CHAPTER 2

Biology of Ebola

Carolyn Dehlinger

"[A] few weeks into this, I've certified the deaths of more patients than in my last two decades. And I'm shocked to the degree to which it has just become part of my daily routine."

—American doctor Joel Selanikio, International Medical Corps, Lunsar, Sierra Leone

In 1976, a deadly viral outbreak occurred near the Ebola River in what is now the Democratic Republic of Congo (at the time known as Zaire). Named for the river, Ebola outbreaks have peppered African history ever since, with most occurring sporadically and temporarily—until now. According to the **World Health Organization**, as of March 22, 2015, the disease has taken an estimated 10,326 lives worldwide. Ebola was previously known as Ebola hemorrhagic fever. While the name has shortened, the signs and symptoms of this disease have not. The words of Dr. Selanikio remind us of the emotional toll and psychological stress this

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disease can bring. These aspects can easily be obscured when we discuss the cold facts of a miniscule yet lethal virus. While reading this chapter whose focus is on the biology of the Ebola virus, or more accurately, viruses—to be sure, more than one strain of Ebola plagues the animal kingdom—it is also important to reflect on the human impact.

QUESTIONS TO CONSIDER

Some questions to consider as you read this chapter include:

1. What are the symptoms of Ebola and how is it contracted?
2. What is the classification of Ebola, and how is the classification determined?
3. What makes Ebola unique from its viral relatives and what makes it similar?
4. How would you describe the replication cycle of Ebola, and how might details of the replication cycle enable the development of effective antiviral medications to combat Ebola infections?
5. What is the probable reservoir organism of Ebola, and have any other candidates emerged since the time of this writing?

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Hemorrhagic Viruses

Hemorrhagic viruses are given their name because they cause **viral hemorrhagic fever (VHF)**, a severe syndrome of the body affecting multiple organ systems with multiple symptoms. Ebola is not the only hemorrhagic virus; there are four families of viruses with this designation. The filovirus family, which includes Ebola, is one. Filoviruses are the focus of this chapter. Arenaviruses, bunyaviruses, and flaviviruses are viral families with hemorrhagic members as well. Arenaviruses are transmitted to humans by rodents (TABLE 2.1). Bunyaviruses infect rodents, plants, insects, and, to a lesser extent, humans. A well known bunyavirus infecting humans is the hantavirus. Human diseases caused by the flaviviruses include hepatitis C, dengue fever, West Nile virus, and yellow fever. They are often transmitted between mammal hosts through a mosquito or tick vector. **Vectors** are organisms that pass infectious agents among different species. The lifecycle of the infectious agent takes place in part within the vector. A few hemorrhagic viruses and their characteristics are described in TABLE 2.2.

The shared symptoms of VHFs typically include:

- Fever
- Muscle aches
- Dizziness
- Fatigue
- Weakness
- Exhaustion

TABLE 2.1 Human Diseases Caused by Arenaviruses

Disease	Arenavirus
Argentine hemorrhagic fever	Junin virus
Bolivian hemorrhagic fever	Machupo virus
Brazilian hemorrhagic fever	Sabiá virus
Chapare hemorrhagic fever	Chapare virus
Lassa fever	Lassa virus
Lujo hemorrhagic fever	Lujo virus
Lymphocytic choriomeningitis	Lymphocytic choriomeningitis virus (LCMV)
Venezuelan hemorrhagic fever	Guanarito virus

Source: Data from Easton, A. J. & Pringle, C. R. (2011). Order *Mononegavirales*. In A. M. Q. King, M. J. Adams, E. B. Carstens, & E. J. Lefkowitz, *Virus Taxonomy—Ninth Report of the International Committee on Taxonomy of Viruses* (pp. 653–657). London, UK: Elsevier/Academic Press.

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TABLE 2.2 Viral Diseases Causing Hemorrhagic Fevers

Disease	Causative Agent	Family	Signs & Symptoms	Transmission	Treatment	Prevention
Yellow fever	Yellow fever virus	Flavivirus	Acute phase: Headache, fever, muscle pain Toxic phase: Severe nausea, black vomit, jaundice, hemorrhaging	Bite from a *Stegomyia aegypti* mosquito	No antiviral medications Supportive care	Vaccination Avoiding mosquito bites in endemic areas
Dengue fever	Dengue fever virus	Flavivirus	Sudden high fever, headache, nausea, vomiting	Bite from an infected *Stegomyia aegypti* mosquito	No specific treatment available	Avoiding mosquito bites in endemic areas
Dengue hemorrhagic fever	A different serotype of dengue fever virus	Flavivirus	Decrease in platelets, skin hemorrhaging	Bite from an infected *Stegomyia aegypti* mosquito with another dengue virus	No specific treatment available	Avoiding mosquito bites in endemic areas
Ebola/ Marburg hemorrhagic fevers	Ebola and Marburg viruses	Filovirus	Fever, headache, joint and muscle aches, sore throat, weakness Internal bleeding and hemorrhaging	Bite of an infected fruit bat Blood transfer through cut, abrasion, or infected animal bite	No specific treatment available	Avoiding dead animals and bats in outbreak areas
Lassa fever	Lassa fever virus	Arenavirus	Severe fever, exhaustion, hemorrhagic lesions on throat	Aerosol and direct contact with excreta from infected rodents	Ribavirin	Avoiding dead or infected rodents Maintaining good home sanitary conditions

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Infected patients often exhibit signs of bleeding, whether beneath the skin, within internal organs, or from body orifices like the mouth, nostrils, ears, or anus. The term *hemorrhagic* refers to leakage such as the bleeding described. As VHF progresses, more severe cases result in delirium, shock, seizures, or coma. The nervous system can malfunction, contributing to the aforementioned conditions. Common symptoms of Ebola include severe stomach pain, vomiting, and diarrhea.

Taxonomy of Filoviruses

The International Committee on Taxonomy of Viruses is responsible for the classification of known viruses. The taxonomy of viruses is based upon their anatomy, or arrangement of physical structures, including the genetic material they carry. Viruses that possess single-stranded, negative-sense ribonucleic acid (RNA) belong to the order known as *Mononegavirales*. The Greek *monos* refers to the single strand of RNA, while the Latin *negare* indicates the negative polarity of the RNA (see Anatomy of Filoviruses section that follows). The order *Mononegavirales* contains a total of five families:

- *Bornaviridae*
- *Nyamaviridae*
- *Rhabdoviridae*
- *Paramyxoviridae*
- *Filoviridae*

TABLE 2.3 provides details on the family members of order *Mononegavirales*. Ebola belongs to the family of viruses known as the *Filoviridae*, or simply the filoviruses. The family is composed of just three genera: *Cuevavirus, Marburgvirus*, and *Ebolavirus*. FIGURE 2.1 shows a phylogenetic tree of order *Mononegavirales*.

TABLE 2.3 The Family Members of Order *Mononegavirales*

Family	Number of Genera	Example Viruses	Host Species
Bornaviridae	1	Borna disease virus Avian Bornavirus	Sheep, horses, cattle, birds, rodents, sometimes humans
Filoviridae	3	*Ebolavirus* *Marburgvirus* *Cuevavirus*	Bats, primates, humans
Nyamaviridae	1	Nyamanini virus Midway virus	Ticks, birds

(*continues*)

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TABLE 2.3 The Family Members of Order *Mononegavirales* (*Continued*)

Family	Number of Genera	Example Viruses	Host Species
Paramyxoviridae	7	Atlantic salmon paramyxovirus Avian metapneumovirus Fer-de-lance virus Hendra virus Human respiratory syncytial virus Measles virus Mumps virus Newcastle disease virus Sendai virus	Humans, most vertebrates
Rhabdoviridae	11	Rabies virus (RABV) Lettuce necrotic yellows virus (LNYV) Bovine ephemeral fever virus (BEFV) Infectious hematopoietic necrosis virus (IHNV) Potato yellow dwarf virus (PYDV) Vesicular stomatitis Indiana virus (VSIV)	Vertebrates, invertebrates, plants

Source: Data from Easton, A. J. & Pringle, C. R. (2011). Order *Mononegavirales*. In A. M. Q. King, M. J. Adams, E. B. Carstens, & E. J. Lefkowitz, *Virus Taxonomy—Ninth Report of the International Committee on Taxonomy of Viruses* (pp. 653–657). London, UK: Elsevier/Academic Press.

Marburgvirus was the first recognized filovirus, named after Marburg, Germany, where in 1967 an index case occurred in humans (FIGURE 2.2). Laboratory workers handling tissue from green monkeys came down with hemorrhagic fever, resulting in 31 cases and seven deaths.

As described earlier, in 1976 the first known cases of Ebola hemorrhagic fever were recorded. In addition to the case in Zaire, another outbreak occurred concurrently in Sudan. These were the initial strains of Ebola recognized, with later outbreaks revealing still more distinctive species. The strain causing the first ever human outbreak is the same one responsible for the most recent outbreaks in Guinea, Sierra Leone, DRC, and

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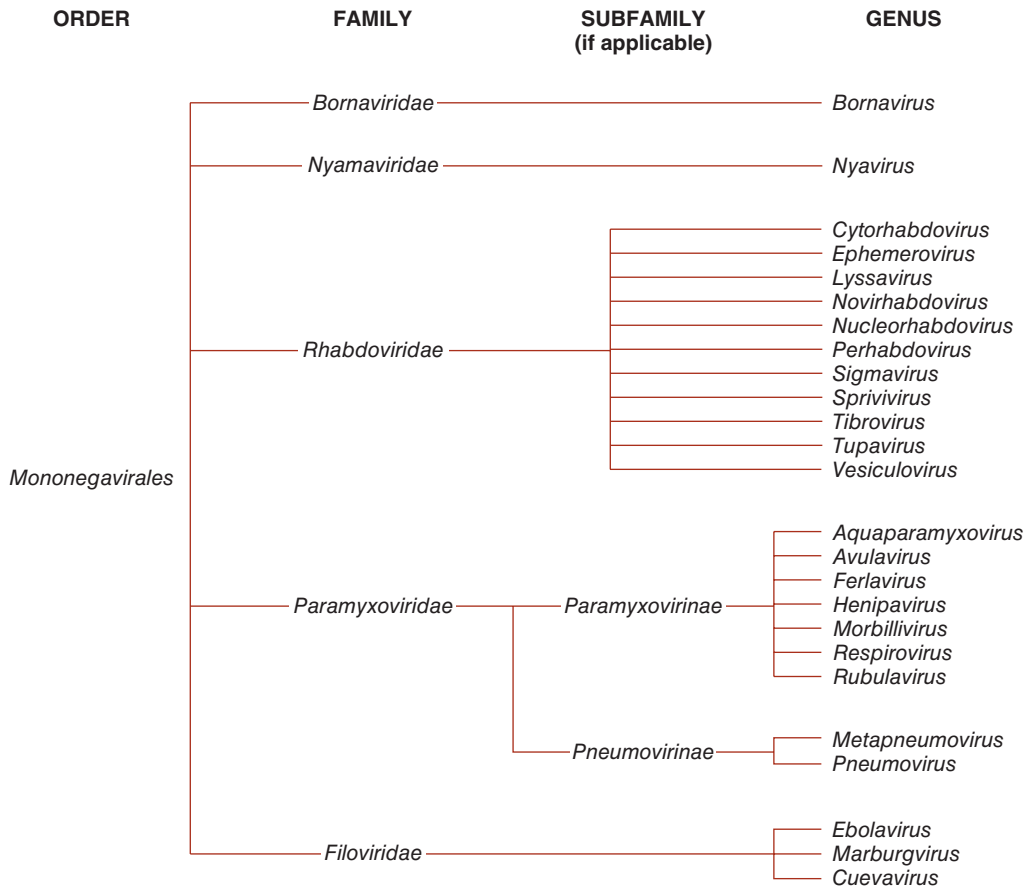

FIGURE 2.1 The phylogenetic tree of order *Mononegavirales* shows the five member families and the genera each contains. Note the location of *Ebolavirus* within the tree.

Source: Data from Easton, A. J. & Pringle, C. R. (2011). Order *Mononegavirales*. In A. M. Q. King, M. J. Adams, E. B. Carstens, & E. J. Lefkowitz, *Virus Taxonomy—Ninth Report of the International Committee on Taxonomy of Viruses* (pp. 653–657). London, UK: Elsevier/Academic Press

Liberia, now considered a widespread epidemic. FIGURE 2.3 shows an image of the Ebola virus.

Cuevavirus is the most recent addition to the filovirus family. Reported in 2010, the single identified species *Lloviu cuevavirus* was discovered when colonies of *Miniopterus schreibersii* (Schreiber's bats) experienced sudden massive deaths in Portugal, France, and Spain. This genus is not known to infect humans and is named for the cave Cueva del Lloviu in Asturias, Spain, where infected bat carcasses were collected for study. In addition to being the most recently discovered filovirus, *Cuevavirus* is also unique with outbreaks limited to Europe. While the other filoviruses, *Marburgvirus* and *Ebolavirus*, have caused infections in Europe and other regions of the world, thus far most of their damage has occurred on the continent of Africa (FIGURE 2.4).

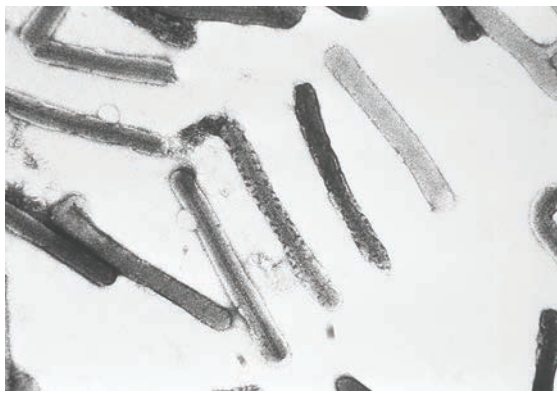

FIGURE 2.2 A transmission electron micrograph showing multiple *Marburgvirus* virions.

Courtesy of R. Regnery; Dr. Erskin L. Palmer/CDC

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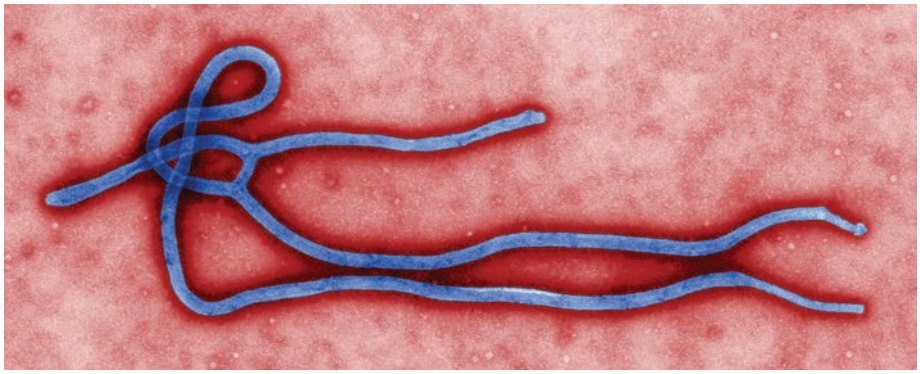

FIGURE 2.3 A colorized transmission electron micrograph of the Ebola virus. This image was created by CDC microbiologist Cynthia Goldsmith.

Courtesy of Cynthia Goldsmith/CDC

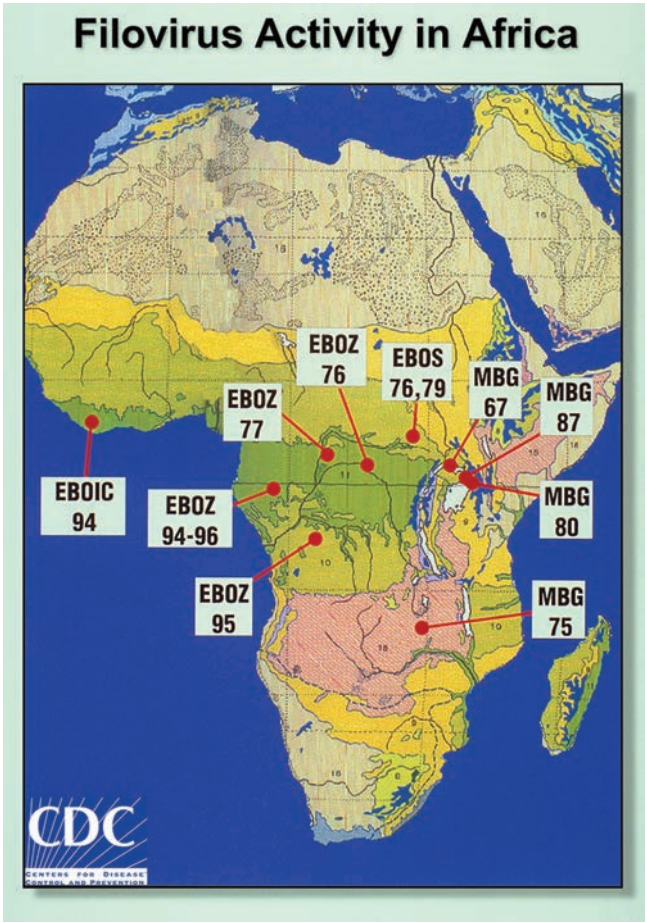

FIGURE 2.4 The distribution of reported cases of *Marburgvirus* (indicated as MBG) and *ebolavirus* (indicated as EBOZ, EBOIC, and EBOS) on the African continent.

Courtesy of CDC

Anatomy of Filoviruses

All viruses share certain anatomical traits. At minimum, viruses possess some form of genetic material enclosed in a protein layer called a **capsid**. Collectively, the capsid and genetic material may be called the **nucleocapsid**. Some viruses also have an additional layer of lipids. These viruses are referred to as enveloped, because the lipid layer is termed an **envelope**. The genetic material of a virus may be deoxyribonucleic acid

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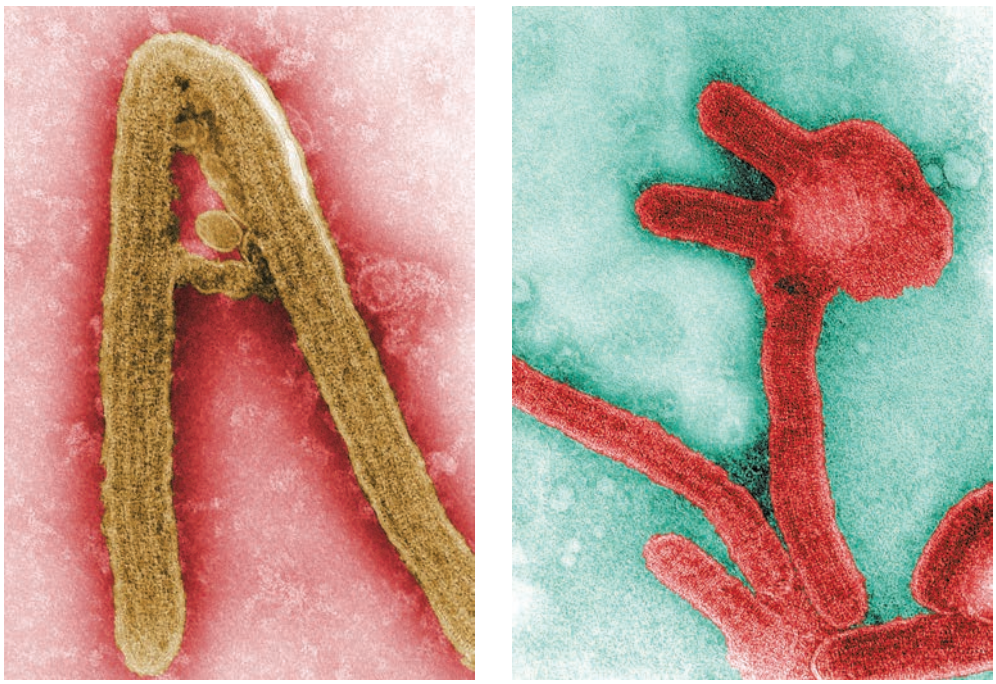
(DNA) or RNA in an assortment of forms. DNA viruses exist in either double-stranded or single-stranded genomes. RNA viruses may also be single or double stranded. When a virus contains single-stranded RNA (ssRNA), the genetic material may be deemed positive sense or negative sense. With **negative-sense** RNA, the molecule possesses a base sequence complementary to the viral messenger RNA (mRNA). In **positive-sense** RNA viruses, the viral genome is identical to its mRNA transcript and thus can undergo direct translation into its protein products. Another way to look at the sense of ssRNA is that it constitutes the polarity of the molecule.

Genetically, filoviruses contain negative-sense, single-stranded RNA. Therefore, during gene expression, a filovirus genome must be transcribed into its complementary mRNA by the enzyme RNA replicase before translation can occur. Filovirus nucleocapsids are enveloped.

Filovirus **virions**, or whole viral particles, take on a number of shapes in their appearance. This trait is known as **pleomorphism**. Filoviruses may be circular, u-shaped, short curlicues, or long, sometimes branched, filaments. The longest observed filoviruses reach up to 14,000 nanometers in length and around 80 nanometers in diameter. Each virion consists of a single-stranded RNA molecule, serving as the viral genetic material (or **genome**) surrounded by a lipid envelope. For example, compare the transmission electron micrograph (TEM) images of *Marburgvirus* (shown previously in Figure 2.2) with FIGURES 2.5a and 2.5b. Even when observing the same genus, pleomorphism is apparent.



(a) (b)

FIGURE 2.5 (a) This transmission electron micrograph depicts a *Marburgvirus* virion. This image was captured in 1968 by F. A. Murphy, who had grown the virus in host cell culture. (b) Another transmission electron micrograph of *Marburgvirus* taken by F. A. Murphy. Compare this image to Figures 2.2 and 2.5a.

Courtesy of Frederick Murphy/CDC

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Transmission of Filoviruses

How does someone contract Ebola or any other filovirus? Direct physical contact between individuals is one way, or, in lieu of direct physical contact, one individual may pick up the virus through contact with the body fluid(s) of an infected person (or animal). There is some experimental evidence that Ebola may be transmitted through small airborne particles, but this has not been directly observed in actual cases. All filovirus outbreaks show the greatest rates of infection occur among patient caregivers, followed by cross-contamination in patient care settings such as hospitals. The latter is known as **nosocomial transmission**. Frequently, these types of transmissions are the result of reusing contaminated needles or syringes. The manner in which filoviruses like Ebola are transmitted makes patient isolation a critical factor in controlling the spread of infection.

Characterization of Ebola

The *Ebolavirus* genus has been characterized based on morphological studies using electron microscopy. These observations lend to the development of diagnostic techniques. At least five species or strains of the *Ebolavirus* genus are known, four of which (indicated here with an asterisk, *) cause disease in humans:

- *Bundibugyo ebolavirus* (BEBOV)*
- *Cote d'Ivoire ebolavirus* (CIEBOV), also known as Taï Forest Ebola*
- *Reston ebolavirus* (REBOV)
- *Sudan ebolavirus* (SEBOV)*
- *Zaire ebolavirus* (ZEBOV)*

Thus, the Reston species of Ebola does not result in disease in humans. Studies of the first outbreaks of Ebola in 1976 determined they were caused by the Zaire and Sudan species, respectively, and indeed these two species are named after the locations of their first appearances in humans. Both of these species are highly lethal to humans, with a 90% fatality rate in Zaire cases and 50% fatality rate in Sudan.

Ebola Morphology and Genetics

Morphological studies show all Ebola viruses are distinct from their closest relatives, the Marburg genus of viruses. Marburg virions obtained through host cell cultures were shorter in length than all Ebola virions. During biosynthesis, *Marburgvirus* develops inclusion bodies within host cells of unique morphology as compared to their *Ebolavirus* cousins. While it is difficult to differentiate *Ebolavirus* variants from one another based on inclusion body morphology, only the *Zaire ebolavirus*

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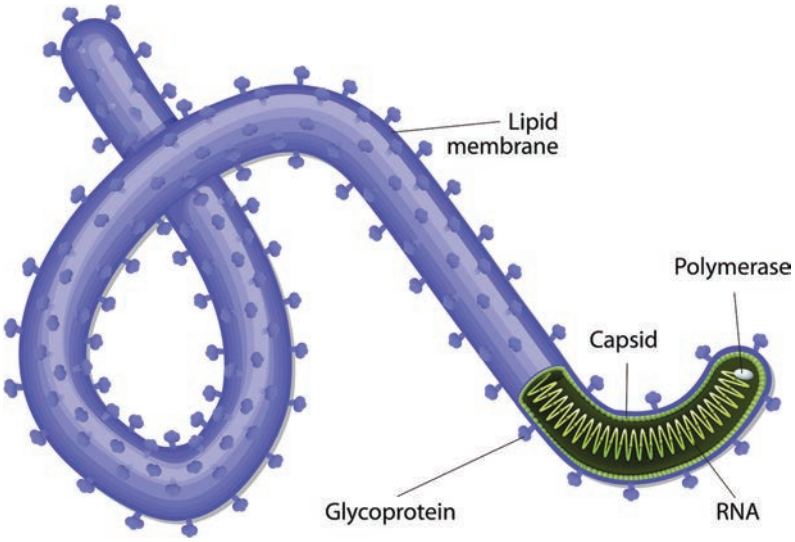

FIGURE 2.6 The generalized anatomy of all Ebola viruses includes a single-stranded RNA genome, one or more RNA replicase enzymes, a lipid envelope, and glycoprotein projections.

© ttsz/iStockphoto

species of Ebola formed inclusion bodies composed of filoviral matrix proteins and nucleoproteins. The association between these proteins continued into the assembly stage of the ZEBOV lifecycle, also unique to this species.

All Ebola species are composed of an ssRNA genome and one or more RNA replicase enzymes surrounded by a lipid envelope studded with glycoproteins (FIGURE 2.6). The Ebola nucleocapsid is intertwined with the viral proteins NP, VP35, VP30, and L. Glycoprotein (GP) spikes are embedded on the Ebola envelope, while the space in between the nucleocapsid and envelope are occupied by the viral proteins VP40 and VP24. There are a total of seven genes in the 19-kilobase Ebola genome, listed in 3′ to 5′ order as follows:

3′-leader-NP-VP35-VP40-GP/sGP-VP30-VP24-L-trailer-5′

Genome sequencing studies conducted by Broad Institute and Harvard University researchers in fall 2014 revealed a number of variations in *Ebolavirus* sequences among a sample of Sierra Leone patients. During the first month of the outbreak in Sierra Leone, 78 patients provided blood samples allowing for a total of 99 genome sequences. For some patients, multiple samples were taken over a span of time in order to observe any changes that may occur during the course of disease progression. A total of 395 mutations were discovered among the sequencing data, indicating rapid change in the viral population. Of these, more than 340 are unique to the current widespread epidemic and 50 are exclusive to the West Africa outbreaks.

The sequencing data prove the ongoing widespread epidemic is genetically distinct from the first Ebola outbreaks in 1976, indicative of mutations accumulating over time. The data also suggest there was a single infection—a common ancestor infecting one person—dating back to 1976. Therefore, although today's strain of Ebola is not genetically identical to the 1976 version, it is likely a direct descendant. Genome sequencing has revealed current *Ebolavirus* populations diverged from the original population sometime within the last decade. Furthermore, the researchers were able to determine the virus was spread from Guinea to Sierra Leone by 12 people, all of whom attended the same funeral.

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Ebola Natural History

An understanding of Ebola ecology may aid researchers in determining the animal origin of the disease (discussed in detail in the paragraphs that follow). Based on outbreak history, Ebola is thought to have the ecological niche of rainforest ecosystems, particularly in the western and central regions of Africa. Transmissions during the past 17 outbreaks (1996–2007) have occurred between humans and nonhuman primates, antelopes known as duikers, and, possibly, bats. This is based on RNA sequence data of *Zaire ebolavirus* infections in human and serological analysis of mammalian **reservoirs**, or animal of origin, for the ZEBOV-specific immunoglobulin G (IgG) antibody. Interestingly, an infographic published by the **Centers for Disease Control and Prevention (CDC)** outlining the ecology of Ebola displays bats as the primary reservoirs of Ebola (FIGURE 2.7). They are the primary candidates at present, as discussed further in this chapter.

A 2004 University of Kansas study by Peterson, Bauer, and Mills comparing the phylogeny and ecological niche characteristics of the filovirus family proposed relationships among the Ebola species. *Zaire ebolavirus* and *Cote d'Ivoire ebolavirus* appear most closely related, with *Sudan ebolavirus* being most distantly related to all other species, followed by *Reston ebolavirus*.

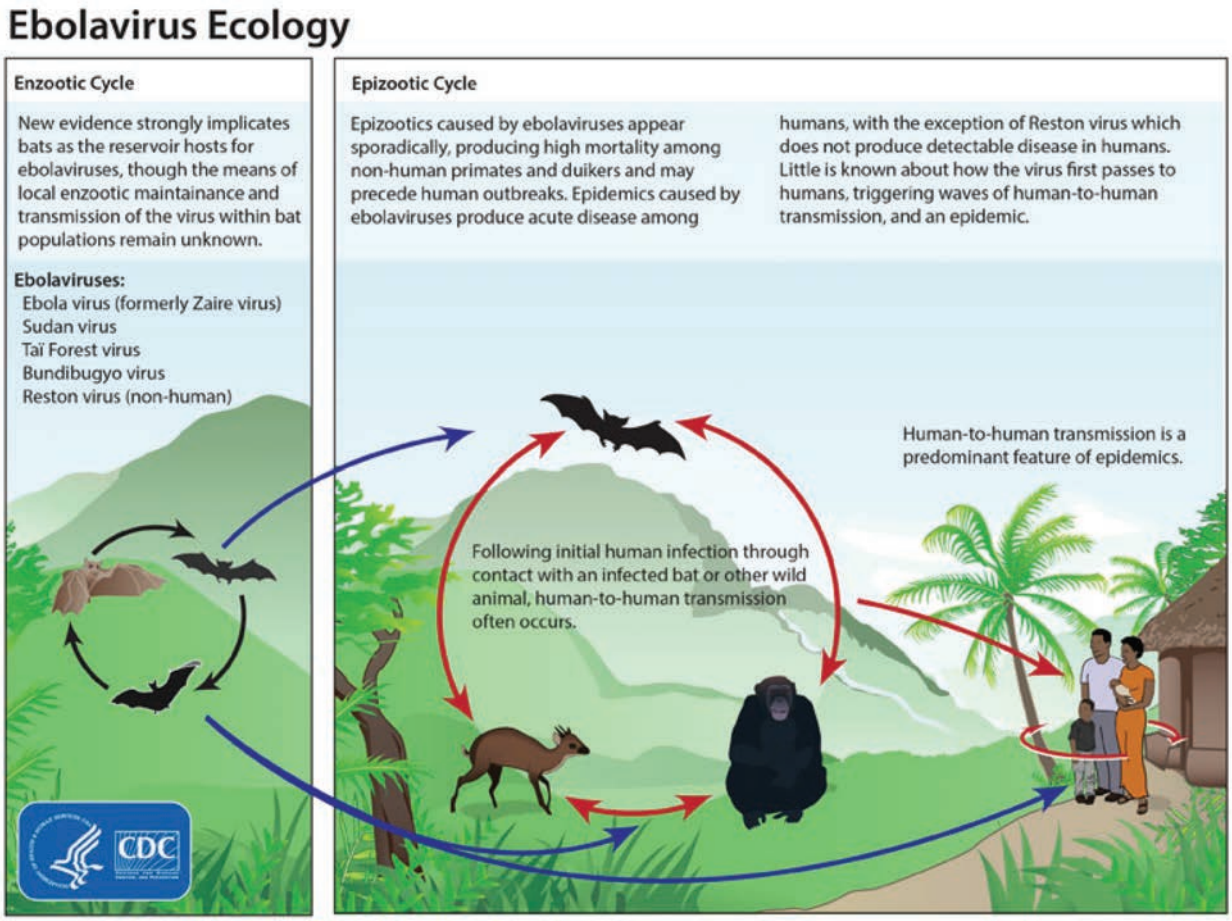

FIGURE 2.7 A recent infographic produced by the CDC shows bats as the reservoir of Ebola viruses.

Courtesy of CDC

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Zaire ebolavirus

Zaire ebolavirus was the first Ebola species discovered, and it also represents the source of the most recent widespread epidemic. FIGURE 2.8 displays a map of West Africa indicating the distribution of cases within the countries of Guinea, Sierra Leone, Liberia, and Côte d'Ivoire. This information was provided by the World Health Organization (WHO) and is current up to October 10, 2014. Epidemiologists utilize maps such as this one to observe trends in the spread of infection. Treatment strategies can also be influenced by these distribution maps. Note the locations of hospitals, laboratories, transit centers for patients, and WHO-designated Ebola Treatment Units (ETUs). The opening image of this chapter depicts the view from inside one ETU looking out through the entrance.

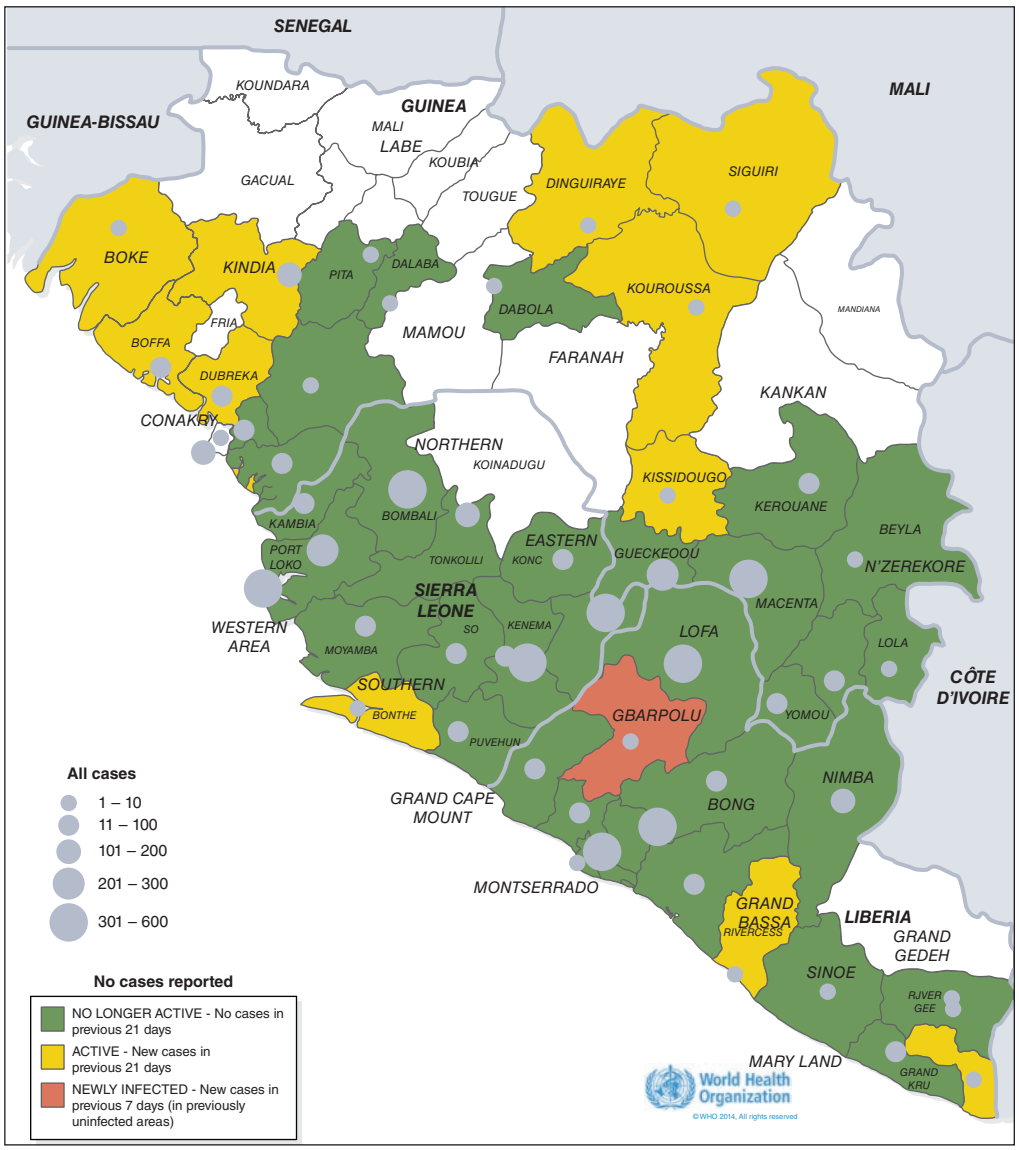

FIGURE 2.8 The distribution of Ebola infections within West Africa as of October 10, 2014, as reported by the World Health Organization.

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The Zaire strain of Ebola is historically the most virulent. *Zaire ebolavirus* has resulted in 17 separate outbreaks since the initial outbreak in 1976. Multiple outbreaks have taken place in Zaire (now DRC) and Gabon, with additional outbreaks in South Africa and twice in Russia. The latest outbreak has spread infections into Sierra Leone, Guinea, and Liberia as well. West African countries experience repeated outbreaks that seem to indicate a connection to ecology for this species, whereas infections in South Africa and Russia were caused by exposure to healthcare workers and laboratory researchers, respectively. The widespread nature of the most recent outbreaks has reached epidemic proportions.

Sudan ebolavirus

The second species of Ebola recognized, *Sudan ebolavirus* was first seen in the country of Sudan in 1976, not long after ZEBOV made its appearance in Zaire. Compared to *Zaire ebolavirus*, SEBOV is less fatal, yet more fatal compared to *Bundibugyo ebolavirus*. A total of eight outbreaks of *Sudan ebolavirus* have taken place since 1976, with all but one occurring in either Sudan or Uganda. The lone exception was an isolated infection of a laboratory worker in England, which happened in 1976. This patient survived.

Cote d'Ivoire ebolavirus

Also known as Taï forest virus, *Cote d'Ivoire ebolavirus* was not observed until 1994 in Côte d'Ivoire (Ivory Coast). The lone human case was a scientist who performed an autopsy on a wild chimpanzee in the Taï forest region of Côte d'Ivoire and soon became ill. The patient was treated in Switzerland and survived the infection.

Reston ebolavirus

This species is perhaps the most enigmatic in terms of its origin, as it represents the only Ebola species initially observed outside of the African continent. *Reston ebolavirus* was found in 1989 when captive macaques came down with hemorrhagic fever. The macaques originated from a breeding facility in Luzon, Philippines, but resided in Reston, Virginia, lending this species of Ebola its name. It is the only Ebola species that has yet to infect humans. After seven separate contamination events, there have been 13 instances of exposure in humans. One laboratory worker who handled the infected macaques postmortem developed Ebola antibodies, but none of the exposed individuals ever showed any signs of disease. All cases took place in the United States, Italy, or the Philippines. The question remains whether REBOV emerged in the similar ecological conditions of the Philippines (i.e., tropical rainforests) or if this species migrated from somewhere else.

Bundibugyo ebolavirus

The most recently discovered species is *Bundibugyo ebolavirus*. Isolated from a 2007–2008 outbreak in the Bundibugyo district of western Uganda, BEBOV is the least fatal within the genus with a mortality rate of 34%. Despite this, BEBOV results in twice the rate of bleeding manifestations in patients compared to other strains

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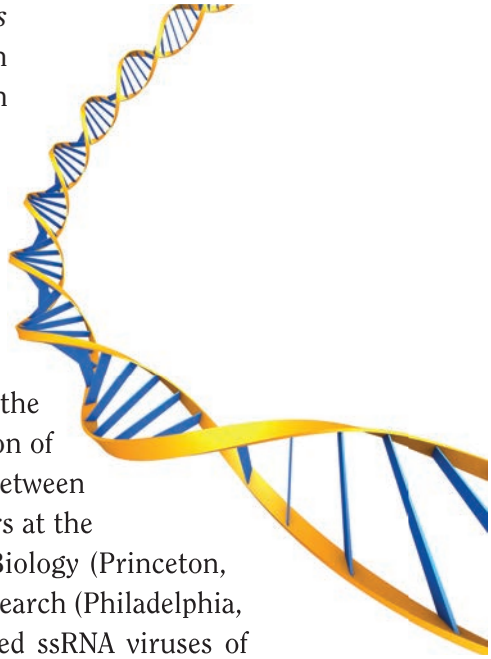
of Ebola. Only one other outbreak of *Bundibugyo ebolavirus* has taken place. In 2012, 36 individuals became infected with BEBOV in DRC. Although this occurred concurrently with an outbreak of *Sudan ebolavirus* in Uganda, there is no epidemiological evidence linking the two events.



Paleovirology of *Mononegavirales*

During the course of more than 3 billion years of evolution of life on this planet, species have accumulated snippets of viral genes within their genomes. Think of them as footprints implanted into the host cell DNA, left behind by viral invaders of long ago. Observation of these viral remnants offers insight into the ancient relationships between viruses and their hosts. One collaborative study among researchers at the Institute for Advanced Study at the Simons Center for Systems Biology (Princeton, New Jersey); the Fox Chase Cancer Center, Institute for Cancer Research (Philadelphia, Pennsylvania); and the University of California, Irvine determined ssRNA viruses of the order *Mononegavirales* have deposited artifacts of their past presence in 19 vertebrate species (Belyi, Levine, & Skalka, 2010). Within the order, the families *Filoviridae* and *Bornaviridae* are almost exclusively represented in these 19 species at nearly 80 separate genome locations. The earliest integration event is estimated at approximately 40 million years ago. This tells us that members of order *Mononegavirales* have been infecting vertebrate species for at least that long. Based on sequence data, the integrated viral artifacts are linked to genetic coding for matrix proteins, glycoproteins, viral nucleocapsids, and RNA replicase. These are the very genes expressed in the filovirus lifecycle (discussed later). Why would vertebrate genomes hold onto relics of viral RNA? Based on the principles of natural selection, it is a reasonable conclusion that they must provide some biological benefit. Perhaps their presence somehow influences their relative virulence upon an infected host in present time, or even whether a species is resistant or susceptible to infection. If there does prove to be a correlation between the presence of endogenous *Mononegavirales* sequences and virulence, this would give the virus an advantage, too. A species resistant to infection would serve the critical function of acting as the viral reservoir, thus allowing the persistence of the virus.

Research Classification

The CDC (2009) classifies Ebola viruses as Biosafety Level 4 (BSL-4) infectious agents, a class of "dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission" (p. 45). There are a total of four safety levels in ascending order, placing Ebola among infectious agents with the highest amount of risk posed to researchers handling this genus of viruses. Hemorrhagic viruses like Ebola are investigated by the Viral Special Pathogens Branch of the CDC (FIGURE 2.9). Their handling requires the most stringent protocols to prevent contamination.

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FIGURE 2.9 In this image from the Centers for Disease Control and Prevention, a microbiologist looks out from a Biosafety Level 4 (BSL-4) laboratory.

Courtesy of James Gathany/CDC

The Replication Cycle of Filoviruses

Viruses are **obligate parasites**: They require a host for their survival. However, we refer to them as parasites in a loose sense, because they are not technically alive at all. As a matter of course, they are acellular (not made of cell[s]) and thus violate the cell theory of biology, which states:

1. All organisms are composed of at least one cell.
2. All cells come from preexisting cells.
3. The cell is the smallest form of life.

In order to replicate, viruses must associate with a host cell. Generally, a virus penetrates the host cell with its genetic material, which may be DNA or RNA (either single or double stranded), and takes advantage of gene expression mechanisms present within. A gene in the most basic sense provides information to create a protein; think of it as a recipe. When a gene is expressed, the cell is "cooking" the recipe. Gene expression involves two steps: transcription and translation. Transcription creates a messenger RNA molecule from a DNA, or sometimes RNA, template. Translation turns the mRNA molecule into a protein product. These two steps comprise what is referred to as the central dogma of molecular biology (FIGURE 2.10). By hijacking the host cell, it unwittingly creates copies of the virus, resulting in its own death as the newly formed virions burst through in their escape, onward to infect new host cells and repeat the whole process.

Viruses have a number of replicative strategies that depend primarily on the type of genetic information they carry. As viruses rely on host cell machinery to complete their

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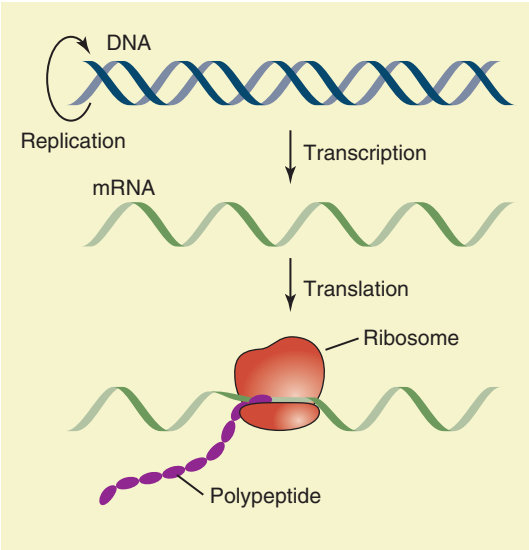

FIGURE 2.10 The central dogma of molecular biology describes the flow of genetic information from DNA to messenger RNA during transcription, then from messenger RNA to a polypeptide (or protein) product during translation.

replication cycles, their mode of replication is dependent upon the presence of or, as we shall see in the case of filoviruses, the lack of necessary enzymes integral to carrying out expression of the viral genome.

Attachment and Entry into Host Cell

Electron microscopy studies of different Ebola species revealed the virus enters host cells via endocytosis. **Endocytosis** is the engulfment of a substance into a cell (FIGURE 2.11). The endocytosis is cell-receptor mediated, which requires the virus to attach to the host cell membrane via receptors on its surface. Specifically, the virus exhibits glycoproteins that enable binding. The host cell membrane begins to invaginate, creating a cavity that eventually pinches off to enclose the virus in a vesicle. The virus has now entered the host cell. From this point, the viral membrane fuses with the vesicle membrane, releasing the viral genome into the host cell's cytoplasm (liquid interior).

FIGURE 2.11 This model demonstrates the process of endocytosis. The large white cell is surrounding the smaller red substance with its membrane. Ultimately, the red substance will become completely engulfed and pinch off into the white cell.

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Research has shown that *Ebolavirus* preferentially targets cells of the immune system, including macrophages and dendritic cells. Once these cells are infected, they release glycoproteins synthesized by the infecting virions. The secreted glycoproteins (sGPs) mimic the behavior of the viral glycoproteins embedded on their envelopes. In this way, *Ebolavirus* confuses the immune system into essentially disarming itself. Dendritic cells normally undergo what is known as maturation during times of infection, during which they release cytokines to promote an adaptive immune response and also trigger the activation of helper T-cells that fight pathogen invasions. Ebola is capable of deregulating dendritic cells and preventing maturation and the associated adaptive immune response. Furthermore, infected macrophages overexpress and release cytokines, which promote an inflammatory response. As this proceeds unchecked, the cytokine storm causes the associated symptoms of VHF such as fever, aches, and leakage of blood into tissues.

Negative-Sense RNA Replication

Filovirus host cells do not normally undergo RNA replication, because their genomes are composed of DNA. The RNA replicase enzyme needed for the virus to complete its replication cycle is lacking. Negative-sense ssRNA viruses such as *Ebolavirus* circumvent this roadblock by carrying one or more copies of the RNA replicase enzyme with them. Upon entering a host cell, the virion piggybacks with the RNA replicase enzyme(s), which then commences making positive-sense strands using the negative-sense strand as a template. These positive-sense strands act as complementary mRNA copies of the viral genome. The genetic information carried within the positive strands can now be used as templates to synthesize more RNA replicase enzymes as well as other viral proteins necessary for reproduction. Indeed, the synthesized positive strands serve as intermediates in the creation of new negative-sense ssRNA offspring (FIGURE 2.12). It is believed the individual genes of the viral genome are transcribed in sequential order by the action of RNA replicase recognizing start and stop regions flanking each gene. FIGURE 2.13 summarizes these steps of the filovirus lifecycle. As noted, this lifecycle is common to negative-sense ssRNA and double-stranded RNA viruses.

Release

Once new progeny are produced in the host cell, they move toward the host cell membrane. In *Zaire ebolavirus,* the matrix protein VP40 appears to play a critical role in interacting with the host cell membrane beneath its surface, allowing viral exit by budding (FIGURES 2.14a and 2.14b). The virions become enveloped in a lipid membrane of their own and are now ready to infect neighboring cells of the host organism. VP40 is experimentally linked to at least two host cell **endosomal sorting complexes required for transport (ESCRT)**. In the broadest sense, ESCRTs are involved in any activity that requires remodeling of the cell membrane.

The tumor susceptibility gene 101 protein is encoded by the human gene *TSG101* and is part of the ESCRT-I complex. This particular ESCRT aids in the regulation

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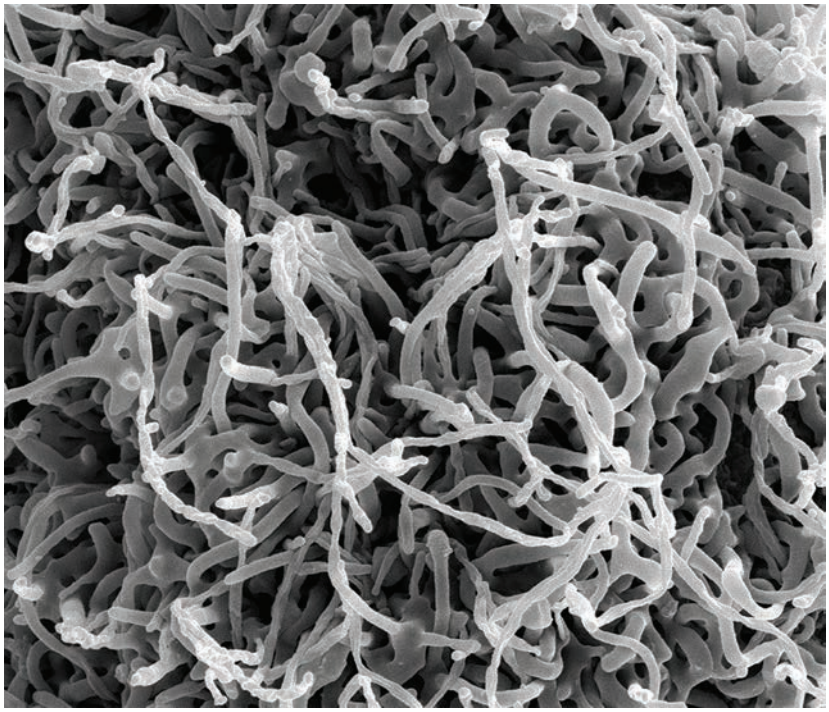

FIGURE 2.12 This scanning electron micrograph (SEM) from the National Institute of Allergy and Infectious Diseases (NIAID) shows a multitude of newly replicated *Ebolavirus* virions within a cultured VERO E6 cell. VERO E6 is a cell line derived from kidney epithelial cells of green monkeys. The image is magnified 50,000×.

Courtesy of National Institute of Allergy and Infectious Diseases (NIAID)/CDC

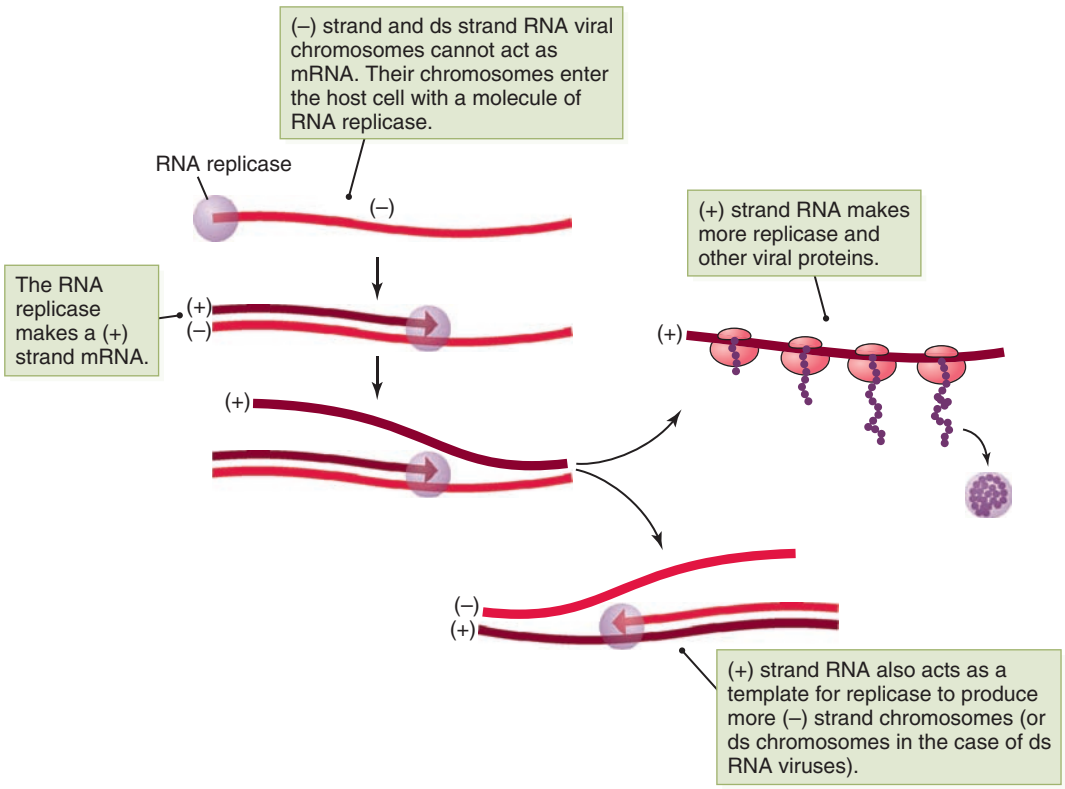

FIGURE 2.13 The replication cycle of negative-sense, single-stranded RNA viruses requires transcription of positive-sense strands before translation can occur. The positive-sense strands act as mRNA transcript templates, which are translated into gene products.

Note: ds stands for double-stranded.

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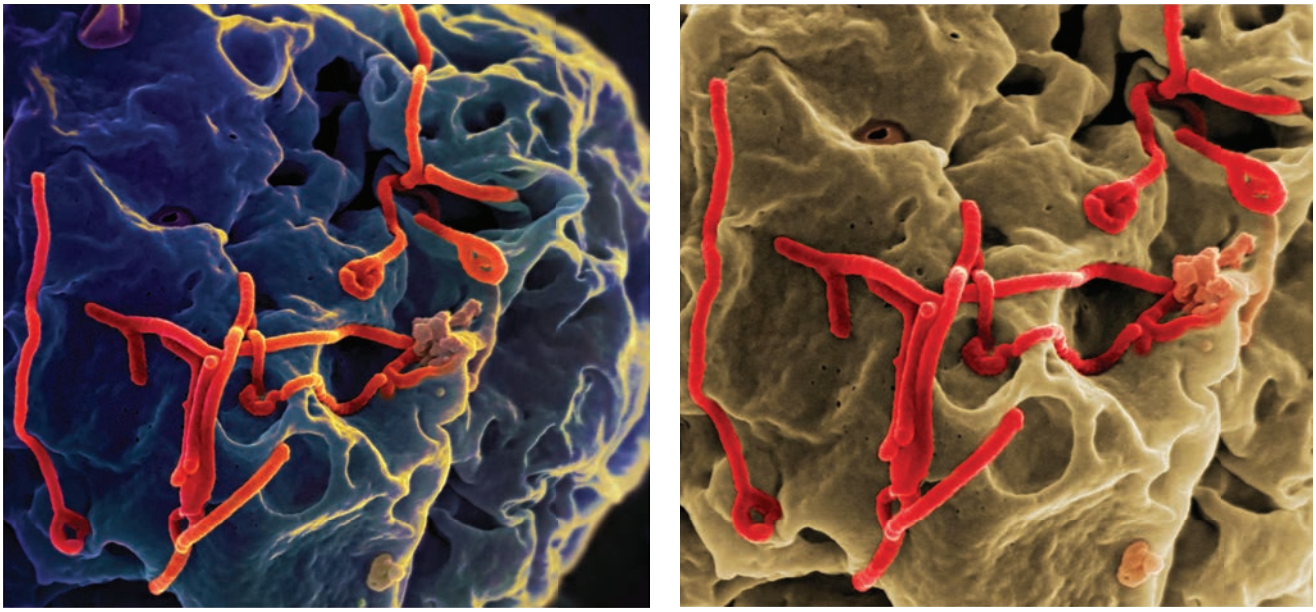

(a) (b)

FIGURE 2.14 (a) This scanning electron micrograph (SEM) has been digitally colorized to differentiate Ebola virions (in red) budding from the surface of a VERO cell (in blue). This SEM was produced under high levels of magnification by the National Institute of Allergy and Infectious Diseases (NIAID). (b) This SEM is an up-close version of Figure 2.14a. The Ebola virions are still displayed in red; however, in this image the VERO cell is now a taupe coloration.

Courtesy of National Institute of Allergy and Infectious Diseases (NIAID)/CDC

of the vesicular trafficking process. In essence, it behaves as a gatekeeper and facilitates the escort of materials in and out of the cell through cargo-carrying vesicles. The human gene *NEDD4* encodes the other protein associated with ZEBOV's VP40. Known as the E3 ubiquitin ligase NEDD4 protein, it releases ubiquitin proteins that appear to aid in viral budding.

As mentioned earlier, a separate protein product is also released from certain Ebola-infected cells. The *Zaire ebolavirus* genome contains the GP gene which encodes a short, nonstructural glycoprotein known as sGP that is never incorporated into the freshly assembled ZEBOV virions. Further investigation of the mechanisms involved in release of Ebola virions into their host organism is integral to the development of antiviral treatments.

Ebola Reservoirs

Ebola is a **zoonotic disease**, meaning it can be spread from animals to humans. One unknown in our understanding of Ebola is the animal origin, or reservoir, of transmission. How exactly the virus first made contact with *Homo sapiens* is still a mystery; however, particulars of *Ebolavirus* outbreaks have revealed distinct clues.

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It Is What It Isn't

First, index cases of Ebola are relatively rare compared to other viruses. Furthermore, the index cases of filoviruses tend to be isolated rather than occurring in multiples at once. While some more common viral infections are transmitted through insect vectors, it is not likely for a member of the arthropods to harbor and pass on Ebola. If that were so, we would expect more index cases simply because human contact with insects is so frequent.

In addition, phylogenetic analyses of the filovirus family show a positive correlation to geographic region, meaning each viral strain is strongly associated with a particular location. This observation suggests a degree of stability for the Ebola reservoir. It is likely the reservoir maintains permanent residence or else we would expect there to be a wider geographic distribution of each strain. Building on this logic, if a reservoir is firmly rooted in its habitat, then there is an increased probability of resistance or immunity to the virus and therefore low death rates of the reservoir species. As infected nonhuman primates exhibit high mortality rates, they are generally not considered prime reservoir candidates.

Experimental studies have attempted infecting various species with Ebola. A summary of their results is presented in TABLE 2.4. Species that suffer high rates of death are probably not a reservoir, because a reservoir needs to survive in order to continuously introduce the disease to new individuals.

The Leading Hypothesis

One of the primary suspects is the fruit bat. Often, disease reservoirs are species hunted by humans as food sources, and fruit bats are commonly hunted in Africa. Bolstering

TABLE 2.4 Experimental Ebola Infections of Various Organisms

Organism	Inoculation	Resulting in Infection?
Plants	*Zaire ebolavirus*	No
Arthropods	*Zaire ebolavirus, Reston ebolavirus*	Very little
Reptiles	*Zaire ebolavirus*	Single infection; low levels of further circulation within the population
Rodents	*Zaire ebolavirus*	Yes
Bats	*Zaire ebolavirus*	Yes; many do not become ill, no deaths
Nonhuman Primates	*Zaire ebolavirus*	Yes; highly lethal

Source: Swanepoel R, Leman PA, Burt FJ, Zachariades NA, Braack LEO, Ksiazek TG, Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis. 1996; 2:321–5.

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this hypothesis is the recent discovery of Ebola nucleic acid sequences residing in DNA samples derived from at least three fruit bat species. This is indicative of prior contact, because Ebola viruses have the potential to leave behind bits of RNA in its hosts (see the section Paleovirology of *Mononegavirales*, earlier in this chapter). Furthermore, experimental studies have shown bats are capable of surviving Ebola infection.

Typically, a reservoir is identified when an outbreak of disease is observed in a particular wildlife population. Indeed, previous Ebola outbreaks were observed in tandem with outbreaks in other species. Scientists from the Robert Koch Institute and the Wild Chimpanzee Foundation recently monitored wildlife communities surrounding southeastern Guinea, where the outbreak likely originated in humans (FIGURE 2.15). While they have not seen any signs of decline in any local populations of bats or other species, such as chimpanzees and antelopes, they did find a suspicious clue in the village of Meliandou. Just 50 meters from where patient zero, a young boy, lived, a burnt out tree stands (FIGURE 2.16). Villagers told the Robert Koch researchers this tree once contained a population of insectivorous Angolan free-tailed bats (*Mops condylurus*), which local children would play with, as well as capture and barbeque (FIGURE 2.17).

Could the transmission of Ebola to the 2-year-old patient zero have taken place in this tree? Fruit bats are not found in Meliandou, suggesting other potential candidates for transmission are nonexistent. Additionally, out of 169 fruit bats captured in surrounding areas and tested for Ebola by the research team, none carried the virus. Lastly, the first individuals to contract Ebola in Meliandou were children and women, increasing the likelihood that hunting and contact with infected **bushmeat**, usually carried out by men of the village, was not how transmission occurred. All of these indicators combined pointed away from fruit bats. The researchers went on to consider the smaller, insect-eating *Mops condylurus* described by villagers. Previous studies have found Ebola antibodies in this species, which may be evidence of infection. FIGURE 2.18 shows a map of Gabon and DRC, two countries southeast of Guinea, where this discovery was made. Furthermore, sampling the remains of the tree revealed DNA fragments of the Angolan free-tailed bats, confirming villager

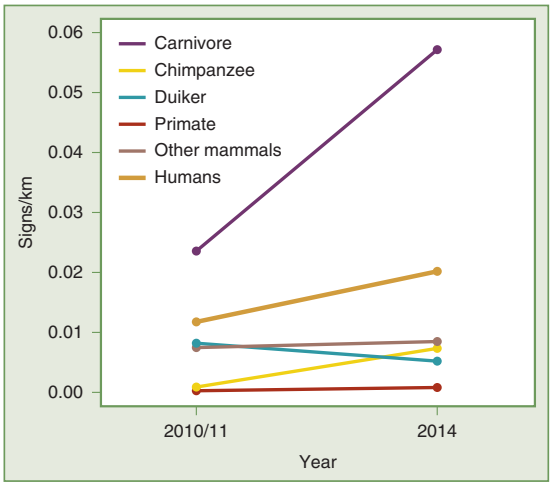

FIGURE 2.15 Densities of various species observed in southeast Guinea, comparing 2010–2011 with 2014.

Robert Koch Institute and the Wild Chimpanzee Foundation. http://embomolmed.embopress.org/content/early/2014/12/29/emmm.201404792.figures-only

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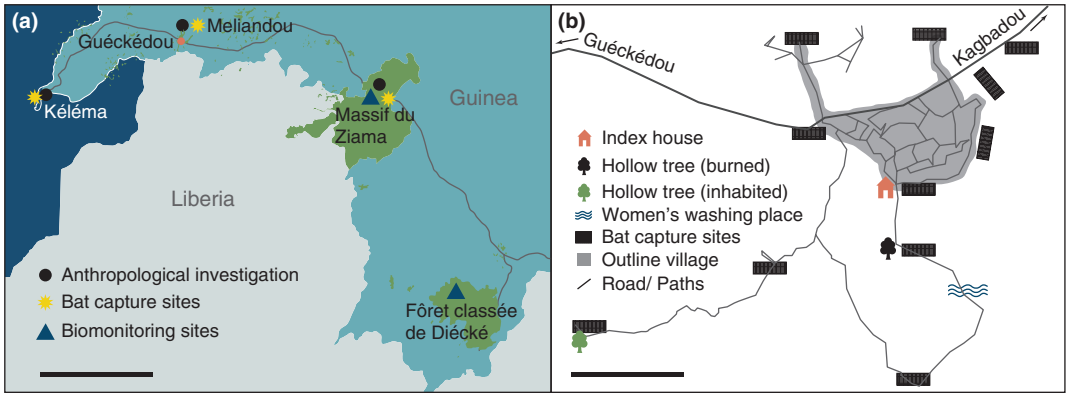

FIGURE 2.16 These maps show: (a) the location of Meliandou, Guinea, in relation to investigative sites within West Africa, and (b) the relative placement of patient zero's home ("index house") and the burnt out tree suspected of harboring Angolan free-tailed bats within the village of Meliandou.

Robert Koch Institute and the Wild Chimpanzee Foundation. http://embomolmed.embopress.org/content/early/2014/12/29/emmm.201404792.figures-only

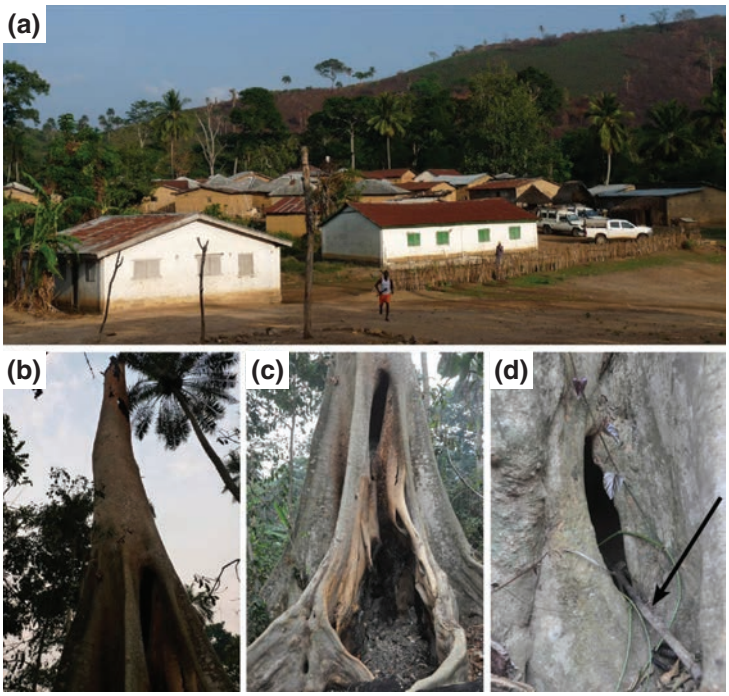

FIGURE 2.17 (a) The village of Meliandou, Guinea. (b–d) The burnt out tree that may have housed Angolan free-tailed bats. (d) A stick near the opening of the tree, which may have been left there by village children who once played close by.

Robert Koch Institute and the Wild Chimpanzee Foundation. http://embomolmed.embopress.org/content/early/2014/12/29/emmm.201404792.figures-only

accounts. The first Ebola patient in this epidemic may have played with an infected bat or accidentally ingested the droppings of an infected bat. However, this cannot be definitively proven without further investigation. It is important to note the insectivorous bat hypothesis is still just that.

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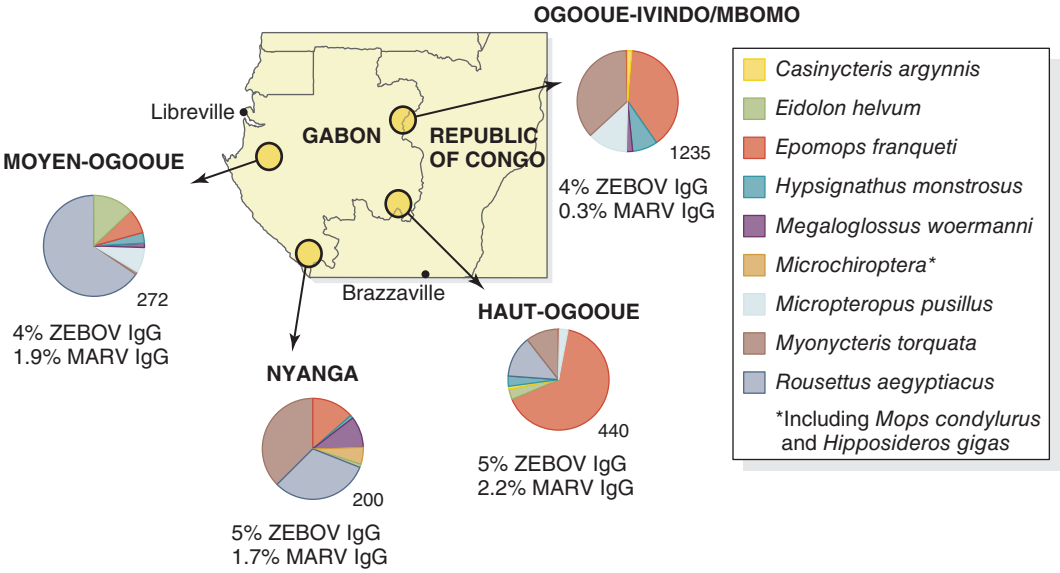

FIGURE 2.18 A map of Democratic Republic of Congo and Gabon, two countries southwest of Guinea, which shows a sampling of bat populations across the region. The pie charts indicate the relative proportions of filovirus antibodies observed in bat specimens, where MARV represents *Marburgvirus* and ZEBOV represents *Zaire ebolavirus*. *Mops condylurus* (Angolan free-tailed bat) is represented in grey within the *Microchiroptera* lineage.

Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in *Rousettus aegyptiacus*. Xavier Pourrut, Marc Souris, Jonathan S Towner, Pierre E Rollin, Stuart T Nichol, Jean-Paul Gonzalez and Eric Leroy, *BMC Infectious Diseases* 2009, 9:159 doi:10.1186/1471-2334-9-159. © 2009 Pourrut et al.; licensee BioMed Central Ltd.

Courtesy of Dr. Heidi Soeters/CDC

CRITICAL THINKING QUESTIONS

1. How might the infection mechanisms displayed by Ebola virions affect the progression of symptoms in viral hemorrhagic fever?
2. What is the relationship between the magnitude of the current widespread epidemic and the conditions present in affected countries, and how might these correlations offer suggestions for improvement in response efforts and/or prevention of future outbreaks?
3. How can understanding the evolution of order *Mononegavirales* and its member family *Filoviridae* aid in the development of treatments and/or vaccines? Or understanding Ebola genomics?

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