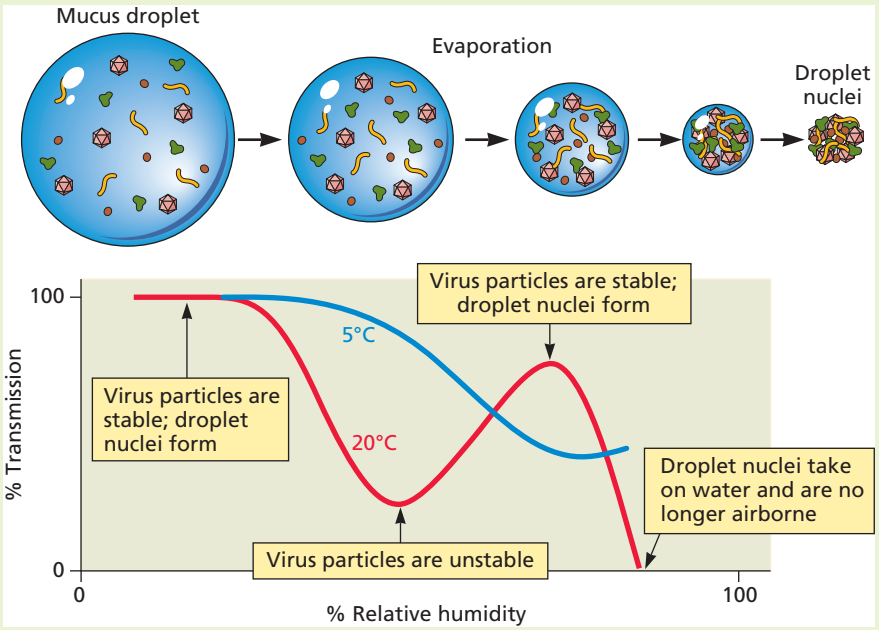
In contrast to cultured cells that grow under conditions of stable temperature and humidity, or laboratory animals that live in strictly controlled enclosures, humans and other animals exist in ever-changing environments that directly influence viral biology. These changes include normal seasonal variations as well as progressive changes, such as climate change.

Climate, including temperature and humidity, can have a profound influence on viral infections of populations. Indeed, there is a striking seasonal variation in the incidence of most acute viral diseases (Fig. 1.10). Respiratory virus infections occur more frequently in winter months, whereas infections of

BOX 1.10

EXPERIMENTS

Temperature influences the transmission of influenza virus



Model for the effect of humidity on the transmission of influenza virus. (Top) Virus particles can be contained in airborne aerosols that are produced from coughs or sneezes. The water in these small droplets evaporates, concentrating the particles into droplet nuclei (defined as droplets <5 mm in diameter and so small and light that they may remain suspended in the air for several hours). **(Bottom)** Transmission efficiency at 20°C (dashed line) or 5°C (solid line) is shown as a function of percent humidity. At 20°C, transmission is highest at low humidity, conditions that favor conversion of exhaled droplets into droplet nuclei. Reduced particle stability at intermediate humidity is the cause of poor transmission. At high humidity, the conversion from droplets to droplet nuclei is inhibited, and the heavier droplets fall from the air, reducing transmission. At 5°C, transmission is more efficient than at 20°C, but there is a gradual loss of transmission with increasing humidity, presumably also as a consequence of the reduced formation of droplet nuclei. Adapted from Lowen AC et al. 2007. *PLoS Pathog* 3:1470–1476, under license CC BY 4.0. © 2007 Lowen et al.

Seasonality is a familiar feature of influenza: in temperate climates, the infection occurs largely from November to March in the Northern Hemisphere and from May to September in the Southern Hemisphere. There have been many hypotheses to explain this seasonality, but none were supported by experimental data until a guinea pig model was used to show that spread of the virus in aerosols is dependent upon both temperature and relative humidity.

Transmission experiments were conducted by housing infected and uninfected guinea pigs together in an environmental chamber. Transmission of infection was most effective at humidities of 20 to 35%, and blocked at a humidity of 80%. In addition, transmission occurred with greater frequency when guinea pigs were housed at 5°C than at 20°C. The authors conclude that low temperature and humidity, conditions found during winter, favored influenza virus spread. The dependence of influenza virus transmission on low humidity might be related to the size of the droplets produced by coughing and sneezing.

BOX 1.11

BACKGROUND

Quiz: The origins and veracity of urban legends about infections

Which of these statements about colds and the flu are true, and which are myths?

1. You can catch the flu from a flu shot.
2. Stress increases your chances of getting ill.
3. Wearing a hat will help protect you from a cold.
4. Flying on an airplane will increase your risk of getting sick.
5. Pregnant or breastfeeding women should not get vaccinated.
6. Increasing how much you sweat (for example, using lots of blankets) will speed up how quickly you resolve an infection.
7. "Feed a cold; starve a fever."
8. Your grandmother's chicken soup can help.
9. Eating garlic can help to prevent you from getting ill.
10. Over-the-counter cold "prevention" tablets or drinks are effective.

Answers

1. ***Myth:*** As discussed in Chapter 7, the injected flu vaccine is an inactivated virus; it is therefore impossible to get the flu from the shot itself. The nasal flu mist contains a live, but drastically weakened, virus, and it is highly unlikely that someone will get influenza from the nasal vaccine.
2. Not yet proven, but probably ***fact:*** stress alters hormones, hormones affect immunity, and immunity controls your response to viral infections, so it is quite possible that stress can affect your ability to respond to an infection.
3. ***Myth:*** Wearing a hat will keep your head warm, but that's it.
4. ***Fact:*** Recirculated air combined with a large number of people in close quarters is a perfect recipe for transmission of respiratory infections from person to person.
5. ***Myth:*** Flu symptoms are generally worse in pregnant women than in nonpregnant women, so it is of added importance that pregnant women be vaccinated. Many studies have shown that there are no adverse consequences of maternal vaccination to the fetus or the nursing neonate.
6. ***Myth:*** While piling on the blankets may make you feel better, it will not make the cold go away faster; the only thing proven to alter the duration of an infection is the use of antivirals within a short (1- to 2-day) window after symptoms appear.
7. ***Myth:*** How much you eat, or what you eat, will not influence how quickly you will resolve an infection. However, drinking lots of fluids **will** help, as staying hydrated, especially if you have a fever, will keep the mucus in respiratory passages loose. Moreover, colds and flu tend to cause a transient lack of appetite, so choosing food wisely (for example, protein-rich) when recovering will hasten feeling better.
8. ***Fact!:*** It is a fact that warm liquids open up nasal passages and keep the mucus moving (a good thing), and chicken soup has also been proposed to mobilize neutrophils, important virus-fighting immune cells.

9. ***Fact:*** Garlic has powerful antioxidant activity, which boosts immunity. Eating **lots** of garlic will also repel potentially infected friends and colleagues.
10. ***Myth:*** The small bottles that often appear at checkout lines in supermarkets and that promise protection from catching a cold are primarily just a large dose (usually 1,000 mg) of vitamin C. While it remains controversial whether vitamin C is beneficial, daily multivitamins (or, better still, a healthy diet) can provide as much of the key ingredient and for less money.

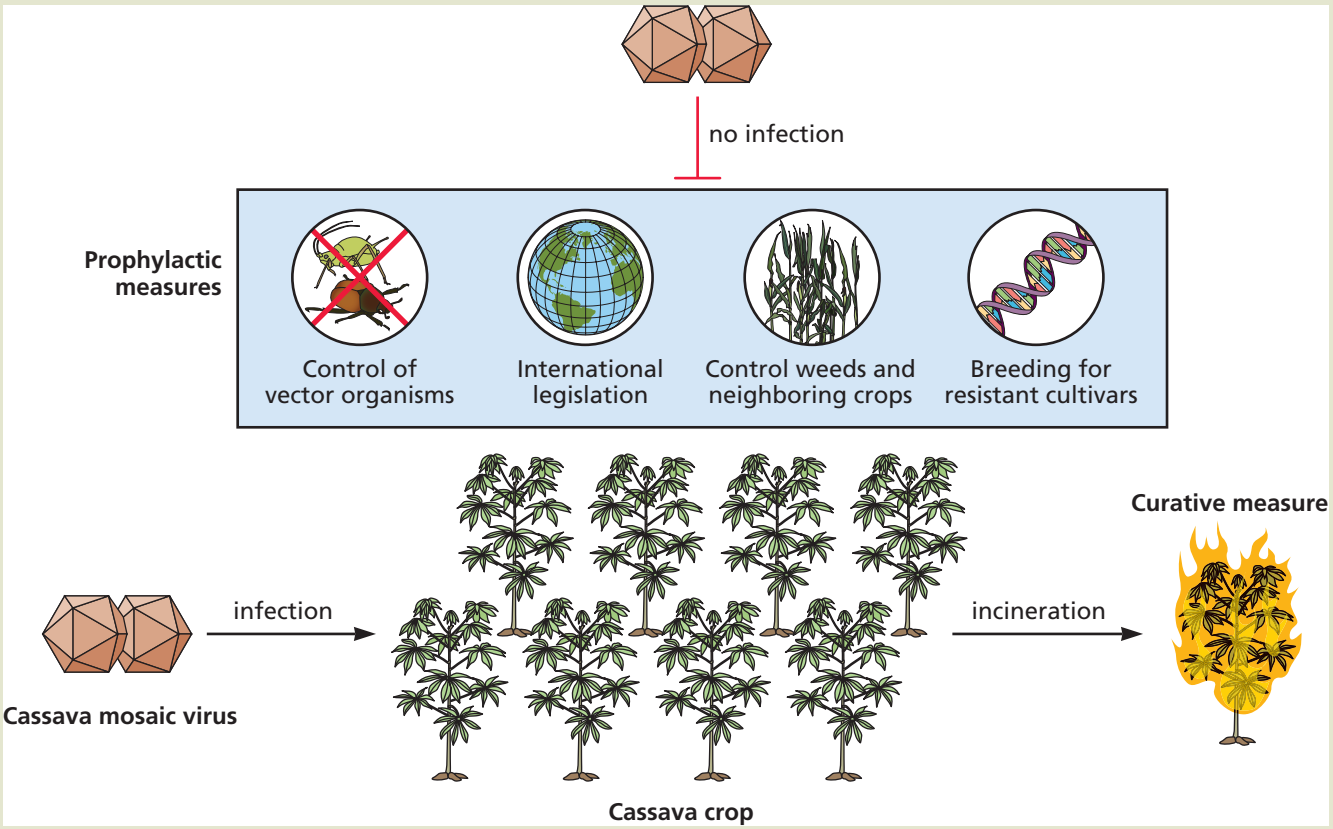
the gastrointestinal tract predominate in the summer. Seasonal differences in diseases caused by arthropod-borne viruses are clearly a consequence of the life cycle of the insect vector; when there are fewer mosquitos, there is a parallel reduction in the prevalence of the viruses that they harbor. However, the basis for the seasonal nature of infections by viruses that are not transmitted by arthropod vectors is less obvious. It has been suggested that the seasonality of some infections is attributable to temperature- or humidity-based differences in the stability of virus particles. For example, poliomyelitis was known as a summertime disease in New England; in Hawaii, however, with its stable and temperate climate, poliovirus infections occurred throughout the year. The prevailing view is that poliovirus is inactivated during winter months when humidity is low. In contrast, other viruses, such as influenza virus, remain infectious through the drier winter months.

A widely held belief is that large changes in temperature will increase a host's susceptibility to infection. In fact, as a parent likely warned you, transmission of "the flu" (specifically, influenza A virus particles) is more efficient at low temperature and humidity, and this property could contribute to increased rates of influenza in the winter months (Box 1.10). However, epidemiological studies with rhinoviruses that are also anecdotally associated with cold temperatures have failed to support any relationship between being cold and getting a cold; whether the "urban legends" associated with respiratory viral infections are true thus appears to depend on the virus in question (Box 1.11).

BOX 1.12

DISCUSSION

Plant virus epidemiology



Approaches to prevent crop infections are shown at top, and include vector control, policy changes, and development of resistant species. When crops do become infected, incineration of the population is usually the only real alternative.

Natural physical barriers of plants, such as the cuticle and cell wall, normally preclude viruses from gaining access to permissive cells. Viruses are delivered into plants when these barriers are breached, through wounds or the action of vectors (insects, nematodes, fungi) that feed on the plants. Agricultural viruses can cause epidemics with far-reaching implications for both food security and the economy. For example, cassava mosaic begomoviruses cause more than 25 million tons of losses per year in Africa, India, and Sri Lanka. Because the cassava crop represents the daily staple for more than 500 million people, epidemics are often linked to famine events.

Viral diseases are difficult to control once they have begun. Although farmers are well educated to detect signs of infections, because the plants are sown in close proximity, by the time an infection is noted, it has often already spread throughout the crop. When infections occur, destruction of the entire crop (including both infected and uninfected plants, usually by burning) is the only certain strategy to end an agricultural viral epidemic. Prophylactic control measures are therefore crucial to prevent or restrict these infections. Historically, farming strategies have included pesticide management of vector insects and the use of non-host "trap plants" that attract the vector but cannot be infected. Because epidemics can arise from viruses that spill over from adjacent reservoir species, parcel placement and meticulous weeding restrict viral spread. The use of genetically resistant plants is one of the most efficient and sustainable strategies to control virus infections in fields. With increased understanding about the reproduction cycles of plant viruses, resistant plant varieties can be developed. For example, as RNA interference is a major immune strategy for plants, developing crop species that encode a viral gene allows complexes to be formed between RNA transcribed from the inserted gene and RNA of the invading virus, resulting in the degradation of the latter.

Climate-based variations in viral disease may also be caused by bodily changes in the host that influence susceptibility. Such changes might be linked to **circadian rhythms**, or be governed by alterations in the thicknesses of mucosal surfaces, production of virus receptors, or immune fitness. For example, if the mucosa is thinner in the winter or the skin is drier and cracked, the protective barriers that normally block viral entry into a host can become compromised.

Although this text focuses primarily on those viruses that cause disease in humans and animals, plants and crops, and the viruses that infect them, are subjected to many of the same variables (Box 1.12).

Perspectives

A fundamental principle of virology is that for a virus to be maintained in a host population, sufficient quantities of infectious virus particles must be released from one infected host to infect another. This process of serial infection, while simple in principle, is difficult to study in natural systems given the mind-boggling number of host, viral, and environmental variables. Nevertheless, epidemiology, the study of this process, is evolving rapidly as new ways to track and identify infectious agents are developed. To thwart a potential epidemic, viral epidemiologists must master diverse skills. In tracking the origins of infection, epidemiologists must consider simultaneously multiple variables and clues, some of which are false leads. They must understand the dynamics of the animal or human populations at risk and how aspects of behavior, social structure, and environment might influence the potential for infection. Epidemiologists must then integrate these diverse pieces of information. Furthermore, as investigation often begins only after an epidemic is under way and victims have been identified, they must be able to work under great pressure, within a constrained time frame, and often under intense media scrutiny and dangerous work conditions. We are witnessing these pressures and challenges in real time, as epidemiologists struggle to contain the worldwide pandemic caused by the SARS-Coronavirus-2 (Box 1.13). Individuals working in computer science, bioinformatics, frontline health care delivery, public policy, and international medicine also are crucial for successful control of a viral outbreak. Containing an epidemic under diverse, often unknown, pressures is a monumental challenge, especially when no certain therapy exists that can be offered to patients, and when, as Zinsser first surmised in the 1930s, the "enemy" may lurk in a ubiquitous and minuscule organism, such as the mosquito.

BOX 1.13

DISCUSSION

This moment in time: the SARS-CoV-2 pandemic

It is 5:31 AM on Sunday, March 1, 2020, and 12 hours ago the first death in the United States was confirmed due to the pandemic coronavirus, SARS-CoV-2. Worldwide, tens of thousands are infected and thousands have died, and most believe that we've yet to see the peak impact of this pandemic. Beyond the impact of the virus on these lives, the worldwide outbreak is on the front page of every newspaper, facemasks are flying off the shelves, the stock market has plunged (and may continue to do so), a Vice President with no scientific background was appointed head of the United States Task Force, tourist destinations are at record low attendance, schools in China and Japan are closed, and there is even some discussion about cancelling the Olympics, which do not begin until the end of July. Adding to a sense of global anxiety, some individuals are being diagnosed as coronavirus-positive with no recent history of travel to countries severely affected by the outbreak, and no known contact with infected individuals. (Of course, this is to be expected, as asymptomatic individuals can be efficient vectors for transmission). Many professionals are reminding the public that this is a lower respiratory tract infection much like influenza A virus, and underscore that many of the same people who are worried about COVID-19 (the name for the disease caused by SARS-CoV-2) are among those who, paradoxically, do not routinely get their flu shot. Despite such comparisons to influenza A virus, however, there are many things we do not know about this virus and the disease it can cause. For example, there are suggestions that the case fatality ratio is similar to influenza, but data collection varies in reliability, and appears to differ based on region and time of the outbreak (the case fatality rate appears to have been higher earlier in the outbreak than now). Also, although most deaths occur in the elderly or severely immunocompromised (as with flu), some otherwise young and immunocompetent individuals, including the Chinese physician who was among the first to sound the alarm about this virus, are also succumbing. Moreover, while initially it was presumed that infections of humans originated in a fish market in Wuhan, China, there are now doubts that this is true, and while bats are presumed to be the vector for spread to humans, until recently some purported that pangolins—animals resembling scaly anteaters—may be a source of zoonotic transmission. A silver lining is that at least we now know what pangolins are.

At this moment, of course, no one knows what comes next. Like every pandemic that has come before, the number of cases of SARS-CoV-2 infection will eventually abate as individuals develop immunity, prophylactic measures (such as preventative quarantining of people who recently traveled to high risk countries) begin to take effect, and eventually, antivirals and vaccines are developed. But surely the fear, confusion, uncertainty, and conspiracy theories of today must echo what occurred in Philadelphia in the summer of 1793, when people were inexplicably dying on the streets of what we would later learn was yellow fever.

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This study presents a useful, real-world description of the challenges of epidemiological fieldwork.

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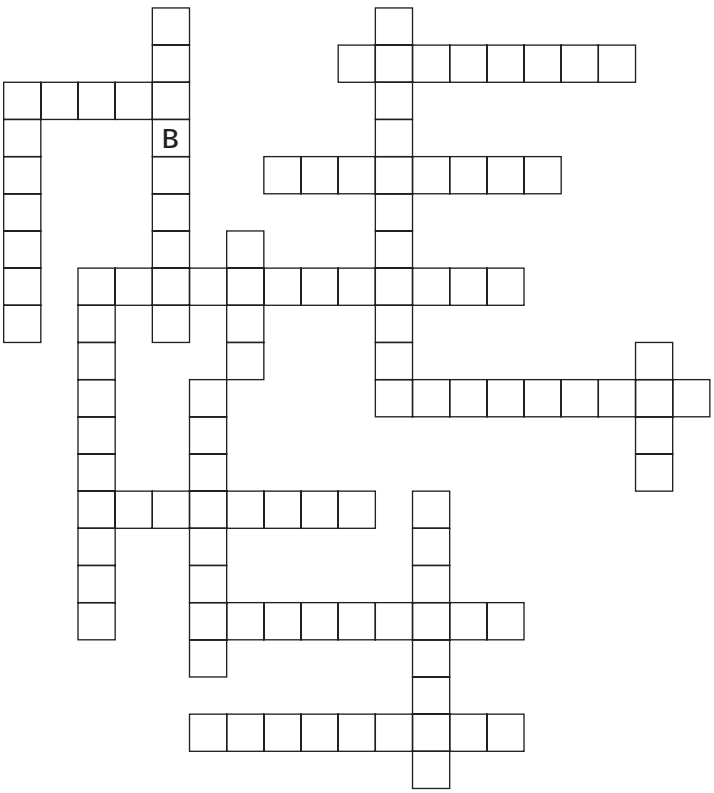
A historical account of the origins of the Zika virus epidemic that led to widespread infection and microcephaly in neonates.

STUDY QUESTIONS

1. Imagine that you are an epidemiologist in a small, remote village in Epidemistan. Epidemistan is located in the high mountains, and the roads to access it are treacherous. Therefore, it takes over a week to reach it by car, and no other transportation in or out of the village is available.

On Tuesday of your worst week ever, a child experiencing high fever, a bright pustular rash, and unremitting diarrhea comes into your clinic. You've seen these symptoms before, but not all at once, and not to this degree. You hydrate the child, provide fever-relieving drugs, and put the child under watchful surveillance. On Thursday, three more children appear with the same symptoms, and on Friday, one of the villagers who provides care to the children of the community also develops the same symptoms. The village leaders come to you for guidance.

You have all the equipment for a modest molecular biology laboratory. You call the nearest clinic for help, and they are on the way, but because of your remote location, you are on your own for at least a week. What is your first step? Of all the things you could consider in this emergency, defend why this action is more paramount than others.



PUZZLE CLUES

He developed the postulates that prove causality between a microbe and disease…except perhaps for viruses (4 letters)

An animal used to assess potential outbreaks (8 letters)

Disease manifestation of a virus infection (12 letters)

The probability that a meaningful difference or effect would be detected if it occurred (5 letters)

The number of new cases in a population in a given period (9 letters)

Disease outbreak of worldwide proportions (8 letters)

The first human virus to be identified (11 letters)

Disease transmitted from other animals to humans (8 letters)

The percentage of deaths in a specified population of infected individuals (9 letters)

A virus transmitted by mosquitos, and associated with severe birth defects (4 letters)

The host population in which a viral population is maintained (9 letters)

The cause or causes of disease (8 letters)

The total number of infected individuals in a population or area (10 letters)

The founder of vaccination, with apologies to Jenner (7 letters)

The percentage of individuals in a specified population who show symptoms of infection within a given period (9 letters)

An event when a viral disease affects a greater number of people than is usual for the area, or when a disease spreads to a new area (8 letters)