CHAPTER 1

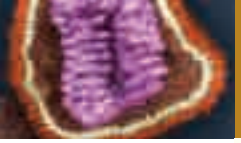
Introduction to Viruses

On April 30th, 2009, newspaper headlines in Mexico City announced that the number of H1N1 influenza deaths had risen to 236 in Mexico. Mexico was the epicenter of the 2009 H1N1 (Swine flu) pandemic.

"Nothing brings us so close to the riddle of Life—and its solution—as viruses."

Wolfhard Weidel, virologist

OUTLINE



CASE STUDY: HEMORRHAGIC FEVER

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 fever, you found a ProMED mail post entitled "Undiagnosed Hemorrhagic Fever—Angola: Request for Information." It was dated March 15, 2005. The post describes the death of two nurses working at the same Huige Province hospital. At the time of the post, 56 people with similar symptoms had died in Angola. Hospital workers told journalists that the illness began with a 2-day fever. Then the patient would cough up blood, lapse into a coma, and die within 4 days. The World Health Organization was planning on investigating the outbreak.

Coincidentally, you have learned that a college friend has joined the Peace Corps. At the end of the semester, he will graduate and then be deployed to Angola to work on HIV/AIDS awareness and youth outreach. You have asked him if he is aware of the viral hemorrhagic fevers such as Ebola, Lassa, or any new hemorrhagic fever viruses in Angola (**FIGURE CS 1-1**). He said he is very naïve about hemorrhagic fever viruses and would appreciate it if you would do some more research on this subject on his behalf.

FIGURE CS 1-1 Angola is located on the Atlantic coast of southern Africa. It is bordered by Namibia to the south and Zambia and Zaire to the east and north. Angola's 994 mile-long coastline and its four major ports make it a natural trans-shipment point for the entire region. Angola has one of the world's worst child mortality rates, with one in four failing to live beyond their fifth birthday. Routine immunizations rarely happen. Yellow fever, Dengue fever, and Crimean Congo hemorrhagic fever outbreaks are **endemic** to Angola.

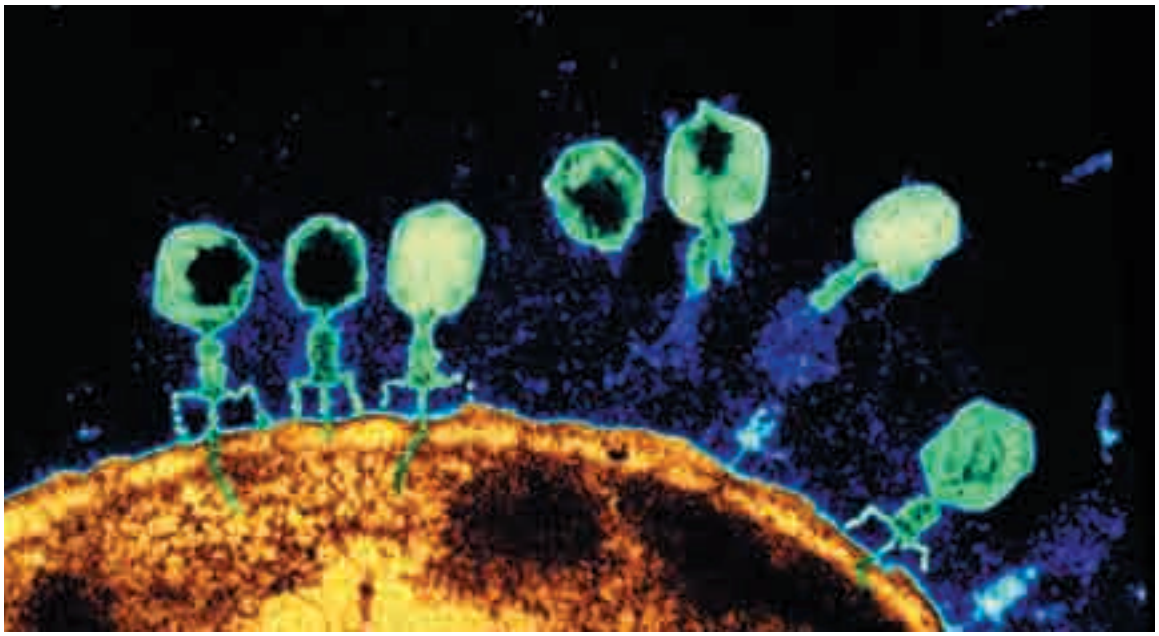


FIGURE 1-1 Colored electron micrograph of viruses (referred to as bacteriophages) attacking the bacterium *Escherichia coli*. Many bacteriophages have a "lunar lander" or head-and-tail structure. The bacteriophage infects the bacterium by attaching to the cell wall. Subsequently, its genetic material enters the cell.

When you think of the word *virus,* what comes to mind? The 2009 H1N1 influenza virus? An invisible entity responsible for the common cold? The cause of sexually transmitted diseases? A mysterious and insidious killer, such as the one that causes Ebola hemorrhagic fever? Biological agents that look like "lunar lander" spaceships (**FIGURE 1-1**)? Have you ever been sickened by images of animals or humans suffering from viral diseases?

This chapter begins your journey through the invisible world of viruses. The word *virus* has been used in the medical world for almost 200 years. In the early 1790s, it simply meant *poison* or *poisonous slime.* A short definition of viruses describes them as submicroscopic agents that are capable of growth in living cells. These biological entities are both beneficial and harmful to humankind. This overview of viruses provides a basis for understanding their impact on all living organisms.

1.1 Viral Impact on the Environment, Research, and Disease

Viruses and Aquatic Ecosystems

Viruses are usually associated with negative effects. Without viruses, though, the earth would be a very different place—maybe even a planet without life! For example, did you know that viruses are the most abundant biological entity in both freshwater and seawater? More than one million viruses are present in a teaspoon of water. Most of these viruses are known as **bacteriophages** (viruses that infect bacteria). For each bacterium in the water there are 15 to 25 virus particles. Bacteria and microscopic plankton are food for nearly all aquatic animals. The discovery of viral abundance in natural water has led to the conclusion that viruses are significant biological agents involved in both the mortality (death) of aquatic microorganisms and in the building of aquatic communities. How can this be?

Dramatic experiments led by Gunnar Bratbak in 1990 were performed in which bacteriophages were selectively removed from seawater and the growth rates of the remaining bacterial and planktonic organisms measured. It was expected that the bacterial and planktonic populations would dramatically increase because they *were freed from viral infection*. Surprisingly, the bacterial populations stopped growing completely, because they depended upon nutrients released as the bacteria were killed by viruses (**FIGURE 1-2**). Without the death of these microbes via viruses, there was no "fuel" to keep the aquatic community running. These viruses are essential for regulating both saltwater and freshwater ecosystems.

During 2009, scientists studying the ecosystems of the pristine freshwater lakes of Antarctica

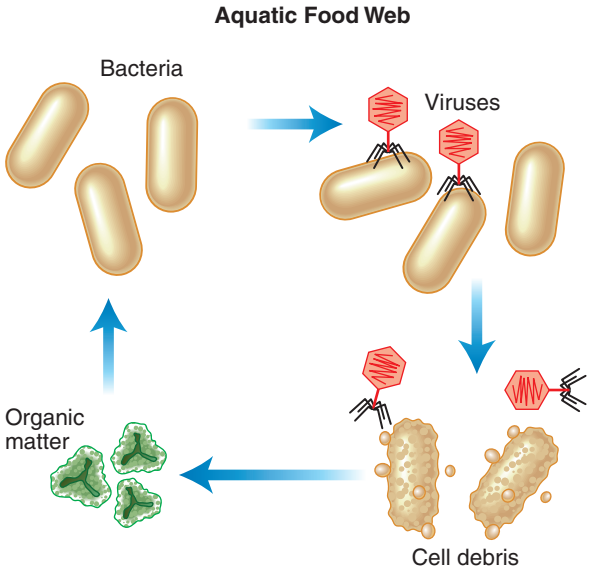


FIGURE 1-2 Viruses free up organic matter so that new life can be generated.

found incredibly diverse types and high numbers of previously unidentified viruses present in the icy lakes. These lakes contained very little animal life (**FIGURE 1-3**). They were void of penguins or seals. The vast majority of life present in the lakes was microbes: bacteria, algae, protozoa, and zooplankton. The microscopic communities adapted to very extreme conditions. In the winter, there was nearly complete darkness and the lakes remained frozen. During the summer, the ultraviolet radiation was intense and the ice melted, resulting in open water in parts of the lake. In contrast to what scientists found in other aquatic systems, the viruses most abundant in the Antarctic lakes during the summer ranged from 50–150 nanometers in diameter. These viruses were mainly tailed 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 nanometers 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.

The Hershey-Chase Blender Experiment

Since their discovery, bacteriophages have taught us many things about the molecular biology of cells. Viruses of bacteria were termed bacteriophages (the suffix "phage" comes from the Greek for "eating") because of their ability to eat, or **lyse**, bacteria. One of the first bacteriophages used in the laboratory, T2, infects the host bacterium *Escherichia coli*. The T2 bacteriophage consists almost entirely of a tightly condensed piece of DNA that is surrounded or packaged by a protein coat. The

FIGURE 1-3 Researchers' tents are dwarfed by Lake Bonney and the Taylor Glacier, one of Antarctica's dry valleys. Scientists are trying to find out what viruses can survive such extreme conditions.

bacteriophage infects *E. coli* and utilizes the cell to reproduce more T2 bacteriophages (**FIGURE 1-4**).

In 1952, geneticists Alfred Hershey and Martha Chase 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 coat of the bacteriophage was labeled with radioactive sulphur [^{35}S]. The second *E. coli* flask was infected with T2 bacteriophages in which the genetic material (DNA) of the virus had been labeled with radioactive phosphorous [^{32}P].

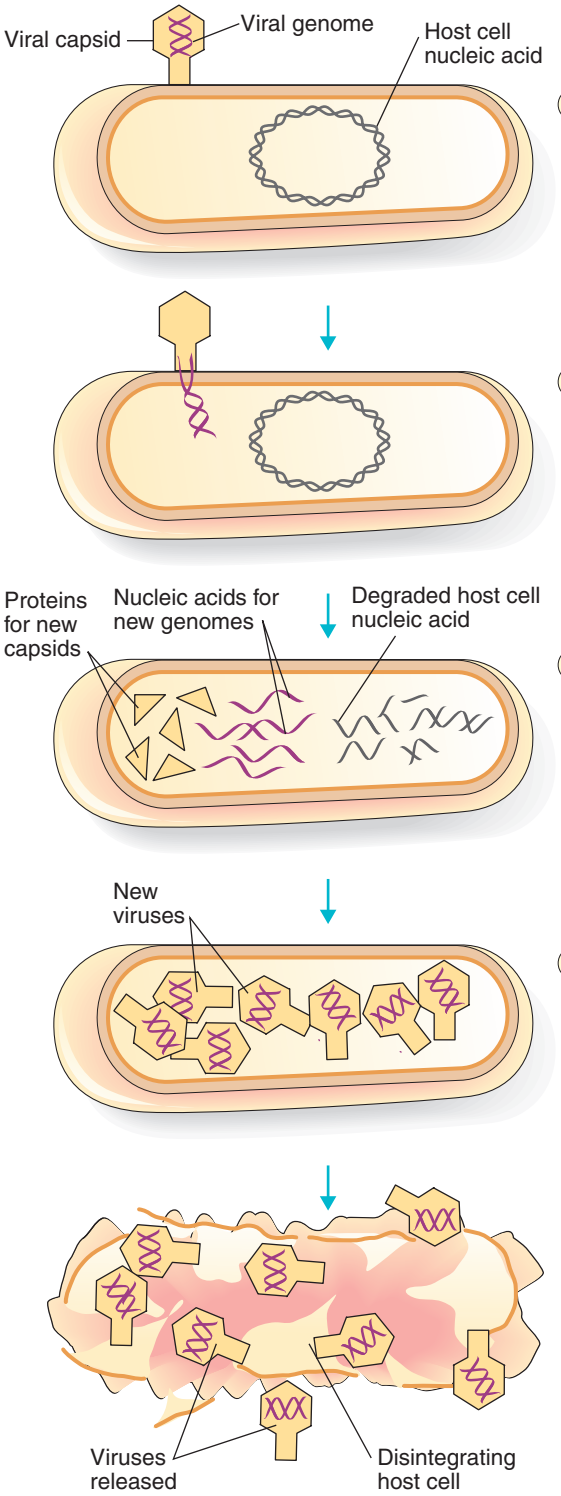


FIGURE 1-4 Diagram of a lytic bacteriophage infection.

(A) **Attachment:** The bacteriophage (virus) attaches to its host cell (bacterium) at a complementary receptor site.

(B) **Penetration:** The capsid remains outside the cell, while the viral genome enters the cell's cytoplasm. (This phase varies among viruses.)

(C) **Biosynthesis:** The viral genome encodes proteins for the synthesis of new viral parts. The cell's nucleic acid degrades.

(D) **Maturation:** New viruses are assembled from the newly synthesized capsids and genomes, usually in a step-by-step process.

(E) **Release:** New viruses are released from the host cell to infect adjacent cells and begin a new cycle of replication.

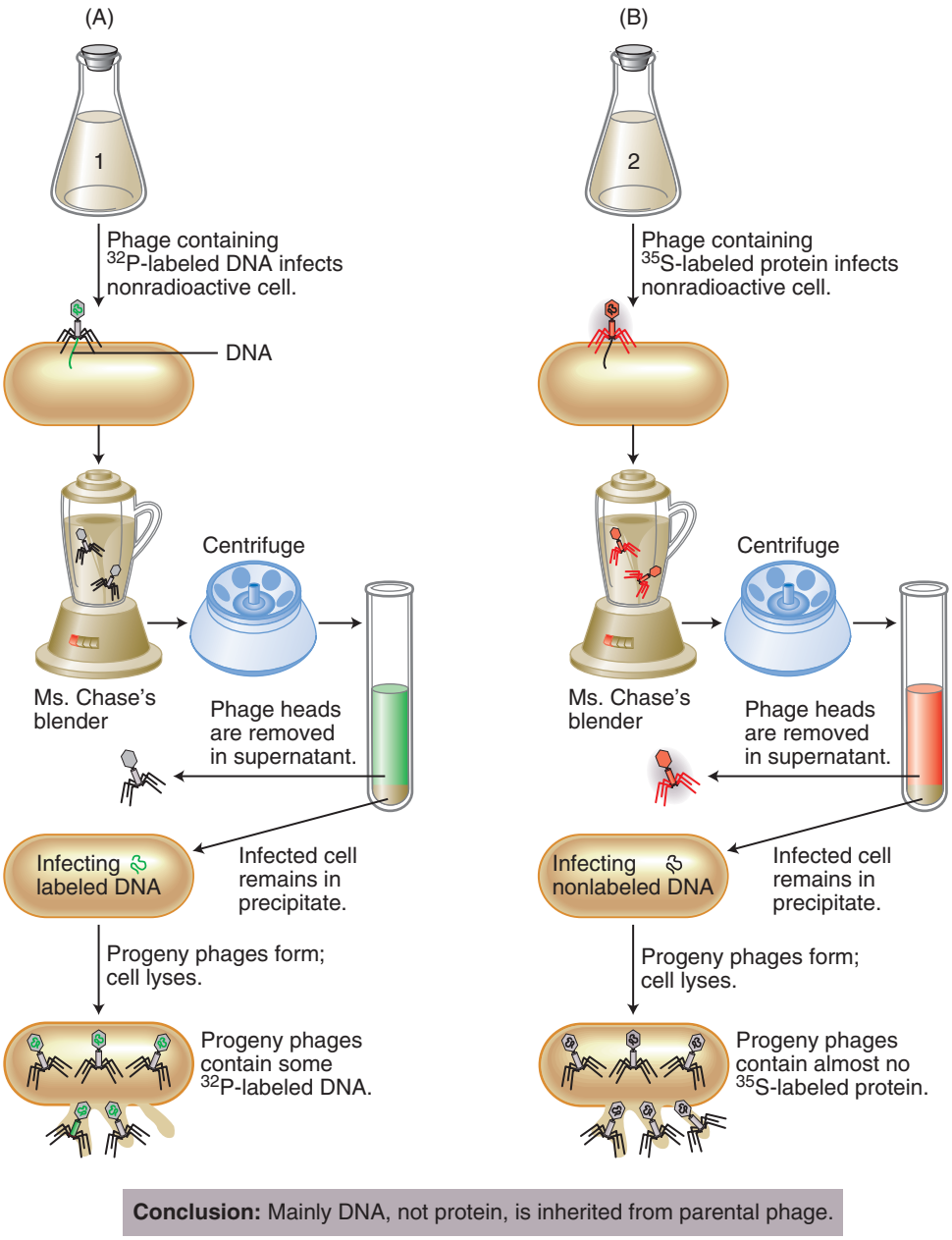


FIGURE 1-5 Diagram depicting the design of Alfred Hershey and Martha Chase's experiment. It provided evidence that DNA was associated with the genetic material of the bacteriophage.

The radioactive T2 bacteriophages were allowed to attach and infect the *E. coli* (**FIGURE 1-5**). After infection, the *E. coli* radioactively labeled T2 bacteriophage mixtures were poured into separate blenders. The blender dislodged the bacteriophage particles from the bacterial host cells in each mixture. The mixtures were then centrifuged (concentrated). This separated the bacteriophage particles in the supernatant liquid from the bacterial cells in the precipitate. Hershey and Chase found that the supernatant contained [^{35}S] (the viral protein) and the precipitate (cell portion) contained the [^{32}P] (the viral DNA). This experiment concluded that the DNA labeled with [^{32}P] transmitted the infective component of the bacteriophage. The DNA was the genetic material that specified all of the information needed to synthesize new T2 bacteriophages (Figure 1-5).

Bacteriophages and other types of viruses continue to be used as molecular biology tools to study host-cell interactions and the molecular biology of cells. Viruses have taught us much of what we know about the processes of replication, transcription, and translation.

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 erythromycin to treat the infection. Before penicillin's discovery in the 1940s, though, the medical community was

focusing its attention on bacteria-killing research (i.e., the use of bacteriophages to kill bacteria pathogenic to humans). Felix Twort (England) and Felix d'Herelle (Canada) first described bacteriophages ("phages" for short) 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 phage. In other words, phages exhibit a very narrow host range: a phage that infects the bacterium *E. coli* will not infect the *Streptococcus pneumoniae* bacterium.

The idea of phage therapy formed the basis for applied medical research as well as for the 1924 Pulitzer Prize-winning novel *Arrowsmith* by Sinclair Lewis. Researchers in Europe began to use bacteriophages to treat infections. In some cases, a liquid containing bacteriophages was poured onto an open wound; in others, the bacteriophages were given orally, via aerosol, or injected. The results were varied, and when antibiotics came into the mainstream, phage therapy largely faded in the West. Countries in the East such as Poland and Russia, however, continued to keep phage therapy research alive (see Chapter 21).

Phage therapy is now being reconsidered as a weapon against antibiotic-resistant "superbugs." These superbugs are lurking in hospitals and cause deadly infections that cannot be treated with the strongest antibiotics currently available. Research and development of alternative therapies are urgently needed to combat this superbug crisis.

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 disease. Most of us do not suffer any harmful effects from defective genes because we carry two copies of nearly all genes (one inherited from each parent). This redundancy exists so that if one of our genes is defective, the second (functional) gene compensates for the defect.

In 1990, W. French Anderson, R. Michael Blaese, and Kenneth Culver—American 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 four-year-old girl. ADA deficiency is a rare but very serious defect that causes immune deficiency, resulting in the lack of normal protection against bacterial and viral infections.

How was a good copy of ADA delivered to the girl? The hero was a retrovirus. The retrovirus was genetically engineered to carry a working ADA gene so that the body could produce functioning adenosine deaminase and consequently eliminate the root cause of the disease. To date, the girl is alive and well.

At least 2000 laboratories are engaged in gene therapy research worldwide. In addition to retroviruses, adenoviruses are being used in clinical gene therapy trials to deliver corrected genes. The history of gene therapy has been a roller-coaster. Gene therapy patient Jesse Gelsinger died in 1999 at the University of Pennsylvania while undergoing gene therapy. New regulations were established to patrol experiments, and new protocols were created. Even though the public is slow to hear about gene therapy developments, steady research progress continues. Gene therapy is discussed in greater detail in Chapter 9.

Vaccine Development

Vaccine development is one of the greatest advances in the history of medicine. The word vaccine is from *vacca*, the Latin word for cow. This is because the material of cowpox (a 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 caught cowpox, a mild disease, didn't get smallpox. They also had beautiful complexions in contrast to others who had pitted faces after contracting smallpox. Jenner deduced that cowpox induced immunity against smallpox. To test his theory, on May 14, 1796, he removed matter from a cowpox pustule on the hand of milkmaid Sarah Nelmes and inserted it into a cut on the arm of eight-year-old James Phipps. James became mildly ill and developed a fever, but recovered after nine days.

On July 1, 1796, Jenner injected smallpox matter 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 for schoolchildren in the United States. The list includes polio, diphtheria, measles, mumps, rubella (German measles), tetanus, pertussis, hepatitis B, and a few others. Most of these vaccines were the result of biomedical research after WWII. Read more about vaccines in Chapter 7.

Most people have experienced a cold, influenza, a cold sore on the lips, or plantar warts, many of which were caused by viruses. During the golden age of microbiology (1857–1914), rapid advances in microbiology, mainly spearheaded by scientists

a

b

FIGURE 1-6 **(a)** This young girl in Bangladesh was infected with smallpox in 1973. The World Health Organization'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!

Robert Koch (Germany) and Louis Pasteur (France), determined that microorganisms cause **infectious diseases**. Additionally, Koch developed a set of experimental steps for determining which specific bacterium causes a particular disease. These steps are referred to as Koch's Postulates.

Bacteria or protozoa were identified to be the cause of certain diseases. They did not, however, cause other diseases such as measles, yellow fever, polio, and smallpox. Today we know that viruses can infect virtually every organism on earth (**FIGURE 1-6**).

Viruses and Cancer

Viral infections are linked to approximately 15% 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 viruses are thought to be one factor that acts at an early stage in the process that leads to cancer.

Cancer has afflicted humans throughout recorded history. Over one 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 (refer to Chapter 10 for more information on this phenomenon). 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. A safe and effective vaccine against the most common high-risk types of human papillomaviruses is available.

1.2 Early Virus Studies

Before the invention of the electron microscope in 1931, viruses could not be seen or grown in the laboratory. *Most importantly, viruses were defined as agents so small they could pass through filters that trapped most known bacteria.* Scientists had to develop ways to observe and grow these invisible agents in the laboratory. The initial observations and methods developed to study viruses involved bacterial and plant systems. The first virus discovered was actually a plant virus known as tobacco mosaic virus (TMV), a disease that destroys tobacco crops. In 1892, the Russian botanist Dimitri Iwanowski demonstrated that extracts from diseased tobacco plants could transmit disease to healthy tobacco plants after passage through ceramic filters known to trap most bacteria. Iwanowski didn't understand the full significance of this result. In 1898 Martinus Beijerinick extended Iwanowski's experiments and was the first to develop the idea of the virus, which he called a *contagium vivum fluidum* (contagious living fluid).

Bacteriophages were first isolated from natural sources (such as human sewage) and studied by scientists in the early 20th century. Bacteriophages grow by inserting their DNA into a host bacterium, directing the host's biosynthetic machinery to make copies of the bacteriophage 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 fresh 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 are allowed to grow. At this point, the bacteriophages lyse bacteria that are present on the surface of agar. The holes (called **plaques**) in the bacterial lawn are areas where the bacteria have been killed by bacteriophages.

Major advances in animal virology did not occur until 1952, when Renato Dulbecco modified the bacteriophage assay to work on animal cell cultures. **FIGURE 1-7** shows a 6-well dish containing crystal violet-stained monolayers of monkey kidney cells that were infected with vaccinia virus. Similar to the 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 left-hand side of the dish than the wells on the right side of the dish. Dulbecco was awarded a Nobel Prize for the development of animal plaque assays in 1975.

Characteristics of Viruses

Today we know that viruses share a number of common features. First of all, they are small. As previously mentioned, viruses are 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 viruses. Typically, bacteria range from 1 to 10 micrometers (μm) in length. A virus would fall in the range of 0.03–0.1 μm in length (or 30–100 nm). Of course there are always some exceptions: Some viruses, such as poxviruses, can be 200 to 400 nanometers (nm) in length, and filoviruses (such as Ebola) can be up to 1000 nm in length. **FIGURE 1-8** provides size comparisons of biological molecules, viruses, bacteria, cellular organelles, and eukaryotic cells.

A second feature used to define a virus is its complete dependence upon the 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 rebuild a virus. As a result, a virus must use its host cellular protein synthesis machinery to synthesize viral proteins. The genome or genetic material of a virus consists of one species of nucleic acid, DNA, or RNA. The DNA or RNA genome of a virus can be single- or double-stranded.

The outside of the virus particle contains a receptor-binding protein or viral-attachment protein that will allow the virus to adhere to **receptors** present on the surface of cells. For example, the common cold virus (also known as rhinovirus)

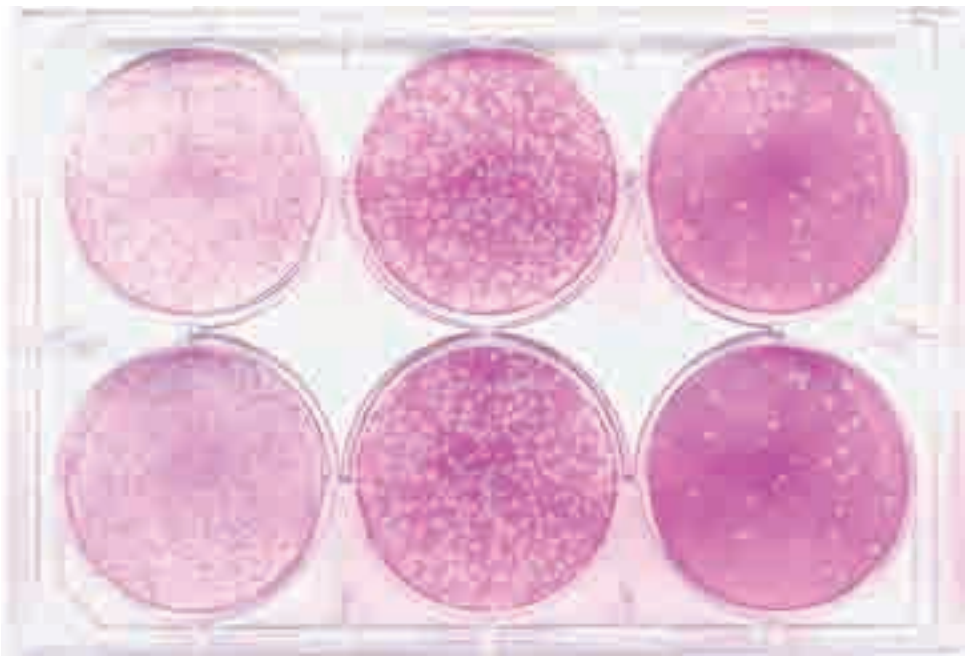


FIGURE 1-7 Plaque assays can be used to study animal or human viruses. The 6-well dish contains crystal violet-stained monolayers of monkey kidney cells that were infected with vaccinia virus.

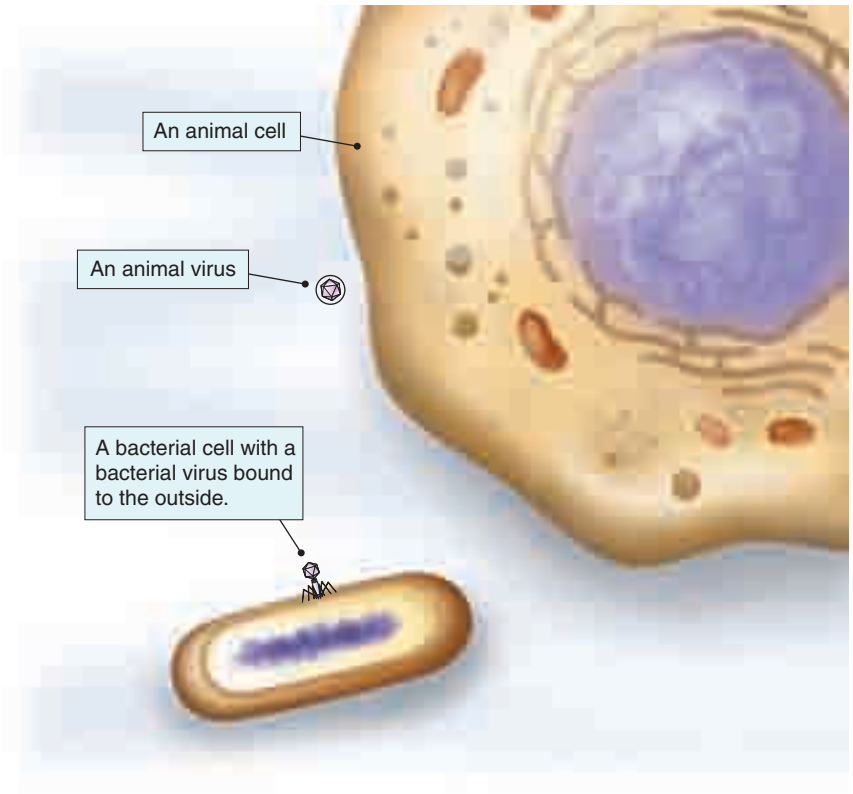


FIGURE 1-8 Viruses are smaller than their host cells. This illustration demonstrates size comparisons of a eukaryotic cell, a bacterium, and a virus.

binds to a receptor on the outside of cells called the **I**nter**c**ellular **A**dhesion **M**olecule-**1** (ICAM-1).

Unlike other unicellular microorganisms, some human or animal viruses can reproduce themselves even if nothing but the viral genome is introduced into the cell. In other words, the viral genetic material is infectious. **FIGURE 1-9a** demonstrates that the RNA genome of poliovirus can be introduced into cultured cells (by a method called **transfection**) and viruses will be produced from that RNA within the cell. In this case, the polioviruses are not required to enter the cell to make more copies. Retroviruses can persist in cells by integrating their own DNA (or a copy of their RNA) into the genome of the host cell (Figure 1-9b).

Review of Virus Characteristics

- Small (nm in size)
- Pass through filters that trap most known bacteria
- Completely dependent on the host cell
- Contain one species of nucleic acid
- Contain receptor-binding protein
- Genome may be infectious
- Some viruses can persist by integrating genome into the cellular chromosome

Visualizing Viruses: Electron Microscopy

Electron microscopes were originally invented in the early 1930s 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 1000 times.

Instead of light rays, electron microscopes use a beam of electrons focused by magnets to resolve minute structures. With electron 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. Kausche, Pfankuch, and Ruska published the first electron micrograph of TMV in 1939. Today electron microscopes continue to be a powerful tool in studying how viruses are assembled within the cell, the structure of fragile viruses, and the rapid detection and diagnosis of viral infections (especially viruses that cannot be cultivated in the laboratory). The electron micrograph image in **FIGURE 1-10a** represents the first isolation and visualization of Ebola virus in 1976. Some of the filamentous particles are fused together, end-to-end, giving the appearance of a "bowl of spaghetti." The electron microscope was instrumental in the initial identification of the new coronavirus, now known as SARS-CoV. Biologist Cynthia Goldsmith is observing a viral isolate via the electron microscope from the 2003 SARS outbreak in Figure 1-10b.

Are Viruses from Outer Space? Theories of Viral Origin

Viruses are everywhere. Wherever there is life, there are viruses! Evidence of viral infections can

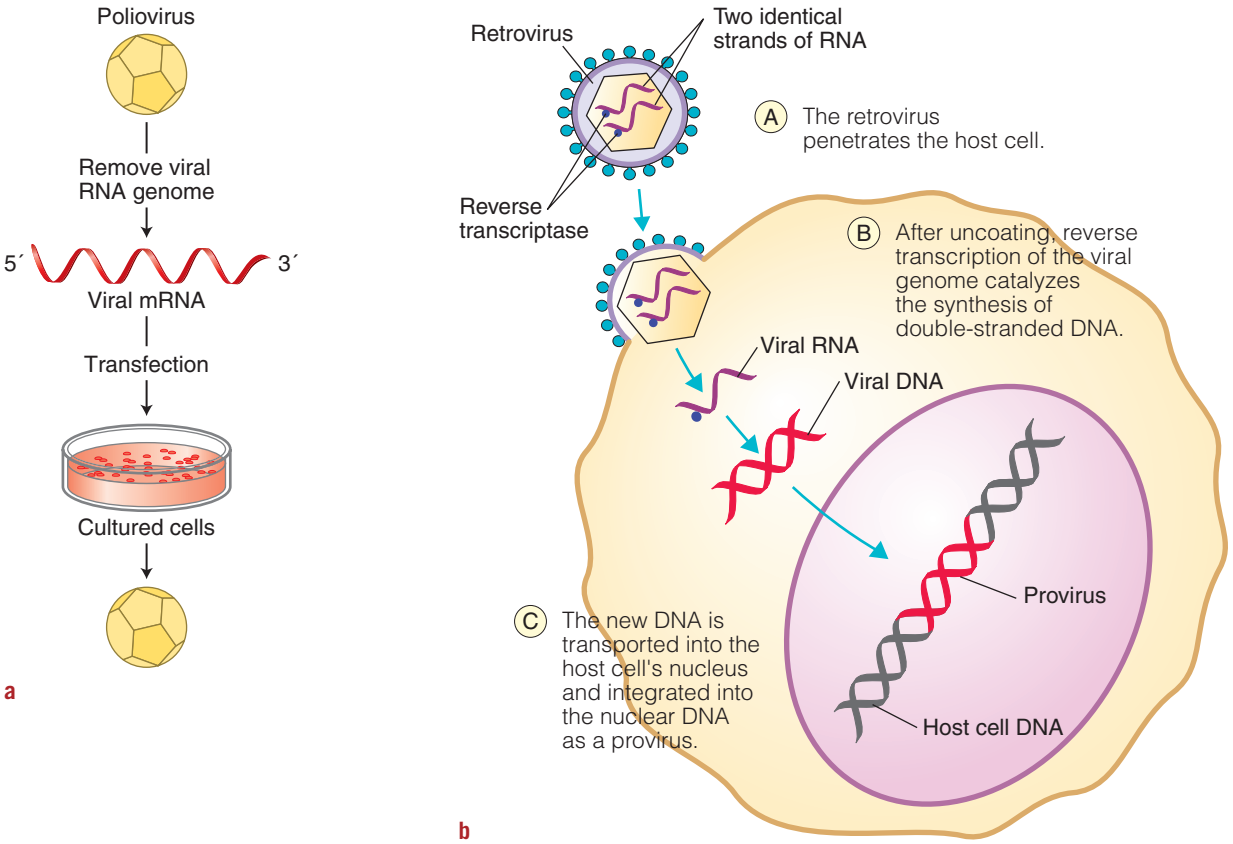


FIGURE 1-9 **(a)** Poliovirus RNA is infectious! Infectious particles of poliovirus can be produced even if only the genetic material (RNA) of the virus is introduced into cells growing in a culture dish. **(b)** Retroviruses can persist in cells by integrating their own DNA (or a copy of their RNA) into the genome of the host cell. The integrated viral genome is called a **provirus**. Adapted from Flint, S. J., et al. *Principles of Virology: Molecular Biology, Pathogenesis, and Control of Animal Viruses,* Second Edition. ASM Press, 2003.

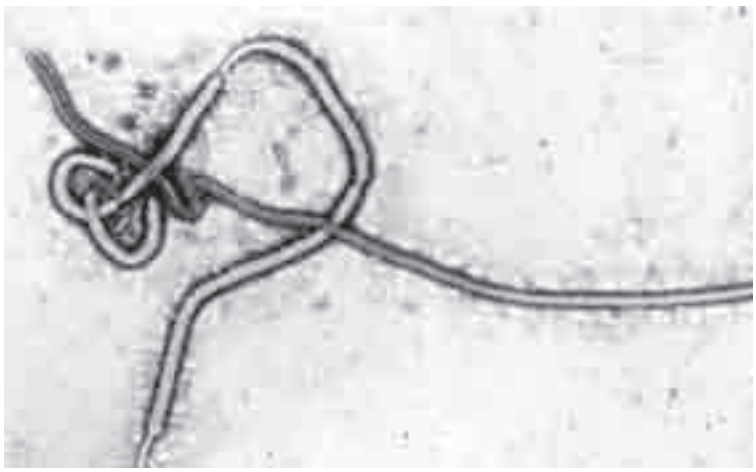


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FIGURE 1-10 **(a)** Transmission electron micrograph of Ebola virus particles that were isolated from a human diagnostic specimen and then cultured in Vero cells. Magnification 40,0003. **(b)** Visualization of SARS-CoV via the electron microscope.

FIGURE 1-11 Egyptian priest from the 18th dynasty (14th century B.C.) with foot-drop deformity.

be found among the earliest recordings of human activities. For example, an ancient Egyptian stele tomb carving depicting a polio-afflicted priest (circa 14th century B.C.) is shown in **FIGURE 1-11**. The foot-drop deformity is characteristic of residual paralysis due to poliomyelitis.

Where did these 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 C. 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 were responsible for originating life on earth and cause massive contagion flowing in from space. They speculated that influenza pandemics occurred in our history when solar winds during sunspot peaks caused the 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 had two trains of thought based on results from research published on molecular virology studies of the 1980s. The first take was that viruses were precursors of the earliest cells. The other take 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 a 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 termed as 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 don't share any common genes, ruling out the idea that viruses have a common origin. Sequence analysis of viral genes reveals at least five classes of viral genes (**TABLE 1-1**).

The classes of genes the viruses possess strongly depend on viral genome size. For example, 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**. ORFans are open reading frames that have no known homologs and no known function. ORFan genes have been found in prokaryotes such as *E. coli*. Daubin and Ochman theorize the ORFan genes were acquired from bacteriophages.

The discovery of the giant Mimivirus called to question the definition of a virus. Mimivirus was first thought to be a new legionella-like bacterium

TABLE 1-1 Classes of Viral Genes

Class	Gene Description
1	Virus genes that have closely related **homologs** in cellular organisms (especially, the host of a given virus) present in a narrow group of viruses.
2	Virus 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.

Source: 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.

isolated from a cooling tower in Bradford, England. It was discovered during an investigation to find the source causing a pneumonia outbreak in 1992. About ten years later, it was determined that it was not a bacterium, but instead a giant virus that was able to grow inside of an amoeba. Its entire DNA genome was analyzed. The structure and genome of Mimivirus were not only large in size, but it contained genes that were not found in any other known viruses. These novel genes were homologs of genes involved in protein synthesis, a process that occurs only in cellular organisms! In 2008, it was discovered that a virus 50 nm in size infected and replicated inside of Mimivirus. It was classified as a **virophage** and named Sputnik. This was the first time virologists had determined that a virus could infect a virus! These findings put another spin on the origin of large DNA viruses. Raoult and Forterre suggested that biological entities be divided into two groups: ribosome-encoding organisms and capsid-encoding organisms that include viruses.

A very crude model of the evolution of life and current viral genomics is shown in **FIGURE 1-12**. This figure represents an emerging concept. It begins with the **hydrothermal origin hypothesis**, also called the iron-sulfur (FeS) world theory that postulates the first organic chemical structures were formed at warm alkaline thermal vents or fissures (long, narrow openings) found in the ocean seafloor. Hydrothermal vents leaked hot sulfuric acid into the surrounding environment. Supporters of this theory claim that a gradient formed between the hydrothermal vent water and extremely ice cold water that surrounds the vent at the bottom of the ocean. The temperature at the cooler temperatures would be suitable for organic chemical synthesis to occur. The vents contained FeS and iron nickel sulfide (Fe-Ni-S) 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. These early viruses must have self-replicated even if very poorly. The RNA viruses evolved first, followed

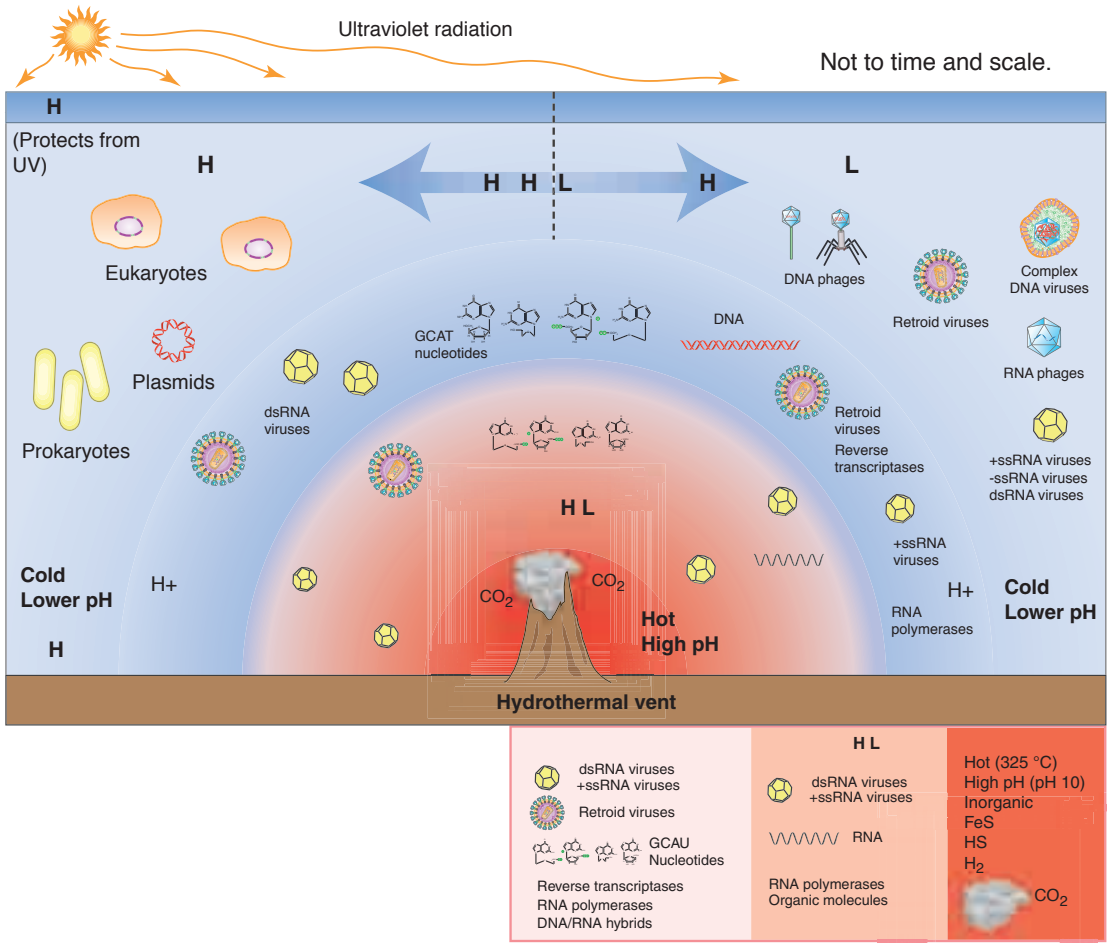

FIGURE 1-12 Model of the evolution of life and current viral genomics.

VIRUS FILE 1-1 Use of PubMed, CDC Publications, and ProMED to Research Specific Viruses or Viral Outbreak Information

FIGURE VF 1-1 PubMed interface. PubMed is the most frequently used search engine for medical research journal articles.

Each chapter of *Understanding Viruses* will contain 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 will be provided at the end of each 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. It includes over 20 million citations for biomedical articles 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 their website (which can be found easily through a Web search engine).

Many Virus Files may cite reports published by the Centers for Disease Control and Prevention (CDC), such as *Morbidity and Mortality Weekly Report* (*MMWR*) and *Emerging and 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 email list posts a variety of information, including on-the-ground observations, media stories, and government reports. ProMED members have helped to diagnose everything from camel pox in Saudi Arabia to measles in military men in Kazakhstan to severe acute respiratory syndrome (SARS) in China. Today there are more than 30,000 subscribers to ProMED. This early warning disease reporting often beats local authorities in disease reporting. Archives of reports can be searched via the mail site within the ProMed website (http://www.promedmail.org).

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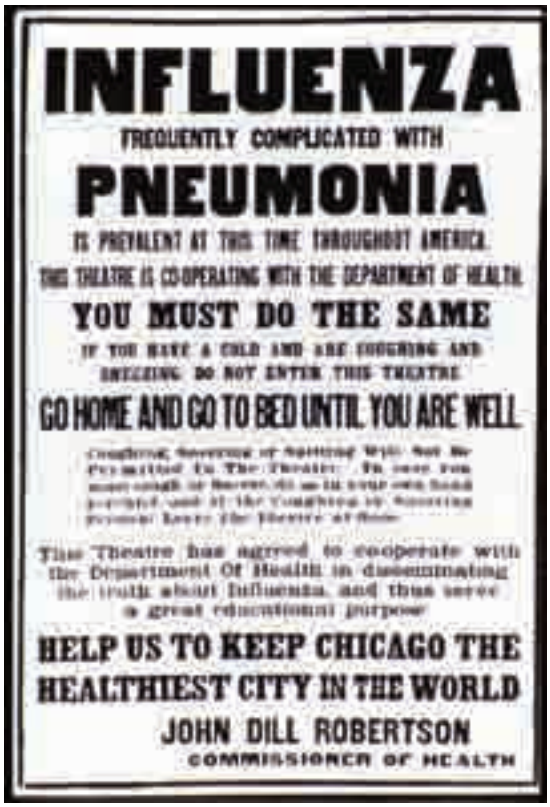
by **retroid viruses** and subsequently DNA viruses. Retroid viruses use reverse transcriptase to replicate their genomes (see Chapters 3, 16, and 17 for more information about retroid viruses and agents). Over the course of 10–100 million years, complex gene assemblies evolved during the DNA stage that resulted in the emergence of new compartmentalized cells and the large DNA viruses. During the evolution of the DNA viruses, there was an explosive evolution of eukaryotic cells. Between the viral and cellular worlds, there was continuous horizontal gene transfer between cells and viruses. Keep in mind that this is a new developing model. Empirical studies are needed to validate the model. The study of the evolution of viruses is entering a new and exciting stage of development.

1.3 Viruses in History: Great Epidemics

Influenza

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



a

b

FIGURE 1-13 **(a)** Influenza placard placed on the front doors of homes to quarantine individuals infected with influenza. **(b)** A New York City policeman wearing a cloth mask to protect him from the Spanish flu in 1918.

whether it was a parent, child, or grandparent who had recently died from a contagious disease. Schools, churches, theaters, libraries, and most restaurants and stores are closed (**FIGURE 1-13a**). There are no public gatherings, not even funerals. The few people you see have a cloth mask covering their face (Figure 1-13b). 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. The Spanish flu was associated with high rates of morbidity, mortality, social disruption, and high economic costs, and was to be the most destructive pandemic ever known.

The Spanish flu incubation period and the onset of symptoms were so short 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. Severe headache, pains in muscles and joints, hair loss, and acute congestion accompanying temperatures of 101°F to 105°F occurred. 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 of secondary bacterial 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

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

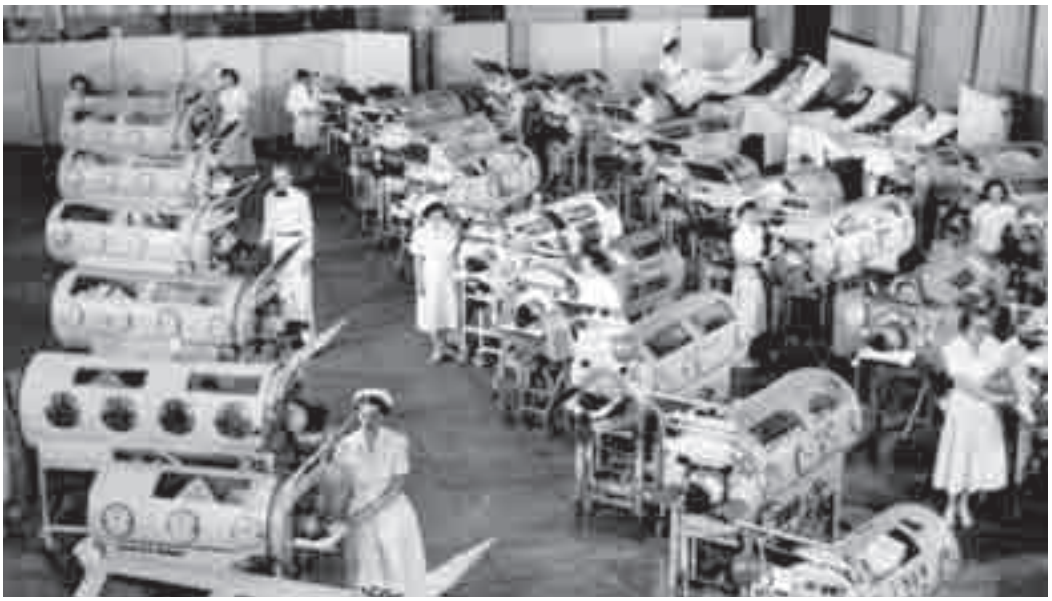


FIGURE 1-14 Polio ward of iron lungs at Rancho Los Amigos Hospital in Amigos, California, 1953.

a role in epidemics. It occurred in a nation thriving with new technologies that would lead to man's 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.

Polio's transmission was a mystery. Cities responded with methods that had met with success in ridding epidemics of typhus, cholera, and diphtheria. New York City officials had the streets doused with 4 million gallons of water a day to flush the streets of their germs intentionally killing 72,000 stray cats that were thought to be virus carriers. Many cities were fogged with insecticides. None of these measures worked, though: polio was spread through human feces. At the time, 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 1-14**).

Franklin Delano Roosevelt 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 polio. 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 1-15a**). He was not photographed being carried or wheeled about.

a **b**

FIGURE 1-15 **(a)** FDR at Warm Springs, Georgia, in 1924. **(b)** The press portrayed Roosevelt as a robust, physically strong leader.

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 in those of his advisors, a robust appearance was necessary to portray a physically strong leader, and the press cooperated (Figure 1-15b).

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 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 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. (Read more about polio vaccination in Chapter 11.)

Today, polio is rare due to the worldwide efforts of the World Health Organization (WHO), CDC, Rotary International, and United Nation's Children Fund (UNICEF) to eradicate it. In 2010, endemic areas of polio were reduced to four countries: India, Pakistan, Nigeria, and Afghanistan. Some countries in Africa have a "re-infected" status today.

Acquired Immunodeficiency Syndrome (AIDS)

In June of 1981, a group of physicians in Los Angeles, California, reported five unusual cases of *Pneumocystis* pneumonia in the CDC's *Morbidity and Mortality Weekly Report*. All patients were young men, and all were sexually active homosexuals. Two of the patients died from this pneumonia and all patients had experienced other rare infections such as candidiasis, a fungal infection of the throat, mouth, or in women, the vagina. *Pneumocystis* pneumonia 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 AIDS (see Chapter 8, Virus File 8-1).

As cases were reported to the CDC, a pattern characteristic of an epidemic emerged, but the culprit was a mystery until Robert Gallo (United States) and Luc Montagnier (France) discovered the human immunodeficiency virus (HIV). Today AIDS is a worldwide epidemic, with the population in Africa being the most severely affected. There are several antiviral drugs available to prolong the lives of those suffering from AIDS, but there is no cure or vaccine. Education promoting AIDS prevention has reduced cases in some countries. (Read more on AIDS in Chapters 8 and 16.)

1.4 Recent Viral Outbreaks

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

At the end of the typical 2009 influenza season, health authorities in Mexico City recognized an unusual pattern of influenza-like illness. Dozens of individuals were suffering from an atypical flu during the middle of March. 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 flu outbreak spreading in La Gloria, Mexico. The data mining company tracked thousands of website searches daily for early signs of medical problems or civil unrest anywhere in the world. The company advised the CDC that the Mexican outbreak reports had potential of a public health emergency and international concern. On April 18, Mexican health officials sent the CDC 14 mucus samples from patients suffering from severe flu or pneumonia-like infections for testing. The CDC 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 at the CDC suggested that the infections were caused by a swine Influenza A (H1N1) based on genetic testing (see Chapter 12, Influenza Viruses, for an explanation of the "H1N1" designation). The viral isolate characterized had never been detected in humans or pigs.

That same day, the President of Mexico, Felipe Calderon, advised citizens to wear face masks when using public transportation, stay indoors and avoid crowded places, exercise frequent handwashing, cover mouths when coughing, cough or sneeze into the crook of the arm or a tissue, and 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." Two days later, schools and

universities were closed country-wide 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 1-16**). 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. dollars (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 2155 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 Houston, Texas, hospital. On April 27, Spain declared the first confirmed case of 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. The CDC 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 boarders for travel to and from Mexico. The Philippines, China, and Indonesia banned the importation of pork from Mexico and certain states within the United States (e.g., border states like Texas and California but also Kansas). By the next day, there were confirmed or suspected cases of H1N1 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 Forth Worth, Texas, school districts due to suspected H1N1 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 Christian pig farmers clashed with the police.

The first wave of influenza continued in 2009. On June 11, WHO Director Dr. Margaret Chan declared the world situation an H1N1 pandemic that was "unstoppable." She also stated that this influenza strain was a very different virus than what we had been used to from season to season. The 2009 H1N1 influenza virus 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 the obese. The H1N1 virus spread rapidly through frequent international travel. Acting Director of the CDC's Emergency Response Team Dr. Richard Besser served as the public face of the

FIGURE 1-16 All restaurants were closed except for take-out orders during the H1N1 outbreak in Mexico City, Mexico (April 29, 2009).

CDC's response to H1N1. As of June 12, 2009, the virus had spread to 74 countries around the world. Over 29,000 cases were reported, including 145 deaths. By July 1, H1N1 cases were reported in all 50 states. It was estimated that more than 1 million people had been infected.

Within a month of this outbreak, sequence data for the new virus became available in public-access databases. A report was published in *ScienceExpress* on May 22, 2009. Researchers discovered the H1N1 influenza virus contained a unique combination of gene segments from both classic Northern American and Eurasian pig influenza strains as well as gene segments from human and avian influenza strains. It was a "mutt" of a virus. The most critical gene segments that pandemic strains contain are novel H and N gene segments (refer to Chapter 12 for further explanation of influenza virus genes and pandemic strains). The 2009 H1N1 virus contained an H gene from classic American pig influenza viruses and the N gene from a Eurasian pig influenza virus. Three gene segments of the 2009 virus shared common sequences from the 1918 pandemic influenza 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 flu season could be of the same severity experienced in 1918. Like the 1918 influenza 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 strain in history. Having had two "near misses," the emergence of severe acute respiratory syndrome caused by a new coronavirus (2002–2003) and the spread of H5N1 avian influenza in the Middle East, nations began working diligently on pandemic action plans in 2005. These plans provided guidance, resources, and checklists intended to help every sector of society to reduce the impact of a pandemic on businesses, hospitals, schools, and the community. Global vaccine manufacturing and stockpiling of influenza antivirals such as Tamiflu became a priority. Regulatory authorities licensed pandemic influenza 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 (refer to Chapter 12, Influenza Viruses, about the 2009 seasonal and H1N1 vaccines). Healthcare facilities stockpiled medications, masks, gowns, and other supplies. Some hospitals suspended the use of student volunteers to limit patients' possible exposure to the H1N1 virus since the influenza virus spreads quickly in academic and university settings.

The 2009 H1N1 virus started a second wave in the fall. On October 24, President Obama declared H1N1 influenza a state of national emergency. This waived certain regulatory requirements for healthcare facilities in response to emergencies and access to the experimental drug peramivir to treat severe cases of H1N1. Responding to a pandemic in the 21st century had some technological advantages. The media responded with public service announcements. It updated the international situation; educated the public, especially on cough etiquette, frequent handwashing, school closings, staying home when sick, and the availability of seasonal and H1N1 vaccine; and corrected disinformation (e.g., it was safe to eat pork). Internet sites contained accurate up-to-date information for citizens and healthcare professionals (**TABLE 1-2**). Some Website H1N1 health maps and cell phone

TABLE 1-2 Pandemic Influenza Resource and Surveillance Information

Internet Resources	URL
Comprehensive U.S. Government Information	http://www.flu.gov/
CDC Seasonal Flu Information	http://www.cdc.gov/flu/
WHO Global Alert and Response: Influenza	http://www.who.int/csr/disease/influenza/en/
European Commission: Influenza Information	http://ec.europa.eu/health/ph_threats/com/Influenza/h1n1_en.htm
Pandemic Influenza: Canada	http://www.pandemicflu.ca/
Public Health Agency of Canada: Influenza	http://www.phac-aspc.gc.ca/influenza/index-eng.php
Google Flu Trends Around the World	http://www.google.org/flutrends/
Tracking the Progress of H1N1 Swine Flu	http://flutracker.rhizalabs.com/

applications tracking influenza cases were created. Users could track outbreaks reported in their region and set alerts notifying a user on their device by email when new cases were reported in their region (Table 1-2).

Like in 1918, different snake oil or quack remedies were advertised. The fear of an H1N1 pandemic resulted in Internet advertisements for a plethora of fraudulent products that cure, treat, or prevent H1N1 infection. Products included shampoos, air purifiers, herbal supplements, inhalers, and even body washes. The Food and Drug Administration 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 protection or treatment. More on the epidemiology of the H1N1 pandemic is found in Chapter 12.

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 food animals, or prepared and served food. They experienced influenza-like symptoms during the first week of illness: fever greater than 100.4°F (38°C) for more than 24 hours, headache, and body aches. During the second week of illness, sick individuals initially developed a dry cough and many developed diarrhea. Most sick individuals rapidly deteriorated to an **atypical pneumonia**. The fever followed by the rapid progressive respiratory compromise were the key signs and symptoms from which the syndrome derived its name as severe acute respiratory syndrome (SARS).

On February 16, 2003, a nephrologist (physician specializing in kidney diseases) working at a Chinese hospital began experiencing the early signs and symptoms of SARS. Five days later, the physician traveled from Guangzhou to Hong Kong, Special Administrative Region of China, and stayed at a hotel there. 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 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 returning to a family reunion in Hong Kong. His stay at the hotel overlapped with that of the nephrologist for one 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.*

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 where a SARS patient infected many more persons than would normally be expected. What made SARS notorious—in contrast to other infectious diseases like influenza—was its *propensity to cause hospital outbreaks*. Healthcare workers accounted for 21% of all SARS cases during this 2002–2003 outbreak. SARS transmission studies led to a new approach to manage patients that was termed **"respiratory hygiene/cough etiquette."** SARS-CoV also infected a mobile population of people who were able to travel for several days before the onset of severe symptoms. Other emerging viral infections such as Ebola, hantavirus pulmonary syndrome, and Nipah do not spread by travelers as rapidly as SARS because 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 28 regions around the world, resulting in 8096 cases and 774 deaths (**TABLE 1-3**). The SARS outbreak is a good example of how modern technology enabled laboratory investigators to determine that SARS was caused by a new coronavirus and not a novel influenza virus or an agent of bacterial origin such as *Mycoplasma pneumoniae, Chlamydia pneumoniae,* or *Legionella pneumophila*. Refer to Chapter 3, Virus File 3-1 for "The Race to Characterize SARS-CoV," Chapter 5, Virus File 5-2 for the "Rapid Diagnosis of SARS," and Chapter 12, section 12.15 regarding "Lessons Learned from the Severe Acute Respiratory Syndrome (SARS) Outbreak."

TABLE 1-3 Summary of SARS Cases (November 1, 2002–July 31, 2003)*

Location	Female	Male	Total Cases	Number of Deaths	Average Age	Date(s) of First and Last Probable Cases
China	2674	2607	5327**	349	N/A	Nov. 16, 2002–June 3, 2003
China, Hong Kong Special Administrative Region	977	778	1755	299	40 (0–100)	Feb. 15–May 31, 2003
Vietnam	39	24	63	5	43 (20–76)	Feb. 23–April 14, 2003
Canada	151	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***	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 Adminstrative Region	0	1	1	0	28	May 5, 2003
TOTALS			**8096**	**774**		

*WHO statistics

**Case classification by sex is unknown for 46 cases.

N/A (not available)

***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.

Source: Table adapted from: http://www.who.int/csr/sars/country/table2004_04_21/en/index.html

The mystery as to the origin of the SARS-CoV is still not fully resolved. Early investigations suggested the virus originated from animals because the first persons infected were traders or animal food handlers at live markets, 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 the SARS-CoV compared to vegetable traders. The presence of antibodies indicated the traders had been infected by the SARS-CoV probably through capturing and marketing wild animals. For this reason, scientists concentrated their investigations toward animals being sold at markets as a likely 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.

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 young (litter of 1–4) 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 in 660 farms all over China. The farms started from either the capture and breeding of local wild civets or from 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 or Hong Kong. They trapped hundreds of bats in their natural habitats for **zoonotic pathogens**. Their logic for testing bats was that these mammals are often persistently infected with many viruses (e.g., Nipah, Hendra, Ebola viruses) but never exhibit symptoms. In other words, they are healthy carriers of certain 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 often in the same cages at food or traditional medicine markets, contributing to the conditions that led to the SARS outbreak.

Foot and Mouth Disease, 2001

Anyone who was in Great Britain in 2001 will remember one of the world's worst foot and mouth disease (FMD) epidemics. Tourists were prompted to follow these rules:

- Do not go near cattle, sheep, pigs, or deer and never feed farm animals.
- Do not go on farmland that has been or is being used by livestock.
- Do not attempt to walk on footpaths that are closed.

Five days before returning home from FMD-infected areas, people were asked to avoid farms, zoos, sale barns, stockyards, animal laboratories, meatpacking plants, fairs, and other animal facilities. It was suggested to bathe and launder or dry-clean all clothing, including outerwear, and to remove any dirt or soil from shoes, followed by wiping them with a cloth that was dampened with a bleach solution.

The media stories showed photographs of individuals bleaching their shoes before entering the airports. All luggage and personal items (including watches, cameras, laptop computers, CD players, and cell phones) were supposed to be cloth-dampened with a bleach solution. Once inside the airport, travelers were questioned about where they had been during their stay and if they were carrying any food products of pigs or ruminants.

Epidemiologists determined how FMD spread to farms and the market. The outbreak began at Burnside farm, Heddon-on-the-Wall, Northumberland, England. The FMD outbreak spread to nearby farms, markets, and meat-packing facilities via vehicles transporting infected animals (**FIGURE 1-17**). Road sites were set up with automatic vehicle-spray devices to contain the spread of FMD (**FIGURE 1-18a**).

Foot and mouth disease is highly contagious among animals and causes loss of production that can have grave economic implications for the meat and dairy industries (Figure 1-18b). FMD has occurred in most parts of the world, causing extensive epidemics in cloven-hoofed animals (that is, cattle, pigs, sheep, goat, and deer). The last outbreak of FMD in the United States occurred in 1929. Slaughtering infected animals or animals in contact with infected animals, vaccination, and public cleansing and disinfection centers can control the disease. Agricultural officials use serology testing to monitor healthy animals for FMD antibodies.

Cases of FMD in humans are rare, and the symptoms are few and/or mild. Human cases have resulted from close contact with animals suffering from FMD, with the virus entering through broken skin, being ingested in unpasteurized milk, or being inhaled. FMD has not been beaten. The Ministry of Agriculture, Fisheries, and Food in England prefers to slaughter livestock during an outbreak instead of vaccinating, and as a result the scourge of FMD continues. Refusal to vaccinate is driven by the market. Animals that test positive for FMD antibodies cannot be sold to other countries. Vaccination would provide immunity to these animals, but because they would then test positive for FMD antibodies it depreciates their market value. This is a controversial issue affecting

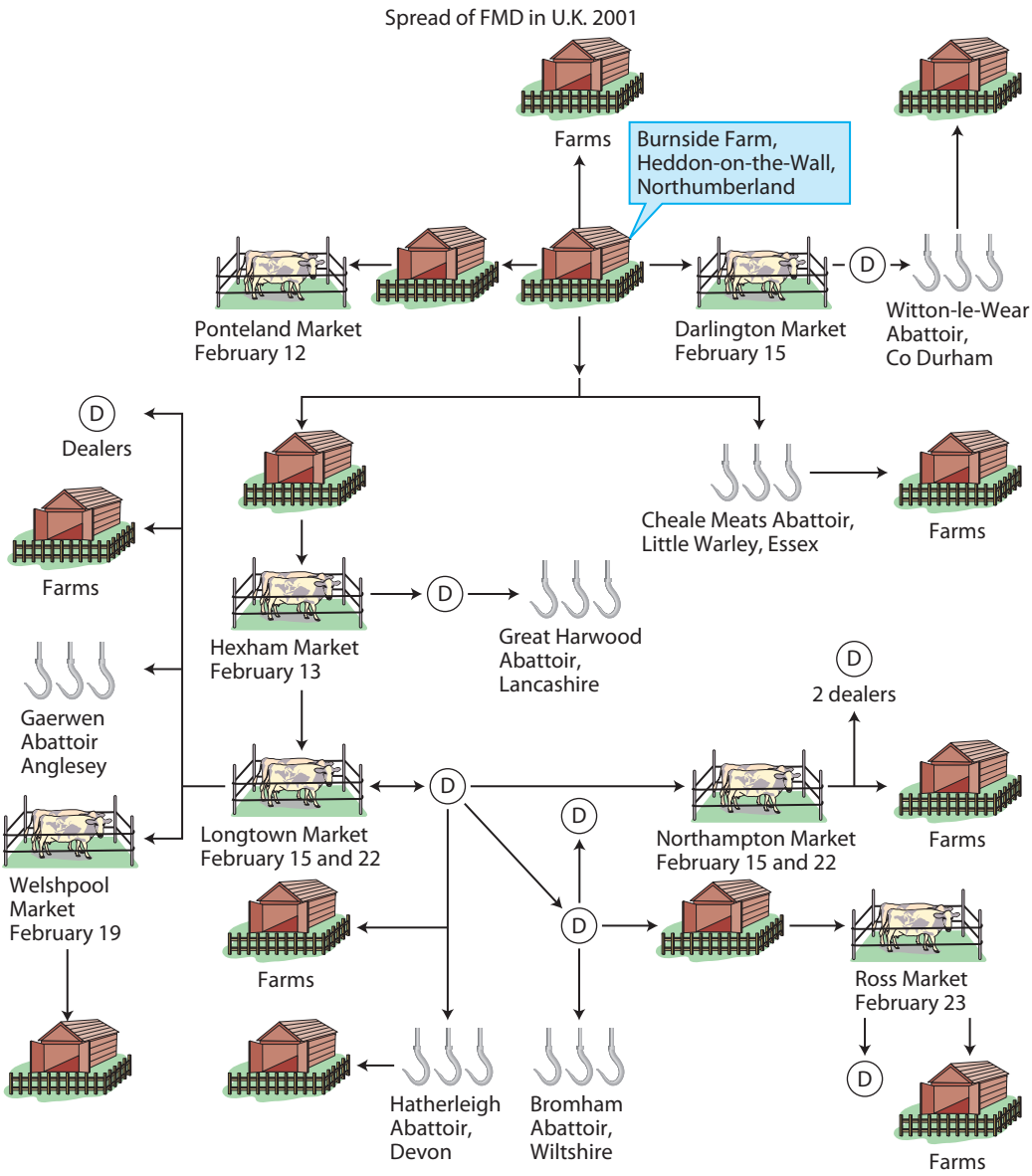


FIGURE 1-17 Epidemiologists determine how FMD spread to farms and the market during the 2001 outbreak in England.

farmers, politicians, scientists, veterinarians, and the consumer.

Hantavirus: Four Corners Disease, 1993

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 fiancé died of the same mysterious respiratory illness. Both victims died from fluid buildup in their lungs. Normally with each breath, the air sacs (aveoli) of the lungs take in oxygen and release carbon dioxide. In these circumstances, the lung aveoli were filled with fluid instead of air, preventing oxygen from being absorbed into the bloodstream, resulting in death. On May 17, the Indian Health Service 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 Four Corners region of Colorado, Utah, Arizona, and New Mexico.

This virulent new disease baffled medical examiners and the CDC. The CDC finally identified the virus as a strain of the hantavirus, an "old world" virus carried by rodents known to only cause hemorrhagic fever and kidney failure. *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. Hantavirus pulmonary syndrome is a rare disease that is sometimes caused by the hantavirus called sin nombre virus (no-name virus in

a

b

FIGURE 1-18 **(a)** This site was located on the edge of the restricted area in Northumberland, England, in 2001. **(b)** The British Ministry's strategy for containment of FMD is to slaughter and burn the infected animals. This photograph shows smoke rising from pyres of burning cows slaughtered to prevent the spread of FMD.

English). Several different types of wild mice, such as deer mice and rats, can be infected with hantavirus and pass it in their droppings, urine, or saliva. Once humans come in contact with fomites such as contaminated blankets or food storage areas, the virus then enters directly into the respiratory system through breathing contaminated air particles. It kills 50% of its victims.

Why did sin nombre virus strike the Four Corners region in 1993? It is likely that the virus had long been present in rodent populations. Officials believe that the unusually mild winter and spring of 1993, the rainfall, and the abundance of pinyon nuts on which the rodents feed led to an increased rodent population in the summer and greater opportunities for people to come in contact with infected rodents. 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 sin nombre virus (**FIGURE 1-19**). The detective work of the CDC led to a course of action, treatment, and prevention, thus thwarting a nationwide epidemic.

FIGURE 1-19 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 and analyzing deer mice.

West Nile Virus, 1999

The next time you are outdoors where there are mosquitoes, you may change your behavior after reading this section. You might wear clothing with long sleeves and apply a bug repellent that includes the ingredient DEET (meta-N, N-diethyl toluamide) over any exposed skin. You may find yourself removing any standing water in your neighborhood (for example, water that has accumulated in old tires, buckets, and wheelbarrows). You may start reporting any dead birds you find.

During the summer of 1999 elderly people in Queens, New York, were getting sick with headaches, fever, weakness, and confusion. Routine tests from the medical laboratory came back negative for bacterial or fungal pathogens. Given the symptoms of the patients, it was suspected that they were suffering from **encephalitis** (inflammation of the brain) of viral origin. Samples of blood, spinal fluid, and tissues from those who had died after falling ill were shipped to the Vector-Borne Diseases Laboratory at the CDC for analysis. Based on a routine antibody test, the disease was first diagnosed as encephalitis caused by the St. Louis Encephalitis Virus (SLE). The positive test result, however, was a very weak reaction. Nevertheless, helicopters began hovering over major highways and residential areas, spraying misty clouds of insecticides such as malathion and pyrethroids. The City Health Commissioner advised individuals to avoid contact with mosquitoes and exposure to insecticides.

Simultaneously, people started finding more than the usual number of dead crows in the area, and several exotic species of birds at the Bronx Zoo died. Birds don't die from SLE. They can, however, die from West Nile Virus (WNV). The CDC did not test the samples for WNV because that virus had never appeared in the United States before. No one had made the connection between the human epidemic and the wildlife epidemic.

As more cases appeared, the CDC conducted additional tests on the brain tissue of deceased individuals, the dead crows, and exotic birds from the zoo. Officials at the CDC attempted to isolate and study the virus taken directly from these tissues. At the same time, the laboratory at the New York State Department of Health in Albany began sequencing the RNA genome of the virus they had isolated from infected brain tissues. These results were shared among the scientific community via a special Internet site called ProMED (Program for Monitoring Emerging Diseases). ProMED shares results about epidemics occurring anywhere in the world, and scientists and physicians who specialize in infectious diseases visit the site on a daily basis. The ProMED post of their results matched with outbreaks of encephalitis in Romania, Egypt, Israel, Italy, and South Africa. It matched exactly with a strain of WNV in Israel from an epidemic in 1998. This turned out to be the final piece of the puzzle. The disease is spread via mosquitoes that have fed upon sick birds that harbor WNV. The mosquitoes then acquire WNV from the birds and transmit the disease via biting humans or animals.

The final toll from the 1999 WNV epidemic in the New York City metropolitan was 62 cases of encephalitis and 7 deaths. The virus was believed

to have infected as many as 1900 unknowing Queens residents who did not develop encephalitis. Exotic zoo birds, American crows, at least 20 other North American wild bird species, and horses were infected, and quite a few died. Why did the Israeli strain of WNV enter the United States? Scientists have suggested several reasons why WNV enters new regions. The most supported hypothesis is that migratory birds play a role in transporting the virus. Other hypotheses are:

- During migration, birds can be displaced by storms.
- Legal and illegal importation of birds (bird and pet trade).
- Birds are vagrants on ships or other transportation.

Most mammals become infected by mosquito bites. The virus is transmitted when the mosquitoes are feeding (taking a bloodmeal). Most non-bird species, including humans and horses, are dead-end hosts and do not transmit WNV to mosquitoes. Mosquitoes will feed on alligators resulting in an infection. However, outbreaks on alligator farms have also been linked to alligators being fed WNV-infected horse meat and ground beef contaminated with feces from WNV-infected wild birds. Reports have shown that birds shed WNV through their **cloaca**. The cloaca is a cavity at the end of the digestive tract into which the intestinal, genital, and urinary tracts open. It is found in birds, reptiles, most fish, and amphibians. Direct transmission has been reported between alligators in close contact, possibly by fecal contamination. Alligators shed WYN in their feces. Chipmunks and squirrels also secrete WNV in feces and urine, and might be able to spread WNV horizontally (**FIGURE 1-20**). Humans do not shed WNV in their secretions or excretions, but the virus can be transmitted by blood transfusions and in organ transplants. **TABLE 1-4** lists species found positive for WNV through surveillance efforts.

Norovirus Outbreaks, 1972 and 2002

Today, noroviruses (formerly called Norwalk-like viruses) may be the most commonly identified cause of infectious intestinal diseases. Much publicity has been given to outbreaks on cruise ships,

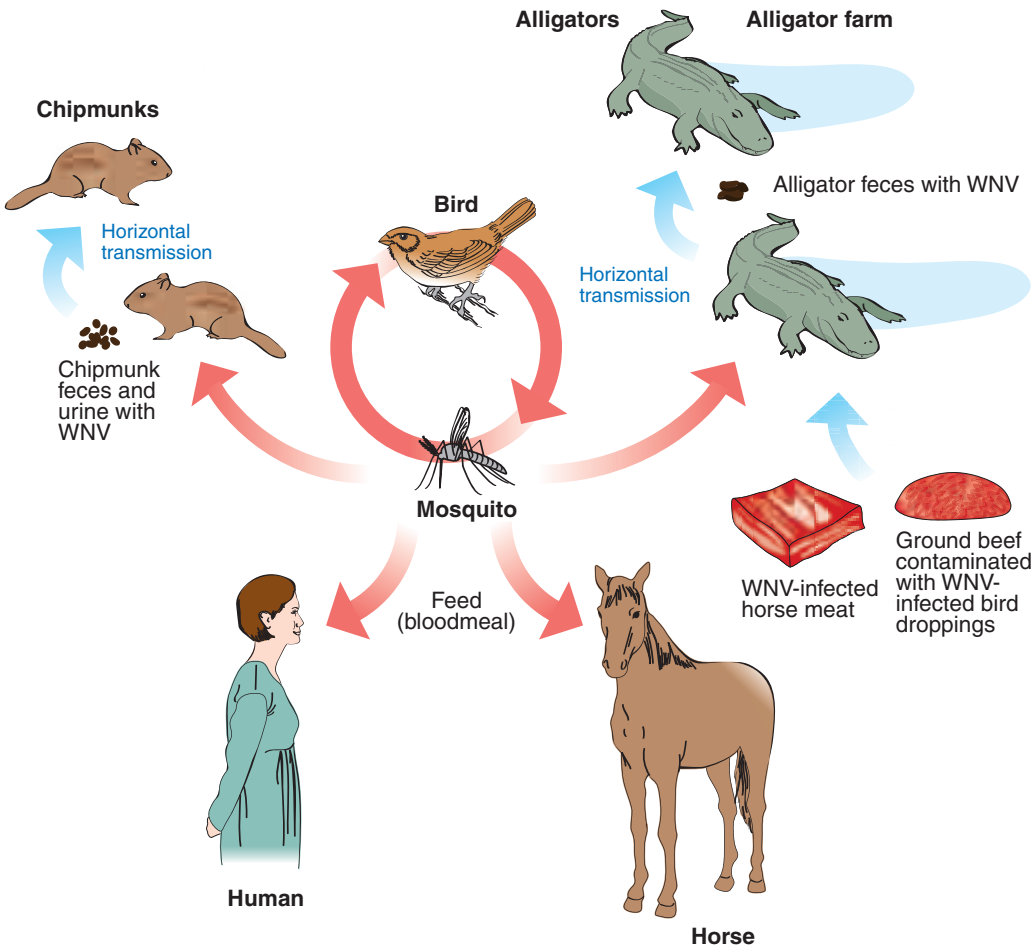


FIGURE 1-20 Diagram showing how WNV spreads from infected birds to mosquitoes to humans or other hosts such as horses, alligators, and chipmunks. Horizontal transmission may occur in chipmunks and alligators through fecal shedding.

TABLE 1-4 Species Found Positive for West Nile Virus in Surveillance Efforts

Birds	White-crowned pigeon	Demoiselle crane (c)(a)
Wood duck	Rock dove (feral pigeon)	West African crowned (a)
Eurasian wigeon (c)	Mauritius pink pigeon (c)(a)	Wattled crane (c)(a)
Mallard	Common ground-dove	Whooping crane (c)(a)
Bronze-winged duck (spectacled duck) (c)	Eurasian collared-dove	Mississippi sandhill crane (c)
Domestic goose (c)(a)	White-winged dove	Red-crowned crane (c)(a)
Canvasback	Mourning dove	Siberian crane (c)(a)
Canada goose	Luzon pigeon (bleeding heart pigeon) (c)(a)	Hooded crane (c)(a)
Barnacle goose (c)(a)	Inca dove	White-naped crane (c)(a)
Emperor goose (c)	Belted kingfisher	Black-necked crane (c)(a)
Greater Magellan goose (Andean goose) (c)(a)	Yellow-billed cuckoo	Virginia rail
Abyssinian blue-winged goose (c)(a)	Cooper's hawk	Lady Ross' turaco (lantain-eater) (c)(a)
Tundra swan (c)	Northern goshawk	Cedar waxwing
Trumpeter swan (c)(a)	Sharp-shinned hawk	Northern cardinal
Mute swan	Golden eagle	Blue grosbeak (a)
Rosy-billed duck (c)(a)	Red-tailed hawk	Rose-breasted grosbeak
Ruddy duck	Rough-legged hawk (c)	Dickcissel
Chimney swift	Red-shouldered hawk	Western scrub
Ruby-throated hummingbird	Broad-winged hawk	American crow
Common	Swainson's hawk	Common raven
Emu (c)	Northern harrier	Fish crow
Ruddy turnstone	Swallow-tailed kite	Blue jay
Killdeer	Bald eagle	Steller's jay
Piping plover	Mississippi kite	Black-billed magpie (c)
Herring gull	Osprey	Song sparrow
Laughing gull	Harris' hawk (c)	Savannah sparrow
Ring-billed gull	Merlin	Fox sparrow
Great black-backed gull	Prairie falcon	Eastern towhee field sparrow
Black skimmer	Peregrine falcon	Zebra finch (c)
Grey gull (c)(a)	American kestrel	American goldfinch
Inca tern (c)(a)	Crested guineafowl	House finch
Yellow-crowned night-heron (c)	Northern bobwhite	Purple finch
Black-crowned night-heron (c)	Chukar (c)(a)	Evening grosbeak
Great blue heron	Ruffed grouse	European goldfinch (c)
Green heron	Domestic chicken (red junglefowl) (c)	Barn swallow
Least bittern	Green junglefowl (c)(a)	Purple martin
Turkey vulture	Impeyan (Himalayan) pheasant (c)	Tree swallow
Black vulture	Bulwer's wattled pheasant (c)(a)	Red-winged blackbird
King vulture (c)(a)	Turkey (domestic and wild)	Rusty blackbird
Saddle-billed stork (c)(a)	Ring-necked pheasant	Brewer's blackbird
Marabou stork (c)(a)	Mount peacock (c)(a)	Baltimore oriole
Lesser Adjutant stork (c)(a)	Crested partridge (c)(a)	Brown-headed cowbird
Chilean flamingo (c)	Blyth's tragopan (c)	Boat-tailed grackle
Greater flamingo (American) (c)	Argus pheasant (c)(a)	Great-tailed grackle
Scarlet ibis (c)	Greater sage grouse	Common grackle
Waldrapp (c)(a)	Common loon	Loggerhead shrike

(continued)

TABLE 1-4 Species Found Positive for West Nile Virus in Surveillance Efforts (continued)

Gray catbird	Eastern kingbird	**Mammals**
Northern mockingbird	Black-whiskered vireo	Domestic cattle (c)
Brown thrasher	Warbling vireo	Mountain goat (c)
Tufted titmouse (c)	Red-eyed vireo	Domestic (Suffolk) sheep (c)
Black-capped chickadee	American white pelican	Llama (c)
Carolina chickadee	Brown pelican (c)(a)	Alpaca (suri) (c)
Black-throated blue warbler	Double-crested cormorant	White-tailed deer
Yellow-rumped warbler	Guanay cormorant (c)	Reindeer (c)
Yellow warbler	Red-headed woodpecker	Mule deer
Blackpoll warbler	Downy woodpecker	Babirusa (c)(a)
Common yellowthroat	Yellow-bellied sapsucker	Domestic dog (c)
Kentucky warbler	Pied-billed grebe	Timber wolf (c)
Northern parula	Cockatoo (unspecified) (c)	Domestic cat (feral)
Ovenbird	Cockatiel (c)	Striped skunk
Northern waterthrush	Red-crowned parrot (c)	Harbor seal (c)
Nashville warbler	Macaw (unspecified) (c)	Red panda (c)(a)
Canada warbler	Budgerigar (c)	Black bear (a)
Hooded warbler	Lorikeet (c)	Big brown bat
House sparrow	Black-footed penguin (c)	Little brown bat
White-crested laughingthrush (c)(a)	Magellan penguin (c)(a)	Domestic rabbit (c)
White-breasted nuthatch	Northern saw	Domestic horse (c)
European starling	Boreal owl (c)	Donkey (c)
Palm tanager (c)	Short-eared owl	Mule (c)
Carolina wren	Verreaux's eagle owl (milky eagle owl) (c)(a)	Great Indian rhinoceros (c)(a)
Winter wren	Great horned owl	Barbary macaque (c)
Veery	Snowy owl (c)	Ring-tailed lemur (c)
Hermit thrush	Eastern screech owl	Indian (Asian) elephant (c)(a)
Gray-cheeked thrush	Tawny owl (c)	Gray squirrel
Swainson's thrush	Great gray owl (c)	Fox squirrel
Wood thrush	Spotted owl (c)	Eastern chipmunk
Eastern bluebird	Barred owl	Human
American robin	Northern hawk owl (c)	**Reptiles**
Traill's flycatcher	Barn owl	American alligator (c)
Eastern phoebe	Ostrich (c)(a)	Crocodile monitor (c)(a)
Scissor-tailed flycatcher		

Note: Species included in this list are wild animals unless followed by a "c," which denotes either a captive or farmed animal(s). Virus or viral RNA was detected in animal tissue unless followed by an "a," which denotes detectable antibodies only have been reported.

Source: Adapted from National Wildlife Health Center. Madison, Wisconsin. Updated 9/2004.

but these viruses also pose problems in hospitals, nursing homes, schools, and homes. Norwalk-like viruses were first characterized and described in 1972 during an epidemic of diarrhea and vomiting involving students at an elementary school in the town of Norwalk, Ohio. These viruses thrive in places where humans are in high concentrations. Cruise ships happen to provide the perfect breeding environment for viruses: they provide an enclosed area with up to several thousand people in close contact with each other.

The virus is spread person to person via feces and sometimes by eating contaminated food (especially oysters) or drinking contaminated water. The intestinal illness is usually short lived (2–3 days). Norovirus outbreaks reached an all-time high in the

United States in 2002. The most important thing that anyone can do to keep from getting sick is to wash his or her hands. By frequently washing your hands, you wash away germs that you have picked up from other people, or from contaminated surfaces, or from animals and animal waste.

1.5 Smallpox: Ancient Agent of Bioterrorism

The use of biological agents in terrorism is not new. It dates back as early as the 6th century, when the Assyrians poisoned the wells of their enemies with rye ergot. Warring tribes would catapult diseased carcasses over castle walls to infect their enemies. On several occasions, smallpox was used as a biological weapon. Pizarro presented South American natives with clothing and gifts laden with the smallpox virus in the 15th century and Sir Jeffrey Amherst provided Native Americans (who were loyal to the French) with blankets tainted with smallpox during the French and Indian War (1754–1763). The epidemic killed most of the tribe, resulting in a successful British attack on Fort Carillon.

The term *smallpox* was introduced during the 16th century to distinguish it from the "great pox," or syphilis. Smallpox has been one of humankind's greatest scourges. It is believed that this virus appeared in agricultural settlements in Africa around 10,000 B.C. The disease left many disfigured with pox scars; some were blinded and many died. It nearly wiped out the Native American population in the United States, which had been free of infectious diseases before the Europeans came. Native Americans were not resistant to smallpox and other European diseases because they had never been exposed to those viruses. Yet few doctors today have seen a case of smallpox.

In 1967, the WHO launched an aggressive program to eradicate smallpox. It was very cost effective. In 1977, the last naturally occurring case was reported in Somalia. In 1980, the WHO declared the world free of smallpox and recommended to cease vaccination. Today we are once again vulnerable to this disease. It is speculated that immunity is not lifelong and many individuals have not been vaccinated. (Read more on smallpox in Chapter 14.)

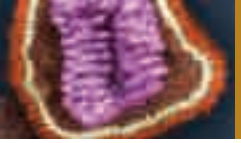
Other Bioterrorism Concerns

Bioterrorism is still a clear and present danger. A series of anthrax cases, some of which resulted in death, occurred during the weeks following the terrorist attacks on the United States of September 11, 2001. As a result there has been increased security and concern over the possibility of additional attacks. The scientific community is working as a team to be prepared for any future attacks. This preparedness involves the production of vaccines, drugs, and diagnostic tests that will play a major role in reducing the threat of bioterrorism.

Summary

Viruses impact all forms of life. They play an important role in ecosystems by affecting population growth in both positive and negative ways. The origin of viruses remains a debatable topic. Early pioneers of virology studied bacteriophages, plant viruses such as TMV, and smallpox virus that caused large numbers of outbreaks and mortalities throughout recorded human history.

Gene therapy, bacteriophage therapy, and vaccine development utilize viruses in applications toward health problems. Influenza, poliomyelitis, AIDS, foot and mouth disease, West Nile encephalitis, hantavirus pulmonary syndrome, smallpox, and norovirus-related gastrointestinal distress are examples of viral diseases that have had historical and recent relevance. Biological weapons have been used for centuries. Disease-causing viruses can be effective weapons used to infect and potentially kill large populations. Understanding the biology of viruses will help the scientific community prepare for a biological attack. The production of vaccines, drugs, and diagnostic tests will play a major role in reducing the threat of bioterrorism.



CASE STUDY QUESTIONS

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

1. To find general information about viral hemorrhagic fevers via the World Wide Web, what search engines and sites would you use? What keywords would you use in your searches?
2. How would you find research papers pertaining to viral hemorrhagic fever? List your resources and the titles of the journal articles. Where was the research conducted?

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