CHAPTER 1

Introduction to Viruses

"Infectious disease is one of the greatest tragedies of living things—the struggle for existence between different forms of life. . . . Incessantly the pitiless war goes on, without quarter or armistice—a nationalism of species against species."

—Hans Zinser, noted American physician, bacteriologist, and author/historian, in Rats, Lice, and History *(1934)*

This sign located at a boat launch on Lake Winnebago, Wisconsin, warns fishermen to not take minnows from the lake for use in other lakes in order to prevent the spread of viral hemorrhagic septicemia (VHS). VHS is a deadly fish disease that was first discovered in the United States in 1988 and has caused massive die-offs of fish in the Great Lakes region since 2005.

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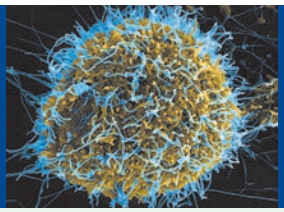
LEARNING OBJECTIVES

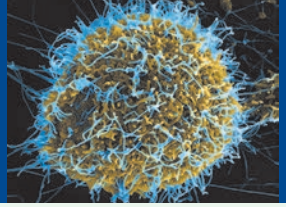
1. List three advances in techniques/technology that were needed in order to study viruses in the laboratory.
2. Define *virus*.
3. Summarize a theory about the origin of viruses.
4. Explain how viruses are transmitted and cause disease.
5. Discuss an example in which a viral infection is beneficial to the host.
6. Describe at least three applications of viruses in treating health problems.
7. Identify important historical and contemporary viral epidemics.



CASE STUDY 1: VIRAL HEMORRHAGIC SEPTICEMIA: A MAJOR THREAT TO FISH

Recently you were inspired by an instructor in a virology course to subscribe to **ProMED-mail**. While searching the ProMED-mail archives for outbreaks of **viral hemorrhagic septicemia** (**VHS**), you found a ProMED-mail post titled "Viral Hemorrhagic Septicemia, Fish—USA: Lake Superior." It was dated May 14, 2010. The post described the disease in symptomatic fish and how the virus can be spread by **carriers** (fish without symptoms that are infected with the virus that causes VHS) or by infected fish that are symptomatic or sick.

Coincidentally, you learned that a college friend is going on a fishing trip to the Great Lakes at the end of the semester. You asked him if he was aware of fish diseases such as VHS, infectious hematopoietic necrosis (IHN), or infectious pancreatic necrosis (IPN). He said that he had noticed signs posted at boat launches on Lake Winnebago, Wisconsin, about a VHS alert and how to prevent spread of fish diseases (see the **Chapter Opener Figure** and **FIGURE 1**). He mentioned his ignorance about fish viruses and said he would appreciate it if you would do more research on VHS on his behalf.



CASE STUDY 1: VIRAL HEMORRHAGIC SEPTICEMIA: A MAJOR THREAT TO FISH (continued)

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FIGURE 1 Sign posted near a boat launch at Lake Winnebago, Wisconsin, reminding anglers on how to prevent the spread of invasive species (e.g., milfoil) and fish diseases such as VHS.

See Case Study 1 Questions at the end of the chapter.

The study of viruses, known as **virology**, began in the 1890s when scientists began studying filterable infectious agents that sickened tobacco plants. *The importance of viruses lies not only in the diseases they cause but also in their intimate relationship with living cells*. Viruses have played a key role in the evolution of life, from its very beginnings on Earth to the diversity we see today. The mysterious and insidious nature of many viruses has drawn scientists in as they study viruses in the laboratory. It is no surprise that viruses have become essential parts of the plots of many popular movies and TV series (**TABLES 1-1** and **1-2**). In the 2013 movie *World War Z*, Dr. Andrew Fassbach, a Harvard virologist (played by Elyes Gable), has a conversation with Gerry Lane, a retired United Nations investigator (played by Brad Pitt), in which Fassbach describes viruses as follows:

> *"Mother Nature is a serial killer.... No one's better ... more creative. Like all serial killers she can't help but the urge to want to get caught ... and what good are all those brilliant crimes if no one takes the credit ... sometimes the thing you thought was the most brutal aspect of the virus, turns out to be the chink in its armor ... and she loves disguising her weaknesses as strengths."*

The viruses in horror movies, TV dramas, and thrillers are scarier than the influenza virus that caused a pandemic in 2009. Reports of **emerging (new) viruses** and **reemerging viruses** continue to appear in the mass media. At the end of the chapter, you will find a **Viral Pestilence Timeline** of epidemics and pandemics that occurred from 1518 through 2016. At the time of this writing, the World Health Organization (WHO) declared a Zika virus epidemic that was racing through Brazil and making its way into the United States, a "public health emergency of international concern." Only 1 in 5 infected individuals experience symptoms. However, for pregnant women, Zika virus infections were correlated with a spike in birth defects: **microcephaly** and eye abnormalities in newborns. Zika virus infection was also associated with a rare autoimmune reaction resulting in temporary paralysis known as **Guillain-Barré syndrome** in adults. Zika virus is transmitted through the bite of *Aedes* mosquitoes, by sexual intercourse (the virus is present in semen), through mother-to-fetus transmission, and by blood transfusions. For nearly seven decades, Zika virus was merely a virological curiosity. There was no need to develop antiviral therapies to treat Zika virus disease or a vaccine to prevent infection by Zika virus.

Table 1-1 "Viral" Television Series

Series	Description
Containment	Premiered on April 19, 2016 on The CW network. A deadly epidemic breaks out in Atlanta and the government orders containment, creating a quarantine zone, which becomes chaotic while officials search for a cure.
Helix	Premiered on SyFy in the United States on January 10, 2014. The plot involves scientists investigating a viral outbreak at a research facility in the Arctic.
The Last Ship	Premiered on TNT in the United States on June 22, 2014. The series is based on the 1988 novel *The Last Ship* by William Brinkley. A viral disease kills 80% of the world population. A crew aboard the USS *Nathan James* naval destroyer is isolated and not exposed to the deadly virus. The crew must try to develop a cure or vaccine to save humanity.
The Walking Dead	Premiered on AMC in the United States on October 31, 2010. Humans are infected by a virus that turns them into flesh-eating zombies. Some episodes of the series include epidemics caused by influenza viruses. The series is based on the graphic novels by Frank Darabont.
ReGenesis	Premiered in Canada on Movie Central on October 24, 2004, and aired for four seasons. Many episodes focused on the investigation of mysterious viral diseases and the ethical issues related to the science at hand.
Survivors	Premiered in 2008 on the BBC. A highly lethal "European influenza virus" wipes out most of the human population. The survivors struggle to overcome the difficulties of a collapsed society.

As Brazil is preparing to host the Summer Olympics, researchers and vaccine makers in the Americas are racing to develop countermeasures.

Also notable is the worst Ebola epidemic in human history, which occurred in West Africa in 2014. Ebola affected multiple countries, including the United States with a handful of **imported cases** that included coverage of a news cameraman who was at the front lines of the epidemic in Liberia and the first **natural cases** at home: two healthcare workers tasked with caring for an imported Ebola case at a hospital in Texas. According to a CNN poll, the public feverishly consumed Ebola coverage, with 49% of Americans closely following news about the Ebola outbreak and a mere 6% not following it closely. A poll by YouGov revealed that 72% of Americans were very worried or somewhat worried when asked "Would you be afraid to sit next to a person who had recently been in any of the countries in Western Africa?"

Table 1-2 Viruses in Movies

Year	Movie Title	Virus
2013	*Dallas Buyer's Club*	HIV
2013	*World War Z*	Rabies-like virus
2011	*Contagion*	A highly lethal airborne virus
2011	*Rise of the Planet of the Apes*	Viral gene therapy
2010	*The Crazies*	"Trixie" virus
2009	*Carriers*	A highly contagious lethal virus
2007	*I Am Legend*	Genetically modified measles virus
2007	*28 Weeks Later* (sequel to *28 Days Later*)	Highly contagious rage-inducing virus
2002	*28 Days Later*	Highly contagious rage-inducing virus
2002, 2004, 2007, 2012	*Resident Evil* movie series	A virus that has escaped from a secret facility called The Hive
1995	*Outbreak*	Ebola virus

Americans watched as medical missionary workers **Dr. Kent Brantly** and **Nurse Nancy Writebol** endured a 14-hour flight aboard a private plane to the United States for treatment after they were infected with Ebola virus while treating patients in Liberia. They were transported to Emory University Hospital in an **Aeromedical Biological Containment System** (**FIGURE 1-1A**). The mini-quarantine ward looks like a framed tent enclosed by thick, clear plastic. The unit has **negative air pressure** and **HEPA-filtered air**. The mini-containment system was originally built to transport SARS patients during the 2003 pandemic but was never used. The same unit was used to transport the two nurses who were infected with Ebola virus while taking caring of Ebola patient **Thomas Eric Duncan** at a Texas hospital. The nurses were transported to different hospitals for treatment: the National Institutes of Health in Bethesda, Maryland, and Emory University Hospital in Atlanta, Georgia.

The 2014 Ebola epidemic was unprecedented in many ways. It changed international air travel and healthcare protocols in the United States (**FIGURE 1-1B**). During the height of the epidemic, entry screening was enhanced for travelers coming into the United States

a Courtesy of the CDC.

b Courtesy of the CDC/Daniel DeNoon.

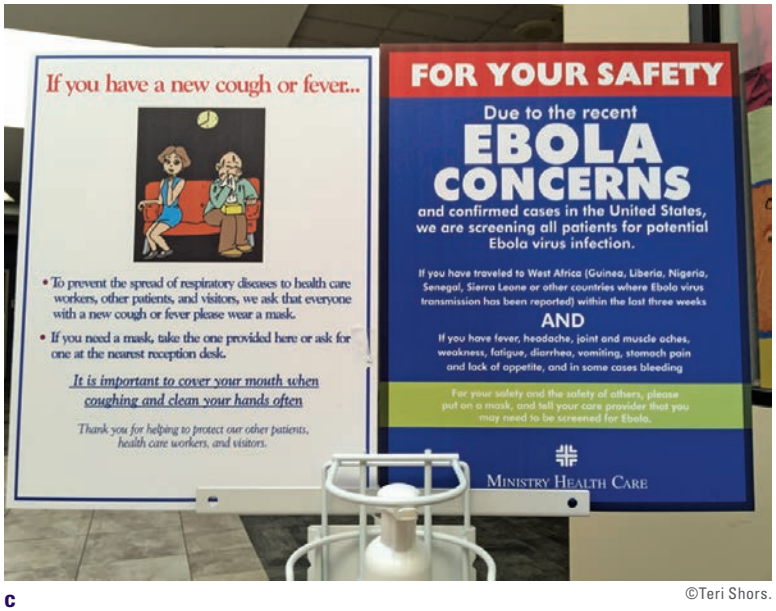

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FIGURE 1-1 **(a)** Aeromedical Biological Containment System used to transport an Ebola patient from an aircraft to a hospital isolation ward. It is a mini-quarantine structure built with internally controlled airflow to ensure that infectious agents remain inside of the unit. **(b)** A scene at Sierra Leone's Freetown-Lungi International Airport in 2014. Airport workers are in the process of constructing a new Port Health Screening Room designed to identify passengers infected by Ebola virus. This is one example of a control measure used to curb the spread of Ebola virus disease from one country to another. **(c)** During the Ebola epidemic in West Africa, Ebola information and a sanitizing station were placed within the entrance of a walk-in clinic in Oshkosh, Wisconsin. The station contains hand sanitizer, child- and adult-size face masks, facial tissues, and a container for used tissues. The photograph was taken March 2015.

from Liberia. New patients at clinics and hospitals were questioned about their travel history to West Africa. Stations with soap dispensers, face masks, and posters about Ebola were placed in lobbies and entrances of walk-in clinics and hospitals (**FIGURE 1-1C**). According to the February 3, 2016, **World Health Organization (WHO) Ebola Situation Report**, since the beginning of the outbreak there had been 28,639 cases, including 11,316 deaths. In particular, healthcare workers were up to 42 times more likely to be infected with Ebola virus in the Ebola-intensive countries than people in the general adult population. The WHO Ebola Situation Report listed 881 confirmed cases and 513 deaths of health workers. Of the health workers, more than 50% of those infected by Ebola virus were nurses and nurse aides. Other categories of health workers infected were doctors, medical students, laboratory and pharmacy staff, midwives, ambulance workers, janitors and maintenance staff (referred to as elementary workers) of Ebola isolation wards. More information about the Ebola epidemic in West Africa is provided later in this chapter and in subsequent chapters. (See Appendix C Bonus Ebola Case Study).

This chapter begins your journey into the invisible world of viruses. The word **virus** has been used in the medical world for over 200 years. In the early 1790s, it simply meant poison or poisonous slime. A brief contemporary definition describes viruses as submicroscopic agents capable of directing their replication inside living cells but that are *not* cells. *Viruses are both beneficial and harmful to humankind.* This overview provides a basis for understanding the impact of viruses on all living organisms.

1.1 Characteristics of Viruses

Today we know that the *majority* of viruses share a few common features. First of all, they are small and cannot be seen by the naked eye. Viruses were originally defined as infectious agents that were able to pass through filters that retain or trap most known bacteria. Hence, viruses are smaller than bacteria. As a rule, most bacteria are 100 times larger than the viruses that infect them. Typically, bacteria range from 1 to 10 micrometers (μm) in length. A virus would fall in the range of 0.03–0.1 μm (or 30–100 nanometers [nm]) in length. Of course, there are always some exceptions. For example, poxviruses can be 200–400 nm in length, filoviruses (such as Ebola virus) can be up to 1,000 nm in length, and the giant **pandoraviruses** that infect amoebae are 600 nm in diameter (comparable to some bacteria). **FIGURE 1-2A** provides size comparisons of viruses to bacterial or eucaryotic host cells and the organelles of eucaryotic cells. **FIGURE 1-2B** compares the sizes of atoms, biological molecules, viruses, bacteria, eucaryotic cells, and multicellular organisms.

A second feature used to define a virus is its complete dependence upon a **host cell** to reproduce itself. Viruses do not have functional organelles or ribosomes. Viruses are too small to carry enough genetic material to code for all of the gene products necessary to assemble a virus particle. As a result, a virus must direct the host's cellular protein synthesis machinery to synthesize viral proteins. The genome, or genetic material, of a virus consists of one type of nucleic acid, DNA or RNA. The DNA or RNA genome of a virus can be single or double stranded. **TABLE 1-3** compares the characteristics of living cells and viruses. Viruses are not alive because they are not cells, they require a host, and they do not use energy.

A **receptor-binding protein**, or viral-attachment protein, is present on the outer surface of virus particles. The viral receptor-binding protein adheres to **receptors** present on the surface of host cells. Cellular receptors function in processes such as chemical signaling to direct cells to divide, die, or allow certain chemicals to enter or exit. For example, rhinoviruses that cause the common cold bind to intercellular adhesion molecule-1 (ICAM-1). Within the host cell, ICAM-1 plays an important role in inflammation and intracellular signaling.

1.2 Early Virus Studies

Before the invention of the transmission electron microscope in 1931, viruses could not be visualized or grown in the laboratory. *Most important, viruses were defined as biological agents so small they could pass through* ***Chamberland porcelain ultrafilters****, which trapped most known bacteria* (**FIGURE 1-3**). The desire to discover viruses drove the early pioneers in virology to develop ways to observe and grow the invisible biological entities in the laboratory.

Bacteriophages and Plant Viruses: 1880s to 1930s

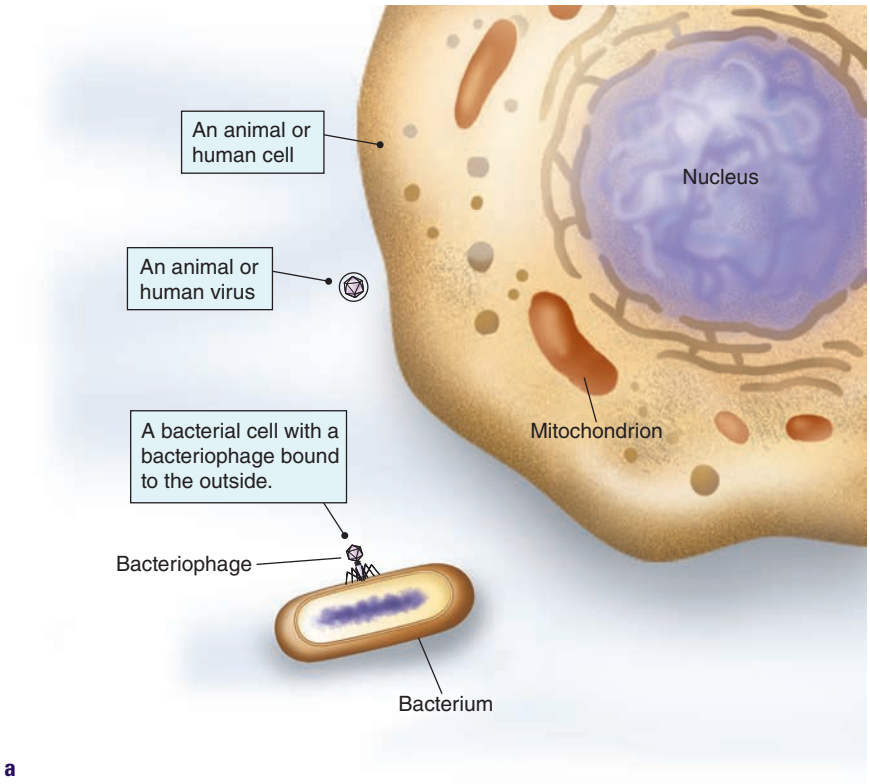
The initial observations and methods developed to study viruses involved bacterial and plant systems. The first virus that was discovered was **tobacco mosaic virus (TMV)**, which causes a disease that destroys tobacco crops. In 1892, the Russian botanist Dimitri Ivanovski (1862–1920) demonstrated that extracts from infected tobacco plants could transmit disease to healthy tobacco plants after passage through the Chamberland porcelain ultrafilters known to trap most bacteria (Figure 1-3). However, Ivanovski did not understand the full significance of this result. In 1898, Martinus Beijerinck (1851–1931) extended Ivanovski's experiments and was the first to develop the idea of a virus, which he called a *contagium vivum fluidum* ("contagious living fluid").

Bacteriophages, or *phages*, were first isolated from natural sources such as human sewage and studied by scientists in the early 20th century. Viruses that infect bacteria were named *bacteriophages*. The suffix *phage* comes from the Greek for "eating" because of their ability to eat, or *lyse*, bacteria. Bacteriophages replicate by introducing their genetic material into a host bacterium, directing the host's biosynthetic machinery to make copies of the bacteriophage genome (usually DNA) and protein coat, and then destroying the host bacterium. The destruction of the host bacterium releases new bacteriophages, which then go on to infect susceptible hosts.

Bacteriophage **plaque assays** are used to quantify the number of infectious bacteriophages in a given phage-containing sample. Briefly, bacteriophages are allowed to adsorb to host bacteria in a test tube. The mixture is then poured onto a solid agar plate of medium, and the bacteria and bacteriophages are allowed to replicate. The bacteriophages lyse the bacteria that are present on the surface of agar. The clearings (called **plaques**) in the bacterial lawn are areas where the bacteria have been killed by bacteriophages (**FIGURE 1-4**). When the bacteria are lysed during phage infection, it is said to be a **lytic infection**, in contrast to a **lysogenic infection**, in which infected host cells are not lysed and do not die during infection.

Visualizing Viruses with Transmission Electron Microscopy: 1930s

The **transmission electron microscope** was invented in 1931 to overcome the limitations of light microscopes to visualize nonbiological materials such as metals and small electronic parts. Light microscopes at that time could magnify specimens as high as 1,000 times. Instead of light rays, electron microscopes use a beam of electrons focused by magnets to resolve fine details of structures as small as 0.2 nm. With transmission electron



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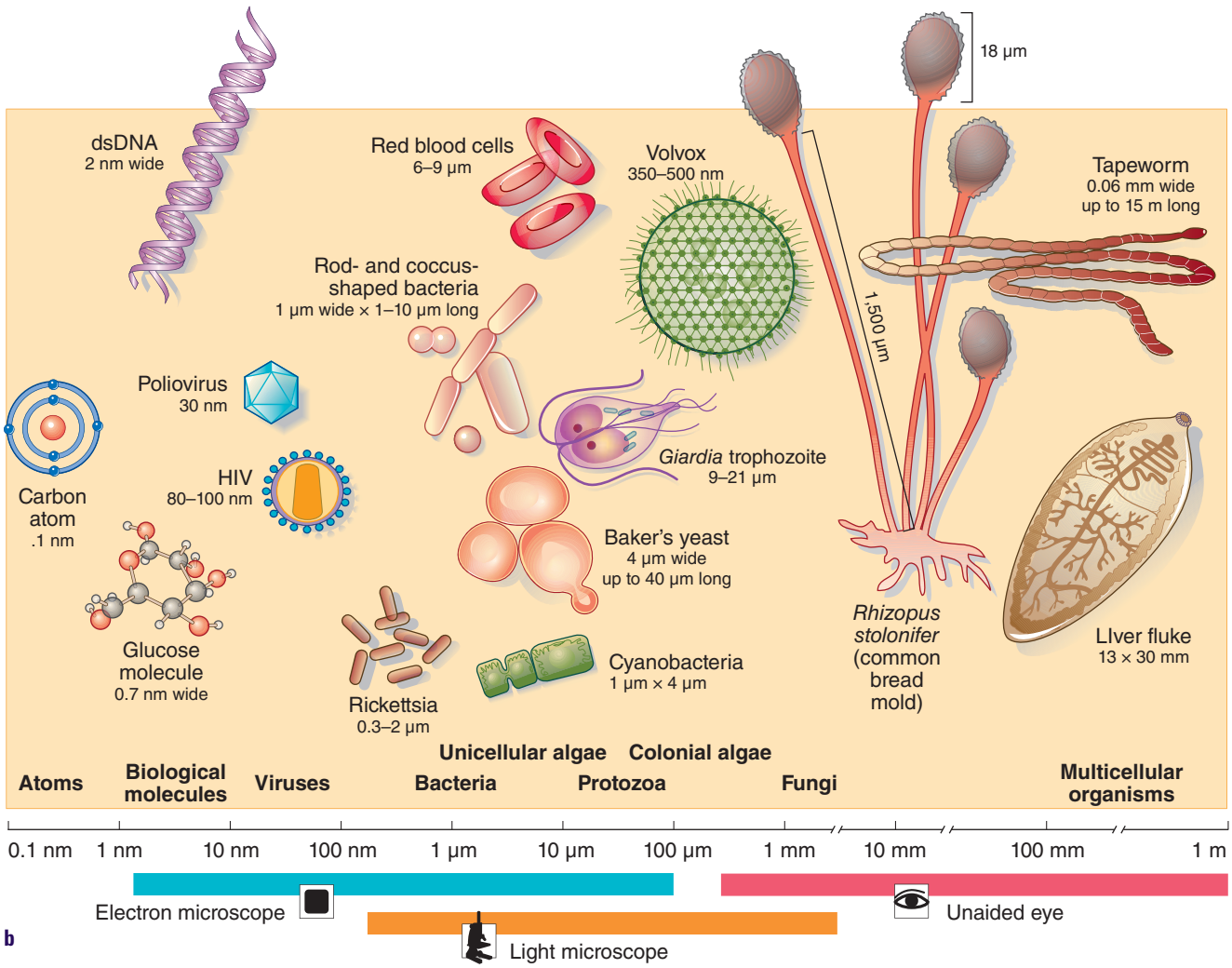

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FIGURE 1-2 **(a)** Viruses are smaller than their host cells. This illustration demonstrates size comparisons of a eucaryotic cell and organelles, a bacterium, an animal or human virus, and a bacteriophage. **(b)** Comparison of the sizes of atoms, molecules, viruses, the different kinds of host cells, and multicellular organisms. To see anything smaller than 500 nm in size, an electron microscope is needed.

Table 1-3 Comparison of Viruses and Cells

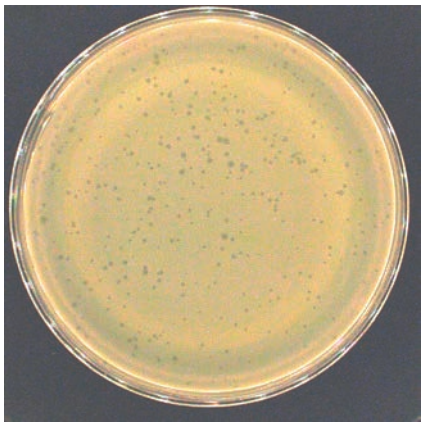
Characteristic	Virus	Cell
Structure/organization	Virus particles contain an RNA or a DNA core protected by a protein coat (capsid). The core/capsid of certain particles is wrapped by an additional membrane stolen from the host cell.	The cell is the basic unit of life. Organisms are unicellular or multicellular. Cells contain a cell membrane, cytoplasm, genetic material, and organelles.
Genetic code	DNA *or* RNA	DNA *and* RNA
Use and need energy (metabolize "food")	No	Unicellular and multicellular organisms require energy and a biochemical strategy to meet the energy requirement.
Irritability (response to environment)	No	Unicellular and multicellular organisms respond to internal and external stimuli.
Growth and development (increase in size or shape)	No	Unicellular and multicellular organisms grow and develop each new generation; specialization and differentiation occur in multicellular organisms.
Maintain homeostasis (ability to control internal environment)	No	Unicellular and multicellular organisms maintain a stable internal environment (e.g., maintain body temperature by sweating or shivering).

microscopy, it is possible to magnify structures 100,000 times and resolve them at 0.5 nm.

Virologists were quick to take advantage of this new, powerful tool. Bodo von Borries, Ernst Ruska, and Helmut Ruska published the first paper that clearly showed electron micrographs of ectromelia and vaccinia virions in 1938. Subsequently, Gustav Kausche, Edgar Pfankuch, and Helmut Ruska published the first transmission electron micrographs of TMV in 1939. Today, transmission electron microscopy continues to be a powerful tool in studying the structure of fragile viruses and how viruses are assembled within the host cell and in assisting in the rapid detection and diagnosis of viral infections (especially viruses that cannot be cultivated in the laboratory). The transmission electron micrograph in **FIGURE 1-5A** represents an image of the first isolation

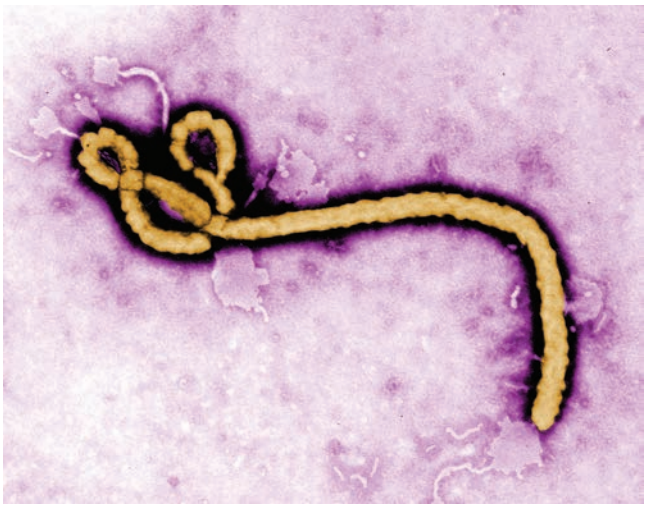
Courtesy of the National Museum of American History.

FIGURE 1-3 Chamberland porcelain ultrafilters were used to retain or remove bacteria from drinking water (1884). Pasteur and others discovered that *unfilterable germs* or bacteria could be retained by the filters, visualized by light microscopy, and grown on solid bacteriological media containing agar and as cultures in broths. Independent observations by Ivanovski (1892, 1899), Beijerinck (1898), and others demonstrated that infectious agents now known as *viruses* present in material responsible for causing a disease of tobacco plants passed through the porcelain filter to the bottom of the flask. The infectious agents were not visible by light microscopy and could not be grown on solid media containing agar or as broth cultures. The infectious agents in the bottom of the Chamberland filter remained infectious and caused disease in healthy tobacco plants. Chamberland filters were produced in 13 types: L1–L13; L1 filters had the coarsest pore size, L13 the finest.



Courtesy of Giles Scientific Inc., CA, www.biomic.com.

FIGURE 1-4 This is a bacteriophage plaque assay. Note the lawn of bacteria growing on the surface of the medium. Circular clearings or plaques present within the lawn are areas in which the bacteria were lysed or killed by bacteriophages.



a Courtesy of Frederick A. Murphy/CDC.

b Courtesy of James Gathany/CDC.

FIGURE 1-5 **(a)** Colorized transmission electron micrograph of Ebola virus particles isolated from a human diagnostic specimen and then cultured in Vero cells. Magnification 60,000X. **(b)** Visualization of SARS-CoV via the transmission electron microscope.

and visualization of Ebola virus in 1976. Some of the filamentous viral particles are fused together, end to end, giving it the appearance of a bowl of spaghetti. The transmission electron microscope was instrumental in the initial identification of the human coronavirus, the causative agent of **severe acute respiratory syndrome** (**SARS**), now known as **SARS-CoV**. **FIGURE 1-5B** shows **Centers for Disease Control and Prevention** (**CDC**) biologist Cynthia Goldsmith observing a viral isolate from the 2003 SARS outbreak through a transmission electron microscope.

Advances in Cultivating Viruses: 1930s to 1950s

As of 1930, only two vaccines had been developed to prevent viral diseases: smallpox and rabies. Both of these vaccines were created decades before the discovery of viruses. **Edward Jenner** used cowpox scabs to inoculate people in 1796. Cowpox causes a benign infection in humans that immunologically cross-reacts with Variola virus, the virus that causes smallpox. Immunity against cowpox also prevented or provided protection against smallpox. Years later, like cowpox, vaccinia virus was grown in the skin of calves to produce a vaccine.

Louis Pasteur succeeded in cultivating rabies viruses in 1885 by developing a method to obtain a consistent source of the "rabies agent." He removed pieces of spinal cord from a rabid street dog and inoculated it by trepanation under the dura mater into the cranium of a rabbit, followed by passing it from rabbit to rabbit about 20–25 times. In doing so, he had spinal cord material that was consistently virulent (i.e., infectious). He then removed 1-inch (3-cm) strips of spinal cord from the infected rabbits. He placed the strips in a vial that contained potassium on the bottom and gradually *attenuated* (weakened) the rabies viruses in the spinal cord material by exposing them to air. In Pasteur's application, inoculation with spinal cord extracts prepared from rabbits infected with attenuated rabies viruses prevented the signs and symptoms of rabies disease.

In the years following the pioneering work of Jenner, Pasteur, and others, microbiologists were able to grow bacteria in pure cultures on a solid or in liquid medium. This same method could not be used to propagate viruses, however, because viruses require a host to replicate. The only host to date was a living animal host, and virologists were unable to produce large amounts of a virus source that was free of contaminating bacteria and fragments of animal tissue.

In 1931, Ernest Goodpasture and the husband–wife team of Eugene and Alice Woodruff developed a way to produce fowlpox virus outside of a live chicken. Fowlpox is a relative of the viruses that cause smallpox and cowpox but cannot infect humans. They were able to cultivate the fowlpox virus in embryonated chicken eggs. This breakthrough allowed researchers to produce the first vaccines to prevent infection by yellow fever and influenza viruses in the 1940s. Most seasonal influenza vaccines are still produced in eggs today.

More advances in animal virology occurred from 1948 to 1960. In 1948, John Enders, Thomas Weller, and

Fredrick Robbins showed for the first time that polioviruses could be grown in non-nervous tissue. This was a significant contribution to the field of virology, because injecting humans with nervous tissue was a potential hazard. Renato Dulbecco and Marguerite Vogt followed their experiments by developing a protocol to cultivate large quantities of polioviruses in cell cultures and modified the bacteriophage plaque assay to a virus plaque assay using cell cultures. Plaque assays are used to quantitate preparations of human or animal viruses. The application of cell cultures to cultivate polioviruses resulted in the development of the Salk and Sabin poliovirus vaccines in the 1950s.

Renato Dulbecco was awarded a Nobel Prize in Physiology or Medicine for the development of animal virus plaque assays in 1975. **FIGURE 1-6** is a photograph of a laboratory technician in a Biosafety Level 4 (BSL-4) laboratory at the CDC counting plaques in a six-well dish containing crystal violet-stained monolayers of monkey kidney cells that were infected with viruses. Similar to bacteriophage assays, plaques, or clearings in the cell monolayer, are visualized where infected cells have been destroyed by viral infection. More viruses were used to infect the cell monolayers in the wells on the technician side of the dish than the wells of the dish on the side farthest away from the technician. Selected milestones in virology are presented in **TABLE 1-4**.

Courtesy of Dr. Scott Smith/CDC.

FIGURE 1-6 Plaque assays can be used to study animal or human viruses. The laboratory technician is counting the number of plaques (clearings) present in crystal violet-stained monolayers of monkey kidney cells that were infected with viruses.

1.3 Learning from Viruses

The Hershey-Chase Blender Experiment

Since their discovery, bacteriophages have taught us about the molecular biology of cells. One of the first bacteriophages used in the research laboratory, T2, infects the host bacterium *Escherichia coli*. The T2 bacteriophage consists almost entirely of a tightly condensed double-stranded DNA that is surrounded or packaged by a protein coat. The bacteriophage infects *E. coli* and utilizes the bacterial host cell to produce more T2 bacteriophages during a lytic infection (**FIGURE 1-7**).

In 1952, geneticist Alfred Hershey (1908–1997) and laboratory assistant Martha Chase (1927–2003) provided evidence that DNA was the hereditary material. They set up an experiment in which they asked the question: "What component of a T2 bacteriophage (protein or DNA) enters the host bacterium?" In their experiment, Hershey and Chase grew two cultures of *E. coli* in the laboratory. One flask of *E. coli* was infected with T2 bacteriophages in which the protein capsid of the bacteriophage was labeled with radioactive sulfur (^{35}S). The second *E. coli* flask was infected with T2 bacteriophages in which the genetic material (DNA) of the T2 phages was labeled with radioactive phosphorous (^{32}P). The radioactive T2 bacteriophages were allowed to attach and infect the *E. coli* (**FIGURE 1-8**).

After the phage infection was allowed to proceed over a defined period of time, the *E. coli* cultures infected with the two different radioactively labeled T2 bacteriophages were poured into separate blenders. The blender dislodged the bacteriophage particles from the bacterial host cells in each mixture. The mixtures were centrifuged (concentrated). This separated the bacteriophage particles in the supernatant liquid from the bacterial cells in the precipitate/pellet. Hershey and Chase found that the supernatant contained ^{35}S (the viral protein) and the precipitate (cell portion) contained the ^{32}P (the phage DNA). Based on these results, they concluded that the DNA labeled with ^{32}P transmitted the infective component of the bacteriophage. Thus, DNA was the hereditary/genetic material that specified all of the information needed to synthesize new T2 bacteriophages (Figure 1-8).

Bacteriophages and eucaryotic viruses continue to be used as biological tools, or our "eyes into cells," to study virus–host cell interactions and the molecular biology of cells. Viral infections are dynamic, involving different steps and interactions with a variety of cellular structures. Researchers study the fate of virus particles as they enter and direct cellular machinery to synthesize new virus particles. In doing so, we learn about the molecular processes of virus–host cell interactions, such as viral entry, internalization, intracellular trafficking, viral genome release and synthesis, particle assembly,

Table 1-4 Selected Milestones in the Field of Virology

Year(s)	Virologists/Investigators	Discovery
1884	C. Chamberland	Development of the porcelain ultrafilter, key to the discovery of viruses.
1885	L. Pasteur and E. Roux	Development of rabies vaccine.
1892–1898	D. Ivanovski and M. Beijerinck	First demonstrations of filterable plant virus (TMV), using Pasteur-Chamberland filter.
1898	F. Loeffler and P. Frosch	First demonstration of a filterable animal virus, foot-and-mouth disease virus, which causes disease in cattle.
1915	F. Twort	Bacteriophages.
1917	F. d'Herelle	Bacteriophages, plaque assay.
1931	J. Furth	Use of mice as a viral host.
1931	A. Woodruff and E. Goodpasture	Use of embryonated eggs as a viral host.
1933	W. Smith, C. Andrewes, and P. Laidlaw	Isolation of human influenza virus.
1938–1942	B. von Borries, E. Ruska, H. Ruska, G. A. Kausche, and E. Pfankuch	First transmission electron micrographs of viruses.
1948–1955	J. Enders, T. Weller, F. Robbins, and H. Eagle	Routine use of tissue cultures to cultivate and study viruses; development of optimal media for growing tissue cultures.
1954	J. Salk, J. Youngner, T. Francis, and others	Development of inactivated polio vaccine.
1954–1961	J. Salk, A. Sabin, H. Koprowski, J. Enders, S. Katz, and S. Krugman	Development of live polio vaccine, measles vaccine; produced in tissue culture (monkey kidney cells)
1957	A. Isaacs and J. Lindenmann	Discovery of interferon.
1969–1976	R. Huebner, P. Vogt, M. Bishop, H. Varmus, R. Weinberg, and others	Work on oncogenes.
1975	B. Blumberg, B. Larouze, W. London, and others	Relationship of hepatitis B virus with hepatocellular carcinoma.
1976	K. Johnson, P. Webb, J. Lange, F. Murphy, J. McCormick, and others	Discovery of Ebola virus.
1977	WHO, D. A. Henderson, F. Fenner, and many healthcare workers and virologists	Eradication of smallpox, a disease that killed more than 300 million people in the 20th century.
1970–2010	R. Zinkernagel, P. Doherty, M. Oldstone, B. Fields, B. Moss, A. Notkins, R. Ahmed, F. Chisari, N. Nathanson, and others	Major histocompatibility restriction and cytotoxic T lymphocytes, immune-mediated viral diseases, molecular pathogenesis.
1980–2010	H. zur Hausen, D. Galloway, D. Lowy, I. Frazer, and others	Recognition of subtypes of papillomaviruses associated with cervical and penile cancer and development of papillomavirus vaccine.
1981–1984	R. Gallo, F. Barre-Sinoussi, L. Montagnier, Y. Hinuma, J. Chermann, and others	Human retroviruses, including HIV.
1985	K. Mullis and others	Invention of polymerase chain reaction (PCR).
1991	C. Venter and H. Smith	Invention of shotgun cloning methods.
1994–2010	R. Webster, Y. Kawaoka, and others	Development of reverse genetics for influenza A viruses.
1990–present	W. I. Lipkin and others	Modern detection of new and emerging viruses.
2005	J. Taubenberger, P. Palese, T. Tumpey, A. Garcia-Sastre, Y. Kawaoka, and others	Molecular reconstruction and sequencing of the 1918–1919 influenza A virus; determination of viral genes associated with pathogenesis.

Information from Murphy, F. A. 2012. *The Foundations of Virology: Discoverers and Discoveries, Inventors and Inventions, Developers and Technologies.* West Conshohocken, PA: Infinity Publishing; Oldstone, M. B. A. 2014. "History of virology." *Reference Module in Biomedical Research*, 3rd ed. Boston: Elsevier.

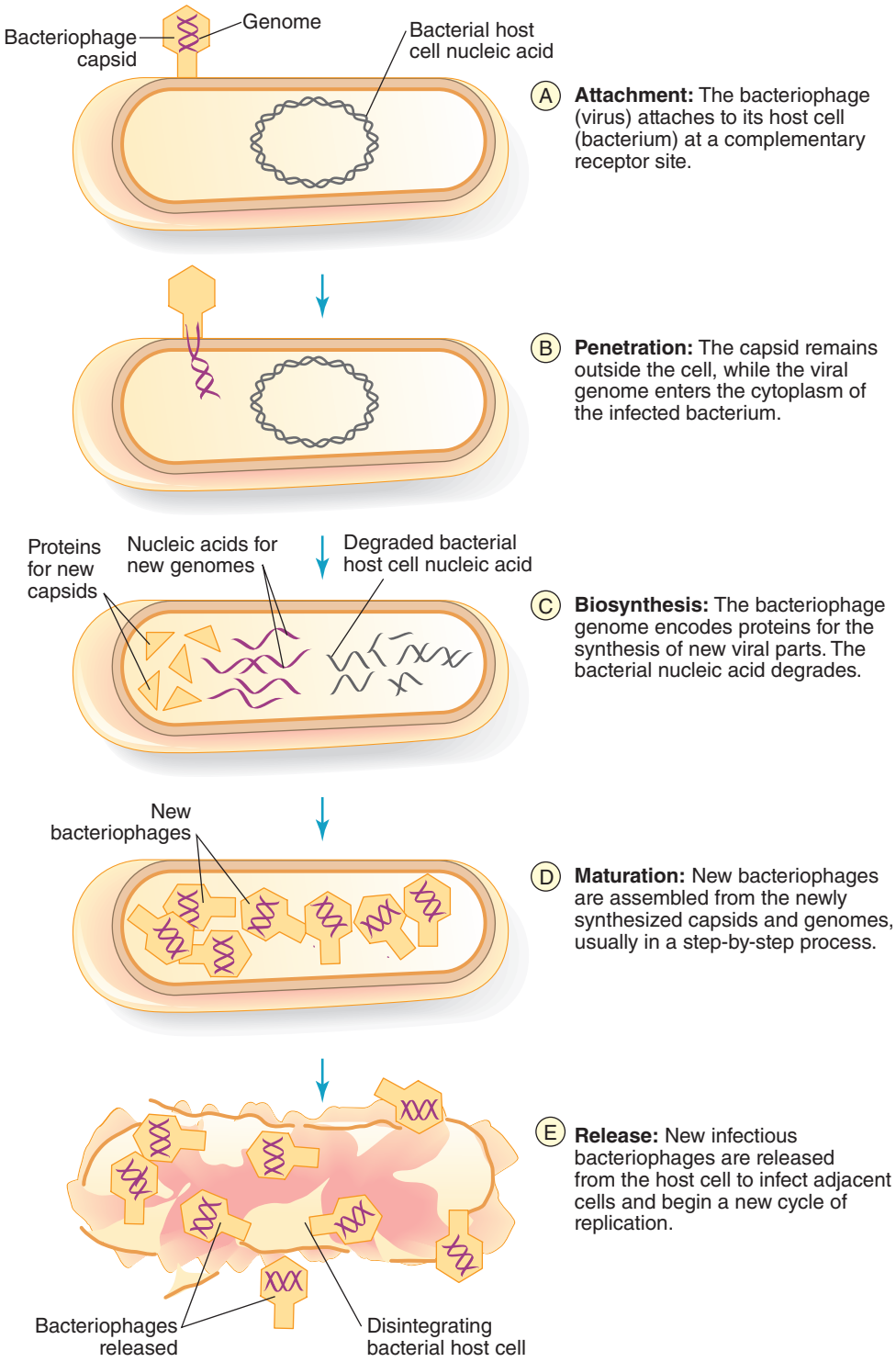


FIGURE 1-7 Diagram of the steps of a lytic bacteriophage infection.

and cell-to-cell transmission. We also learn more about basic cellular processes such as DNA replication, mRNA synthesis and regulation, protein synthesis, cytoplasmic transport by actin or microtubules, membrane formation, cellular transformation, proteolytic cleavage, and glycoprotein biosynthesis.

Today, these insights are accomplished by **single-virus tracking**, or **live cell imaging**, a microscopy method developed in 2006 in which viral components and relevant cellular structures are labeled with fluorescent probes to track the fate of individual virus particles or viral components inside of live host cells in **real time**. At the time of this writing, a new method was being developed and described by a team of researchers in Switzerland in *Cell Host and Microbe* in which researchers were able to visualize and quantify adenovirus genomic

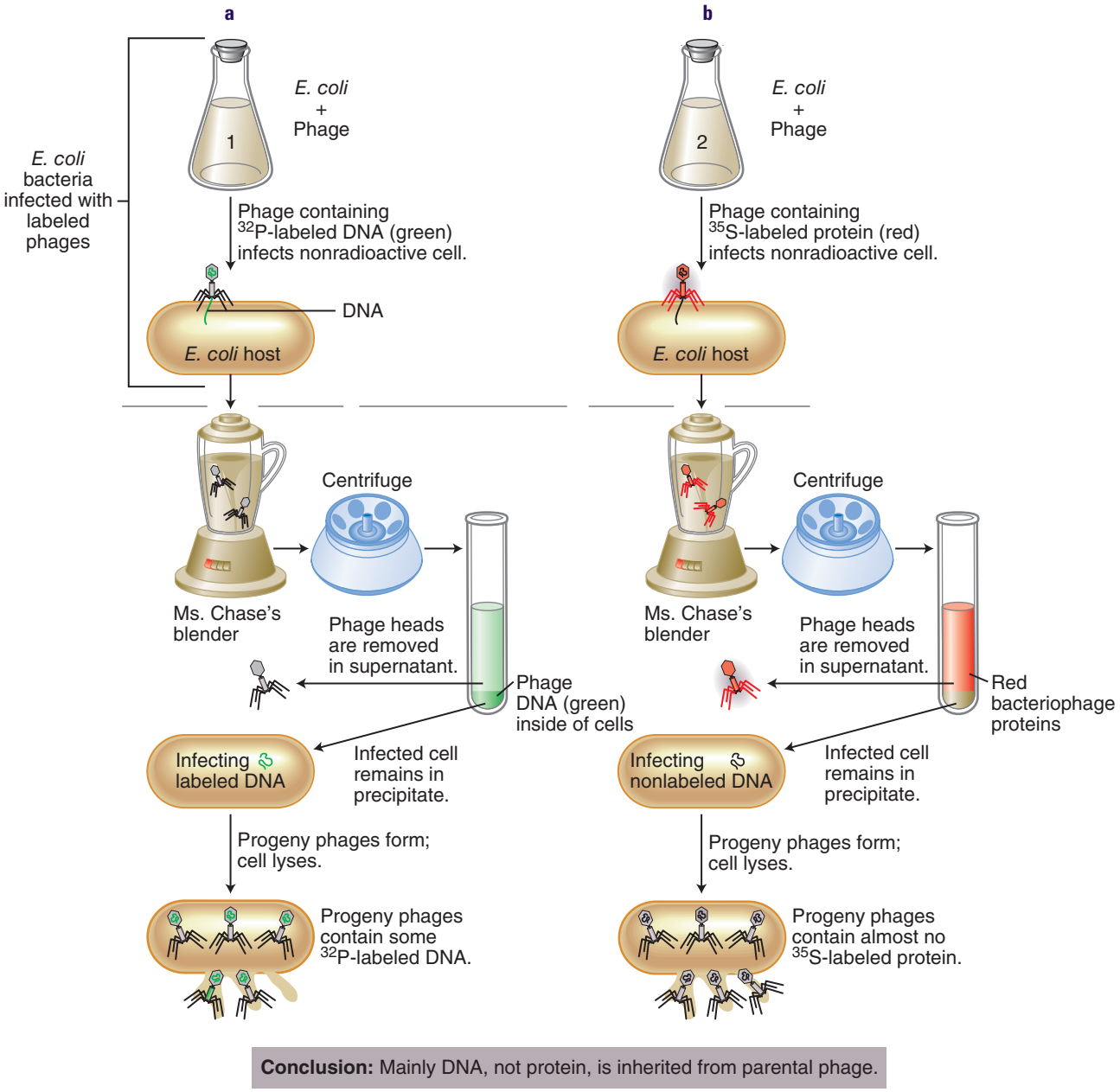


FIGURE 1-8 Diagram depicting the design of Alfred Hershey and Martha Chase's experiment. The ^{32}P-labeled phage DNA was found inside of the *E. coli* cells after infection, which provided the evidence that DNA was associated with the hereditary material of the bacteriophage.

DNA trafficked through a cell at single-molecule resolution. This method could be used to study the entire replication cycle of viruses containing DNA genomes. **TABLE 1-5** is a short list of viruses used in research to study different molecular processes of host cells and the steps that occur during virus trafficking in live cells.

Tracking Cold Sores and Human Migration

A number of pathogens have coevolved with their human hosts, such as *Helicobacter pylori* (a bacterium found in the gastrointestinal tract that can cause peptic ulcers), *Mycobacterium tuberculosis* (a bacterium that causes tuberculosis), and John Cunningham, or JC, virus (a virus found in 70–90% of the human population but that rarely causes disease). **Herpes simplex virus 1** (**HSV-1**) is best known for causing cold sores around the mouth. It is so widespread that nearly everyone has been infected by HSV-1 by the time they are elderly. Once an individual is infected with HSV-1, the virus hitchhikes and coevolves along with the individual for the duration of the person's life. Analyzing the genetic material (DNA) of HSV-1 strains provides an ideal opportunity to study long-term migration patterns of humans for several reasons. For example, HSV-1 forms lifelong latent infections and is spread by close contact, such as kissing or exposure to saliva, and usually family members have the

Table 1-5 Viruses Used to Study Molecular Biology Through Live Cell Imaging or Other Methods

Virus	Examples of Host Cells Used	Viral Steps Studied	Information Obtained About Host Cell Processes
Herpes simplex virus-1	Neural and glioma cells Baby hamster kidney cells	Viral entry	mRNA synthesis and regulation DNA replication Cellular transformation
Poliovirus	HeLa cells	Genome release	Host mRNA translation
Adenovirus	Human epithelial cells Primary baby rat kidney cells	Endocytosis/internalization of viruses	mRNA synthesis and regulation DNA replication Cellular transformation
Human immunodeficiency virus (HIV)	Modified HeLa cells Human primary fibroblasts	Viral entry Genome release Nuclear import Cell-to-cell transmission	Microtubule- or actin-dependent intracellular transport
Vesicular stomatitis virus	African green monkey kidney cells	Viral entry Endocytosis	Membrane formation Glycoprotein synthesis Actin-dependent intracellular transport

Information from Sun, E., Jiang, H., and Zhuang, X. 2013. "Live cell imaging of viral entry." *Curr Opin Virol* 3:34–43.

same strain. The HSV-1 DNA genome also is easy to collect (e.g., swabbing cold sore lesions) and is smaller than the human genome, and thus cheaper and faster to sequence.

Geneticists and evolutionary biologists hypothesize that humans from the earliest migrations out of Africa would have more genetic diversity than populations that have migrated more recently.

A study conducted by researchers at the University of Wisconsin–Madison compared the genomes of 31 distinct HSV-1 strains isolated from Europe, North America, East Asia, and Eastern Africa. The 31 strains were further divided into 6 clades numbered 1–6. In the study, a **clade** represented a group of HSV-1 isolates that had a common ancestor and all of its viral descendants (**FIGURE 1-9**). The clades were determined using **cladistics** software. The researchers used cladistics to analyze the sequences of the HSV-1 DNA genomes to create evolutionary trees representing groupings of the most diverse (oldest) to the least diverse (newest) genetically related HSV-1 isolates.

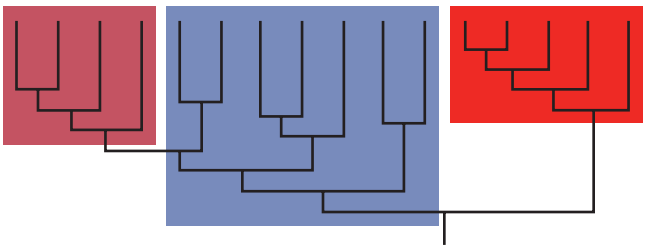
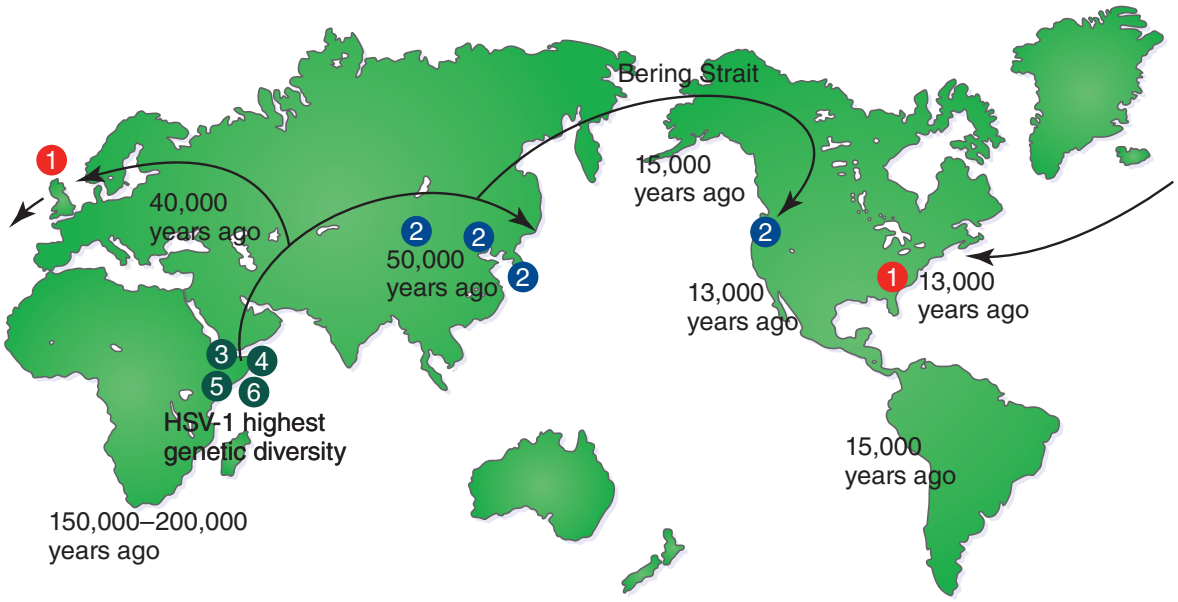


FIGURE 1-9 A clade is a section of a phylogenetic tree that extends to current organisms or, in this example, HSV-1. A clade must include all descendants from a common ancestor. In the diagram, the purple and red boxes are clades. The blue box is not a clade because it does not include all descendants from a common ancestor.

The HSV-1 study confirmed the theory that humans migrated "out of Africa" 2,000 generations ago (tens of thousands of years ago) and moved across the Earth by passing through the Middle East and then moving in separate directions to Europe, Asia, and eventually North and South America. The African strains had more genetic diversity than strains from locations that diverged from Africa. Almost all virus isolates from the United States were genetically similar to European strains, reflecting a European heritage. However, one strain from Texas was more similar to Asian strains, suggesting the possibility that this HSV-1 came during a human migration over a land bridge between Asia and North America (**FIGURE 1-10**). This study suggests that HSV-1 isolated from various populations can serve as a biomarker to study human populations and migration patterns.

1.4 Theories of Viral Origin

Viruses are everywhere. Wherever there is life, there are viruses! Evidence of viral infections can be found among the earliest recordings of human activities. The discovery of lesions present on the mummified body of **Ramses V** implicated smallpox as the young pharaoh's cause of death. Ramses V died approximately 1157 BC. The mummy of Ramses V is believed to be the earliest evidence of smallpox in human history. The mummy has



Information from Kolb, A. W., et al. 2013. "Using HSV-1 genome phylogenetics to track past human migrations." *PLoS ONE* 8:e76267.

FIGURE 1-10 Genetic analysis of HSV-1 isolates from various locations around the world provide support for the "out of Africa" theory of human migration. The 31 strains were further divided into 6 clades, numbered 1–6. The HSV-1 genomes representing clades 3–6 in Eastern Africa had the most genetic diversity, in contrast to clades 1 and 2 that had more recent HSV-1 viruses with less genetic diversity. The dates indicate arrival of modern humans in a given location.

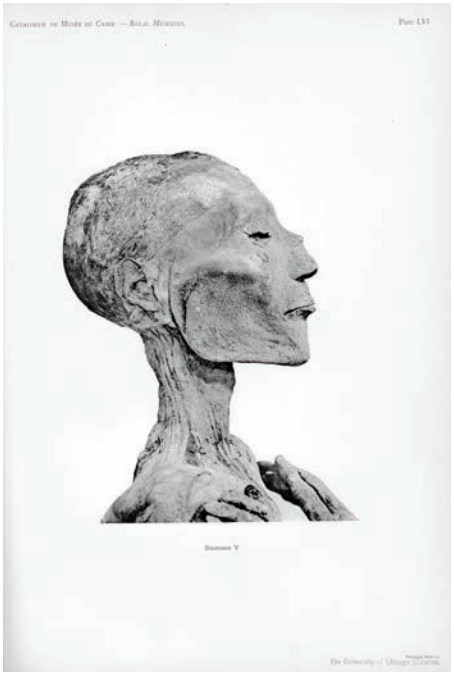
distinct crusts from pustular eruptions characteristic of smallpox present on the lower face, neck, and shoulders, but not the abdomen, giving an appearance of a **centrifugal rash** (**FIGURE 1-11A**). **FIGURE 1-11B** is a photograph of a boy suffering from smallpox in 1974. The rash is of a centrifugal distribution, with more pustules present on the boy's arms than on his abdomen. Another example of viral diseases in human history is that of the ancient Egyptian stele tomb carving depicting a polio-afflicted priest (circa 14th century BC) shown in **FIGURE 1-11C**. The foot drop deformity is characteristic of residual paralysis due to **poliomyelitis**.

Where did viruses come from? Could a cough or sneeze be a sign of a close encounter with a tiny visitor from outer space? The late Sir Fred Hoyle (1915–2001)—a world-renowned astronomer known for being controversial—and his former student Nalin Chandra Wickramasinghe proposed the **panspermia hypothesis**. This hypothesis asserts that viruses or other microorganisms are raining down upon Earth and contaminating it. Hoyle and Wickramasinghe proposed that these outer space microbes cause massive **contagion**, or epidemics, and new diseases. They speculated that influenza pandemics occurred in our history when solar winds during sunspot peaks caused influenza viruses to be swept down through the Earth's atmosphere. Hoyle speculated that diseases tend to strike during the winter season because cooler weather generates stronger downdrafts. Almost all members of the scientific community have dismissed the panspermia theory. Most scientists believe that cosmic radiation would almost certainly destroy germs in space.

Theories about the origins of viruses developed within the last couple of decades were of two trains of thought based on the results of molecular virology studies of the 1980s. The first view was that viruses were precursors of the earliest cells. The other view was that viruses originated from cells that underwent degeneration as a result of viral parasitism. The viruses were gene robbers that "broke away" as genetic elements from cellular genomes.

As technology improved, viral genomes were sequenced. These sequences did not resolve the debate. Instead, they threw considerable new light on viruses. It became evident that the genomes of viruses are so diverse that it is unlikely that all viruses evolved from a common single-celled ancestor named the **last universal common ancestor (LUCA)** that lived perhaps 3 or 4 billion years ago. As more viral sequences became available, especially the larger genomes of Mimivirus, Mamavirus, and Marseillevirus, a quiet revolution was brewing among evolutionary biologists. This is because many virus groups do not share any common genes, ruling out the idea that viruses have a common origin. Sequence analysis of viral genes has revealed at least five classes of viral genes (**TABLE 1-6**).

The classes of genes a virus possesses strongly depend on the size of the viral genome. For example,



a Courtesy of University of Chicago.

b Courtesy of Jean Roy/CDC.

c © SPL/Photo Researchers, Inc.

FIGURE 1-11 **(a)** Mummified head of Ramses V, who is thought to have reigned for only 4 years during the 20th Dynasty of Egypt. Ramses V was the son of Ramses IV and Queen Ta-Opet. Ramses V died in his 30s from smallpox in 1157 BC. The mummy was found in the tomb of Amenophis II by Egyptologists and is now located in the Royal Mummy Hall of the Museum of Egyptian Antiquities in Cairo, Egypt. Ramses V is one of the best-preserved royal mummies. **(b)** Boy suffering from smallpox in a Bangladeshi village in 1974. The photograph shows a centrifugal rash covering the face and extremities as opposed to the entire body. **(c)** Egyptian priest from the 18th Dynasty (14th century BC) with foot drop deformity.

viruses with small RNA genomes often have only a few genes. The majority of these viral genes belong to the hallmark class. Viruses with larger DNA genomes, such as the poxviruses, possess all five classes of genes. More than 80% of the genes found in the moderate and large genomes of bacteriophages and archaeal viruses are **ORFans**. ORFan genes are open reading frames that have no known homologs and no known function. They have been found in prokaryotes such as *E. coli*. Some researchers theorize the ORFan genes were acquired from bacteriophages.

The discovery of the giant Mimivirus called into question the definition of a virus. Mimivirus, which was isolated from a cooling tower in Bradford, England, was first thought to be a new *Legionella*-like bacterium. It was discovered during an investigation to find the source of a pneumonia outbreak in 1992. About 10 years later, it was determined that the source was not a bacterium but rather a giant virus that was able to grow inside of an amoeba. Its entire DNA genome was analyzed. The genome of the Mimivirus was not only large in size *but contained genes that were not found in any other known viruses*.

Table 1-6 Classes of Viral Genes

Class	Gene Description
1	Viral genes that have closely related *homologs** in cellular organisms (especially hosts of given viruses).
2	Viral genes that are conserved within a major group of viruses that have distantly related cellular homologs.
3	Virus-specific genes that have no detectable cellular homologs. These genes are referred to as ORFans.
4	Virus-specific genes that are conserved in a broad group of viruses but have no detectable homologs in cellular life forms.
5	Genes shared by many diverse groups of viruses with only distantly related homologs in cellular organisms. These are referred to as **viral hallmark genes**.

*A homolog is a gene sequence that is similar to a gene sequence in the cellular (host) genome.

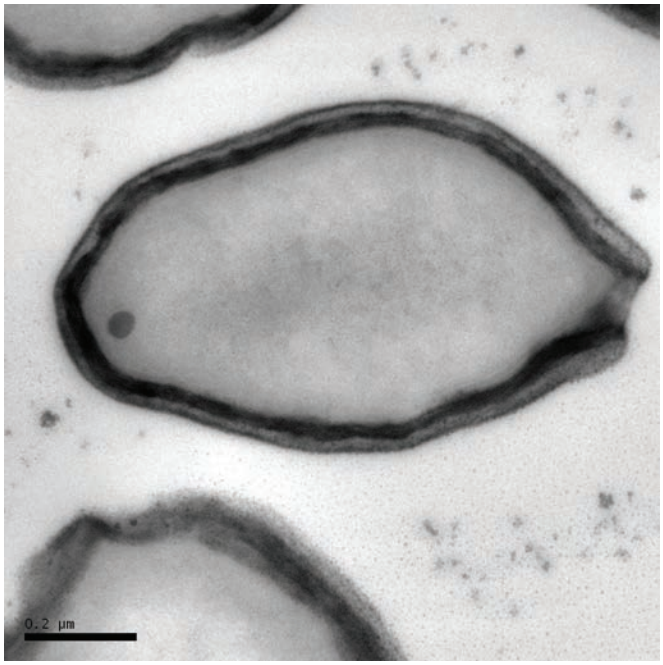
Reprinted from Koonin, E. V., et al. 2006. "The ancient virus world and evolution of cells." *Biology Direct* 1:29; doi10.1186/1745-6150-1-29.

These novel genes were **homologs** of genes. The homolog gene products were involved in protein synthesis, a process that occurs only in cellular organisms! In 2008, it was discovered that a virus 50 nm in size within the viral factories of Mimiviruses was replicating inside of the amoebic host. The virus was classified as a **virophage** (viruses that infect viruses) and named **Sputnik**. *This was the first time virologists determined that a virus could infect a virus!* The findings put another spin on the origin of large DNA viruses. On the basis of these observations, Raoult and Forterre (2008) suggested that biological entities be divided into two groups: ribosome-encoding organisms and capsid-encoding organisms, which include viruses.

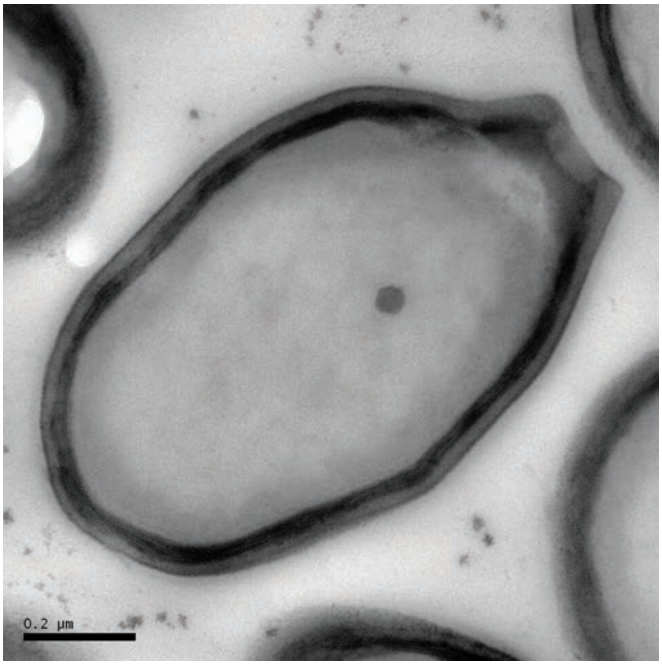
Even larger viruses were discovered in 2013 by researchers at Aix-Marseille University in France. Two new gargantuan viruses were found inside of amoebae. The viruses were isolated from sediment removed from the mouth of the Tunquen River near the coast of central Chile and from sediment located at the bottom of a freshwater pond near Melbourne, Australia. The viruses appeared to be identical (**FIGURE 1-12**). They were ovoid-shaped (more like the shape of a cell), 1-µm particles (the same size as the bacterium *E. coli* and 1,000 times the size of influenza viruses) that contained dsDNA genomes of 2.5 and 1.9 megabases in length. The large particles could be cultivated inside of laboratory-grown amoebae, demonstrated strong cellular lytic activity, and were insensitive to antibiotics, suggesting that they were large viruses. The researchers proposed that these two newly discovered viruses belong to a new genus of viruses called pandoravirus. Out of 2,556 pandoravirus genes, only 200 are familiar genes seen in other viruses or bacteria or eucaryotes. Therefore, more than 93% of the pandoravirus genes are unique; their origin cannot be traced to any known cellular or viral homologs. The researchers state that the genomes of the giant DNA viruses may represent a fourth domain of life.

The **hydrothermal origin hypothesis**, also called the *iron-sulfur (FeS) world hypothesis*, postulates that the first **organic chemical structures** were formed at warm alkaline thermal vents or fissures (long, narrow openings) found in the ocean seafloor (**FIGURE 1-13A**) around 3.7 billion years ago (**FIGURE 1-13B**). A very crude model of the evolution of life and current viral genomics is shown in **FIGURE 1-13C**. Supporters of this theory claim that hydrothermal vents leaked hot sulfuric acid into the surrounding environment, creating a gradient between the hydrothermal vent water and the extremely cold water surrounding the vent at the bottom of the ocean. The temperature at the cooler temperatures would have been suitable for organic chemical synthesis to occur.

The vents contained iron sulfide and iron nickel sulfide that acted as catalysts fueled by chemical energy (H_2 from the hydrothermal environment and CO_2 from the marine environment), resulting in the formation of the organic precursor molecules for the building blocks of life (e.g., amino acids, sugars) in the cooler surrounding environment (about 100°C). As the biological melting pot cooled off, different classes of viruses emerged from different genetic elements at different stages.



Philippe, N., et al. 2013. "Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes." *Science* 341:281–286.



Courtesy of Chantel Abergel.

FIGURE 1-12 Transmission electron micrographs of purified pandoraviruses.

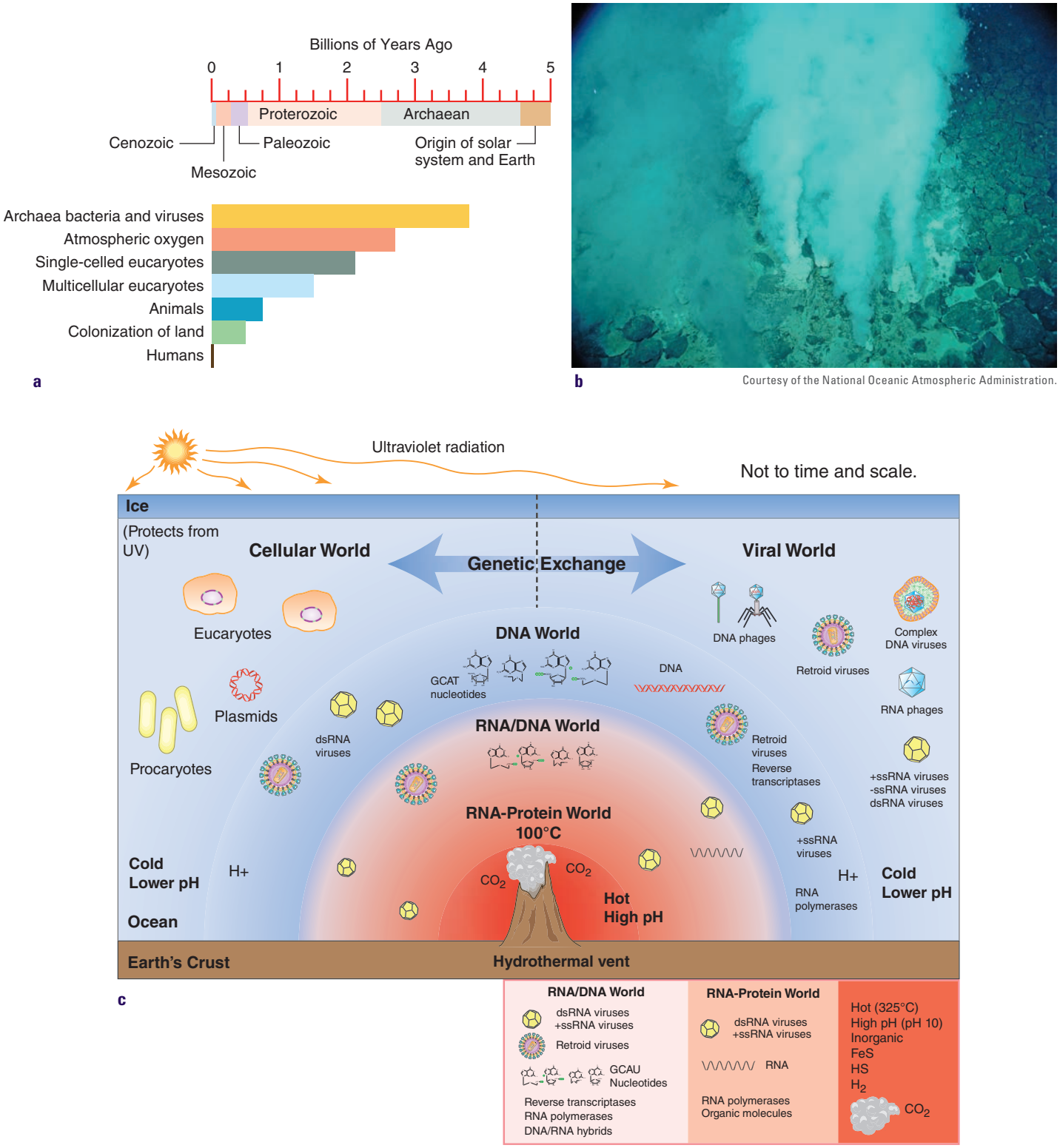


FIGURE 1-13 **(a)** Geologic clock of life. The first life on Earth emerged about 3.7 billion years ago. **(b)** The thriving communities surrounding this hydrothermal vent may represent the environments where life began on Earth. This extremely gassy, "white smoker," high-temperature Champagne hydrothermal vent was found during an NOAA Ocean Explorer expedition at a depth of 1,500 meters (0.93 miles) at NW Eifuku volcano of Japan's Volcano Island chain. **(c)** Model of the evolution of life and current viral genomics. The environmental conditions in porous hydrothermal vents in the crust of the ocean served as a "battery" made from H_2 and CO_2 to fuel the creation of the first chemical precursors of life, organic molecules, which subsequently assembled to form protoviruses and protocells around 3.7 billion years ago. The first organic molecules to form were RNA and proteins, followed by DNA. RNA viruses evolved first. Over time, the formation of DNA led to the coevolution of DNA viruses and their procaryotic or compartmentalized eucaryotic host cells. At the "DNA world" stage, opportunities for genetic exchanges occurred between DNA viruses and procaryotic and eucaryotic cells.

The early viruses must have self-replicated, even if very poorly. RNA viruses (viruses containing RNA genomes) evolved first, followed by **retroid viruses**, and subsequently DNA viruses. Retroid viruses use reverse transcriptase to replicate their genomes. Over the course of 10 to 100 million years, complex gene assemblies evolved during the DNA stage, resulting in the emergence of new compartmentalized cells and large DNA viruses (which contain DNA genomes). During the evolution of the DNA viruses, there was an explosive evolution of eucaryotic cells. Continuous horizontal gene transfer occurred between viruses and their host cells, which may help explain why viral genes can be found in the human genome or other host genomes and why human or other host genes can be found in viral genomes. The study of the evolution of viruses is entering a new and exciting stage of development. Recent advances in genomics and structural biology, along with **bioinformatics** tools, will allow researchers to test alternative evolutionary models.

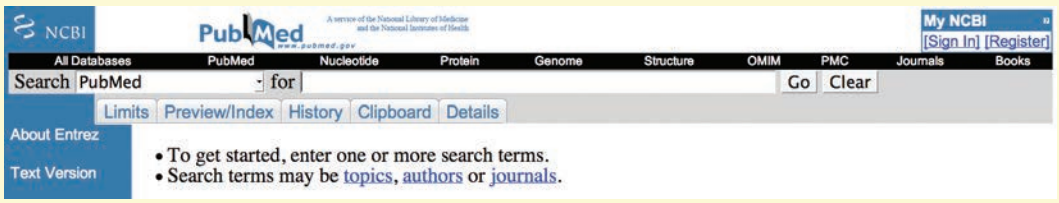
VIRUS FILE 1-1 Use of PubMed, ScienceDirect, CDC Publications, ProMED-mail, and HealthMap to Research Specific Viruses or to Monitor Viral Outbreaks

Each chapter of *Understanding Viruses* contains one or more **Virus Files**. These files or synopses are intended to connect students to research being conducted in the field of virology. References to the original research articles, or **primary literature**, will be provided at the end of each Virus File. Students and instructors interested in exploring topics further may opt to search for more information via **PubMed**, which is a service of the National Library of Medicine (**FIGURE 1**). It includes over 22 million citations for biomedical articles dating back to the 1950s. These citations are from MEDLINE and additional life science journals. PubMed searches can be done by topic, author, journal title, and other parameters. To begin a PubMed query, start at the PubMed website (http://www.ncbi.nlm.nih.gov/pubmed).

A similar leading source for technical and medical research is **ScienceDirect**. It contains articles from more than 2,500 peer-reviewed articles (http://www.sciencedirect.com).

Most Virus Files cite reports published by the CDC, such as *Morbidity and Mortality Weekly Report (MMWR)* and *Emerging Infectious Diseases* (*EID*). The archives of these publications can be quickly searched from the CDC's website (http://www.cdc.gov).

If you are particularly interested in virus-related outbreaks, another valuable resource is **Program for Monitoring Emerging Diseases (ProMED)-mail**. ProMED-mail was established in 1994 by a small group of scientists. ProMED-mail is intended to address the threat of disease outbreaks in remote corners of the world that could spread across continents in days or weeks—much faster than the doctors could spread word about the disease. ProMED-mail is the "CNN" of outbreak reporting. This e-mail list posts a variety of information, including on-the-ground observations, media stories, and government reports. ProMED-mail members have helped to diagnose infectious diseases ranging from camelpox in Saudi Arabia to measles in military personnel in Kazakhstan to SARS in China. Today, ProMED-mail has more than 60,000 subscribers in at least 185 countries. This form of early warning disease reporting often beats local authorities in reporting outbreaks. Archives of reports can be searched via the mail site within the ProMED-mail website (http://www.promedmail.org). ProMED-mail collaborates with **HealthMap** at Children's Hospital in Boston. HealthMap (http://healthmap.org/en/) was created in 2006 as an informal source for disease outbreak monitoring and real-time surveillance of public health threats.



Courtesy of PubMed/NCBI.

FIGURE 1 PubMed interface. PubMed is the most frequently used search engine for medical research applications.

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1.5 The Helpful or Collaborative Viruses

Parasitoid Wasps and Polydnaviruses

In spite of the common perception that viruses are pathogens, more and more evidence is revealing the ways in which viruses benefit their hosts through **mutualistic** interactions (**TABLE 1-7**). The most studied example of this type of relationship is that between **parasitoid wasps** and polydnaviruses. A **polydnavirus** is an insect virus that contains a genome composed of multiple segments of dsDNA, or *polyDNA*. Female parasitoid wasps prey upon caterpillars, "stinging" them when they inject their eggs into the caterpillars (**FIGURE 1-14A**). As the eggs are deposited, the female wasp also deposits polydnavirus particles that only express *wasp genes that paralyze the caterpillar*, allowing the wasp eggs to develop into embryos and then larvae. The larvae feed on the inside of the caterpillar until they break out of the caterpillar carcass to form pupae that develop into adult wasps.

The polydnaviruses have coevolved with the wasp for so long that the viral genes essential for replication and assembly of polydnavirus particles have been inserted into the wasp's chromosomal DNA as a **provirus**. The wasp genes that code for venom-like proteins that inhibit the caterpillar's immune system by paralyzing its ability to encapsulate and kill the wasp eggs have been inserted into the DNA of the polydnavirus "paralysis" particles. The polydnaviruses are dependent upon the wasp to form new virus particles that contain wasp genes that suppress the caterpillar so that the wasp eggs survive. The two make a living off of each other (**FIGURE 1-14B**). In scientific terms this can be referred to as **obligatory mutualism**.

Plant–Cryptic Virus Collaborations

Over the past 125 years, hundreds of viruses from diseased crop plants have been discovered. It was not until the 1960s and 1970s that **cryptic viruses** were isolated from plants. Cryptic viruses can be described as **persistent viruses** that establish lifelong associations with their host but do not cause disease in them. Aphid lethal paralysis virus (ALPV) is an example of a cryptic plant virus linked to a decline in aphids colonizing wheat fields of the Western Province of South Africa in the 1980s. Laboratory experiments showed that aphids infected with ALPV developed unusual behavior, such as moving away from their food source, uncoordinated in their movement, followed by paralysis and death. This plant–virus collaboration is a survival strategy that functions to sicken the aphid predator but extend the life of the plant (wheat) host of ALPV. For this reason, investigations on the application of ALPV as a natural **bioinsecticide** to control aphid pests or the insertion of ALPV genes into plants to create transgenic aphid-resistant crops are under way.

A number of other helpful plant viruses enable their hosts to survive changes in the environment, such as drought or changes in temperature. For example, when rice is infected with brome mosaic virus (BMV), tobacco

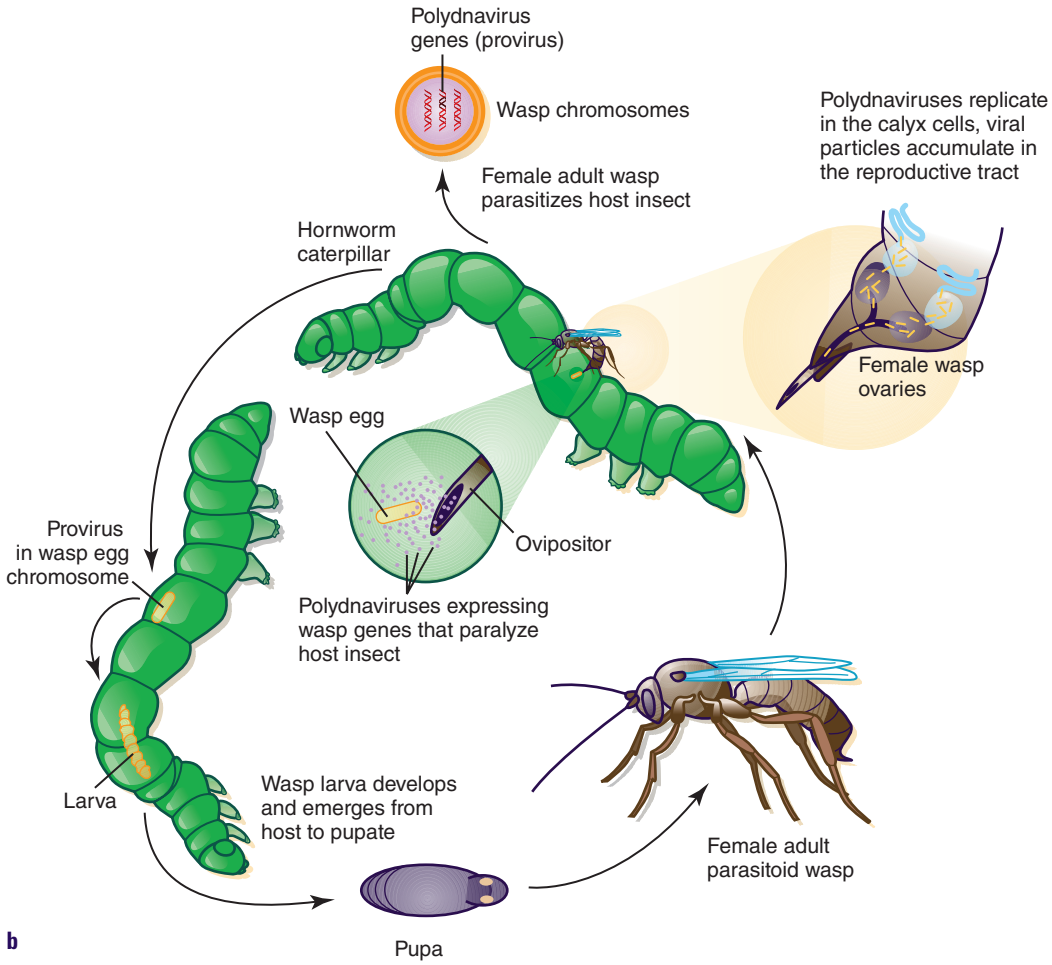
Table 1-7 Mutualistic Viruses

Virus	Host	Benefits
Polydnaviruses	Parasitic wasps	Essential for development/survival of wasp egg in caterpillar.
Bacteriophages	Bacteria	Provides non-host-derived defense to metazoan mucous surfaces while bacteriophage evolves to invade different types of bacteria.
Endogenous koala virus (KoRV)	Koala bears	Koalas that harbor KoRV are protected from lymphomas and leukemia.
Cytomegalovirus	Mice	Protects host from bacterial infections (*Listeria monocytogenes* and *Yersinia pestis*).
Dysaphis plantaginea densovirus	Rosy-apple aphids	Virus is essential for the development of wings, allowing the aphids to colonize new plants when food is less abundant and plants are crowded.
Aphid lethal paralysis virus (ALPV)	Aphids	ALPV is a cryptic plant virus that paralyzes and kills aphids on wheat as a survival strategy (for the wheat and ALPV).
Killer yeast L-A dsRNA virus	*Saccharyomyces cerevisae* (yeast)	Some killer yeast L-A viruses carry a dsRNA molecule that encodes a killer toxin that kills contaminants/competing yeast strains present in wine, beer, or bread production. The toxin does not harm the starter yeast in these fermentations and benefits from this toxin only under certain conditions (e.g., if the pH is high, the toxin loses its function).

Information from Roossinck, M. J. 2011. "The good viruses: Viral mutualistic symbiosis." *Nature Reviews Microbiology* 9:99–108.

a

Courtesy of Alexander Wild.



b

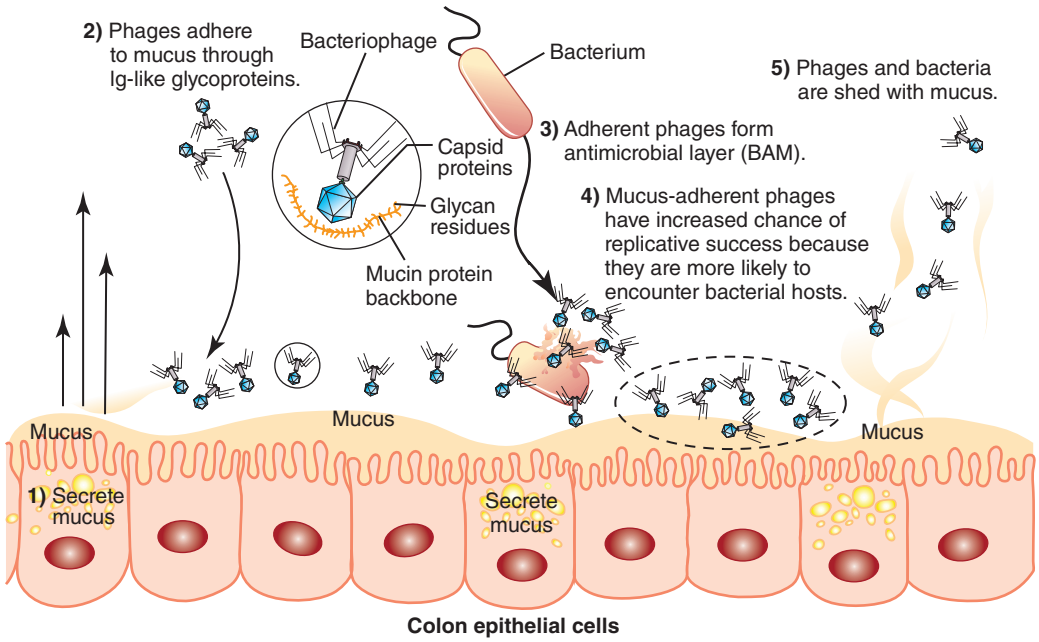
FIGURE 1-14 **(a)** A parasitic *Cotesia* wasp attacking a hornworm caterpillar. **(b)** Illustration showing the mutualistic relationship between parasitoid wasps and polydnaviruses. The adult female parasitoid wasp lays her eggs along with polydnaviruses inside of a hornworm caterpillar. The polydnaviruses contain wasp genes. The wasp genes are expressed in the caterpillar, preventing the caterpillar from encapsulating, walling off, and killing the wasp egg. The wasp chromosome contains the polydnavirus genes involved in viral replication and packaging, allowing new virus particles to be made in the wasp. In order to continue their existence, the polydnaviruses need the wasp, and the wasp needs the polydnaviruses.

is infected with TMV, or cucumbers, peppers, watermelons, squash, beets, and tomatoes are infected with cucumber mosaic virus (CMV), the plants are resistant to drought. Beets infected with CMV survive cold conditions. The mechanisms for these observations are not known.

Bacteriophage "BAM Velcro"

Many **metazoans** (multicellular animals) such as humans, animals, and fish secrete a layer of mucus to protect themselves from invasion by bacterial pathogens. The mucus is continuously secreted and shed, maintaining a thickness of 10–700 µm depending on the body site (e.g., lung, airways, colon). The mucous layers are composed of hundreds of negatively charged glycoproteins, providing both structure and nutrients to support bacterial colonization. Also present in the mucus are bacteriophages that embed themselves head first into the mucus by attaching to host defensive **immunoglobulin (Ig)-like glycoproteins** present in the mucus.

The capsid surface of the bacteriophage head is "sticky." It contains a protein called **highly antigenic outer capsid** (**hoc**) that binds to the Ig-like glycoproteins in the mucus. The protruding bacteriophage tails bind to invading bacteria, infecting and killing them. The **bacteriophage adhering to host mucus (BAM)** community forms an antibacterial defense or "velcro" in the mucous layers of diverse hosts (**FIGURE 1-15**). As the bacteriophages continue their fate within the BAM community, they continue to infect and kill invading bacteria. In doing so, the metazoan host is protected from bacterial infections that could potentially cause damage or harm to it. The chemical properties of the host mucous layer and the sticky bacteriophage hoc proteins appear to have coevolved as a mutualistic relationship between the bacteriophage and its metazoan host.



Information from Barr, J. J., et al. 2013. "Bacteriophage adhering to mucus provide a non-host-derived immunity." *PNAS* 110:10771–10776.

FIGURE 1-15 Model for the formation of a BAM community consisting of mucous layers of the colon or gut embedded with bacteriophages. The mucus of BAM protects the host by reducing infections by bacteria, and the bacteriophages provide an added non-host-derived antibacterial defense that also benefits the bacteriophages by increasing their chances for replication when the phages encounter bacteria trapped by mucus within BAM.

Herpesvirus "Protection"

The literature is replete with examples of viruses that cause disease in humans; however, there are some examples of viruses that are beneficial to their human hosts. All humans become infected with different herpesviruses during childhood. Herpesviruses persist in a dormant state, or **latency**, for the duration of a person's life. During latency, herpesvirus particles are not being produced. It has been suggested by researchers that the lifelong persistence is a symbiotic relationship that confers immune benefits or protection against other pathogens. For example, mice infected with a type of herpesvirus known as a cytomegalovirus are resistant to infection by the bacterial pathogen *Listeria monocytogenes*, a causative agent of serious foodborne illness, and *Yersinia pestis*, which causes the **Black Death**, or **plague**. Cytomegaloviruses stimulate the host's **innate immune response**, protecting the host from subsequent infections. It appears that the latency of the cytomegalovirus is a symbiotic relationship with immune benefits for the host. Additional examples of beneficial viruses can be found in the literature despite the common perception of viruses as pathogens. Some viruses provide a benefit only under certain environmental conditions. Others have adapted to their hosts such that the host and virus cannot survive without each other (Table 1-7). *It is likely that more examples of mutualistic viruses will be discovered in the future, especially if researchers open their minds to the possibility that viruses are not all bad.*

1.6 Human and Aquatic Viromes

Viruses on Human Skin

Human skin is an important physical barrier that provides protection against assault by pathogenic microbes, including viruses, and toxic substances. Conversely, skin harbors various types of microorganisms, especially bacteria, that are referred to as normal flora or **microbiota** (**FIGURE 1-16**). About 2–5 pounds or 1–3% of our body weight consists of microbes that are residents of our skin, intestines, mouth, nose, and genitals. The human **microbiome** is defined as the total of all microbiota that lives on or inside of the human body. The **Human Microbiome Project**, a logical conceptual and experimental extension of the **Human Genome Project**, was launched in 2008 to identify and determine the roles of human microbiota. The human microbiome is quickly becoming an important field of biomedical research.

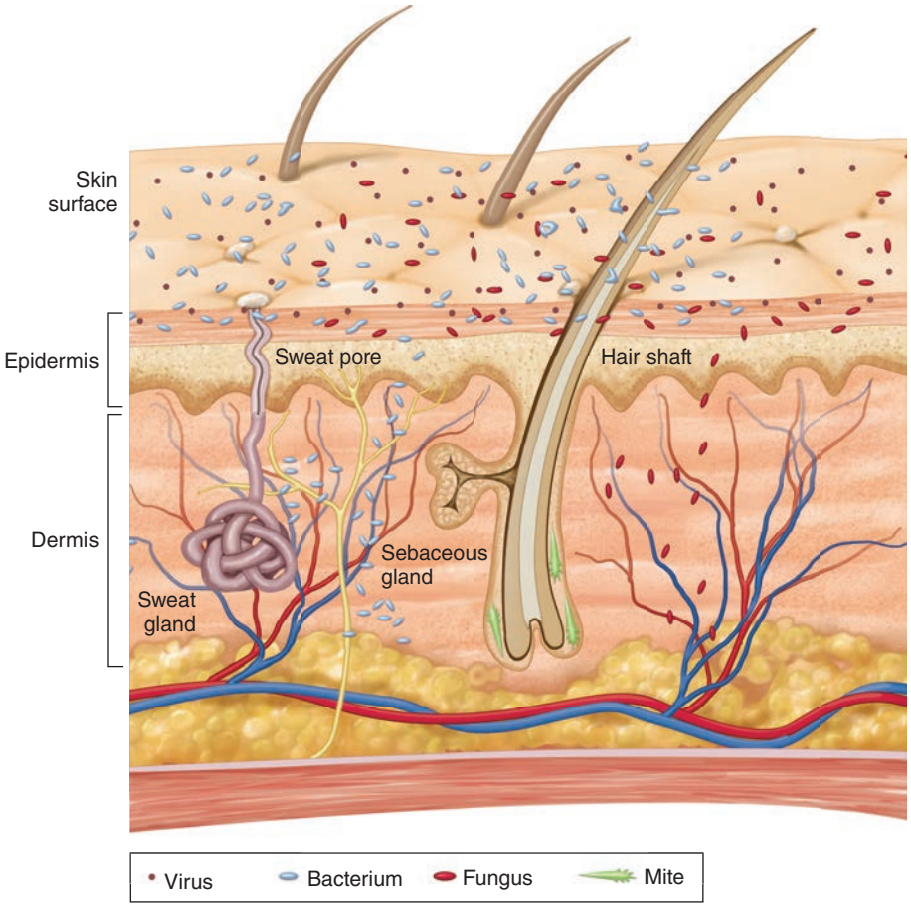
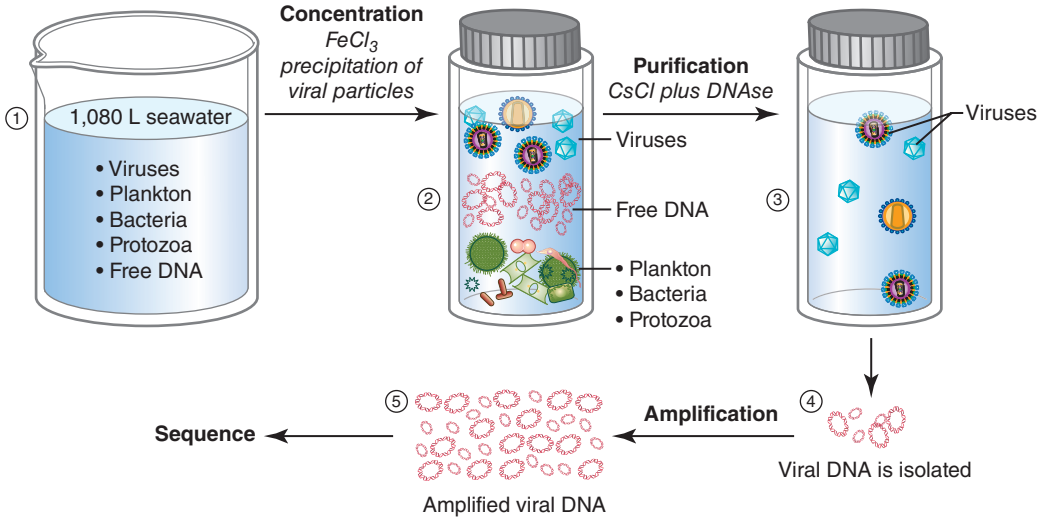


FIGURE 1-16 Cross section of human skin, including hair follicles, sebaceous (oil) glands, and sweat glands, along with the locations of diverse microbiota and viruses. Viruses may be free or found inside of cells. Bacteria, fungi, and viruses are present on the skin surface and upper epidermis. Bacteria and fungi are found in sweat pores as well as in sweat and sebaceous glands. Mites live in or near the hair follicles.

Most of the microbiome studies have focused on developing an understanding of the diversity of bacteria living on and within the human body. The skin is a unique and variable ecosystem that can change in temperature, pH, and humidity. Little is known about human-associated viral communities (the human **virome)** present on skin, mainly because most skin-associated viruses cannot be isolated and grown under laboratory conditions and there is no universal **genetic marker** shared by all viral genomes that can be targeted by high-input molecular methods such as sequencing. In contrast, bacterial diversity is much easier to investigate because all bacteria share a common 16S ribosomal RNA gene marker.

Metagenomics is a fast-growing field in which nucleic acids are isolated directly from environmental samples and sequenced, requiring no culturing or a priori knowledge of what viruses may be present, in order to study microbiomes present in the natural environment (**FIGURE 1-17**). The earliest viral metagenomics research, led by Mya Breitbart, was performed in 2002 and applied to a 200-liter sample of surface seawater collected from Scripps Pier located in La Jolla, California. Subsequently, Breitbart's research team applied viral metagenomics to analyze the uncultured virome from human feces. These early studies revealed that viral diversity had been underestimated. Viral metagenomics was first applied to human clinical research in 2008 when a team of researchers detected a new arenavirus in three patients who died shortly after receiving organ transplants from the same donor. Other new pathogenic viruses have also been found using viral metagenomic studies (**TABLE 1-8**).

Less than a handful of studies have been directed at viruses commonly found on human skin. In 2012, a small study in which the faces of five people with healthy-looking skin and one patient with **Merkel cell carcinoma (MCC)** were swabbed as the source of genetic material to investigate the virome of human skin. MCC is a rare skin cancer caused by Merkel cell polyomavirus and mainly affects elderly and immunocompromised individuals. **Merkel cell polyomavirus** was discovered in 2008 as a new cancer-causing virus. The results of this small study revealed the existence of a diverse array of papillomaviruses, polyomaviruses, circoviruses, and bacteriophages on healthy-looking skin samples. The skin virome of the patient with MCC contained a much higher proportion of Merkel cell polyomaviruses compared to the healthy skin samples and 17 different human papillomavirus strains not identified in the healthy skin samples. The human skin virome requires more investigation in order to determine whether these resident viruses play a role in protection against other



Information from Duhaime, M. B., and Sullivan, M. B. 2012. "Ocean viruses: Rigorously evaluating the metagenomic sample-to-sequence pipeline." *Virology* 434:181–186.

FIGURE 1-17 Overview of the steps of a viral metagenomics procedure. (1) An environmental sample is collected from the ocean. (2) Virus particles in the sample are concentrated away from other organisms and free DNA present in the sample. (3) The viral particles are purified (by removing bacteria and other organic material present). Free organismal DNA is degraded using DNase. (4) The viral DNA (which was protected from DNase inside of the virus particle) is isolated from the virus particles. The viral DNA is amplified using **polymerase chain reaction (PCR)**. (6) Amplified viral DNA is sequenced and analyzed.

pathogens or whether their fluctuations are influenced by the immune status of the host.

It is estimated that about 10^{13} to 10^{15} bacteriophages populate the human body. Bacteriophages may play an important role in regulating bacterial communities by influencing healthy and disease states in humans. In 2009, a metagenomic study of human airway viromes used sputum samples from healthy individuals, individuals who had **cystic fibrosis (CF)**, and an individual with mild asthma. Cystic fibrosis is an inherited disease, sometimes called **mucoviscidosis**, in which thick, sticky mucus builds up in the lungs, digestive tract, and other organs of the body. The strong-mucus phenotype was mapped to the **cystic fibrosis transmembrane conductance regulator** (**CFTR**) gene. CTFR codes for a faulty cyclic adenosine monophosphate

Table 1-8 Clinical Applications of Viral Metagenomics

Year of Study	Disease or Symptoms	New Virus(es) Discovered	Source of Genetic Material
2008	Merkel cell carcinoma	Polyomavirus	Biopsies of skin cancers
2008	Diarrhea	Astrovirus, torque tenovirus, norovirus, picobirnavirus, enterovirus, nodavirus	Stool (feces)
2008	Fatal transplant-associated illness	Arenavirus	Brain, cerebrospinal fluid, serum
2009	Hemorrhagic fever	Arenavirus	Liver biopsies, serum
2009	Acute flaccid paralysis (sudden weakness or loss of muscle tone)	Bocavirus, picornavirus, circovirus, nodavirus, dicistrovirus	Stool
2010	Pandemic influenza	Influenza A virus (2009 H1N1)	Nasopharyngeal swabs
2010	Encephalitis	Astrovirus	Brain biopsy (frontal cortex)
2012	Lower respiratory tract infection	Rhinovirus C	Nasopharyngeal swabs
2012	Tropical fever illness	Circovirus	Serum
2013	Asymptomatic in humans (no illness)	Marseillevirus-like virus	Human blood donations in France

Information from Fancello, L., Raoult, D., and Desnues, C. 2012. "Computational tools for viral metagenomics and their application in clinical research." *Virology* 434:162–174.

(cAMP)-regulated chloride ion transporter that plays a role in decreasing water movement into mucus, leading to dehydrated airway surfaces and concentrated mucus. CF patients suffer from chronic bacterial infections of the lungs commonly caused by the following bacteria: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia*. Significant differences in the bacteriophage communities were observed in CF patients compared to healthy individuals. Antibiotic-resistant genes were also detected in the bacteriophages colonizing CF patients. These genes could potentially be transferred to bacterial communities in the airways, making them resistant.

In 2012, researchers compared the genetic diversity of *Propionibacterium acnes* bacteriophages recently isolated from healthy individuals and those with acne attending a dermatology outpatient clinic in Los Angeles, California, to *P. acnes* bacteriophages isolated over 30 years ago from an acne patient in Philadelphia, Pennsylvania. *P. acnes* is a bacterium that lives in the oily or lipid-rich anaerobic environment deep within hair follicles and pores of skin. During puberty, the presence of *P. acnes* increases as much as 100-fold or more, causing inflammation and acne in about 85% of the population. It was discovered that unlike other well-studied bacteriophages, all of the *P. acnes* bacteriophages lack genetic diversity. *P. acnes* bacteriophages produce a protein called **endolysin**, which breaks down the peptidoglycan component of bacterial cell walls before killing the bacteria. These features may make *P. acnes* bacteriophages an ideal natural predator for the development of a topical phage-based therapy for acne.

Aquatic Viromes

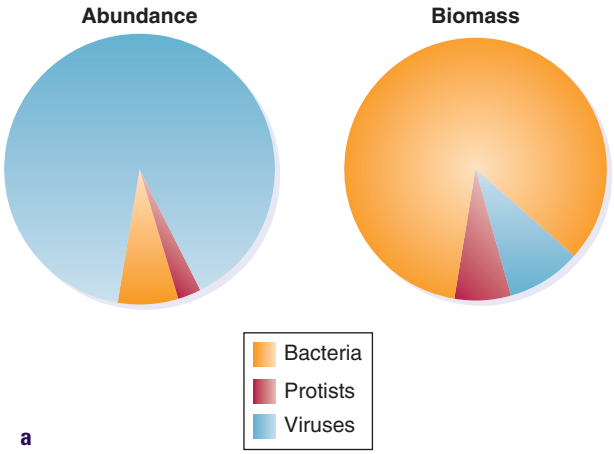
The ecological role of bacteriophages in the ocean has been the subject of intense investigation for nearly two decades. It is estimated that there are about 10^7 bacteriophages present in a milliliter of surface seawater and over 100 quintillion (or 100 billion billion) bacteriophages for every human that has ever lived on Earth. For each bacterium in the ocean there are 15–25 virus particles. Despite their small size, viruses are the most abundant biological entities in the ocean, even though they only comprise about 5% of the biomass of bacteria, protists, and viruses combined in the ocean (**FIGURE 1-18A**).

Before the 1990s, it was expected that bacterial and planktonic populations would dramatically increase if viruses were not present in marine water or freshwater because their host organisms were freed from the pathogenic effects (death) caused by viral infection. Pioneering experiments during the 1990s demonstrated that when bacteriophages were selectively removed from seawater, bacterial populations stopped growing completely, because they depended upon nutrients released from their lysed bacterial hosts killed by the bacteriophages. Without the death of microbes by viruses, there was no "fuel" to maintain the aquatic community. This gave rise to the food loop, or **viral shunt hypothesis** in which viruses are believed to be significant biological agents involved in the mortality (death) of aquatic microorganisms and thus responsible for recycling carbon and other nutrients necessary to support living organisms of aquatic communities (**FIGURE 1-18B**). That is, the bacteriophages *shunt* organic material to stimulate the growth of new bacteria and other organisms in the aquatic ecosystem.

Curtis Suttle at the University of British Columbia estimated that ocean virus may turn over 150 gigatons of carbon every year—more than 30 times the standing abundance of carbon in marine plankton. Viral infections influence microbial ecology and diversity, evolution, and health of all living organisms in the ocean.

In 2009, scientists studying the ecosystems of the pristine freshwater lakes of Antarctica found incredibly diverse types and high numbers of previously unidentified viruses present in the icy lakes. The lakes contain very little animal life (**FIGURE 1-19**) and are devoid of penguins or seals. The vast majority of life present in the lakes is microbes: bacteria, algae, protozoa, and zooplankton. These microscopic communities are adapted to very extreme conditions. In the winter, there is nearly complete darkness and the lakes remain frozen. During the summer, ultraviolet radiation is intense and the ice melts, resulting in open water in parts of the lake. In contrast to what scientists found in other aquatic systems, the most abundant viruses in the Antarctic lakes during the summer ranged from 50 to 150 nm in diameter. The viruses were mainly bacteriophages and algal viruses. During the spring, there was a reduction of the larger viruses and an increase in viruses that were less than 30 nm in diameter. The viruses likely play a role in controlling the microbial populations during the seasonal transition of the ice-covered lakes in the spring to open-water lakes in the summer.

Recently, more sensitive methods have become available to detect plant viruses in water. The survival of plant viruses in water has become of interest because of increased irrigation and use of hydroponic systems (**FIGURE 1-20A**) to grow plants in soil-free cultures. Ponds, lakes, canals, rivers, and reservoirs may serve as the **freshwater** sources for irrigation. Hydroponic systems use freshwater, rainwater, or municipal water (**FIGURE 1-20B**). In hydroponics systems the water remains in the system and is reused. Freshwater sources may harbor viral and microbial pathogens, including plant viruses. Using hydroponic systems and irrigation may be a source of water-transmissible plant pathogens. To date, the following types of plant viruses have been detected in freshwater: carmoviruses, cucumoviruses, dianthoviruses, necroviruses (such as TMV), potexviruses, and tobamoviruses. The majority of these viruses are stable and infectious. More research is needed to determine



Information from Suttle, C. A. 2007. "Marine viruses—major players in the global ecosystem." *Nature Reviews Microbiology* 5:801–812.

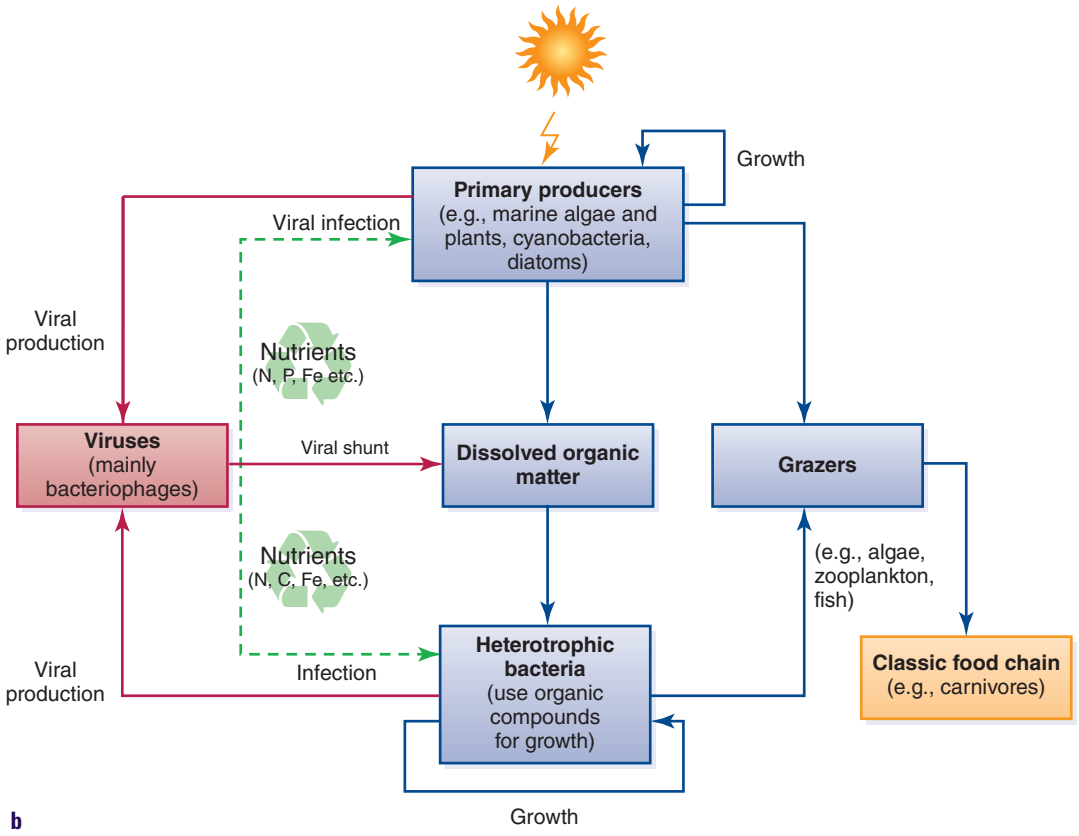


FIGURE 1-18 **(a)** Comparison of biomass and abundance of bacteria, protists, and viruses in the ocean. Viruses are the most abundant biological entity (94%; the majority are bacteriophages) but represent only 5% of biomass. Bacteria represent the most biomass (90%); however, protists can represent 50% of the biomass in the surface waters. **(b)** Simplified diagram of the viral shunt hypothesis incorporated into the marine microbial food chain. In the viral shunt process, viruses lyse bacteria and other microbes, resulting in dissolved organic matter that can be used by bacteria and other microbes for growth and reproduction. Essentially, the viruses are involved in a microbial recycling program that stimulates energy and nutrient cycling.

whether the list of plant viruses found in freshwater is of biological significance.

1.7 Applications of Viruses in Health or Medicine

Bacteriophage Therapy

If you experience a bacterial infection that is not healing on its own, a physician will prescribe an antibiotic such as **penicillin** or amoxicillin to treat the infection. Before the discovery of penicillin in 1939, though, the medical community was focusing its attention on the use of bacteriophages to kill bacteria pathogenic to humans. Frederick Twort (England) and Frederick d'Herelle (Canada) first described bacteriophages in 1915 and 1917, respectively. Before the use of penicillin, it was determined that each type of bacterium can only be infected with a specific type of bacteriophage. In other words, bacteriophages exhibit a very narrow host range: a bacteriophage that infects *E. coli* will not infect *Streptococcus pneumoniae*.

The idea of **bacteriophage therapy** formed the basis of applied medical research and of the 1924 Pulitzer Prize–winning novel *Arrowsmith* by Sinclair Lewis. Researchers in Eastern Europe began to use bacteriophages to treat a wide range of infections caused by bacteria. They

Courtesy of Ken Kloppenberg.

FIGURE 1-19 Researchers' tents are dwarfed by Lake Bonney and the Taylor Glacier, one of Antarctica's dry valleys. Scientists are trying to find out which viruses can survive such extreme conditions.

practiced bacteriophage therapy to treat infections of the skin, mouth, ears, nose, throat, eyes, and urinary tract, as well as postsurgical infections. In some cases, a liquid containing bacteriophages was poured onto an open wound; in others, the bacteriophages were given orally, by aerosol, or injected. The majority of the research in this time period was published in Russian, and therefore was not easily accessible to Western scientists. The results were varied, and when antibiotics came into the mainstream bacteriophage therapy largely faded in the West, but it continued in the East in countries such as Poland and Russia. Because bacteriophages selectively kill bacteria and cannot cause infections in humans, bacteriophage therapy represents a safe form of biological control that encompasses clinical, agricultural, and food-safety applications.

Bacteriophage therapy is now being reconsidered as a weapon against bacterial **superbugs**. Superbugs are lurking in hospitals, causing deadly infections that cannot be treated with the strongest antibiotics currently available. Research and development of alternative therapies are urgently needed to combat antibiotic-resistant bacterial pathogens.

An important clinical application of bacteriophage therapy is in the treatment of CF patients. Individuals who suffer from CF lack the enzymes in their lungs and other body cavities to reduce mucus, resulting in the accumulation of viscous, sticky mucus, which compromises the lungs. People with CF are particularly susceptible to *P. aeruginosa* bacterial infections in the lungs, which cause respiratory failure in 80–90% of CF patients. Many of the clinical isolates from the lungs of CF patients have been shown to be resistant to antibiotic therapy. A research team recently demonstrated that two phages isolated from sewage (ϕMR299-2 and ϕNH-4) were able to infect and kill *P. aeuruginosa* strains isolated from CF patients. The bacteria were marked with a bioluminescent tag and inoculated intranasally into mice. The observation of luminescence indicated intact bacteria. The decrease in luminescence indicated reduced bacterial populations in the mice (**FIGURE 1-21**). This study reinforces the potential to use bacteriophage therapy to treat antibiotic-resistant bacterial infections of CF patients.

Research on bacteriophage therapy is also being directed toward the treatment of bacterial biofilms present in root canals and on medical devices such as

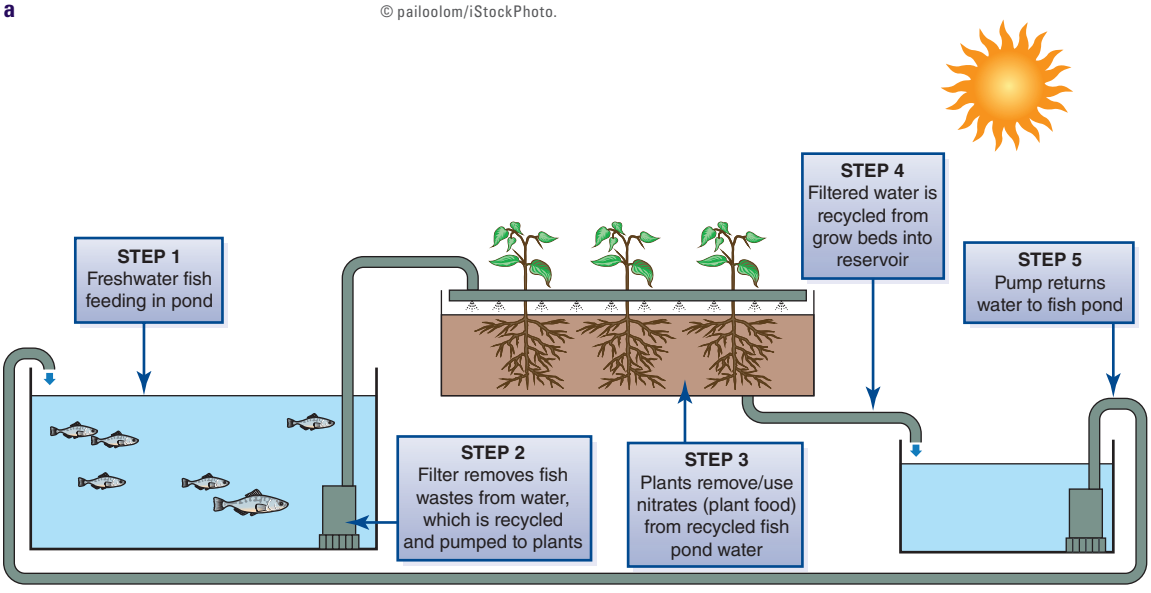
catheters using bacteriophages that attack *P. aeruginosa*. **Biofilms** are bacterial communities containing different types of bacteria that attach to a surface, forming a protective slime layer that resists the effects of antibiotics and the body's defenses (**FIGURE 1-22**). About 65% of all bacterial infections involve biofilms. Because of the time it takes for regulatory approval of bacteriophage therapy clinical applications, many companies and researchers are pursuing bacteriophage therapy for food-safety and agricultural applications. For example, LISTEX P100 and ListShield have been approved as bacteriophage cocktails to control and prevent the contamination of the food-borne pathogen *Listeria monocytogenes* on ready-to-eat meat products. *L. moncytogenes* causes **listeriosis** and presents serious complications in pregnant women, resulting in miscarriages, stillbirths, premature deliveries, or life-threatening infections of the newborn.

Agriphage has been approved as a safe, natural bacteriocide that can be used against agricultural crop pathogens such as *Xanthomonas campestris* and *P. syringae* that cause bacterial spot and speck (especially in tomatoes and peppers). One milliliter of Agriphage contains over 4 billion bacteriophages. It is typically mixed with fertilizers and sprayed on plants in the field and in greenhouses throughout the entire growing season. Agriphage was to significantly increase crop yields. Continued investments in research and development and well-controlled trials in many countries will be needed to overcome regulatory and technical hurdles before bacteriophage therapy will receive acceptance and approval for real-world clinical use in Western medicine.

Gene Therapy

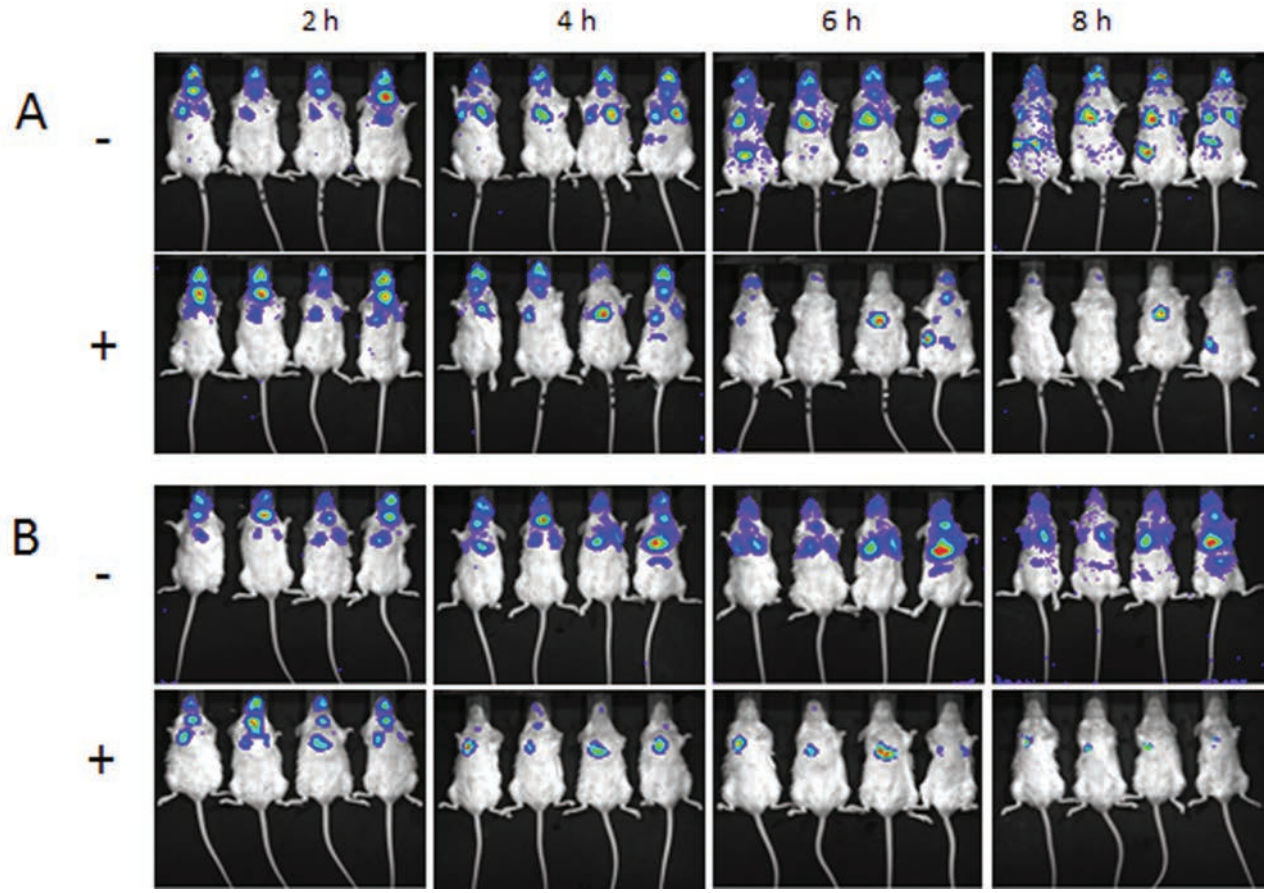
Each of us carries a few defective genes. We remain blissfully unaware of this fact unless one of our close relatives or friends suffers from a genetic or hereditary disease. Most of us do not suffer any harmful effects from a defective gene, because we carry two copies of nearly all genes (one inherited from each parent). If one of our genes is defective, the activity of the second (functional)

a



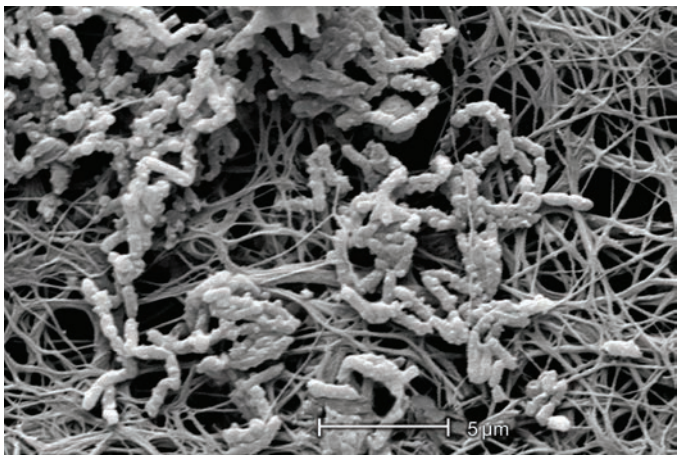
b

FIGURE 1-20 **(a)** Outdoor vegetable hydroponics. **(b)** Diagram of hydroponic system that represents a closed system. Freshwater is efficiently recycled but may also be more vulnerable to infection by viruses not trapped during the filtering process.



Alemayehu, D. et al., 2012. "Bacteriophages ψMR299-2 and ψNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells." *mBio* 3(2). doi:10.1128/mBio.00029-12.]

FIGURE 1-21 Mice were infected with two different strains of *Pseudomonas aeruginosa* and then treated with a bacteriophage mix. Blue luminescence indicates the presence of the *P. aeruginosa* cells. The (+) indicates infected mice treated with bacteriophages, whereas (–) indicates untreated mice. Panel A contains mice infected with a strain of *Pseudomonas* that does not produce any capsular slime on the surface of the bacteria. Panel B represents mice infected with a strain of *P. aeruginosa* that produces a capsular, sticky slime layer on its surface. The mice treated with the bacteriophages show decreased luminescence/growth of *P. aeruginosa*. This represents a potential application of bacteriophage therapy to treat CF patients with *P. aeruginosa* lung infections.



Courtesy of the CDC/Rodney Donian.

FIGURE 1-22 This is a scanning electron micrograph of the segment of a patient's catheter containing a biofilm of rod-shaped bacteria in association with fibrin-like material on the surface of the catheter.

gene product might compensate to reduce the effects of the defect. The redundancy of genes may be beneficial for survival.

In 1990, W. French Anderson, R. Michael Blaese, and Kenneth Culver—pioneer researchers at the *National Institutes of Health (NIH)*—announced results of the first clinical **gene therapy** trial to genetically correct the **adenosine deaminase (ADA) gene** belonging to a 4-year-old girl suffering from **severe combined immunodeficiency disease (SCID)**. SCID is caused by an ADA deficiency; this rare but very serious defect causes immune system deficiencies, resulting in the lack of normal immune system protection against bacterial and viral infections.

How was a functional copy of the ADA gene delivered to the girl? The hero was a retrovirus. A retrovirus was genetically engineered to carry a correct ADA gene producing functioning ADA, consequently eliminating

the root cause of the immune deficiency disease. To date, the girl is alive and well.

In addition to retroviruses, adenoviruses also are being used in clinical gene therapy trials to deliver genes. The history of gene therapy has been on a roller-coaster ride. Eighteen-year-old Jesse Gelsinger died in 1999 at the University of Pennsylvania after receiving gene therapy with an adenovirus infused into his liver. Gelsinger suffered from **ornithine transcarbamylase deficiency (OTCD)**, an inherited liver disease in which ammonia builds up in the blood. He died of multiple-organ failure caused by an immune reaction against the adenovirus. Another troubling setback occurred during French and UK clinical trials that began in 2002. Twenty boys in the trials suffered from severe SCID, in its X-linked form, also known as the **bubble boy disease**. Four of 10 children in the French trial and 1 in 10 children in the UK trial developed leukemia related to integration of the retroviral DNA into the children's chromosomes, which activated a cellular **oncogene** involved in cellular growth.

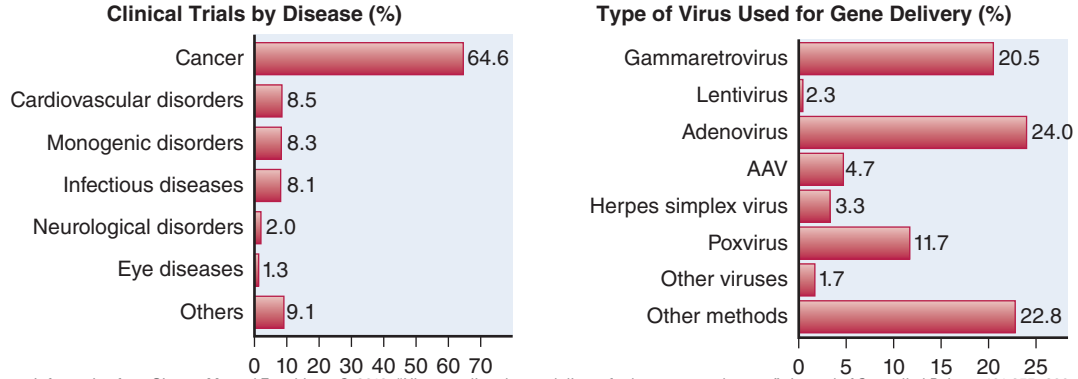
These dark days in the clinical trials led to new gene therapy protocols and regulations to patrol experiments. Even though the public is slow to hear about gene therapy developments, enthusiasm for gene therapy has grown once again. From 1989 to 2012, a total of 1,761 gene therapy clinical trials were approved worldwide. Today, the applications of gene therapy are significantly different from what they were in the early days. Clinical gene therapy trials are now under way for Alzheimer's disease, Parkinson's disease, retinal degeneration, and heart failure. The types of diseases currently being treated by gene therapy and the viruses used to deliver genes are listed in **FIGURE 1-23**.

Vaccine Development

The development of **vaccines** has been one of the greatest advances in the history of medicine. The word *vaccine* is derived from *vacca,* the Latin word for "cow." This is because the cowpox (a viral disease affecting the udders of cows) was injected into people to protect them against smallpox during the 1800s. Attempts to deliberately protect humans against disease have a long history. Edward Jenner's work with cowpox vaccination holds the title as the first scientific attempt to control an infectious disease by means of intentional inoculation. In the late 18th century, Jenner, a rural physician, observed that milkmaids who had acquired cowpox, a mild disease, did not get smallpox. They also had beautiful complexions, in contrast to those who had pitted faces after surviving smallpox. Jenner deduced that the *biological agent that caused cowpox induced immunity against the causative agent of smallpox*. To test his theory, on May 14, 1796, he removed the infectious fluid from a cowpox pustule containing cowpox viruses from the hand of milkmaid Sarah Nelmes and inserted the pus into a cut on the arm of 8-year-old James Phipps. James became mildly ill and developed a fever, but recovered after 9 days.

On July 1, 1796, Jenner injected smallpox scabs (which contained Variola, the virus that causes smallpox) into James and repeated it again several months later. James did not get smallpox as a result of either of these inoculations. He was revaccinated 20 times and later died of tuberculosis at the age of 20. Today, there is a mandatory list of vaccines that must be given to U.S. schoolchildren to prevent viral and bacterial diseases. The list includes vaccines to prevent diseases such as poliomyelitis, diphtheria, measles, mumps, rubella (German measles), tetanus, pertussis (whooping cough), hepatitis B, and a few others. Most of these vaccines were developed as a result of biomedical research following World War II.

A majority of people have experienced a cold, influenza, a cold sore on the lips, or plantar warts, which were caused by viruses. During the golden age of microbiology (1857–1914), rapid advances in microbiology, mainly spearheaded by scientists Robert Koch (Germany) and Louis Pasteur (France), determined that microorganisms cause **infectious diseases**. Koch developed a set of experimental steps for determining which specific bacterium causes a particular disease. The steps are referred to as **Koch's postulates**. Through the application of Koch's postulates, a number of different bacteria and protozoa were identified as the causes of certain illnesses.



Information from Giacca, M., and Zacchigna, S. 2012. "Virus-mediated gene delivery for human gene therapy." *Journal of Controlled Release* 161:377–388.

FIGURE 1-23 The type of diseases being targeted in human gene therapy trials and the most common viruses used to deliver genes.

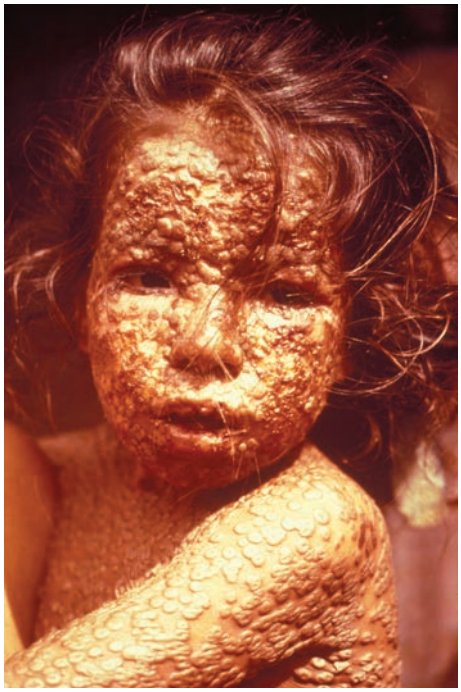
However, scientists did not know the causes of other infectious diseases, such as measles, yellow fever, poliomyelitis, influenza, and smallpox (**FIGURE 1-24**). Today we know that viruses can infect virtually every living organism on Earth, from bacteria to insects to birds, fish, reptiles, plants, and animals, including humans.

Cancer-Causing Viruses and Virotherapy

Globally, viral infections are linked to approximately 12% of all cancers. About 80% of these are cancers of the cervix (caused by papillomaviruses) and the liver (caused by hepatitis viruses B and C). These cancer-causing viruses are thought to be one factor that acts at an early stage in the process that leads to cancer. Currently, more than a dozen human viruses have been classified by the **International Agency for Research on Cancer (IARC)** as group 1, "carcinogenic to humans," or group 2A, "probably carcinogenic to humans," including Epstein-Barr virus (EBV), the hepatitis B and C viruses, human T-cell lymphotropic virus type 1 (HTLV-1), several types of human papillomaviruses, Kaposi's sarcoma–associated herpesvirus (also known as human herpesvirus-8), and Merkel cell polyomavirus. It remains unresolved whether xenotropic murine leukemia virus (XMRV) is related to prostate cancer or whether EBV and mouse mammary tumor viruses influence the development of breast cancer.

Cancer has afflicted humans throughout recorded history. Over a hundred years ago, amateur epidemiologists reported a curious phenomenon: cervical cancer was common among prostitutes but extremely rare in nuns. Some noted that cervical cancer was very high in women who were married to men whose first wives had died of cervical cancer. From these observations and reports, scientists speculated that a sexually transmitted agent caused cervical cancer. In 1986, a paper entitled "Detection of Papillomavirus DNA in Human Semen" was published in *Science*. Today, it is known that human papillomaviruses are responsible for 90% of all cervical cancers. Two safe and effective vaccines for the prevention of the most common high-risk types of human papillomaviruses have been approved for use in the United States: **Gardasil** and **Cervarix**.

Chemotherapy, radiation, and surgery are conventional treatments for cancer. **Virotherapy**, the use of **oncolytic viruses** to kill cancer cells, is an unconventional cancer therapy that is moving rapidly forward in development. Oncolytic viruses are able to selectively replicate inside of cancer cells and kill them but do not harm neighboring, normal cells. A variety of viruses, including adenoviruses, herpesviruses, reoviruses, paramyxoviruses, poxviruses, and flaviviruses, are able to selectively replicate in cancer cells, making them potential tools for virotherapy. Approval has been granted for a modest number of clinical trials using wild-type or genetically modified viruses to treat patients with advanced head and neck cancers. **Reolysin**, a variant reovirus, used in combination with two chemotherapy agents (paclitaxel and carboplatin) is in a Phase III clinical trial in the United States at the time of this writing. In June of 2013, a study using a genetically modified adenovirus was able to be targeted to, infect, and destroy breast cancer stem



a Courtesy of CDC/James Hicks.

b © nazira_g/Shutterstock, Inc.

FIGURE 1-24 **(a)** This young girl in Bangladesh was infected with smallpox in 1973. The WHO's International Commission declared Bangladesh "smallpox free" in 1977. **(b)** Respiratory viral diseases such as influenza and the common cold are spread by airborne transmission. Smallpox can also be transmitted in this way. All you have to do is breathe!

cells in preclinical experiments using athymic nude mice. This approach may one day be an alternative innovative therapy used in combination with chemotherapy to treat aggressive and therapy-resistant breast cancer.

1.8 Viral Infections: A Brief Introduction to Transmission and Pathogenesis

As noted by James Lovelock (1919–), the eminent British scientist and environmentalist, "An efficient virus kills its host. A clever virus stays with it." For viral infections to exist at the community level, a chain of linked factors is required. These factors are a reservoir, a mode of transmission, a portal of entry, a portal of exit, a susceptible host, and the infectious agent/virus (**FIGURE 1-25**). An understanding of these factors is imperative to attempt to break the cycle somewhere along the path. For example, if insects are involved in transmission, then controlling their population is a target; for those viruses transmitted by food or water, providing safe water or food is a goal. Shrinking the **reservoir** (i.e., where viruses accumulate and persist in nature) is a potential target for other diseases. In some situations, a combination of targets is preferable.

Transmission is the bridge between the reservoir and the portal of entry. The **mode of transmission** is the mechanism by which a virus is spread through the environment to another person. Simply put, transmission answers the question, "How do you get the disease?" Viruses can be transmitted to humans by **direct transmission** from person to person, whereby the virus is directly and immediately transferred from a portal of exit to a portal of entry through contact with airborne droplets from a sneeze or cough containing influenza viruses or through touching (including sexual contact). Another type of direct transmission is through animal bites, with rabies being the most common example. The rabies virus is directly transmitted from the saliva of a rabid animal to the skin and underlying tissues of another animal. Viruses gain access into (or onto) the body through a **portal of entry** (e.g., through body orifices such as the mouth, nose, ears, eyes, anus, urethra, and vagina). Once the virus has gained access into the body and completes its cycle of disease, the virus requires a **portal of exit** for the disease to spread into the community.

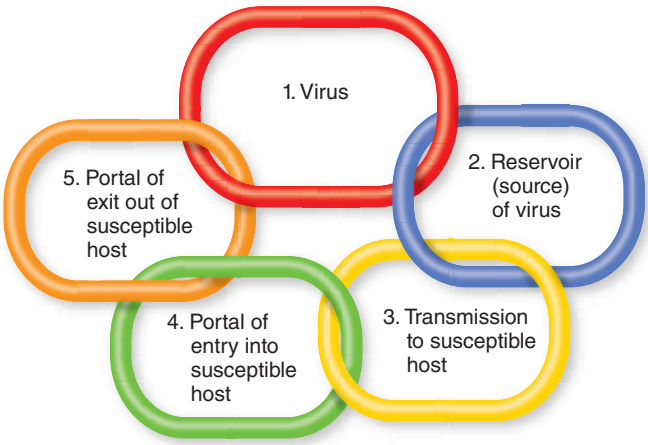


FIGURE 1-25 The cycle or chain of viral infection.

Indirect transmission involves the passage of the virus from a reservoir or source to an intermediate agent and then to a host. The intermediate agent can be living or nonliving. Indirect transmission of viruses can occur through contaminated food, water, blood, aerosols (e.g., hantavirus infections can be traced to individuals inhaling aerosols containing hantaviruses present in mouse droppings while sweeping a dusty summer cabin), blood products, and fomites. **Fomites** are inanimate objects, such as desk surfaces, door knobs, facial tissue, or escalator buttons. Another type of indirect transmission is through the bite of an infected insect, such as a mosquito or tick. **FIGURE 1-26** illustrates examples of direct versus indirect transmission.

Pathogenesis is the process by which a viral infection causes disease. Viral infection can cause direct damage to cells through cell death, or **apoptosis**. For example, poliovirus replication causes paralysis that results from the destruction/death of motor neurons associated with apoptosis. The immune deficiency caused by the depletion/death of T-lymphocytes is a direct effect of HIV infection.

Pathogenesis during viral infection is also caused by disruption of normal cellular functions such as translation (protein synthesis) and the process of membrane trafficking used by the cell to distribute proteins and other macromolecules to different parts of the cell. The body's immune response toward the viral pathogen during infection may cause immune cells to release **cytokines** that promote inflammation, damaging cells. For example, the 1918 influenza A virus caused a **cytokine storm** within the lungs of infected individuals. The cascade of cytokines led to the accumulation of fluids and macrophages in the lungs that eventually blocked the airways of influenza victims, resulting in death. **Shock** and death are often associated with the release of cytokines in Ebola patients.

1.9 Viruses in History: Great Epidemics

Viral infections have occurred throughout human history. Epidemics spread easily among humans during the New Stone Age. During this period (approximately

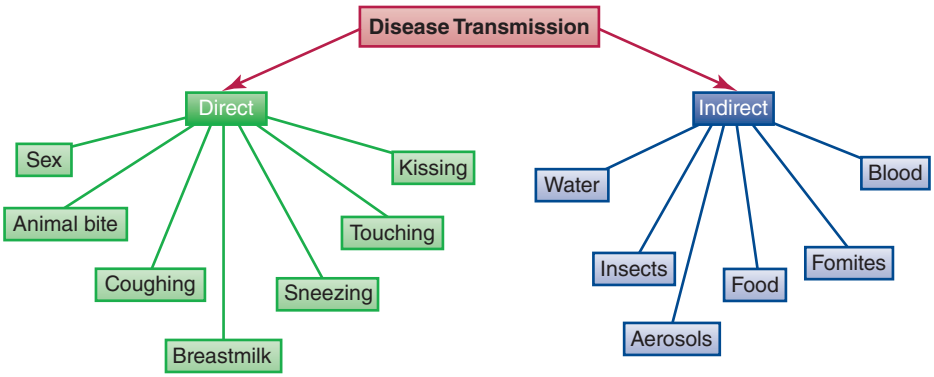


FIGURE 1-26 Direct versus indirect transmission of viral diseases.

10,200 BC), humans began to transition from a hunter-gatherer nomadic existence to living in more densely populated permanent communities. They planted seeds and domesticated animals such as dogs, sheep, and goats. These changes coincided with increased viral infections of plants, livestock, and humans. New viruses were spread to the New World by Europeans during the Spanish Conquest that began in 1492, killing unknown numbers of indigenous people. Viruses have shaped the history of wars and religious pilgrimages.

The American Civil War has often been described as the "bloodiest war" in U.S. history. Union general Carl Schurz from Wisconsin graphically described the surgical aftermath of an injury sustained during the Battle of Gettysburg (**FIGURE 1-27**):

> *"There stood the surgeons, their sleeves rolled up to their elbows, their bare arms as well as their linen aprons smeared with blood, their knives not seldom held between their teeth, while they were helping a patient on or off the table. … The surgeon snatched his knife from between his teeth … wiped it rapidly once or twice across his bloodstained apron, and the cutting began."*

The victorious ending of slavery and the freeing of slaves overshadowed the death of approximately 700,000 soldiers between 1861 and 1865. More soldiers died from camp diseases than from battle wounds. Losses from disease usually exceeded half of the state mortality totals (**FIGURE 1-28**). Many soldiers coming from remote rural areas had not been previously exposed to viruses that caused measles, chickenpox, mumps, rubella, and smallpox, ordinarily common during childhood. Thus, these rural infantrymen were vulnerable to infection by these viruses and were associated with their spread. Measles outbreaks resulted in the suspension of army drills, and entire battalions and regiments were temporarily disbanded.

Measles is the most contagious viral disease suffered by humans, with an **R-nought (R_0)** ranging from 12 to 18. R_0 (basic reproduction number) is a measure of the potential for transmission; it is the mean number of secondary cases occurring in a nonimmune (susceptible) population in the wake of a particular infection. The R_0 determines how fast an epidemic will spread. For an epidemic to spread, the R_0 value must be greater than 1; if it is less than 1, then the epidemic will die out. The greater the R_0 value, the greater the chance of the epidemic spreading, making it more difficult to establish control measures. If you watched the movie *Contagion*

Courtesy of the National Archives and Records Administration (79-T-2265).

FIGURE 1-27 Surgical tent at the headquarters of the U.S. Sanitary Commission at Camp Letterman in Gettysburg, Pennsylvania, in 1863. A Civil War surgeon with a Liston knife prepares for an amputation procedure as a soldier is being held down on a table. Liston knives were renowned for their sharp steel. The knives were named after pioneering Scottish surgeon Robert Liston (1794–1847), who was described as "the fastest knife in the West End." He could amputate a leg in less than 2.5 minutes. He would begin an operation by exclaiming, "time me, gentlemen, time me."

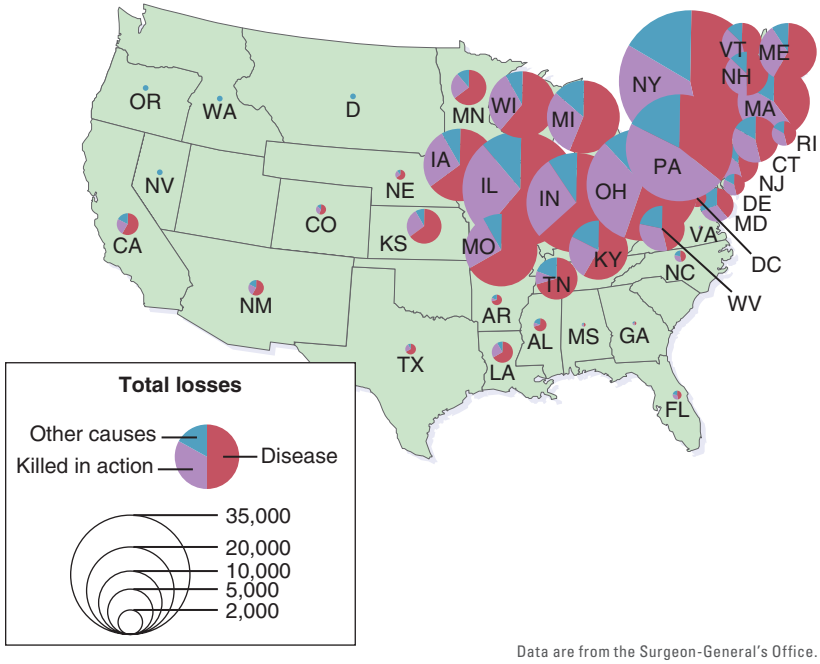


FIGURE 1-28 Geographic distribution of mortalities (killed in action/other causes/disease) plotted by the state of origin of troops during the American Civil War (1861–1865). Losses from disease commonly exceeded 50% of the state totals.

Data are from the Surgeon-General's Office.

(2011), recall that the infectious agent MEV-1 had an R_0 of 4 to 6 on the sixth day of the pandemic, and cases increased exponentially over time.

A person afflicted with measles can spread it to 12–18 susceptible persons. *The MMR vaccine used to prevent infection by measles virus was not available during the Civil War.* Soldiers who had never been exposed to measles were susceptible to it. Measles is spread by person-to-person contact and through the inhalation of infectious droplets containing the respiratory secretions of a person with measles. Measles viruses present in respiratory secretions can remain airborne for up to 2 hours.

Military records of Union soldiers indicate that there were 21,676 cases of measles, including 551 deaths, during the first year of conflict. One of these measles cases was a Wisconsin soldier whose letters to home mentioned illnesses he experienced and the environmental conditions during his 4 years of service in the Civil War (**VIRUS FILE 1-2**). Infectious diseases contracted by the Union and Confederate soldiers during the Civil War are listed in **TABLE 1-9**. A number of factors contributed to the high rates of infectious diseases, including:

- Unsanitary conditions of military camps, battlefields, and abandoned plantations
- Spoiled food and inadequate hygiene
- Fly-infested latrines located next to the cooking tents
- Concurrent illness (soldiers were fighting more than one infection at the same time)
- Dietary deficiencies (malnutrition) resulting in immunosuppression
- Polluted waterways (latrines often dug too close to streams, contaminating water supplies)
- Overcrowding
- Migrations of troops
- Dislocations triggered by emancipation of former slaves
- Unburied bodies of humans and animals (**FIGURE 1-29**)
- Limited medical treatment

At the beginning of the Civil War there were no nurses. Medical care for the soldiers was nonexistent. There was no organized means to evacuate injured or dead troops from the battlefield. Medical officers in the field were surgeons. As it became apparent that medical care required organization, field hospitals were located within a mile of the front line that focused on treating trauma injuries (e.g., amputations of limbs). Disease and mortality rates were rampant in military camps. The camps were the main source of disease transmission. Nearly half of the command was ill during the months of July, August, and September of 1863. Many freed slaves died once they secured refuge behind Union camps.

Soldiers were more likely to die from disease than in the heat of battle. Keep in mind that this is a time period in which soldiers and surgeons had inaccurate views on disease causation. Surgeons had no knowledge of aseptic technique and were using the same unsterile instruments on infected and uninfected wounded soldiers. Soiled bandages were reused! Surgeons thought yellow creamy pus, called **laudable pus**, from a wound or

VIRUS FILE 1-2 "Now I Take My Pen in Hand …": Letters by a Wisconsin Soldier During the Civil War Chronicled Disease*

Edgar Eno (**FIGURE 1**) entered military service in Company 1 of the famed 12th Regiment of the Wisconsin Voluntary Infantry on October 4, 1861. He mustered out of service at the rank of corporal on July 16, 1865. The 12th Infantry was organized at Camp Randall in Madison, Wisconsin, during October and November of 1861. They began marching in Fort Leavenworth, Kansas, were transferred to western Kentucky, joined with other forces in Tennessee and Mississippi, and finally fought with General Sherman until the surrender of Johnston, North Carolina, on April 16, 1865. The 12th Infantry Regiment was then assigned the duty of guarding the railways and accompanying expeditions and was involved in the siege at Vicksburg on June 11, 1863. They remained in the trenches until its surrender on July 4, 1863. The 12th Infantry Regiment was known as one of the "Marching Regiments," having marched 3,838 miles; they also traveled 2,506 miles by railroad and 3,159 miles by steamboat.

The letters Eno wrote during the Civil War were presented along with a history of the 12th Infantry Regiment by the Hillsboro Historical Society in the book *Civil War Letters: Now I Take My Pen in Hand …*, published by Hillsboro Sentry-Enterprise in 1998. Eno mentioned disease and illness in his letters. He stated being ill in the majority of his letters. Excerpts from his letters that chronicled disease symptoms and the conditions that may have contributed to viral, bacterial, or parasitic infections are presented here:

Courtesy of Roger and Sylvia Gasser.

FIGURE 1 Edgar Eno in uniform.

January 5, 1862, Camp Randall, Madison, Wisconsin (this letter was written for Eno by someone else in the hospital)
Dear Father, I am sick in the hospital. I was taken sick two weeks ago with the lung fever (pneumonia). I was just commencing to get around when I was taken down with the measles. They are all out on me all though I am in considerable pain just now but I think I shall soon get over it.
June 17, 1862, Fort Leavenworth, Kansas
Dear Sisters … Thare is a lot of them agoin down thare to never cum back not to die with bullits but with diseas …
January 31, 1863, Collierville, Tennessee
Dear Father … You hav no idea how it is on a march if a fellow gives out without he has a good companion with him he can lay down by the road side and die befor he will be sean to. On this march I hav notast a good many as we hav been marching along laying on the wet ground, seamenly burning up with the feaver and no one to se him for it wont do for a fellow to leave the ranks. So than they lay perhaps git better and go on or perhaps die …
April 28, 1863, Memphis, Tennessee
Dear friends at home … I am well at present with the exception of a prety bad cold …
May 7, 1863, Memphis, Tennessee
Dear Sister … I was pretty sick for two or three days but am giting better now. I caut cold and it throwed me into a feavor but I am all right now …
June 18, 1863, Vicksburg, Mississippi
I havnt been very well for the last two weeks. The ague got ahold of me and shook me pretty hard … we lay in a rifle pay all day … I don't know how many mules and horses we killed we knoct them over as fast as they cum thre the works. It will be a nice smelling place for the rebs in a few days. They cant bury them for they dasent cum out.

(*continues*)

VIRUS FILE 1-2 "Now I Take My Pen in Hand …": Letters by a Wisconsin Soldier During the Civil War Chronicled Disease* (continued)

August 12, 1863, Vicksburg, Mississippi
There is such a stench around here of dead animals that it is no wonder that the boys are sick. Say nothing about the hot weather and sickly season. I think if we git down to Natchez where there has been no troops camped it will be much healthier. This month is going to be hard one on the boys but the only way for us is to be carfle what we eat and keep clean and I think we will wory it through.
August 29, 1863, Natchez, Mississippi
Dear friends at home … I am not very well and hav ben for some time but I think I am better. I hav had the deiriah and it has run me down so I am pretty weak …
August 22, 1864, General Hospital, Marietta, Georgia
Dear friends at home … I have the diareah pretty bad and it makes me feal weak …
October 11, 1864, near Atlanta, Georgia
Dear brother … found me a little unwell but I think by being careful with alittle rest I will soon be as good as ever. We have had a hard and never seasing duty to do for the last three months and it got me wore down. About all the trouble is Direah and over-doing …
October 18, 1864, Hospital, Atlanta, Georgia
Dear Father … I am still in the hospital and don't know as I am any worse or much better. I have got the direah prety bad yet it keeps me pretty weak …

There were a total of 313 mortalities in the 12th Infantry Regiment: 3 officers, 93 enlisted men killed in action or mortally wounded, and 224 enlisted men died of disease (e.g., measles, smallpox, yellow fever, malaria, pneumonia, dysentery, typhoid or "camp fever"). Amputation was a huge source of disease and infection (e.g., gangrene and staphylococcal infections). The Civil War forced Americans to reconsider the appropriate treatment of the dead and the symbolic meaning of the dead body. Death became an urgent public health issue. Disposing of the soldiers who died in action presented a challenge on the battlefield. If you have ever observed tombstones of Civil War soldiers in a local cemetery, you may not realize that the remains of the soldiers buried there were likely soldiers that survived the war (**FIGURE 2A**). Local cemeteries often contain a memorial for those comrades who died in action or of disease during the war (**FIGURE 2B**). Soldiers who died of battle wounds were buried in mass graves or common graves on the battlefield. The losing side of a battle had to retreat, leaving their dead to be disposed of by the enemy.

a

b

FIGURE 2 **(a)** Soldier's burial lot for remains of indigent members/veterans of G.A.R. (Grand Army of the Republic) located in Riverside Cemetery, Oshkosh, Wisconsin. G.A.R. was a veteran's organization founded to provide support for soldiers and their families. In the far right background stands a memorial. **(b)** Photograph taken at sunset of a memorial for Union soldiers who died serving their country in the Civil War, Riverside Cemetery, Oshkosh, Wisconsin.

VIRUS FILE 1-2 "Now I Take My Pen in Hand …": Letters by a Wisconsin Soldier During the Civil War Chronicled Disease* (continued)

Rudimentary graveyards next to hospitals became a more organized way and a new form of sacred space in which to bury soldiers who died of war wounds and/or disease in the hospitals.

These cemeteries, along with cemeteries located near or on major battlefields, are registered today as National Cemeteries to honor those who served in the Civil War. There are approximately 130 sites or registered Civil War cemeteries, such as Forest Hill Cemetery Soldiers' Lot located in Madison, Wisconsin. The remains of 240 Union soldiers who died in the military hospital or Madison's general hospital are interred there in a section referred to as the Union Soldiers' Lot (**FIGURE 3A**). Even though no battles were fought in Madison, Camp Randall was a Union army training facility and a prisoner-of-war camp. Approximately 140 Confederate troops or prisoners of war were buried in this cemetery in a section known as Confederate Rest (**FIGURE 3B**).

*Note: You may notice a lot of misspellings in Edgar Eno's letters. These were retained to preserve the authenticity of his writings. *Direah* refers to diarrhea. *Feaver* refers to fever. *Ague* refers to malaria. Edgar Eno's letters were kindly provided by Roger and Sylvia Gasser. Edgar Eno was Sylvia Gasser's great-grandfather.

a ©Teri Shors. **b** ©Teri Shors.

FIGURE 3 Remains of Civil War soldiers and prisoners of war (Confederate soldiers) buried in Forest Hill Cemetery, Madison, Wisconsin. **(a)** Union Soldiers' Lot . **(b)** Confederate Rest.

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Table 1-9 Civil War Infections

Disease	Causative Agent	Number of Cases and Deaths Among Union Soldiers*
Influenza and bronchitis	Bacterium and/or virus	1,765,000 cases and 45,000 deaths
Typhoid	Bacterium	149,000 cases and 35,000 deaths
Severe diarrhea/dysentery	Virus, bacterium, or parasite	360,000 cases and 21,000 deaths
Malaria	Parasite	1,316,000 cases and 10,000 deaths
Epidemic jaundice	Virus	71,691 cases with a 0.5% mortality rate
Tuberculosis	Bacterium	29,510 cases and 6,946 deaths
Venereal diseases (e.g., syphilis, gonorrhea)	Bacterium	182,482 cases and 158 deaths
Staphylococcus aureus infections (e.g., boils)	Bacterium	Prevalent but rarely caused death
Smallpox	Virus	18,952 cases and 7,058 deaths
Measles	Virus	76,318 cases and 5,177 deaths
Yellow fever	Virus	1,371 cases and 436 deaths
Meningitis	Bacterium or virus	3,999 cases and 2,660 deaths
Scarlet fever	Bacterium	696 cases and 72 deaths
Diphtheria	Bacterium	8,053 cases and 777 deaths

*These values represent infections reported among Union forces. Similar data for mortalities of Confederate soldiers are lacking, but cases and death rates are likely proportional.

Information from Sattin, J. S. 2007. "Civil War medicine: The toll of bullets and bacteria." *Gundersen Lutheran Medical Journal* 4:79–83; Bollett, A. J. 2004. "The major infectious epidemic diseases of Civil War soldiers." *Infectious Disease Clinics of North America* 18:291–309.

surgical amputation was essential to the healing of bone infection, so they transferred pus from the wounds of soldiers that had it to wounds of soldiers who did not have it, infecting more soldiers. The application of laudable pus was based on the observation that soldiers with laudable pus that formed after a wound or surgical amputation had a better chance of surviving than those soldiers with a thin watery discharge, who usually died of sepsis within days. The yellow discharge was likely a bacterial (*Staphylococcus* sp.) infection. Staphylococcal infections of wounds flourished. Virtually all wounds became infected.

Courtesy of the Library of Congress.

FIGURE 1-29 Bodies of dead Union soldiers on the field after the first day of battle at Gettysburg.

Louis Pasteur believed that germs caused disease. He was performing laboratory research to determine the cause of a disease of silkworms during the time period of the Civil War. By 1867, Lister's use of phenol or carbolic acid as an antiseptic and the practice of handwashing led by Hungarian physician Ignaz Semmelweis to prevent childbed fever (a streptococcal infection of pregnant women) in hospitals were becoming better known. During the 1880s, Robert Koch formalized the **germ theory of disease** in which he isolated a bacterium that caused anthrax in animals and reproduced the disease in experiments with mice. However, this knowledge was too late for those who suffered in the Civil War. Had the war been fought only 10 years later, the mortalities from disease might have been a mere fraction of what they were.

By the 1880s, the germ theory of disease was accepted among doctors. They knew about bacteria, how infectious diseases were spread, and the importance of aseptic technique. However, even as late as World War I conditions at military camps were ideal for the spread of influenza A viruses, which are still considered one of the most formidable infectious agents. In 1918, the influenza A virus might have contributed to the ending of World War I.

Influenza: 1918 Pandemic

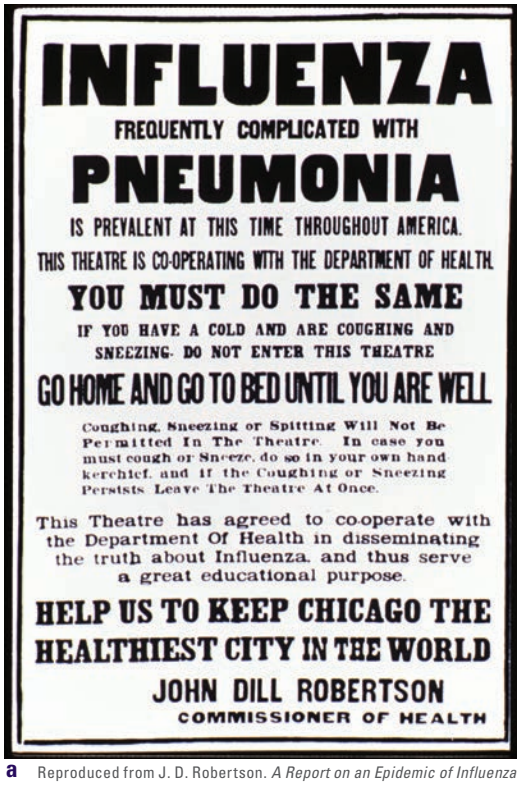
Imagine walking down a street in a city that used to be full of activity but now looks like a ghost town. You see quarantine signs posted on homes; flags or wreaths hanging on the doors indicate whether it was a parent, child, or grandparent who recently died from a contagious disease. Schools, churches, theaters, libraries, and most restaurants and stores are closed (**FIGURE 1-30A**). There are no public gatherings, not even funerals. The few people you see have a mask covering their face (**FIGURE 1-30B**). There is a shortage of coffins, and the use of mass graves is common. This really did happen in 1918. The visitor to this town was the virus named *influenza*.

In 1918 the Spanish flu (also termed ***la grippe***) pandemic charged across America in 7 days and across the world in 3 months. It claimed more American lives than all the major wars of the 20th century combined. Estimates of deaths range from 20 to 50 million—most in the brief period from October through the end of December 1918. The Spanish flu was associated with high rates of morbidity, mortality, and social disruption, as well as high economic costs, and was to be the most destructive pandemic ever known. The Spanish flu got its name from newspaper reports of that period. The government of Spain was neutral during World War I and did not censor the press. There were reports that King Alphonse XIII was seriously ill with influenza.

The onset of Spanish flu symptoms came on so quickly that apparently healthy people in the prime of their lives (ages 20–40) were suddenly overcome, and within an hour could become helpless with fever, delirium, and chills. They suffered from high fevers of 101°F to 105°F, severe headaches, muscle and joint pain, hair loss, and acute congestion. The most unusual pathologic finding was massive pulmonary edema and/or hemorrhage. This was a unique viral pneumonia—a patient could be convalescing one day and dead the next. Those who did not die of Spanish flu often died from secondary bacterial infections resulting in pneumonia. Surprisingly, before the recent concerns of a potential bird flu pandemic, not very many people were familiar with the 1918 Spanish flu. It may be that few survivors wanted to talk to historians about the terrifying and ghastly days when so many were dying that there were bodies in the streets.

Poliomyelitis: 1916 and 1940s and 1950s

Evidence of the viral disease poliomyelitis (polio) dates to the dynasties of ancient Egypt. In the current era, polio epidemics peaked in the United States in 1916 and the mid-1940s and 1950s. Poliomyelitis was quite



a Reproduced from J. D. Robertson. *A Report on an Epidemic of Influenza in the City of Chicago in the Fall of 1918* (Chicago, 1918), p. 103.

b © National Archives and Records Administration.

FIGURE 1-30 **(a)** Poster used to educate Chicago residents during an influenza epidemic that occurred in the fall of 1918. **(b)** A New York City policeman wearing a cloth mask as protection from the Spanish flu in 1918.

frightening because of its mysterious seasonal incidence (July–October). Many children were not allowed to play outside because of the fear of polio. Newspapers included word games and puzzles to occupy children's playtime while they stayed indoors. Some families fled to remote summer vacation homes.

Polio struck an industrialized nation free from the poor sanitation conditions that typically play a role in epidemics. It occurred in a nation thriving with new technologies that would lead to the control over disease. Civil engineers were creating a network of aqueducts and water purification plants to provide clean, safe drinking water for much of the nation.

The **mode of transmission** was a mystery to health officials. Cities responded with methods that had met with success in halting epidemics of typhus, cholera, and diphtheria. New York City officials doused the streets with 4 million gallons of water a day to flush the streets of germs and intentionally killed 72,000 stray cats that were thought to be carriers of disease. Many cities were fogged with the insecticide **DDT** (dichlorodiphenyltrichloroethane; **FIGURE 1-31**) to kill flies, mosquitoes, and other disease-spreading insects. None of these measures worked, however, because the disease spreads through human feces. At the time, health officials did not know that.

Polio broke America's heart. It crippled its victims. Children were lined up in wheelchairs. Paralysis of the muscles used for respiration and swallowing (called *bulbar poliomyelitis*) was sometimes fatal. Those with symptoms of difficulty in breathing and swallowing were put into an **iron lung**, also known as the "Drinker respirator" (**FIGURE 1-32**).

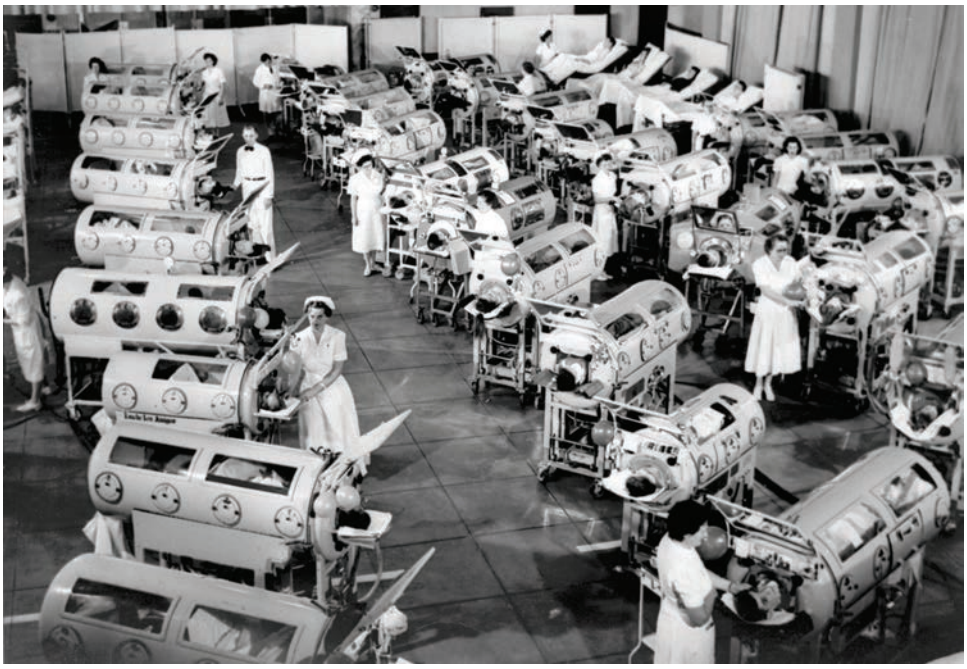
Franklin Delano Roosevelt (FDR) was likely the most famous adult who suffered from the effects of poliomyelitis. Roosevelt contracted polio in 1921, at the age of 39. He spent over half of his personal fortune to purchase Warm Springs, Georgia, a resort with warm natural springs for swimming and rehabilitation, to provide a place for the "polios." Frightened by reminders of this terrible disease, guests not suffering from polio abandoned the resort. Roosevelt did not recover from the initial paralytic effects of poliomyelitis. When he left Warm Springs to run for governor of New York in 1928, and then for the U.S. presidency in 1932, he chose to hide the effects from the public (**FIGURE 1-33A**). He was not photographed being carried or wheeled about. He would lean on a cane or a companion's arm. It appeared to many that he could walk. It was a brilliantly staged deception. In Roosevelt's opinion, and those of his advisors, a robust appearance was necessary to portray a physically strong leader, and the press cooperated (**FIGURE 1-33B**).

The March of Dimes

The March of Dimes began in 1938 as an effort to raise money for polio treatment and research. The organization selected Dr. Jonas Salk to lead research on polioviruses, and in 1941 it provided the first iron lung to assist polio patients. The March of Dimes ran field trials of the Salk vaccine (an inactivated preparation of poliovirus), with 1,830,000 schoolchildren participating in 1954. In

Courtesy of the Los Angeles Times Photography Archives.

FIGURE 1-31 Photograph taken November 4, 1948, accompanying a story in the *Los Angeles Times* on November 5, 1948. The photograph depicts a mobile crew spraying DDT and lephane in a mixture of oil and water in the 100 miles of alleys of Santa Monica after an outbreak of polio.



Courtesy of Los Angeles County DHS-Rancho Los Amigos National Rehabilitation Center, Donney, California.

FIGURE 1-32 Polio ward of iron lungs at Rancho Los Amigos Hospital in Amigos, California, 1953.

1955, the vaccine was declared safe, effective, and potent and was licensed for general use. Later, in 1960, the Sabin vaccine was licensed for use in the United States.

Today, poliomyelitis is rare due to the worldwide efforts of the WHO, CDC, Rotary International, and United Nation's Children Fund (UNICEF) to eradicate it. In 2015, **endemic** areas of poliomyelitis caused by wild polioviruses were reduced to two countries (Pakistan and Afghanistan), down from more than 125 in 1988. The number of poliomyelitis cases globally was 51 from January 1 to November 5, 2015. All of the cases occurred in the endemic countries.

Acquired Immunodeficiency Syndrome (AIDS): 1980s

In June of 1981, a group of physicians in Los Angeles, California, reported five unusual cases of individuals suffering from a rare form of pneumonia in the CDC's *Morbidity and Mortality Weekly Report*. The Pneumocystis pneumonia (PCP), or **pneumocytosis**, was caused by a fungus identified to be *Pneumocystis* (*carinii*) *jiroveci*. All patients were young men, and all were sexually active homosexuals. Two of the patients died from this pneumonia, and all patients experienced more rare infections such

a Courtesy of Franklin D. Roosevelt Presidential Library and Museum.

b Courtesy of Franklin D. Roosevelt Presidential Library and Museum.

FIGURE 1-33 **(a)** FDR at Warm Springs, Georgia, in 1924. **(b)** The press portrayed Roosevelt as a robust, physically strong leader.

as **candidiasis**, a fungal infection of the throat, mouth, or, in women, the vagina. PCP is rare in the United States and almost exclusively found in severely immunosuppressed individuals. This first report recognized a new growing epidemic in the United States that was later termed **acquired immunodeficiency syndrome (AIDS)**.

As cases were reported to the CDC, a pattern characteristic of an epidemic emerged, but the culprit was a mystery until Luc Montagnier (France) discovered a **retrovirus** named human immunodeficiency virus (HIV). Today, AIDS is a worldwide epidemic, with the population in Africa being the most severely affected. However, more than 20 different life-extending antiretroviral drugs are now available that have turned a disease that was once a death sentence into a chronic, manageable condition for those who have access to drug treatment. A few individuals have been cured of HIV infection—their viral loads reduced to insignificant levels and their antiretroviral therapy stopped (e.g., the "**Berlin patient**" treated in 2007 and a French teenage girl treated early with antiretroviral drugs who has been in remission for 12 years as of 2015). There is no vaccine available to prevent HIV infection.

1.10 Recent Viral Outbreaks

Influenza A (H1N1), the First Influenza Pandemic of the 21st Century: 2009

At the end of the 2009 influenza season, health authorities in Mexico City recognized an unusual pattern of influenza-like illness. Dozens of individuals were suffering from atypical pneumonia and flulike symptoms during the middle of March. The adjective **atypical** is applied to the illness in question when no known pathogen during diagnostic testing has been identified as the causative agent. The number of cases increased dramatically within the month. On April 6, a U.S. data-mining biosurveillance company posted an alert on its website warning about a possible severe respiratory illness spreading in La Gloria, Mexico. The data-mining company tracks thousands of Google searches daily for early signs of medical problems or civil unrest anywhere in the world. The company advised the CDC that the Mexican flu outbreak reports could represent a potential public health emergency and warranted international concern. On April 18, Mexican health officials sent the CDC 14 diagnostic specimens from patients suffering from severe flu- or pneumonia-like infections for testing.

CDC officials held a press conference on April 24 to announce that 7 of the 14 Mexican samples contained the same viral strain that was also causing flulike illness in individuals located in adjacent counties in California and Texas. They indicated that containment of this outbreak was "not very likely." Preliminary laboratory tests by scientists at the CDC suggested that the infections were caused by swine influenza A virus (H1N1) based on genetic testing and had never been detected in humans.

That same day, the president of Mexico, Felipe Calderon, advised citizens to wear face masks when using public transportation, to stay indoors and avoid crowded places, to exercise frequent handwashing, to cover their mouths when coughing, to cough or sneeze into the crook of the arm or a tissue, and to avoid sharing food. Schools were closed in Mexico City. People were urged not to go to work and to seek immediate medical attention if they experienced flulike symptoms. These recommendations were repeated daily through public media announcements, including newspapers. The Mexican army distributed 6 million masks, handing many out to citizens at subway stations and Metrobus lines.

Mexican President Calderon invoked emergency powers, giving the government the power to enforce quarantine and conduct home inspections. Public events were canceled on April 25. WHO Director General Dr. Margaret Chan declared the outbreak "a public health emergency of international concern." All countries were requested to report all laboratory-confirmed deaths associated with H1N1 influenza A. Two days later, schools and universities were closed countrywide in Mexico. Members of the public were alarmed by the dramatic changes imposed by the government in response to this epidemic. On April 26, the government ordered all gyms, cinemas, art galleries, restaurants (except for take-out orders), bars, and cantinas to close (**FIGURE 1-34**). By April 29, the Mexican government required that drivers in public transportation wear masks and gloves. The fine for not complying was around $150 U.S. (40 times the daily minimum wage in Mexico). Instead of imposing the fines, the Mexican police enforced regulation by taking bribes from drivers who failed to comply. They also threatened to seize taxis or buses for 5 days for noncompliance. On this same day, the Mexican Ministry of Health reported its April monthly total of 2,155 patients with severe pneumonia and 100 deaths. *These government orders were not very different from the measures applied during the 1918 influenza pandemic.*

Meanwhile the United States declared a public health emergency on April 26. Also, the first U.S. fatality was reported: a 23-month-old child visiting Mexico. The child died at a hospital in Houston, Texas. On April 27, Spain declared the first confirmed case of influenza caused by influenza A virus (H1N1) in Europe. Hong Kong, Thailand, Singapore, Malaysia, Vietnam, and Indonesia issued travel advisories against travel to Mexico. Countries in Southeast Asia, Russia, India, and North and South America initiated airport screenings. CDC experts recommended that U.S. citizens avoid all nonessential travel to Mexico. The European Union Health Commissioner Androulla Vassiliou recommended that individuals postpone nonessential travel to affected parts of the United States and Mexico. Argentina, Ecuador, Peru, and Cuba closed their borders for travel to and

FIGURE 1-34 All restaurants were closed except for take-out orders during the H1N1 outbreak in Mexico City, Mexico (April 29, 2009).

from Mexico. The Philippines, China, and Indonesia banned the importation of pork from Mexico and certain U.S. states (e.g., border states such as Texas and California, but also Kansas). By the next day, confirmed or suspected cases of H1N1 influenza were reported in the United States, Canada, New Zealand, the United Kingdom, and Spain. By the end of April, local officials closed schools in New York City and Fort Worth, Texas, due to suspected H1N1 influenza cases. Egyptian leaders ordered the slaughter of more than 300,000 pigs farmed by the Coptic Christian minority in Egypt. Violence erupted in Cairo as Coptic Christian pig farmers clashed with the police.

The first wave of influenza caused by influenza A virus (H1N1) continued into 2009. On June 11, WHO director Dr. Margaret Chan declared the world was facing an unstoppable pandemic. She also stated that this influenza strain was a very different virus than what we had experienced season to season. The 2009 influenza A virus (H1N1) spread rapidly from person to person. It also targeted unusual risk groups: young people (aged 6 months to 19 years), children with neuromuscular diseases, pregnant women, and obese people. The influenza A virus (H1N1) spread quickly through frequent international travel. Acting director of the CDC's Emergency Response Team Dr. Richard Besser served as the public face of the CDC's response to H1N1 influenza. As of June 12, 2009, the influenza virus had spread to 74 countries around the world. Over 29,000 cases were reported, including 145 deaths. By July 1, H1N1 influenza cases were reported in all 50 states. It was estimated that more than 1 million people were infected.

Within a month of this epidemic, sequence data for the genetic material of the new strain of influenza A virus became available in public-access databases. A report was published in *Science Express* on May 22, 2009. Researchers discovered that the influenza A virus (H1N1) contained a unique combination of gene segments from both classic Northern American and Eurasian pig influenza A virus strains, as well as gene segments from human and avian influenza A virus strains. It was a "mutt" of a virus. The most critical gene segments that pandemic strains contain are novel viral H and N gene segments. The 2009 influenza A virus (H1N1) contained an H gene from classic American pig influenza A viruses and the N gene from Eurasian pig influenza A viruses. Three gene segments of the 2009 influenza A virus shared common sequences from the 1918 pandemic influenza A viral strain. *It contained the genetic characteristics of a pandemic strain and could spread easily from person to person.*

The main challenge for scientists and health officials was to assess how severe the pandemic might be. Researchers were concerned that a second wave of influenza during the fall influenza season could be of the same severity experienced in 1918. Like the 1918 influenza A virus, young people were dying from influenza or complications of it, making it different from typical seasonal influenza that causes complications in infants, the immune compromised, and the elderly resulting in death. In 1918, a milder influenza appeared in the spring of the year that returned in the fall with a vengeance, making it likely the deadliest influenza A viral strain in world history.

Having had two "near misses"—the emergence of SARS caused by a new coronavirus (2002–2003) and the spread of avian influenza A virus (H5N1) in the Middle East—nations began working diligently on pandemic

action plans in 2005. These plans provided guidance, resources, and checklists intended to reduce the impact of a pandemic on businesses, hospitals, schools, and the community. Global vaccine manufacturing and stockpiling of influenza A virus antivirals such as Tamiflu became a priority. Regulatory authorities fast-tracked licensed pandemic influenza A vaccines for a number of countries, including the United States and Canada. Healthcare workers were given first priority to early vaccination. Other high-risk groups were next on the list for vaccination: pregnant women and children. Healthcare facilities stockpiled medications, masks, gowns, and other supplies. Some hospitals suspended the use of student volunteers to limit patients' possible exposure to the influenza A virus (H1N1) because the virus spread quickly in academic and university settings.

The 2009 influenza A virus (H1N1) started a second wave of infections in the fall. On October 24, President Obama signed a national public health emergency proclamation for H1N1 influenza A. This waived certain federal regulatory requirements for healthcare facilities in response to emergencies (e.g., setting up alternative screening locations for influenza A patients away from a hospital's main campus) and access to the experimental drug **peramivir** to treat patients with severe cases of H1N1 influenza A. (Five years later, **peramivir** was licensed by the U.S. **Food and Drug Administration [FDA]** as **Rapivab** to treat influenza.) Two separate influenza A vaccines were distributed in the United States: a seasonal vaccine and an influenza A virus (H1N1) 2009 monovalent vaccine.

Responding to a pandemic in the 21st century had technological advantages. The media responded with public service announcements. It updated the international situation; educated the public, especially on **cough etiquette**, frequent handwashing (**FIGURE 1-35**), school closings, the need to stay home when sick, and the availability of seasonal and H1N1 influenza A vaccine; and corrected false information (e.g., rumors spread that pork was the source of influenza A virus [H1N1], making it unsafe to eat, *but influenza A viruses are not transmitted by eating food, pork, or pork products*). The popular Angry Birds game developed by Rovio Entertainment was inspired by this "swine flu" pandemic. The original idea came from Senior Game Designer Jaakko Iisalo. The developers of the game realized that the angry birds needed an enemy and were influenced by the H1N1 swine influenza A pandemic that was in the news media at the time, inspiring the birds' enemy to be the "pigs" (**FIGURE 1-36A**).

Websites provided accurate up-to-date information for citizens and healthcare professionals. The websites listed in **TABLE 1-10** are still in use today. Online influenza A (H1N1) health maps and cell phone applications tracking swine influenza A (H1N1) cases were created. Users could track outbreaks reported in their region and set alerts to be notified on their devices by e-mail when swine influenza A (H1N1) cases were reported in their region. The CDC reached out to people using new emerging technologies by posting up-to-date information on Facebook and Twitter feeds, creating video podcasts and YouTube videos, and establishing a 24-hour information hotline for texting.

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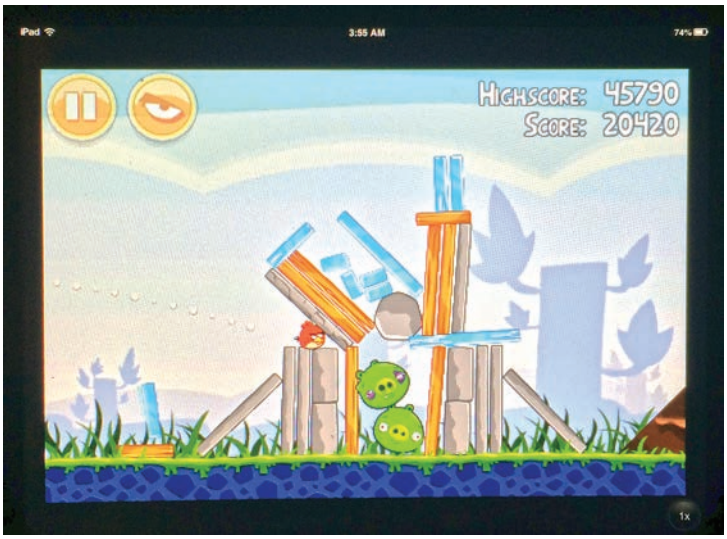
FIGURE 1-35 Poster on handwashing etiquette on a wall in the Halsey Science Center at the University of Wisconsin–Oshkosh.

Table 1-10 Pandemic Influenza Resources and Surveillance Information

Resource	URL
Comprehensive U.S. government information website	http://www.flu.gov
CDC seasonal flu information	http://www.cdc.gov/flu/
WHO Global Alert and Response: Influenza	http://www.who.int/influenza/en/
European Commission: Influenza Information	http://ec.europa.eu/health/vaccination/influenza/index_en.htm
Public Health Agency of Canada: Influenza	http://www.fightflu.ca/index-eng.php
Google Flu Trends Around the World	http://www.google.org/flutrends/
HealthMap: Global Health, Local Information	http://healthmap.org/en/
World Organization for Animal Health: Avian Influenza Portal	http://www.oie.int/animal-health-in-the-world/web-portal-on-avian-influenza/



a ©Teri Shors.

b ©Teri Shors.

FIGURE 1-36 **(a)** Rovio Entertainment's popular Angry Birds game was inspired by and developed during the 2009 H1N1 swine influenza A pandemic. The birds are upset with the pigs (swine). **(b)** Swine flu prevention lollipops that looked similar to the ones in this photograph were popular among people looking for natural products to ward off the swine flu. They were advertised as containing zinc, vitamin C, peppermint, licorice, roasted chicory, eucalyptus, natural menthol extract, lavender, star anise, a dash of ginger oil, and a very exotic variety of *Echinacea*. This product is no longer available for sale.

Like in 1918, different **snake oil** or quack remedies were advertised. The fear of influenza A viruses (H1N1) resulted in advertisements for a plethora of fraudulent products that cure, treat, or prevent infection by influenza A viruses (H1N1). Products included shampoos, air purifiers, herbal supplements, inhalers, body washes, and even swine flu prevention lollipops (**FIGURE 1-36B**). The FDA developed a comprehensive list of websites listing the companies selling various gels, kits, supplements, sprays, and other unauthorized products that made unsubstantiated claims about H1N1 influenza protection or treatment.

A total of 18,500 laboratory-confirmed deaths caused by influenza A virus (H1N1) were reported worldwide from April 2009 to August 2010. The majority of pandemic-associated deaths reported came from high-income locations. Less than 12% of deaths were reported from Africa and Southeast Asia, which are home to more than 38% of the world's population but where countries lack data-collecting capabilities and virological surveillance. Therefore, laboratory-confirmed deaths in these areas represent gross underestimates of influenza A–related deaths because of the lack of routine laboratory testing, difficulty in identifying influenza-related deaths caused by secondary bacterial infections, or complications due to chronic illnesses. Findings reported in the literature confirmed that the 2009 influenza A (H1N1) pandemic was not the doomsday scenario of a 1918-like influenza A pandemic that could have caused millions of deaths worldwide.

Severe Acute Respiratory Syndrome (SARS): 2002–2003

The **SARS coronavirus (SARS-CoV)** emerged from the Guangdong Province of southern China during November and December of 2002. The first infected individuals handled, butchered, or sold birds and animals at **live markets**. They experienced influenza-like symptoms during the first week of illness: fever higher than 100.4°F (38°C) for more than 24 hours, headache, and body aches. During the second week of illness, they developed a dry cough, and many had gastrointestinal distress. Most sick individuals rapidly deteriorated to an **atypical pneumonia**. The fever followed by the rapid shortness of breath were the key signs and symptoms from which the syndrome derived its name as **severe acute respiratory syndrome** (**SARS**). On February 10, 2003, authorities at the WHO Beijing office received an e-mail describing a "strange contagious disease" that had "already left more than 100 people dead" in the Guangdong Province in the short time span of a week. The e-mail embellished further by describing "panic" and people using any kind of pharmaceutical or alternative medicine they thought would protect them from getting sick.

On February 16, a 64-year-old nephrologist (physician specializing in kidney diseases) working at a Chinese hospital in the Guangdong Province began experiencing the early signs and symptoms of SARS. Five days later, the physician traveled from Guangzhou to Hong Kong for a wedding and stayed at the Metropole Hotel. The physician felt well enough to sightsee and shop with his brother-in-law for 10 hours during the day of his arrival but the next day sought medical attention. He was directly admitted to the intensive care unit (ICU) of a hospital with respiratory failure. He later died.

The next person to get sick was his brother-in-law. Subsequently, a nurse in the accident and emergency department at the same hospital became ill. The nurse was present in the same resuscitation room as the nephrologist who died but had had no direct contact with him and she

was wearing a surgical mask at the time. The fourth person to get sick was a Chinese-Canadian businessman attending a family reunion in Hong Kong. His stay at the hotel overlapped with that of the nephrologist for 1 day. There was no direct contact between the businessman and the nephrologist in the common areas of the hotel. The businessman was later admitted to a different hospital than the nephrologist. The next three individuals to contract SARS were nurses who had close encounters with the Chinese-Canadian businessman. They had cleaned him after an episode of diarrhea. These nurses did not wear masks or gowns during their routine care of any patients in the hospital ward. *The SARS-CoV was efficiently transmitted in the healthcare setting among healthcare workers, patients, and hospital visitors.* About 80% of the cases in Hong Kong were traced back to the 64-year-old doctor.

Well-documented outbreaks of SARS transmission occurred in hospitals located in Canada, China, Hong Kong, Singapore, Taiwan, and Vietnam. The concept of **super-spreading** was proposed to explain incidents in which a SARS patient infected many more persons than would normally be expected. What made SARS notorious—in contrast to other infectious diseases like influenza A—was its *propensity to cause hospital outbreaks.* Healthcare workers accounted for 21% of all SARS cases during this 2002–2003 outbreak. SARS transmission studies resulted in a new approach to manage patients with a set of measures called **respiratory hygiene and cough etiquette** to prevent patients with respiratory illness from transmitting infection to others. These measures included patients covering their mouth and nose with tissues and disposing of the tissues properly, wearing masks, handwashing, and standing at least 3 feet from other persons to prevent **droplet transmission**. People infected with SARS-CoV were able to travel for several days before the onset of severe symptoms. The period of time between exposure to an infectious agent and the first signs or symptoms of illness is called the **incubation period**. Other emerging viral diseases, such as Ebola virus disease, hantavirus pulmonary syndrome, and Nipah virus encephalitis, do not spread by travelers as rapidly as SARS because the incubation period is much shorter. Individuals experience severe symptoms quickly, making them less able and likely to travel.

On March 15, 2003, the WHO issued a travel advisory that included emergency guidelines for travelers and airlines. The outbreak spread to 30 countries across 5 continents, resulting in 8,096 cases and 774 deaths (~10% fatality rate; **TABLE 1-11**), including 44 Torontonians. The SARS pandemic is a good example of the use of modern laboratory technologies to determine that SARS was caused by a new coronavirus (SARS-CoV) and not a novel influenza A virus, paramyxovirus, metapneumonia virus, or bacterial pathogen such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, or *Legionella pneumophila.*

Early investigations suggested that SARS-CoV originated from animals because the first persons infected were traders or animal food handlers at live markets (**FIGURE 1-37**), restaurant workers, and butchers of **exotic animals** for culinary purposes. These investigations discovered that blood specimens of wild animal traders at wholesale markets in Guangzhou had significant levels of **antibodies** against SARS-CoV compared to vegetable traders. The presence of antibodies indicated that the marketplace traders were likely infected by SARS-CoV while capturing and marketing wild animals. For this reason, scientists concentrated their investigations toward animals being sold at markets as the potential source of the virus. Traders who only engaged in civet cat trading were much more likely to have been infected with SARS-CoV than traders who only engaged in snake or fowl marketing. All evidence pointed toward masked palm civet cats as playing a role in the transmission of SARS-CoV. It led to a temporary ban on the hunting, sale, transportation, and export of all wild animals in the Guangdong Province. Over 10,000 civets, badgers, and raccoon dogs were **culled** to prevent the spread of SARS-CoV infections.

In the wild, masked palm civets are arboreal, taking shelter in hollow trees in the mountain and hill forests of China. They are solitary and nocturnal predators; the female can bear litters of one to four pups twice per year. Civets eat mainly fruits but will also eat rodents, birds, insects, and roots. The farming of masked palm civets started in the late 1950s. Breeding the civets for use as exotic food became popular in the late 1980s. In 2003, there were about 40,000 masked palm civets, raised on 660 farms all over China. The farms started by capturing and breeding local wild civets or by breeding civets brought in from other farms. Scientists studying farmed civet cats reported few diseases among them. Several research groups suggested that SARS-CoV entered the human population through transmission by infected civet cats but that they were not the natural reservoir of SARS-CoV. In 2005, two independent groups of researchers isolated and identified SARS-CoV from Chinese horseshoe bats in China and Hong Kong. They trapped hundreds of bats in their natural habitats and screened them for the presence of **zoonotic pathogens**. Their logic for testing bats was that these mammals are often persistently infected with many different viruses (e.g., Nipah, Hendra, Ebola) but are asymptomatic healthy carriers of the viruses. Many people eat bats or use bat feces in traditional medicine for asthma, kidney ailments, and general malaise in Southeast Asia. Sanitation in the live markets is often lacking. Bats, civets, and other wild animals were caged together at food or traditional medicine markets, contributing to the conditions that led to the SARS-CoV outbreak.

Middle East Respiratory Syndrome (MERS): 2012–

Ten years after the SARS pandemic, another new human coronavirus emerged in Saudi Arabia. On September 20, 2012, Dr. Ali Mohamed Zaki, a virologist at the virology

Table 1-11 Summary of SARS Cases (November 1, 2002–July 31, 2003)[a]

Location	Female	Male	Total Cases	Number of Deaths	Average Age of Patient (years)	Dates of First and Last Probable Cases
China	2,674	2,607	5,327[b]	349	N/A	Nov. 16, 2002–June 3, 2003
China, Hong Kong Special Administrative Region	977	778	1,755	299	40 (0–100)	Feb. 15–May 31, 2003
Vietnam	39	24	63	5	43 (20–76)	Feb. 23–April 14, 2003
Canada	51	100	251	43	49 (1–98)	Feb. 23–June 12, 2003
United States	13	14	27	0	36 (0–83)	Feb. 24–July 13, 2003
China, Taiwan	218	128	346[c]	37	42 (0–93)	Feb. 25–June 15, 2003
Singapore	161	77	238	33	35 (1–90)	Feb. 25–May 5, 2003
Philippines	8	6	14	2	41 (29–73)	Feb. 25–May 5, 2003
Australia	4	2	6	0	15 (1–45)	Feb. 26–April 1, 2003
Republic of Ireland	0	1	1	0	56	Feb. 27, 2003
United Kingdom	2	2	4	0	59 (28–74)	March 1–April 1, 2003
Switzerland	0	1	1	0	35	March 9, 2003
Germany	4	5	9	0	44 (4–73)	March 9–May 6, 2003
Thailand	5	4	9	2	42 (2–79)	March 11–May 27, 2003
Italy	1	3	4	0	30.5 (25–54)	March 12–April 20, 2003
Malaysia	1	4	5	2	30 (26–84)	March 14–April 22, 2003
Romania	0	1	1	0	52	March 19, 2003
France	1	6	7	1	49 (26–61)	March 21–May 3, 2003
Spain	0	1	1	0	33	March 26, 2003
Sweden	3	2	5	0	43 (33–55)	March 28–April 23, 2003
Mongolia	8	1	9	0	32 (17–63)	March 31–May 6, 2003
South Africa	0	1	1	1	62	April 3, 2003
Indonesia	0	2	2	0	56 (47–65)	April 6–17, 2003
Kuwait	0	1	1	0	50	April 9, 2003
New Zealand	1	0	1	0	67	April 20, 2003
India	0	3	3	0	25 (25–30)	April 25–May 6, 2003
Republic of Korea	0	3	3	0	40 (20–80)	April 25–May 10, 2003
China, Macao Special Administrative Region	0	1	1	0	28	May 5, 2003
TOTALS			**8096**	**774**		

N/A (not available)

[a]WHO statistics.

[b]Case classification by sex is unknown for 46 cases.

[c]Since July 11, 2003, 325 cases have been discarded in Taiwan, China. Laboratory information was insufficient or incomplete for 135 discarded cases, of which 101 died.

Information from: http://www.who.int/csr/sars/country/table2004_04_21/en/index.html.

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FIGURE 1-37 Live market in Shenzhen, China. Some of the first cases of SARS were workers with live animals at the live food markets.

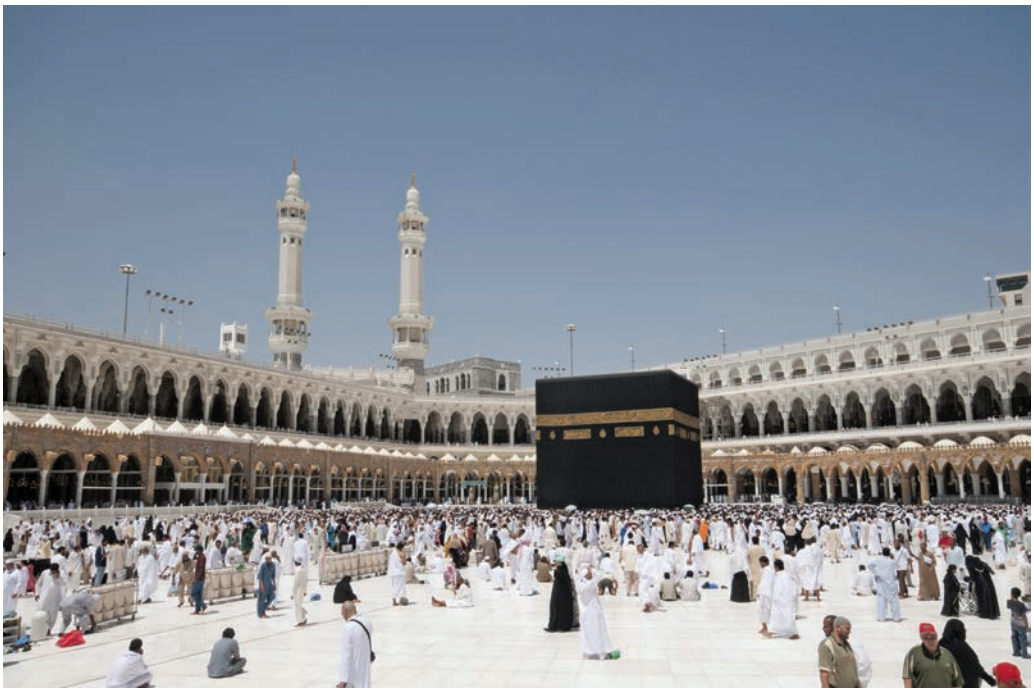
laboratory of Dr. Soliman Fakeeh Hospital in Jeddah, Saudi Arabia, was the first to announce the discovery of the virus on ProMED-mail, the online disease-reporting system. He isolated the coronavirus from a **sputum** sample (mucus coughed up from a patient's lungs) taken from a 60-year-old male patient who died on June 24, 2012, from atypical pneumonia and kidney failure. Dr. Zaki reported that the sputum sample tested negative for the influenza A and B viruses, parainfluenza virus, enterovirus, and adenoviruses. He shipped the genetic material (RNA) of the virus to Dr. Ron Fouchier, a virologist at the National Influenza Centre and Virology Department, Erasmus University Medical Center (EMC), located in Rotterdam, the Netherlands.

Dr. Fouchier is best known for being one of the virologists who created the controversial laboratory-made avian influenza A virus (H5N1) and performed experiments to demonstrate that the influenza A virus could infect laboratory ferrets in 2012. Besides studying influenza A virus zoonoses, his research team focused on the identification and characterization of several "new" viruses, such as the human *metapneumovirus* (hMPV); new human coronaviruses, including SARS-CoV and a human coronavirus isolated from hospitalized Dutch children (hCoV-NL); and a new influenza A virus (H16N3) isolated from black-headed gulls found in city parks of urban areas located in the Netherlands. Asking for Dr. Fouchier's expertise was a logical action for Dr. Zaki. Fouchier's team confirmed Zaki's isolated virus to be a new human coronavirus closely related to coronaviruses isolated from bats. Dr. Zaki's decision to inform the public of the discovery of the new coronavirus online enabled the international scientific and healthcare communities to gain an upper hand in the face of a new deadly virus and potential pandemic.

When the report came out, it caught the attention of doctors in a London hospital who were scratching their heads over a puzzling case of a 49-year-old man from Qatar suffering from atypical pneumonia. The Qatari man was airlifted to the London hospital 8 days prior to Dr. Zaki's post to ProMED-mail about the new coronavirus. The patient became ill while in Qatar but had visited Saudi Arabia in August. While trying to solve the case, a physician and a scientist independently noticed the ProMED-mail announcement of Dr. Zaki's newly discovered virus. Immediately, they decided to test for the new virus and determined that the man had been infected with a human coronavirus. Within a day, the viral genome was sequenced and confirmed to be nearly identical to the new coronavirus discovered by Dr. Zaki.

Dr. Zaki's post on ProMED-mail had set off a global response and alert. Laboratories around the world were equipped with the means to diagnose new cases of the severe respiratory illness. The world was much more prepared than in 2002. When the SARS outbreak occurred, the outbreak came first and SARS-CoV was discovered later. *In 2012, it was the reverse situation. The novel coronavirus was discovered first as virologists and healthcare workers watched and waited for the epidemic to happen or not happen.*

One year later, 130 cases from eight different countries had been reported to the WHO. The initial cases originated in Jordan, Qatar, Saudi Arabia, and the United Arab Emirates, hence the new coronavirus was named **Middle East respiratory syndrome-coronavirus**

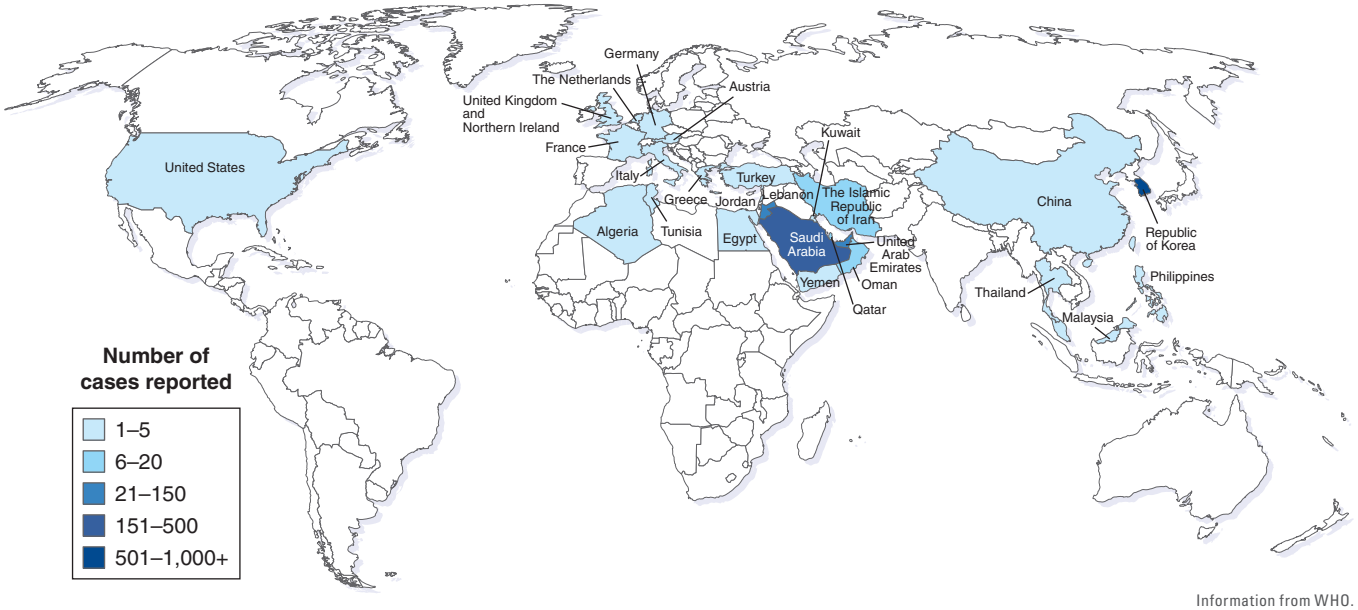


© Ahmad Faizal Yahya/Shutterstock.

FIGURE 1-38 Pilgrims face the Kaaba during prayer time. It is challenging to control sanitation and the spread of epidemics during a mass gathering of visitors from many different countries such as the pilgrimage of the Hajj.

(**MERS-CoV**). Travel-associated cases were diagnosed in France, Germany, Italy, Tunisia, and the United Kingdom. Of these cases, 58 (45%) individuals died. The average age of laboratory-confirmed cases was 50 years. Twenty-three (18%) of the cases were healthcare workers. Despite this evidence of person-to-person transmission, the number of contacts infected by persons with laboratory-confirmed infections was very low. The WHO had enough confidence in the global response to MERS-CoV that no travel restrictions were imposed on pilgrims traveling to Saudi Arabia for the Hajj, which begins during mid-October every year. The Hajj is one of the world's mass gatherings of Muslim people every year (**FIGURE 1-38**).

As of December 10, 2015, a total of 1,621 laboratory-confirmed MERS-CoV infections had been reported in 26 countries, including 584 deaths (**FIGURE 1-39**).



Information from WHO.

FIGURE 1-39 Map of the MERS epidemic, including cases from the start of the epidemic in April 2012 through December 4, 2015. Saudi Arabia and South Korea had the largest number of cases and deaths. Globally, there were 1,621 laboratory-confirmed cases, with 584 deaths, affecting 26 countries.

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FIGURE 1-40 Dromedary camels have one hump and are also called the Arabian camel. Dromedary camels are a possible animal reservoir for MERS-CoV.

Investigations continued to search for an animal reservoir of MERS-CoV. This epidemic was different from the SARS outbreak associated with the live markets in China.

Researchers found that MERS-CoV could replicate in various bat cell lines. Seven teams of researchers collaborated to collect **sera** from **dromedary camels** (**FIGURE 1-40**), goats, sheep, and cattle in the Middle East (Oman), Spain, the Netherlands, and Chile. They discovered that the sera from 50 of 50 (100%) of the camels from Oman and 15 of 105 (14%) of Spanish camels contained neutralizing antibodies against MERS-CoV, indicating infection/exposure to the new coronavirus. Sera from European sheep, goats, and cattle did not contain antibodies. Additional research found antibodies against MERS-CoV in camels in Egypt, Jordan, Saudi Arabia, and the Canary Islands. These results suggested camels are a possible animal reservoir of MERS-CoV, and when people come in contact with infected camels they too can become infected.

Interestingly, the owner of a farm in Qatar and a young man who worked on the farm were reported as new MERS cases in October 2013. Five of 14 camels on the farm tested positive for MERS-CoV RNA, meaning that the camels were infected with the coronavirus. All 14 camels had MERS-CoV antibodies, meaning that all of the camels had been infected at some time. The MERS-CoV genomic sequences for the human and camel samples containing MERS-CoV from the farm were nearly identical. It was impossible to conclude whether the people on the farm were infected by the camels, or vice versa. There is also a possibility that the people and camels were infected from a third, unknown source.

From May 11 through July 28, 2015, the largest outbreak of MERS outside of Saudi Arabia occurred in South Korea. The respiratory disease spread rapidly for several reasons, including the lack of awareness among healthcare professionals and the public about MERS; the weakness in preventing and controlling its spread during close and prolonged contact of infected MERS patients in crowded emergency rooms and multibed rooms in hospitals; and the custom of family members and visitors spending time with infected patients in hospital rooms (instead of isolating patients), facilitating spread among contacts.

The news of the outbreak was quite sudden, triggering an overreaction by the public. At the epidemic's peak, panic buying caused a shortage of face masks, and thousands of schools and kindergartens were closed even though there was no link of MERS-CoV transmission with school attendance in South Korea. Public events were canceled or suspended. The number of foreign tourists decreased by 41% compared to the same month of the previous year. South Korea lost $10 billion (U.S.). The MERS outbreak in South Korea resulted in 186 cases, including 38 deaths.

The MERS epidemic has not been without controversy. When Dr. Zaki provided the coronavirus that was isolated in the laboratory in Saudi Arabia to Dr. Fouchier for testing, he handed over the sovereign and intellectual property rights on the first diagnostic tests or treatments based on the viral sequencing results to the Erasmus University Medical Center (EMC) in the Netherlands. This created tensions among Dr. Zaki, the Saudi Ministry of Health authorities, and some researchers. The EMC had control over requests by researchers to access samples of the virus through **material transfer agreements (MTAs)** related to patent applications. The hospital authorities terminated Dr. Zaki's contract. Dr. Zaki left to work as a microbiologist at Ain Shams University in Cairo, Egypt. Dutch researchers have fulfilled MTA requests by other laboratories but have placed some restrictions on experiments on and applications of the new coronavirus. Who would have thought that a virus is a commodity for which the development of a vaccine or treatment causes conflicts of political and commercial interests that may hamper rather than help the global community prevent potential pandemics?

Hantavirus: 1993 (U.S. Four Corners Region) and 2012 (Yosemite Park)

On May 14, 1993, a Native American marathon runner in rural New Mexico known to be in excellent health collapsed and died of respiratory failure at an Indian Health Service hospital emergency room. Days before his collapse he visited a physician twice with flulike symptoms, but his chest x-ray was normal. He was treated with antibiotics and acetaminophen. Two days before he fell ill, his fiancée died of the same mysterious respiratory illness. Both of them died from fluid buildup in their lungs. Normally, with each breath the air sacs (**alveoli**) of the lungs take in oxygen and release carbon dioxide. The lung alveoli of the marathon runner and his fiancée were filled with fluid instead of air, preventing oxygen from being absorbed into the bloodstream, resulting in their death. On May 17, the Indian Health Service

FIGURE 1-41 Locations of the 24 confirmed cases of HPS during the 1993 Four Corners outbreak in the United States.

reported five similar deaths. All of the individuals were previously very healthy. By June 11, 24 cases of respiratory failure following flulike symptoms were reported in the **U.S. Four Corners region** of Colorado, Utah, Arizona, and New Mexico (**FIGURE 1-41**).

The cause of this **infectious disease** baffled medical examiners and CDC experts. Researchers at the CDC finally identified the infectious agent to be an **Old World hantavirus**. *Old World* refers to those parts of the world known before the voyages of Christopher Columbus. It includes Europe, Asia, and Africa and its surrounding islands. Hantaviruses were not associated with human disease in North America, nor had hantaviruses been associated with respiratory illness. This Old World hantavirus had entered the United States (the *New World*), and it is now referred to as a **New World hantavirus** in scientific and medical reports. The new or emerging infectious disease in the Four Corners region was named **hantavirus pulmonary syndrome** (**HPS**).

Until the 1993 description of HPS in the Four Corners outbreak, most Old World hantavirus outbreaks had been reported by the U.S. military. During the Korean War (1951–1953), U.S. Army physicians were suddenly confronted with what is now referred to as **hemorrhagic fever with renal syndrome (HFRS)**. Soldiers in the field were suffering from sudden fever, hemorrhage, multiple organ failure, and shock. It was not until 1978 that Korean scientist Ho Wang Lee and his colleagues isolated the first hantavirus known to cause **Korean hemorrhagic fever** (**KHF**). Lee's team isolated the hantaviruses from the lungs of striped field mice trapped in fields and near farm dwellings in Korea.

This first identified hantavirus was called *Hantaan*, after the river that runs near the famous 38th parallel between North and South Korea where most of the battles were fought in the Korean War and where most of the KHF cases were recorded. The American TV series *M*A*S*H*, which followed doctors and staff stationed at the 4077th Mobile Army Surgical Hospital, included an episode in its eighth season that aired on November 12, 1979, titled "Mr. and Mrs. Who?" in which Colonel Shaw and several other soldiers in the camp were afflicted with KHF. Hawkeye, B. J. Hunnicutt, Colonel Sherman Potter, and Charles Emerson Winchester III were faced with how to treat Colonel Shaw and other soldiers during the third phase of the illness, in which Shaw's kidneys were failing (**FIGURE 1-42**).

After the 1993 Four Corners hantavirus outbreak, researchers identified at least 20 different New World hantaviruses. Each was associated with a unique rodent host that was persistently infected with hantaviruses without causing disease (**FIGURE 1-43**). *Not all hantaviruses cause disease in humans*. For example, antibodies against the Prospect Hill hantavirus were found in mammals trapped in Minnesota and Maryland but were not

FIGURE 1-42 In an episode of the popular American TV series *M*A*S*H* that aired November 12, 1979, members of the 4077th Mobile Army Surgical Hospital medical staff must confront an outbreak of Korean hemorrhagic fever.

Information from McNeil, A. et al. 2011. "Hantavirus Pulmonary Syndrome." *Virus Research*. 162:138–147.

FIGURE 1-43 The locations and scientific and common names (green text) of the unique rodent reservoirs of New World hantaviruses. Pathogenic hantaviruses are in bold (red text) and contain a rodent next to it. Yosemite Park was the site of a U.S. outbreak in 2012.

associated with any human illness. However, other hantaviruses do cause HPS or HFRS in humans. For example, the Andes hantavirus that caused an HPS outbreak in southwestern Argentina in 1995 was associated with the most HPS cases to date. *Andes hantavirus is the only hantavirus to be associated with human-to-human transmission*.

The new hantavirus in the Four Corners region was isolated from the deer mouse, *Peromyscus maniculatus* (**FIGURE 1-44A**, **B**). It is likely that hantaviruses have been present in rodent populations for a long time in this region, but an unusually mild winter and increased spring rainfall led to an abundance of pinyon nuts on which the deer mice feed. The end result was an increased deer mice population in the summer and greater opportunities for people to come in contact with the infected rodents. The apparently healthy wild rodents harbor hantaviruses in their droppings, urine, and saliva.

The hantaviruses were aerosolized when humans came into contact with contaminated items such as blankets or food storage areas. Hantaviruses entered the respiratory tract through the inhalation of contaminated air particles through the aerosol of mouse droppings. Investigators trapped rodents in barns, woodpiles, and inside of homes.

More than 20% of deer mice subsequently captured in southwestern areas of the United States and as far north as southwestern Montana tested positive for the *Sin Nombre* hantavirus. The mass media focused on the fact that the unknown illness was linked to Navajos, creating fear and a media frenzy. Researchers determined that the hantavirus was not transmitted from person to person. The new hantavirus was originally named the **Four Corners Disease virus**, then Muerto Canyon virus, but the name was later changed to *Sin Nombre* virus, which means "no name virus" in English.

a Courtesy of the CDC/Games Gathany.

b Courtesy of the CDC/Cheryl Tryon.

FIGURE 1-44 (a) A deer mouse, *Peromyscus maniculatus*, is a hantavirus carrier when it enters the areas occupied by humans in rural and suburban areas. An increased population of deer mice led to the new hantavirus in the Four Corners region. The virus was originally named the Four Corners Disease virus and was later changed to *Sin Nombre* virus, or "no name virus" in English. **(b)** Rodents that carry the *Sin Nombre* virus can be deceptively cute, but suspected carriers must be handled with extreme care. This photograph shows scientists wearing protective gear while collecting samples from deer mice for hantavirus screening.

On August 16, 2012, two Californian residents were confirmed to be suffering from HPS after an overnight stay in Yosemite National Park. On September 5, WHO officials issued a global warning to travelers from 39 other countries who may have come into contact with contaminated rodent droppings or saliva while staying at the "signature" tent cabins in the Curry Village of Yosemite National Park. Deer mice infestations were discovered in the insulation of the tent cabins (**FIGURE 1-45**). All 91 of the tent cabins were closed indefinitely on August 28. Trapping/control of deer mice and educational interventions for staff and visitors were implemented.

CDC experts speculated that up to 10,000 park visitors were at risk for exposure to cabin dust containing *Sin Nombre* hantavirus based on the number of cabin reservations. By October 10, 10 laboratory-confirmed cases of hantavirus infection had been identified among visitors from California (8), Pennsylvania (1), and West Virginia (1) who had visited Yosemite National Park. Three cases were fatal. The cluster of cases in Yosemite National Park was unusual. Fortunately, *Sin Nombre* hantavirus is not contagious. Before this outbreak, there had been 61 cases of HPS in California since 1994. Of these, two were visitors of Yosemite National Park before 2012.

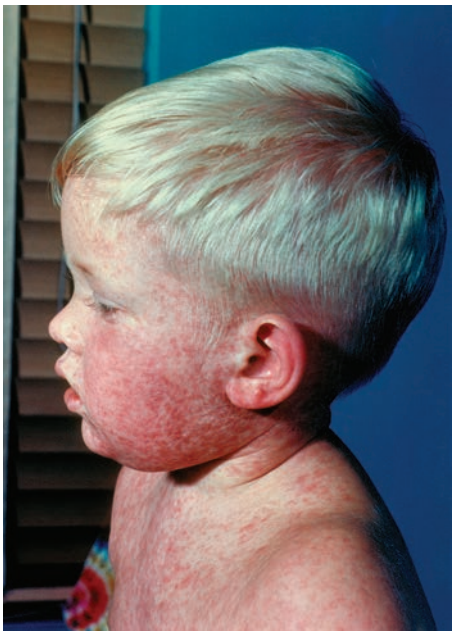
© Eunika Sopotnicka/iStockPhoto.

FIGURE 1-45 Tent cabins with bear lockers in Yosemite National Park at Camp Curry were infested with rodents carrying the *Sin Nombre* hantavirus in 2012.

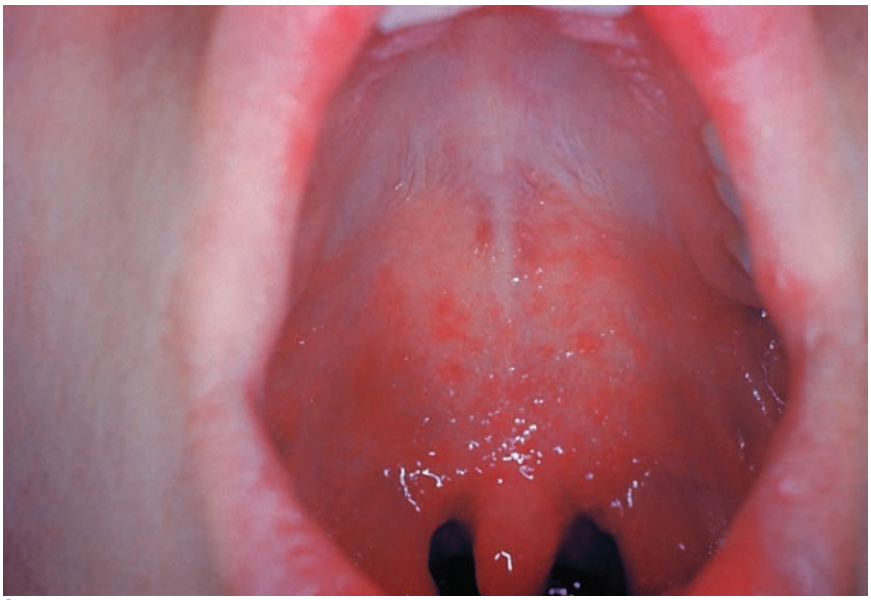
Measles Once Eliminated in 2000 in the United States, Now on the Rise

Prior to 1963, approximately 400 to 500 people died of measles every year. Virtually everyone in the United States contracted measles by the age of 20. The first measles vaccine was licensed for use in the United States in 1963. Today, the measles vaccine is administered with the mumps and rubella vaccines (**MMR**) or as MMR combined with varicella/chickenpox (**MMRV**). *Measles is the most contagious infectious disease; it is estimated that a person has a greater than 98% chance of becoming infected if directly exposed to someone infected with measles virus.* The symptoms are coldlike—cough, runny nose, and **conjunctivitis** (i.e., pink eye)—with the development of **Koplick's spots** in the mouth early in the disease, followed by a red rash that starts on the face and spreads to the extremities and over most of the body (**FIGURE 1-46**). The disease is usually mild and self-limiting, but 1 in 500 children with measles develop potentially serious and even fatal complications, including ear infections, pneumonia, seizures, and brain damage.

The United States was declared free of naturally occurring cases of measles in 2000. Even though an effective vaccine exists, an estimated 20 million cases, including 158,000 deaths, are attributed to measles



a Courtesy of the CDC.



b Courtesy of the CDC/Heinz F. Eichenwald, MD.

FIGURE 1-46 (a) Characteristic diffuse rash (on the third day) covering the face and shoulders of this boy with measles. **(b)** Koplik's spots found inside of the **palate** (roof of the mouth) of patient with measles. Koplik's spots are small, red, irregular-shaped spots with blue-white centers found inside the surface of the mouth (palate and cheeks).

each year worldwide. Measles cases continue to be imported into the United States when unvaccinated residents return to the United States after exposure to measles virus while traveling in Western Europe, Asia, or Africa. From 2001 to 2011, there was an average of 60 measles cases in the United States each year. The common denominators were no measles vaccination, which was frequently due to religious beliefs; overseas exposure to an unvaccinated person with measles; and home schooling. In 2013, the number of measles cases in the United States nearly tripled to 175 cases. CDC director Tom Frieden said, *"A measles outbreak anywhere is a risk everywhere, the steady arrival of measles in the U.S. is a constant reminder that deadly diseases are testing our health security every day."*

In 1998, **Andrew Wakefield**, a British gastroenterologist, was the lead researcher and main author of a study published in *The Lancet* that described a new autism syndrome speculated to be triggered by MMR vaccination. The study lowered parental confidence in vaccination programs and created an MMR vaccination crisis in the United Kingdom. It sparked questions about the measles vaccine in North America. Evidence mounted that there was no increased autism risk associated with the MMR vaccine.

The paper was retracted 12 years later (**FIGURE 1-47**). Britain's General Medical Council ruled that the data presented by Wakefield and colleagues were fraudulent. Wakefield was found guilty of unethical medical practice and scientific misconduct. Despite the retraction, parents and autism advocacy groups continue to support

EARLY REPORT

Early report

Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children

A J Wakefield, S H Murch, A Anthony, J Linnell, D M Casson, M Malik, M Berelowitz, A P Dhillon, M A Thomson, P Harvey, A Valentine, S E Davies, J A Walker-Smith

Summary

Background We investigated a consecutive series of children with chronic enterocolitis and regressive developmental disorder.

Methods 12 children (mean age 6 years [range 3–10], 11 boys) were referred to a paediatric gastroenterology unit with a history of normal development followed by loss of acquired skills, including language, together with diarrhoea and abdominal pain. Children underwent gastroenterological, neurological, and developmental assessment and review of developmental records. Ileocolonoscopy and biopsy sampling, magnetic-resonance imaging (MRI), electroencephalography (EEG), and lumbar puncture were done under sedation. Barium follow-through radiography was done where possible. Biochemical, haematological, and immunological profiles were examined.

Findings Onset of behavioural symptoms was associ... by the parents, with measles, mumps, and rub... vaccination in eight of the 12 children, with measl... infection in one child, and otitis media in a... All 1... children had intestinal abnormalities ranging from lymphoid nodular hyperplasia to g... oid ul...ration. Histology showed patchy chronic infla...tion in 11 children and reactive ileal lymph... perplasia in seven, but no granulomas. B... oural disor... included autism (nine), disintegrativ... sis (one), a... possible postviral or vaccinal encephalitis (...). There were no focal neurological abnormalities and ... and EEG tests were normal. Abno... laboratory results were significantly raised urinary ...thylmal... acid compared with age-matched control... (p<0·003), low haemoglobin in four children, ... low ...n IgA in ... children.

Interpretation We identi... associated gastrointestinal di...e and ...evelopmental regression in a group of previ...ly ...rmal ..., which was generally associated in time ... possible environmental triggers.

Lancet 199...**351:** 637–41

See Commentary page

Inflammatory Bowel Disease Study Group, University Departments of Medicine and Histopathology (A J Wakefield FRCS, A Anthony MB, J Linnell PhD, A P Dhillon MRCPath, S E Davies MRCPath) **and the University Departments of Paediatric Gastroenterology** (S H Murch MB, D M Casson MRCP, M Malik MRCP, M A Thomson FRCP, J A Walker-Smith FRCP.), **Child and Adolescent Psychiatry** (M Berelowitz FRCPsych), **Neurology** (P Harvey FRCP), **and Radiology** (A Valentine FRCR), **Royal Free Hospital and School of Medicine, London NW3 2QG, UK**

Correspondence to: Dr A J Wakefield

Introduction

We saw several children who, after a ... of apparent normality, lost acquired skills, incl... communication. They all had gastrointestinal ...mptoms, ...luding abdominal pain, diarrhoea, and ...ting and, ... some cases, food intolerance. We ...scribe... clinical f...ings, and gastrointestinal featur... of these ch...en.

Patients and meth...s

12 children, con...secutively ...ed to ... department of paediatric gast...erology ... a h...y of a pervasive developmental ...der with los... ...ed skills and intestinal symptoms ...rrh... abdomina... pain, bloating and food intolerance), were inv...gated. All children were admitted to the ward for ...ays, accom...ied by their parents.

Clinical investigations

...took histor... including details of immunisations and ...posure to infec...s diseases, and assessed the children. In 11 cases the histor... was obtained by the senior clinician (JW-S). Neur...ical psychiatric assessments were done by consultant staff (PH, MB) with HMS-4 criteria.[1] Developmental ... included a review of prospective developmental records from parents, health visitors, and general practitioners. Four children did not undergo psychiatric assessment in hospital; all had been assessed professionally elsewhere, so these assessments were used as the basis for their behavioural diagnosis.

After bowel preparation, ileocolonoscopy was performed by SHM or MAT under sedation with midazolam and pethidine. Paired frozen and formalin-fixed mucosal biopsy samples were taken from the terminal ileum; ascending, transverse, descending, and sigmoid colons, and from the rectum. The procedure was recorded by video or still images, and were compared with images of the previous seven consecutive paediatric colonoscopies (four normal colonoscopies and three on children with ulcerative colitis), in which the physician reported normal appearances in the terminal ileum. Barium follow-through radiography was possible in some cases.

Also under sedation, cerebral magnetic-resonance imaging (MRI), electroencephalography (EEG) including visual, brain stem auditory, and sensory evoked potentials (where compliance made these possible), and lumbar puncture were done.

Laboratory investigations

Thyroid function, serum long-chain fatty acids, and cerebrospinal-fluid lactate were measured to exclude known causes of childhood neurodegenerative disease. Urinary methylmalonic acid was measured in random urine samples from eight of the 12 children and 14 age-matched and sex-matched normal controls, by a modification of a technique described previously.[2] Chromatograms were scanned digitally on computer, to analyse the methylmalonic-acid zones from cases and controls. Urinary methylmalonic-acid concentrations in patients and controls were compared by a two-sample *t* test. Urinary creatinine was estimated by routine spectrophotometric assay.

Children were screened for antiendomysial antibodies and boys were screened for fragile-X if this had not been done

© Teri Shors.

FIGURE 1-47 Editors of *The Lancet* retracted this controversial paper 12 years after it was published. The authors suggested a link between the MMR vaccine and autism. Many parents, especially in the United Kingdom and the United States, lost confidence in vaccination because of this research study.

Wakefield. **Paul Offit**, chief of infectious diseases at the Children's Hospital in Philadelphia, said: "*This retraction by* The Lancet *came far too late. It's very easy to scare people; it's very hard to unscare them.*"

That being said, parental opposition to vaccination in the United States continues. In 2013, eight outbreaks accounted for 77% of measles cases. The majority of cases occurred in locations in which there were pockets of unvaccinated children exposed to an active case of measles. The largest outbreak occurred in New York City. None of the sick children had documentation of vaccination at the time of exposure. Parents objected to childhood vaccination because of religious or philosophical beliefs.

Once again, in 2015, 189 individuals from 24 states and Washington, D.C., were reported to have measles. Most cases were a part of a large multistate outbreak linked to exposure to a visitor infected with measles virus who went to Disneyland or Disney Adventure Park in Anaheim, California. The outbreak likely started after an overseas traveler was infected with measles virus and then attended one of the parks. Genetic analysis of the measles virus isolated in this outbreak was identical to a measles virus strain that caused a large epidemic in the Philippines in 2014.

The CDC recommends that children receive two doses of MMR, starting at 12–15 months of age. Vaccination coverage, early detection of cases, and rapid public health response to a case are key factors needed to eliminate measles cases, despite continued imported cases through international travel. Travelers should make sure their vaccinations are up-to-date. *These outbreaks underscore the necessity to maintain a high level of immunity in the population as a preventative measure.*

Schmallenberg Virus in Europe: 2011 to 2013

From August through September of 2011, an unidentified disease of cattle was reported on farms located in Germany and the Netherlands. Disease symptoms included fever (40°C, or 104°F), loss of appetite, up to 50% reduction in milk production, and occasional bouts of diarrhea. Some of the symptoms were similar to those of bluetongue virus infections that caused a major epizooty from 2006 to 2009 in Europe. An **epizooty** is an epidemic among large numbers of animals in a defined location. It was feared that this was a **reemergence** of a bluetongue virus outbreak. Surprisingly, the cattle tested negative for endemic or **emerging viruses**, such as bluetongue virus, bovine herpesvirus type-1, malignant catarrhal fever virus, bovine diarrhea virus, foot-and-mouth disease virus, epizootic hemorrhagic disease virus, Rift Valley fever virus, and bovine ephemeral fever virus.

Blood samples collected and pooled from three sick cows on a farm located near the city of Schmallenberg (North Rhine-Westphalia, Germany) along with a blood sample for comparison from a healthy cow located on a different farm were sent to a team of scientists led by Dr. Martin Beer of the Friedrich Loeffler Institute (FLI) in Greifswald-Insel Riems, Germany, in October 2011. They performed sequencing and metagenomic analyses. The blood samples from the sick cows contained sequences of a new virus related to a Shamonda-like virus that had been detected in cattle in Japan in 2002. Prior to that, the **Shamonda virus** had been isolated from cattle and *Culicoides* spp. **biting midges** in Nigeria in the 1960s. Transmission of these viruses was known to occur mainly through biting midges and mosquitoes. The new viral sequences were not detected in the blood samples of the healthy cow.

The scientists at FLI developed a diagnostic test based on the new viral sequence information to screen blood samples collected from more sick cattle on six different farms in North Rhine-Westphalia located in close proximity to farms in the Netherlands that had sick cows, including some stillborn calves.

The new Shamonda-like virus was isolated from a blood sample of a diseased cow from a farm near Schmallenberg and was shown to cause **cytopathic effects** in an insect cell line, *Culicoides variipennis* larvae cells. Four different healthy cows were inoculated with the new viral isolate or blood from sick cows that tested positive for the new virus. The cows developed similar symptoms. Concurrently, *Culicoides* spp. biting midges were trapped near sheep flocks on three dairy farms in the Netherlands. Genetic material (RNA) of the new Shamonda-like virus was detected in a high proportion of different species of biting midges collected—*C. scoticus*, *C. obsoletus*, *C. dewulfi*, and *C. chipterus*—helping to explain the transmission of the newly identified virus (**FIGURE 1-48**). Biting midges trapped in Denmark, Norway, Poland, and Sweden also tested positive for the virus. Field-trapped common European mosquito species tested negative for the virus. Scientists also screened archived blood samples collected from livestock in Europe before 2011, none of which tested positive for Schmallenberg virus. This new or emerging virus was named Schmallenberg virus after the place of origin of the original blood samples collected from sick dairy cows.

During the months after the primary onset of the disease, more severe symptoms were observed. Large numbers of malformed lambs (**FIGURE 1-49**) born dead or that died after birth with defects such as malformations of the skull; spinal column (e.g., **kyphosis**, or hunchback); and brain, including **hydrocephalus**, were reported from farms in the Netherlands in November and December of 2011. This raised concerns that Schmallenberg virus might cause **congenital defects**, because related viruses were known to cause **arthrogryposis hydranencephaly syndrome** (**AHS**). AHS results in **stillbirths** and congenital defects in fetuses of cattle, sheep, and goats following infection during pregnancy. The brain of a malformed lamb in the Netherlands tested positive for Schmallenberg virus. Surveillance was stepped up across

a Specimen and image provided by Purdue Entomological Research Collection, Jennifer M. Zaspel and Gareth Powell.

b Specimen and image provided by Purdue Entomological Research Collection, Jennifer M. Zaspel and Gareth Powell.

c Courtesy of Scott Bauer, United States Department of Agriculture/Agricultural Research Service Photo Gallery.

FIGURE 1-48 (a) *Culicoides* spp. biting midges are very small and sometimes called "no-see-ums." The adults are usually less than 1/8th inch (3.2 mm) long. Photograph shows *Culicoides varipennis*. Scale bar added to demonstrate relative size. **(b)** Up-close image of a mosquito (*Culex pipiens*). Scale bar added to demonstrate relative size. **(c)** Up-close image of female biting midge that is 1/16th inch (1.6 mm) long feeding on blood delivered through an artificial membrane.

Courtesy of Rachael Tarlinton, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough, UK.

FIGURE 1-49 Malformed lamb associated with Schmallenberg virus infection. The lamb has an extreme curvature of the upper spine, also known as hunchback or kyphosis.

Northern Europe, with the UK's Department for Environment, Food and Rural Affairs (Defra) and Animal Health and Veterinary Laboratories Agency (AhVLA) raising awareness among veterinarians.

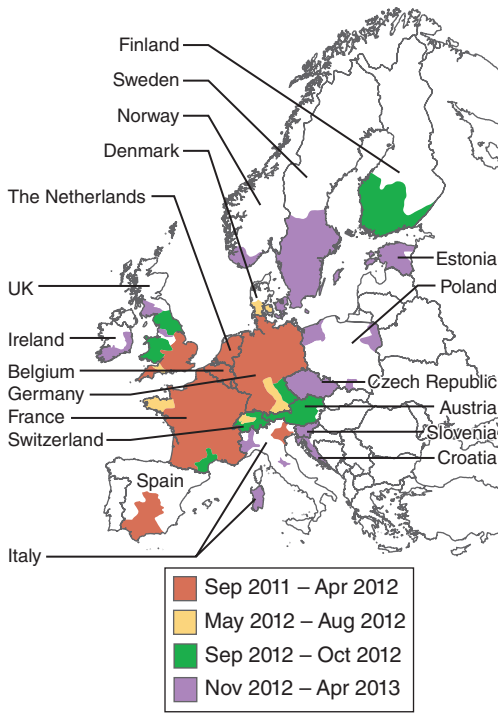
The United Kingdom is the largest producer of sheep meat in Europe and the fifth largest producer worldwide; outbreaks caused by Schmallenberg virus had the potential for considerable impact (economic and otherwise) on farmers, the meat industry, and the food supply, both in the United Kingdom and on mainland Europe.

The semen of naturally infected bulls was shown to contain Schmallenberg virus, raising concerns that semen could be infectious and have an impact on fertility. The emergence of Schmallenberg virus infections had a major impact on the international trade of susceptible animals and animal products such as semen and embryos. More than 15 countries imposed restrictions on cattle imports from the European Union. The importation of embryos and semen of livestock was restricted by the United States, Mexico, and Japan.

Schmallenberg virus spread rapidly in cattle, sheep, and goats in vast parts of Europe from its first identification in September 2011 through April 2013. Within a few

months, it spread over a large area, including Belgium, France, Germany, Luxembourg, the Netherlands, the United Kingdom (south and east of England), and Switzerland. Sporadic infections were reported from Italy, Spain, and Denmark. By October 2012, antibodies to Schmallenberg virus were detected in goats, sheep, and cattle in Poland, Austria, Scotland, Norway, the Czech Republic, Slovenia, and Estonia (**FIGURE 1-50**). To date, the Schmallenberg virus has spread to most countries of Europe, with a few exceptions, including Cyprus, Lithuania, and Portugal.

Serological surveys were used to screen livestock, farmers, and wildlife. Antibodies against Schmallenberg virus were found in red and roe deer, mouflon, and alpacas, indicating their exposure to Schmallenberg virus, but there was no evidence of outbreaks or congenital malformations of animals in the wild. Viruses related to Schmallenberg virus, such as the Akabane and Shamonda viruses, are known to also infect buffalo, pigs, horses, camels, and zebras. Two related viruses cause fevers in humans: Iquitos (first isolated from a patient in Peru in 1999) and Oropouche (originally isolated from a patient in Trinidad in 1954 and a public health concern in Central and South America today). No evidence of antibodies was found in humans (e.g., farmers working near or with sick livestock) or zoonotic transmission to humans by Schmallenberg virus. Therefore, this epizootic outbreak was not considered a public health risk.



Information from The European Food Safety Authority Technical Report "Schmallenberg virus: Analysis of the epidemiological data." (May 2013).

FIGURE 1-50 Rapid geographic spread of Schmallenberg virus infection for all ruminants (cattle, sheep, and goats) in Europe from September 2011 through April 2013.

How did Schmallenberg virus enter Europe? The rapidly spreading bluetongue virus outbreak that had recently emerged in Europe was also transmitted by *Culicoides* spp. The geographic locations affected by Schmallenberg virus in 2011 overlap many of the same areas affected by bluetongue virus in 2006. Some scientists think that changes in climate and land use and the influence of wind may favor transmission. Midges can be blown by wind for hundreds of miles. A 2013 study by Sedda and Rogers suggested that midges arriving by downwind movements could explain 62% or a mixture of downwind and random movements could explain 38% of Schmallenberg infections during dusk when the midges were most active. Another possibility was the presence of midges in shipments of plants. The Netherlands is a major center for international flower imports (e.g., from sub-Saharan Africa), which are cut and packed at night under bright lights. Midges are attracted to bright lights and could be trapped within shipments and then released upon unpacking.

Midges are one of the most difficult insects to control. Chemicals to control midges may harm aquatic environments and nontarget organisms. They breed over large areas, making chemical control impractical. The best method to prevent bluetongue virus outbreaks was through vaccination. Based on the similarities between bluetongue virus and Schmallenberg virus regarding the insect vector involved and the affected hosts (livestock), vaccination against Schmallenberg virus was considered to be one of the most promising ways to control the disease from breaking out on farms. Two inactivated vaccines for cattle and sheep became available in 2013: SBVvax (Merial) developed in France and Bovilis SBV (MSD) developed in the United Kingdom.

Ebola Virus Disease in West Africa: 2014–2016

As noted by the American author and journalist David Quammen, "*Ebola doesn't disappear. It just goes into hiding.*" **Patient zero** of the Ebola epidemic in West Africa was 2-year-old Emilo Ouamiouo. The toddler suffered from fever and vomiting and had black stool. The boy died in the Meliandou village of Gueckedou, Guinea, on December 6, 2013. A colony of thousands of Angolan free-tailed bats roosted in a hollow tree where Emilo and other children from the village played. The boy's family hunted bats and grilled and ate them. Past human Ebola epidemics were associated with epizootics in wildlife, such as those affecting duikers (small antelope), gorillas, and chimpanzees, or were traced to hunters who butchered animals found dead in the rainforest. Bats are thought to be a reservoir for the Ebola virus. The hollow tree hosting the bats was only 50 yards (about 150 feet) from the house where the boy lived. The first individuals to be infected in this new outbreak were women and children rather than hunters. It is possible that the toddler was infected when playing in the tree with an

infected bat or by ingesting a small quantity of bat droppings contaminated with Ebola virus.

The child's sister, mother, and grandmother; a village midwife; and the nurse who cared for them were infected and suffered from similar symptoms, in addition to bleeding. Between December 29, 2013, and January 25, 2014, they all died. The villagers were frightened and baffled. This area was notorious for cholera epidemics and many other infectious diseases. *Early symptoms of the disease that killed the family and their caregivers were camouflaged by those similar to many other endemic diseases.*

Meanwhile, the disease continued to spread, its causative agent unknown. An infected health worker from Gueckedou likely triggered the transmission of Ebola viruses to people in nearby districts and to patients in Gueckedou's Macenta hospital where she died.

The Ebola epidemic that started in Guinea was unprecedented for many reasons. This was the first time that an Ebola outbreak had occurred in West Africa. Medical teams struggled to curb human-to-human transmission of Ebola virus. Superstition, misinformation about how to care for people infected by Ebola virus, and poor public health infrastructure contributed to the spread of disease. There was a severe shortage of doctors. Many of the new cases of Ebola virus disease were hospital acquired or acquired when family members and relatives of the dead touched the bodies during funeral practices. Residents strongly opposed aid from healthcare workers. *An unprecedented number of health workers were infected and died.* Supplies of personal protective equipment and disinfectants were inadequate.

Ebola spread to Liberia by a symptomatic traveler. *The cases in Liberia increased exponentially and were by far the most worrisome* (**FIGURE 1-51A**). Bodies in the poor, overcrowded, and filthy West Point slum in the capital, Monrovia, were simply thrown into two nearby rivers. The West Point slum had more than 70,000 people crowded on a peninsula with no running water, sanitation, or garbage collection. According to the head of the WHO Emergency Assessment Team, Dr. Rick Brennan, delivering a baby in Liberia was "one of the most dangerous jobs in the world." Some pregnant women were infected with Ebola virus, but neither they nor the doctors were aware of their infection. Several obstetricians isolated themselves when they left the wards, forgoing any contact with families, neighbors, or friends. Some of the "medical giants" became infected and died of Ebola virus disease. One of the most emotionally, gut-wrenching jobs was guarding the gates to overflowing treatment centers in Monrovia where desperate families and their sick loved ones were turned away. **Doctors Without Borders** staff watched helpless, hopeless patients die alone and without dignity outside of the locked gates.

Five months into the outbreak, *the disease spread to Conakry, the capital of Guinea, a city that has a population of 2 million people* (**FIGURE 1-51B**). *It was the first time in human history that Ebola infections occurred in such a highly populated location, as opposed to remote areas and small villages, with an international airport.* Ebola cases and deaths in Conakry created a serious situation in West Africa. Poverty, weak governance, limited health infrastructure, and political instability stood in the way of its rapid containment. The most severely affected countries—Guinea, Liberia, and Sierra Leone—were only recently returning to political stability following civil war and conflict, with healthcare systems largely destroyed or severely disabled (**FIGURE 1-52A**). There were hundreds of new cases each week, and the world was terrified. *The exponential growth of cases raised concerns about whether the rising number of cases of Ebola virus disease could be reversed.*

At the time of the epidemic, there were no FDA-approved postexposure treatments for Ebola virus infections for human use, no vaccines to prevent the viral disease, and no standard assays to predict how infected individuals would fare after becoming infected. The standard protocol for treatment of Ebola patients is called **best available supportive care (BASC)**. BASC is palliative care that focuses on providing the patient with relief from EVD symptoms that are life threatening. It involves balancing the patients' fluids and electrolytes, maintaining their oxygen status and blood pressure, and treating the patient for any complicating infections.

In order to slow the spread of the epidemic, the WHO and the United Nations Mission for Ebola Response established the **70-70-60 plan**, with the goal of isolating and treating 70% of cases and burying 70% of Ebola victims safely within 60 days from October 1 until December 1, 2014. The international support to meet this target was unprecedented, and resources were pooled to set up more isolation centers. Intense networking was used to address the collapse and state of health infrastructure. Mobile laboratories with new rapid tests to diagnose cases of Ebola virus disease were established. Safe burial practices were widely adopted. Worldwide, there were 28,639 cases of Ebola virus disease and 11,316 deaths as of February 3, 2016. Health workers were at highest risk of infection, resulting in 881 cases, including 513 health worker deaths. As the Ebola epidemic fades from the world's attention, we must learn from it. As noted by CDC director Tom Frieden on June 30, 2015, "We've made great progress, but we can't let our guard down. There will continue to be cases and clusters of Ebola, but an epidemic of this kind we've had in the past year never has to happen again" (**FIGURE 1-52B**).

a Courtesy of Sally Ezra/CDC.

c Courtesy of Dr. Heidi Soeters/CDC.

b Courtesy of Sally Ezra/CDC.

FIGURE 1-51 **(a)** An automobile that was draped with a banner over the hood that read "EBOLA IS HERE: LET'S STOP THE SPREAD OF EBOLA." The vehicle was being used by the zonal head for the Kings Gray community near Eternal Love Winning Africa (ELWA) hospital in Monrovia, Liberia. **(b)** Apartment complexes in Conakry, Guinea, photographed during the 2014 Ebola outbreak. The Ebola virus disease spread to Conakry, a city with 2 million people. **(c)** Red and green gloves propped up on sticks to dry after being washed in a bleach solution to kill infectious Ebola viruses. The gloves somewhat resembled a garden outside the grounds of Donka Hospital in Conakry.

a Courtesy of Daniel J. DeNoon/CDC.

b Courtesy of Sally Ezra/CDC.

FIGURE 1-52 **(a)** Site outside of a village in West Africa where a mattress was burned after an Ebola patient laid on it during his illness. **(b)** Dr. Tom Frieden, director of the CDC, dressed in personal protective equipment (PPE) while undergoing decontamination procedures as he is prepared to exit ELWA 3 (located in Monrovia, Liberia), which opened as an Ebola treatment center on August 17, 2014. It was staffed by members of Doctors Without Borders.

Summary

Viruses are submicroscopic agents capable of directing their own replication inside of living host cells, but they are *not* cells. The majority of viruses share a few common features. Viruses are smaller than their host cells, are not alive, and do not use energy. Early pioneers of virology studied bacteriophages; plant viruses such as TMV; and viruses that caused large numbers of outbreaks and mortalities throughout recorded human history, such as Variola, the virus that causes smallpox.

Bacteriophages and other viruses are used as biological tools—our "eyes into cells"—to study virus–host cell interactions and the molecular biology of cells. The origin of viruses is a topic of continuous debate. Recent advances in genomics and structural biology, along with bioinformatics tools will allow researchers to test alternative evolutionary models.

Viruses have long had a bad rap. However, more and more evidence is unraveling ways in which viruses benefit their hosts through mutualistic interactions. For example, parasitoid wasps depend upon polydnaviruses for survival and development of their eggs, with the wasp providing essential genes for the replication of polydnaviruses. Cryptic plant viruses paralyze aphid pests on their plant hosts. Other plant viruses enable their hosts to survive environmental changes, such as drought or changes in temperature. Bacteriophages produce BAM as an antibacterial defense. Some viruses may even be beneficial to their human hosts. For example, infection with one type of virus may induce immunity that protects an individual from infection by a different virus or bacterial pathogen. Metagenomics has allowed researchers to study the virome present on human skin. Papillomaviruses, polyomaviruses, circoviruses, and bacteriophages exist as diverse viral residents on human skin. Some viral residents may play a role in protection against other pathogens, and fluctuations in their numbers may be influenced by the immune status of the host.

Bacteriophages play a role in aquatic viromes, aiding in the recycling of carbon and other nutrients necessary to support living organisms. They influence microbial ecology and diversity, evolution, and the health of all living organisms in the ocean. Stable plant viruses have been detected in freshwater, but their biological significance is unknown.

Viral diseases can be mild or severe. The transmission of viruses and interactions with their hosts are briefly introduced. Viruses are transmitted to humans through direct or indirect transmission. Viruses gain access into the body through portals of entry. After completing their cycle of disease, viruses require a portal of exit to spread infections into the community. Pathogenesis toward the host cells, tissues, and/or organs is caused by the disruption of normal cellular functions at the molecular level. The body's immune response toward

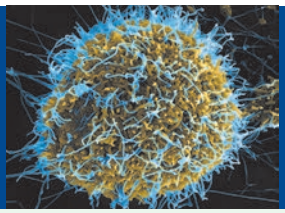
viral infection (e.g., cytokine storm) may cause cellular damage as well.

Viruses are also utilized in the healthcare arena. Gene therapy, bacteriophage therapy, vaccines, and virotherapy to treat cancers are just a few of the applications of viruses in fighting disease.

Viral outbreaks have impacted humans throughout history. As humans shifted from hunter-gatherer lifestyles to living in permanent settlements, epidemics began to spread more easily. The American Civil War occurred before health officials accepted the theory that germs cause disease. Soldiers' camps became a major source of disease transmission during the war. Soldiers from remote, rural areas were more vulnerable to viral infections such as measles, chickenpox, mumps, rubella, and smallpox than soldiers who had grown up in cities and been exposed to these viruses and developed immunity toward them during childhood.

In the 20th century, influenza, poliomyelitis, and HIV/AIDS all captured news headlines, and victims. Today, new and reemerging viruses continue to impact humans. The first viral pandemic of the 21st century was caused by a swine influenza A virus (H1N1). Human, bird, and swine influenza A viruses continue to circulate around the world. The main challenge for scientists and health officials has been to assess how severe a particular influenza A epidemic may be. Other emerging viruses causing severe respiratory illness in humans are SARS-CoV, MERS-CoV, and New World hantaviruses. Although measles was eliminated in the United States in 2000 because of an effective vaccination program, it is now on the rise. Today, most new cases of measles in the United States are attributed to parental resistance to vaccination for religious or philosophical reasons and overseas exposure to an unvaccinated person with measles. The recent Ebola and Zika virus crises presented new challenges at an international level. Countermeasures such as vaccines and antiviral therapeutics are not available to date.

Emerging viral diseases of animals are also a cause for concern. For example, bluetongue and Schmallenberg viruses transmitted to livestock by biting midges spread rapidly in Europe, causing major epizootic outbreaks on farms. Continued effective surveillance is necessary to predict viral outbreaks so that measures can be put in place to coordinate their control and prevention.



CASE STUDY 1: QUESTIONS

These questions relate to the Case Study 1 presented at the beginning of the chapter.

1. To find general information about viral fish diseases using the Internet, what search engines and websites would you use? What keywords would you use? (Hint: Read Virus File 1-1.)
2. How would you find primary literature and reviews pertaining to VHS? List at least five scientific journal articles and two reviews on VHS. Where was the VHS research conducted?
3. Can VHS cause die-offs of freshwater and marine fish? How many species of fish are susceptible to infection by VHS virus?
4. List the parts of the world where VHS can be found, and note where it is predominant.
5. What environmental conditions are optimal for VHS outbreaks among fish in the wild? In the aquaculture industry?
6. What are the signs and symptoms of VHS?
7. How long can VHS virions retain their infectivity in water?
8. Which of the following vectors can cause VHS transmission or spread?
   - Boating
   - Ballast water
   - Fishing tackle
   - Crustaceans
   - Leeches
   - Turtles
   - Birds
   - Fish-to-fish transmission

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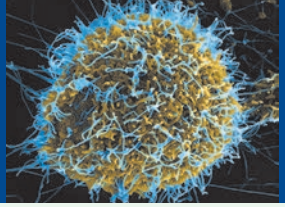

CASE STUDY 1: QUESTIONS (continued)

9. Create a list of measures that anglers can take to avoid spreading VHS to other bodies of water.
10. When was VHS first discovered in European aquaculture? What European countries are affected by VHS?
11. Do VHS virus strains differ in their virulence for different fish species? Compare VHS in rainbow trout to VHS in lumpfish.

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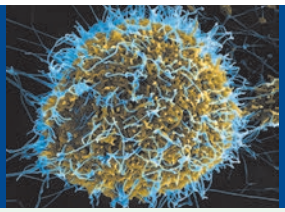
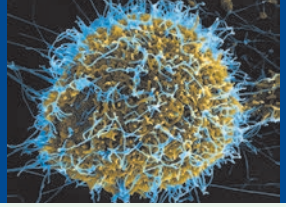


CASE STUDY 2: TICKBORNE HEARTLAND VIRUS

In June 2009, two middle-aged farmers living 60 miles apart in northwestern Missouri independently presented symptoms of fatigue, fever, diarrhea, **anorexia**, headache, and nausea. Both men spent most of their days working in fields of a wooded area where they had gotten a lot of tick bites, but neither one had a rash or itching on body sites where they removed embedded ticks. One of the men received an average of 20 tick bites daily for about 2 weeks. About a week after their symptoms began, both were hospitalized. Laboratory testing revealed both had **thrombocytopenia** (low platelet count). The farmers were promptly treated with antibiotics for suspected ehrlichiosis because of the wide variety of symptoms and the confirmed contact with ticks. Ehrlichiosis is a bacterial flulike illness transmitted by ticks. The antibiotics did not quell the illness in either farmer, although all symptoms abated for both farmers 4–6 weeks after their symptoms began.

It was later discovered that the farmers had contracted an **arthropod** virus carried by a tick now tentatively known as the **Heartland virus**. Dr. Scott M. Folk, an internal medicine physician with an infectious disease subspecialty who treated the farmers at the Heartland Regional Medical Center in St. Joseph, Missouri, named the Heartland virus. Dr. Folk drew blood and bone marrow samples from the farmers and sent the samples to CDC experts working at the Viral Special Pathogens Branch and the Infectious Disease Pathology Branch for more elaborate testing, because he suspected that a new infectious agent had caused the farmers' illnesses. The CDC experts confirmed that the culprit was, in fact, a novel virus. Although the farmers were able to improve on their own, they were sick for weeks. Both returned to their normal daily routines, but the patients complained of fatigue, recurrent headaches, and short-term memory loss even 2 years after their illness. However, it is not clear whether these symptoms can be attributed to the Heartland virus infection.

The doctors treating the farmers believed that there were more cases that had not been reported because of the wide range and different degrees of severity of symptoms associated with the new suspected tickborne infectious disease. CDC epidemiologists continued to work with state and local partners in order to define the ecology and modes of transmission of the Heartland virus, to develop diagnostic assays, and to identify additional cases. From this work, it was discovered that the Heartland virus was found in the **lone star tick** (*Amblyomma americanum*). The lone star tick has a characteristic yellow "lone star" on its back (**FIGURE 1**). It is also an important vector of the bacterial pathogens *Ehrlichia chaffeensis* and *Ehrlichia ewingii*, causing ehrlichiosis in humans and



CASE STUDY 2: TICKBORNE HEARTLAND VIRUS (continued)

Courtesy of James Gathany/CDC.

FIGURE 1 The lone star tick (*Amblyomma americanum*). Note the characteristic "lone star" on its dorsal surface.

dogs. The geographic distribution of the established breeding sites of the lone star tick is shown in **FIGURE 2**. This map does not represent the risk areas of tickborne disease.

Six additional cases of Heartland virus disease were identified during 2012–2013. Four of the individuals were hospitalized, including one who died. Five of the patients were from Missouri, and one was from Tennessee. All of the patients were male and reported spending several hours a day outside; five of the six patients confirmed they had experienced tick bites 2 weeks before the onset of their illness.

Courtesy of the CDC.

FIGURE 2 Established/breeding sites of the geographic distribution of the lone star tick in the United States. *This map does not represent the risk of contracting any specific tickborne illness.*

No vaccine or medication is available to prevent or treat Heartland virus disease. Prevention depends on using insect repellents; wearing long sleeves and pants; avoiding bushy, wooded areas; and performing tick checks after spending time outdoors.

A clinical case of Heartland virus disease is defined by the following symptoms:

- Fever ≥ 100.4°F (38°C).
- Leukopenia: White blood cell count < 4,500 cells/mm^3 (normal range is 54,500–12,000 cells/mm^3).
- Thrombocytopenia: Platelet count < 150,000/mm^3 (normal range is 150,000–400,000/mm^3).
- Evidence of a recent Heartland virus infection through RT-PCR detection of viral RNA in blood or tissues or viral neutralization assays using patient convalescent serum.

The CDC is working hard with state health departments and hospitals to educate healthcare workers about the potential impact of the Heartland virus on public health and how to protect people from infection, especially during "tick season." To learn more about the ticks that may be present in your particular area, contact your local health department or United States Department of Agriculture (USDA) extension office.

1. The Heartland virus is transmitted by ticks and is related to viruses in the *Bunyaviridae* family. Perform a PubMed or ScienceDirect search and create a list of other members of this family of viruses, the diseases they cause, and the insects/arthropods that transmit them.
2. What are the physical characteristics of viruses in the *Bunyaviridae* family (e.g., type of genome, size of particles)?
3. Do you think the farmers were contagious? Explain your answer.
4. Create a table listing at least five infectious diseases in humans transmitted by insects other than ticks. Include the disease, along with its insect vector.
5. What precautions or measures could you take to prevent infection by the Heartland virus?
6. What types of ticks are abundant in Missouri that could be the potential vector for this new disease?
7. What kinds of surveillance or screening could be done to find more individuals who have been exposed to or infected by the Heartland virus? What groups are more likely to have been exposed or infected, and why? (Consider location, occupation, daily routines, gender, age, etc.)

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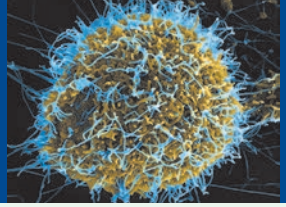

CASE STUDY 2: TICKBORNE HEARTLAND VIRUS (continued)

8. Blood samples collected from farm animals in Minnesota were recently found to contain antibodies that react with the Heartland virus. Can members of the *Bunyaviridae* family infect animals? Is it possible that the farmers in this case study were infected through cattle or other livestock on the farm? Explain your answer. List ways in which zoonotic transmission might occur.
9. New research has correlated tick bites and meat allergies. Use the references following the case study (such as Saleh, H., et al. 2012) to write a summary of these findings. In your summary, address which ticks are correlated with meat allergies, observed symptoms, and clinical management of meat allergies.
10. During 2004–2013, about 60 cases of Powassan virus disease were reported in the United States. The **Powassan virus** is transmitted by ticks primarily in the northeastern United States and the Great Lakes region. Compare and contrast the Heartland virus and the Powassan virus. Are they similar in structure? Are their viral genomes similar? Do they cause similar signs and symptoms in the diseases they cause?
11. In 2014, there was a case of Bourbon virus disease in eastern Kansas. The individual died. Research the signs and symptoms of Bourbon virus disease. The **Bourbon virus** is associated with ticks found over a wide geographic area. List additional regions where this virus has been isolated. Compare and contrast the genomes and virion structures of the Heartland, Powassan, and Bourbon viruses (*Hint*: Begin your research with the article by Kosoy et al. listed in the References.)

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Resources

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Saving Dr. Brantly: The Inside Story of a Medical Miracle. NBC News Special with Mark Lauer. September 5, 2014.
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Building the Perfect Bug: Virology, Rogue Science, and Bioterrorism. 2012. Insight Media.
Changing Planet: Past, Present, Future. 2012. Howard Hughes Medical Institute.
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"The Age of AIDS." 2006. *Frontline*, PBS.
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"The Boy in the Bubble." 2006. *American Experience*, PBS.
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A World Without Polio. 2005. Films for the Humanities.
Warm Springs. 2005. HBO.
Understanding Viruses. 2004. Insight Media.
Bioterror, The Invisible Enemy. 2004. Films for the Humanities.
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The Emerging Viruses. 1998. Films for the Humanities.
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The Boy in the Plastic Bubble. 1976. Made for TV movie.

*Also refer to Tables 1-1 and 1-2 for viruses in TV series and movies.

VIRAL PESTILENCE TIMELINE

1500–2015

1500
1550
1600
1650
1700
1861
1918
1947
1954
1960
1976
1993
1999
2003

1518–1520 Smallpox outbreaks, Aztec empire (Mexico)

1527–1530 Smallpox outbreaks, Inca empire (Peru)

1616 Smallpox, New England, Native American population hit hard

1657 Measles, Boston

1759 Measles, most places inhabited, North America

1818 First recorded epidemic of Dengue fever, Peru

1847–1848 Influenza pandemic

1894 First Polio outbreak in the U.S. (Vermont)

1952 U.S. highest record of Polio epidemics

1954 Zika virus first isolated from humans in Nigeria

1957 Asian influenza pandemic

1962–1965 Rubella (German measles) pandemic

1993 *Sin Nombre* (hantavirus) outbreak, Four Corners disease (U.S.)

2003 SARS pandemic (first appeared in southern China in November 2002, recognized as a global threat in March 2003)

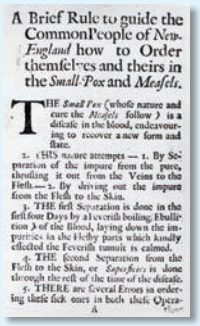
2003–present Avian influenza, rare but highly lethal in humans, Hong Kong, China, Indonesia, Vietnam, Thailand, Cambodia, Turkey, Iraq, Egypt, Azerbaijan, Djibouti

2005 Largest and deadliest outbreak of Marburg hemorrhagic fever, Angola

First outbreak of Polio in the U.S. in 26 years, unvaccinated children in an Amish community, Minnesota

© AbleStock.

Courtesy of the CDC.



A Brief Rule to guide the Common People of New-England how to Order themselves and theirs in the Small-Pox and Measels.

THE Small Pox (whose nature and cure the Measels follow) is a defaile in the blood, endeavouring to recover a new form and state.

2. THIS is in three attempts — 1. By Separation of the impure from the pure, expelling it out from the Veins to the Flesh. — 2. By driving out the impure from the Flesh to the Skin.

3. THE first Separation is done in the first four Days by a feverish boiling, Ebullition of the blood, laying down the impurities in the Fleshy parts which kindly effected the Feverish tumult is calmed.

4. THE second Separation from the Flesh to the Skin, or Superficies is done through the rest of the time of the disease.

5. THERE are several Errors in ordering these sick ones in both their Opera-

© National Library of Medicine.

1649 Smallpox, Boston hit hard

1794, 1796–1797 Yellow fever outbreaks, Philadelphia

1798 Yellow fever outbreak, Philadelphia (one of the worst)

1861–1865 American Civil War soldiers suffered from viral diseases in camps

Courtesy of Roger and Sylvia Gasser.

1912–1916 Dengue fever pandemic

1918 Spanish flu epidemic

1918–1919 Spanish influenza pandemic

© National Library of Medicine.

1947 Zika virus first isolated from Rhesus monkey in Zika forest, Uganda

1967 First Marburg hemorrhagic fever outbreak. Initial cases were laboratory workers handling infected African green monkeys imported from Uganda; Marburg, Germany; and the former Yugoslavia

1968 Hong Kong influenza pandemic

1976 First Ebola outbreaks, Democratic Republic of Congo

Courtesy of Dr. Lyle Conrad/CDC.

1997 Avian influenza, Hong Kong

1999 First recognized West Nile virus outbreak in the United States (New York City)

2000–2001 Ebola outbreak, Uganda (highest mortality of all Ebola outbreaks)

2001 Foot and Mouth disease virus outbreak, United Kingdom (no human cases)

Courtesy of Frank Collins, Ph.D./CDC.

2006
2006 Mumps outbreaks, midwestern U.S.

2006 Chikungunya virus outbreak,La Reunion Island (France)

2006–2007 Porcine reproductive and respiratory syndrome virus (also called blue ear pig disease), China

2007
2007 First outbreak caused by Zika virus in the South Pacific, Yap Island, Micronesia

2007 Foot and Mouth disease virus outbreak, Southern England

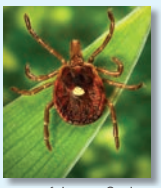
Viral hemorrhagic septicema spreads to the Great Lakes region, U.S.

2009


Specimen and image provided by Purdue Entomological Research Collection, Jennifer M. Zaspel and Gareth Powell.

2009 H1N1 influenza pandemic, epicenter: Mexico

2009 First cases of the Heartland virus transmitted by the lone star tick in the U.S.

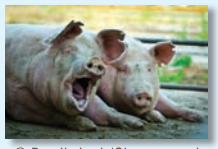


Courtesy of James Gathany/CDC.

2011
2011 Schmallenberg virus quickly spreads in Europe; transmitted by biting midges

2011 Many imported Measles outbreaks,often spread by air travel; U.S.

2011 H3N2v Swine influenza, county fairs; U.S.



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2012


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2012 Hantavirus outbreak; Yosemite National Park, U.S.

2012 MERS outbreak; started in Saudi Arabia and spread by travel

2012 Mysterious Polio-like illness caused by Enterovirus D68 in California

2012 H7N9 influenza; China

2012 Ebola outbreaks in Uganda and Democratic Republic of the Congo

2013
2013 Measles cases triple; unvaccinated children exposed to active case of measles; U.S.

2013 Unusual bottlenose dolphin die-offs caused by morbillivirus; dophins washed up from North Carolina to New York mid-Atlantic coastline

2013 Polio epidemic associated with reduced vaccination; Syria

2013 Chikungunya virus outbreak in the Caribbean Islands for the first time (epicenter: St. Martin)

2013 First new subtype of Dengue virus in 50 years

2013 Bald eagle die-off caused by West Nile virus; Utah

© Teri Shors.

2014
2014 Chikungunya in Mexico

2014 Caribbean Princes cruise ship worst cruise ship norovirus outbreak in 20 years

2014 Ebola outbreak (epicenter: Guinea, West Africa). First natural case of Chikungunya in Texas

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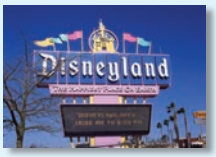
2015


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2015 Measles outbreak traced to infected child at Disneyland, California

2015 H5N2 Avian influenza outbreaks on poultry farms in the Midwest

2015 Canine H3N8 influenza outbreak in Chicago

2015 Emerging Powassan virus transmitted by deer ticks in Northeast and Midwest

2015 Zika virus first time detected in the Americas (in Brazil)

2015 New virus found in old human blood samples (Hepegivirus-1)

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