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CHAPTER

4

Minus-Strand RNA Viruses

INTRODUCTION

Seven families of viruses contain minus-strand RNA [(−)RNA], also called negative-strand RNA, as their genome. These are listed in Table 4.1. Included in the table are the names of the genera belonging to these families and the hosts infected by these viruses. Six of the families are known to contain members that cause epidemics of serious human illness. Diseases caused by these viruses include influenza (*Orthomyxoviridae*), mumps and measles (*Paramyxoviridae*), rabies (*Rhabdoviridae*), encephalitis (several members of the *Bunyaviridae*), upper and lower respiratory tract disease (numerous viruses in the *Paramyxoviridae*), and hemorrhagic fever (many viruses belonging to the *Bunyaviridae*, the *Arenaviridae*, and the *Filoviridae*), as well as other diseases. Bornavirus, the sole representative of the *Bornaviridae*, also infects humans and may cause neurological illness, but proof of causality is lacking. Many of the (−)RNA viruses presently infect virtually the entire human population at some point in time (e.g., respiratory syncytial virus, influenza virus), whereas others did so before the introduction of vaccines against them (e.g., measles virus and mumps virus). These viruses are thus responsible for a very large number of cases of human illness. The diseases caused by such widespread viruses are usually serious but have a low (although not insignificant) fatality rate. In contrast, some (−)RNA viruses, such as rabies and Ebola viruses, cause illnesses with high fatality rates but (fortunately) infect only a small fraction of the human population. The (−)RNA viruses are major causes of human suffering, and all seven families and the viruses that belong to these families will be described here.

OVERVIEW OF THE MINUS-STRAND RNA VIRUSES

Viruses belonging to four families of (−)RNA viruses, the *Paramyxoviridae*, the *Rhabdoviridae*, the *Filoviridae*, and the *Bornaviridae*, contain a nonsegmented RNA genome having similar organization. They are grouped into the order *Mononegavirales* (*mono* because the genome is in one piece, *nega* for negative-strand RNA). This was the first order to be recognized by the International Committee on Taxonomy of Viruses and still is one of only three orders currently recognized. Viruses belonging to the other three families, the *Arenaviridae*, *Bunyaviridae*, and *Orthomyxoviridae*, possess segmented genomes with two, three, and six to eight segments, respectively. Regardless of whether the genome is one RNA molecule or is segmented, the genomes of all (−)RNA viruses possess a similar suite of genes, as illustrated in Fig. 4.1. In the *Mononegavirales*, the order of genes along the genome is conserved among the viruses (although the number of genes may differ). In the viruses with segmented genomes, the genes can be ordered in the same way if the segments are aligned as shown. In addition, many features of virion structure and of replication pathways are shared among the (−)RNA viruses.

Structure of the Virions

All (−)RNA viruses are enveloped and have helical nucleocapsids. The different families encode either one or two glycoproteins (called G in most of the families but called HA, NA, F, or HN in some, after hemagglutinating, neuraminidase, or fusion properties). These glycoproteins are present in the viral envelope. In most cases, cleavages

TABLE 4.1 Negative-strand RNA Viruses

Family/genus	Genome size (in kb)	Type virus[a]	Host(s)[b]	Transmission
***Mononegavirales* (nonsegmented)**				
Rhabdoviridae	13–16			
Vesiculovirus		VSIV	Vertebrates	Some arthropod-borne
Lyssavirus		Rabies	Vertebrates	Contact with saliva
Ephemerovirus		BEFV	Cattle	Arthropod-borne
Novirhabdovirus		IHNV	Fish	
Two genera of plant viruses				Arthropod-borne
Filoviridae	13			
Marburgvirus		Marburg	Vertebrates	?
Ebolavirus		Zaire Ebola	Vertebrates	?
Paramyxoviridae	16–20			
Respirovirus		Sendai	Vertebrates	Airborne
Morbillivirus		Measles	Vertebrates	Airborne
Rubulavirus		Mumps	Vertebrates	Airborne
Henipavirus		Hendra	Vertebrates	Airborne
Avulavirus		Newcastle disease	Birds	Airborne
Pneumovirus		HRSV	Vertebrates	Airborne
Metapneumovirus		TRTV	Turkeys	Airborne
Bornaviridae				
Bornavirus	~9	BDV	Vertebrates	Contaminated forage
Segmented Negative Strand RNA Viruses				
Orthomyxoviridae	10–14.6			
Influenzavirus A	8 segments	Influenza A	Vertebrates	Airborne
Influenzavirus B		Influenza B	Vertebrates	Airborne
Influenzavirus C	7 segments	Influenza C	Vertebrates	Airborne
Thogotovirus	6 segments	Thogoto	Vertebrates	Arthropod-borne
Isavirus	8 segments	ISAV	Fish	Waterborne
Bunyaviridae	11–20 in 3 segments			
Orthobunyavirus		Bunyamwera	Vertebrates	Mosquito-borne
Hantavirus		Hantaan	Vertebrates	Feces–urine–saliva
Nairovirus		Dugbe	Vertebrates	Tickborne
Phlebovirus		Rift Valley fever	Vertebrates	Arthopod-borne
Tospovirus		TSWV	Plants	Thrips
Arenaviridae	10–14 in 2 segments			
Arenavirus		LCMV	Vertebrates	Urine–saliva

[a] Abbreviations of virus names: VSIV, vesicular stomatitis Indiana virus; BEFV, bovine ephemeral fever virus; IHNV, infectious hematopoietic necrosis virus; HRSV, human respiratory syncytial virus; TRTV, turkey rhinotracheitis virus; BDV, Borna disease virus; ISAV, infectious salmon anemia virus; TSWV, tomato spotted wilt virus; LCMV, lymphocytic choriomeningitis virus.
[b] In all cases, "Vertebrates" includes humans.

are required to produce the mature glycoproteins, such as cleavage to release a signal peptide, cleavage to separate two glycoproteins produced as a common precursor, or cleavage to activate viral infectivity. The glycoproteins project from the lipid bilayer as spikes that are visible in the electron microscope (see, e.g., Fig. 2.18D).

All (−)RNA viruses have a single major nucleocapsid protein (called N) that encapsidates the virion RNA to form the helical nucleocapsid. Also present in the nucleocapsid is a phosphorylated protein that is required for RNA synthesis, variously called P (for phosphoprotein) or NS (for nonstructural protein because it was not originally known to

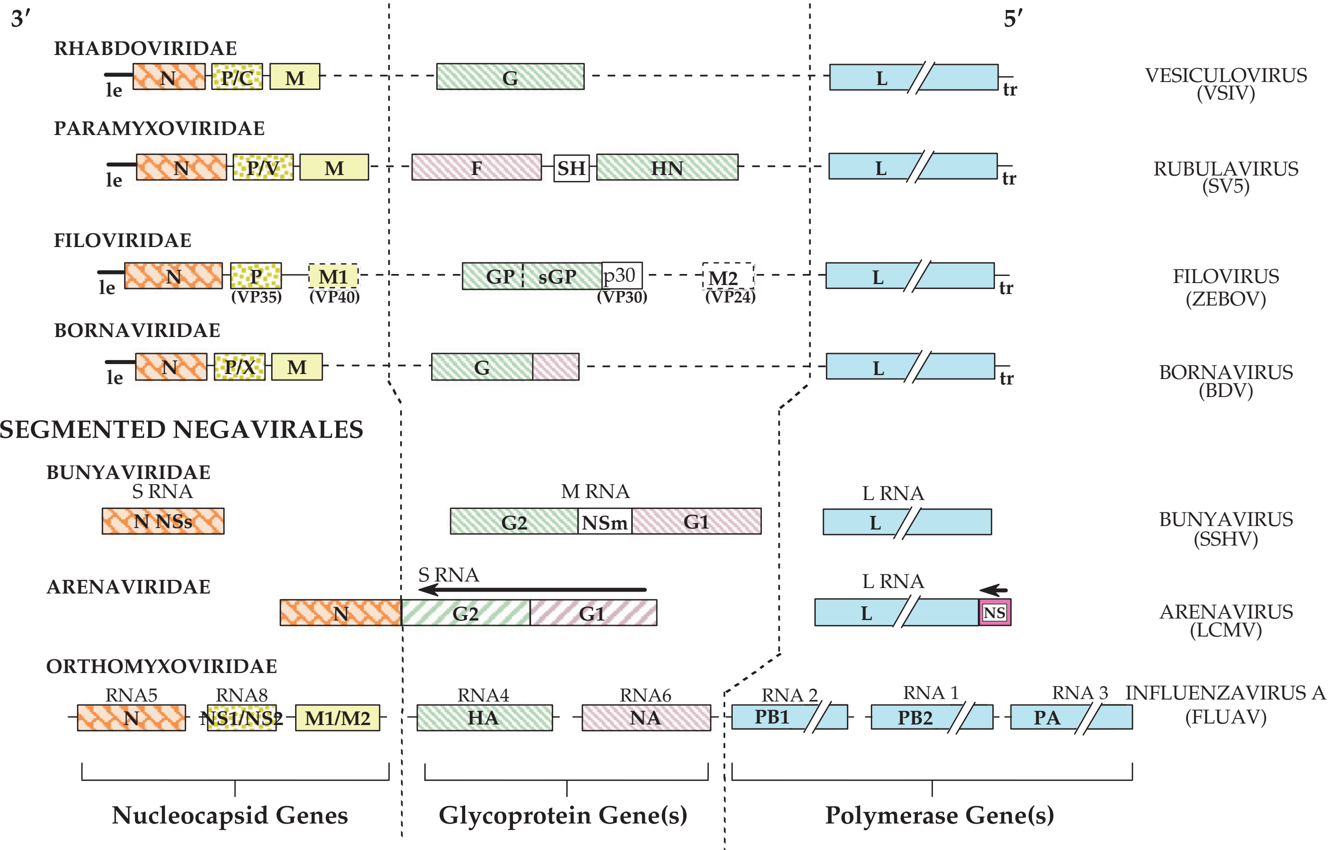


FIGURE 4.1 Genome organizations of the *Negavirales*. The genomes of representatives of the four families of *Mononegavirales* have been aligned to illustrate functional similarity between gene products. The individual gene segments of the representatives of the three families with segmented genomes, *Bunyaviridae*, *Arenaviridae*, and *Orthomyxoviridae*, have been aligned according to similarity of function with those of the *Mononegavirales* above. Gene expression strategies for the other genera of *Bunyaviridae* vary (see Fig. 4.21). Abbreviations of virus names are as follows: VSIV, vesicular stomatitis Indiana virus; SV5, simian virus 5; ZEBOV, Zaire ebolavirus; BDV, Borna disease virus; SSHV, snowshoe hare virus; LCMV, lymphocytic choriomeningitis virus; FLUAV, influenza A virus. The gene products are abbreviated as follows: le is a leader sequence; N is the nucleoprotein; P is the phosphoprotein; M (M1, M2) are matrix proteins; G (G1, G2) are membrane glycoproteins; F is the fusion glycoprotein; HN is the hemagglutinin-neuraminidase glycoprotein; L is the RNA polymerase; NA is the neuraminidase glycoprotein; HA is the hemagglutinin glycoprotein; NS (NV, SH, NSs, NSm) are nonstructural proteins; PB1, PB2, and PA are components of the influenza RNA polymerase; tr is the trailer sequence. Within a given genome, the genes are drawn approximately to scale. mRNAs for most genes would be synthesized left to right; however, an arrow over a gene means that it is in the opposite orientation (ambisense genes). Redrawn from Strauss *et al*. (1996), Figure 5.

be a component of the virion), as well as a few molecules of an RNA-dependent RNA polymerase. The polymerase is a large, multifunctional protein called L in most families but is present as three proteins in the *Orthomyxoviridae*. L and P form a core polymerase that replicates the viral genome and synthesizes mRNAs.

A matrix protein (M) is present in all of the viruses except the bunyaviruses and the arenaviruses. M underlies the lipid bilayer where it interacts with the nucleocapsid. M also inhibits host transcription and shuts down viral RNA synthesis prior to packaging.

The (−)RNA virions are heterogeneous to a greater or lesser extent. Members of five families often appear roughly spherical in the electron microscope. The example of influenza virus is shown in Figs. 2.1 and 2.22D, and the paramyxovirus measles virus is shown in Fig. 2.22C. The compositions of these virions are not rigorously fixed and some variability in the ratios of the different components, particularly in the glycoprotein content, is present. The rhabdoviruses are bullet shaped or bacilliform and appear more regular (Fig. 2.23), but even here variations in the composition of the glycoproteins in the envelope can occur. The filoviruses are filamentous (Fig. 2.23). Orthomyxoviruses and paramyxoviruses also produce filamentous forms as well as round virions (see Fig. 2.25E). In fact, clinical isolates of influenza viruses and human respiratory syncytial virus are predominantly filamentous.

Synthesis of mRNAs

For all (−)RNA viruses, the first event in infection is the synthesis of mRNAs from the minus-strand genome by the RNA polymerase present in the nucleocapsid. Because this polymerase is necessary for the production of the mRNAs, and because the proteins translated from the mRNAs are required for replication of the genome, the naked genomes of (−)RNA viruses are not infectious, nor are complementary RNA copies of the genomes. It has been possible, nonetheless, to rescue virus from cDNA clones of viral genomes by using special tricks, as described in Chapter 11.

Multiple mRNAs are produced from minus-strand genomes. By definition, each region of the genome from which an independent mRNA is synthesized is called a gene. In (−)RNA viruses with segmented genomes, it is obvious that multiple mRNAs are produced (the number of mRNAs produced actually exceeds the number of segments, as described later). In the *Mononegavirales*, multiple mRNAs arise from the use of a single polymerase entry site at the 3′ end of the genome. The polymerase then recognizes conserved start and stop signals at the beginning and end of each gene to generate discrete mRNAs. The amount of mRNA produced for any given gene is controlled by the location of the gene relative to the single polymerase entry site, because mRNA synthesis is obligatorily sequential and attenuation occurs at each gene junction. Thus, more mRNA for the proteins encoded 3′ in the genome is made and more protein is thus translated from these genes. The N protein, required for encapsidation of both genome and antigenome, is thereby produced in the largest quantities and the RNA polymerase, needed in the smallest quantities, is made in the smallest quantities. The synthesis of mRNAs is described in more detail in the sections on *Rhabdoviridae*.

Most of the mRNAs are translated into a single protein, but a few of the genes produce mRNAs that are translated into more than one product. Multiple products can be produced from the same gene by the use of alternative translation initiation codons during translation of an mRNA; by the introduction of nontemplated nucleotides during mRNA synthesis, which results in a shift in the reading frame; or by splicing of an mRNA. The P genes, in particular, of most of the (−)RNA viruses are translated into multiple products, and two of the segments of influenza virus, which replicates in the nucleus, can be spliced to produce a second mRNA encoding a different product. In no case are the mRNAs exact complements of virion RNAs. This is obvious in the case of the *Mononegavirales*, where as many as 7–10 mRNAs are produced from a single long genomic RNA, but is also true of the segmented (−)RNA viruses, where the mRNAs lack *cis*-active sequences required for encapsidation and replication that are present near the ends of the antigenome segments. Thus, the mRNAs of (−)RNA viruses do not replicate nor are they packaged into virions.

In contrast to the translation strategy used by the (+)RNA viruses, the (−)RNA viruses do not produce polyproteins that require processing by virally encoded enzymes, and virus-encoded proteases are unknown among them. However, most of the glycoproteins of the (−)RNA viruses are produced as precursors that are processed by cellular enzymes, and some of these precursors can be considered to be polyproteins.

Replication of the Genome

Replication of the (−)RNA genome requires the production of a complementary copy of the genome, called an antigenome or virus-complementary RNA (vcRNA), which is distinct from the mRNAs (schematically illustrated in Figs. 1.11C and D). Neither the genomic (−)RNA nor the antigenomic template produced during replication is ever free in the cytoplasm. Instead, replication of the genome, as well as the synthesis of mRNAs, takes place in nucleocapsids (sometimes referred to as ribonucleoprotein or RNP), which always contain the phosphoprotein and the polymerase as well as N and the viral RNA. Replication can only occur in the presence of ongoing protein synthesis to produce the new proteins required to encapsidate the genome or antigenome. The mRNAs can be synthesized in the absence of viral protein synthesis and lack encapsidation signals, so that they are released into the cytoplasm where they can associate with ribosomes and be translated. Thus, early after infection, mRNAs are synthesized. After translation of the mRNAs, which leads to production of sufficient amounts of viral proteins, a switch to the production of antigenomes for use as templates occurs, followed by production of genomic RNA from the antigenomic templates.

The genomes (or genome segments) of all (−)RNA viruses have sequences at the ends that are complementary (so-called inverted terminal repeats). In the bunyaviruses, the RNAs form panhandles, circular structures that are visible in the electron microscope. Panhandles have also been reported for influenza A virus. In other viruses, circles have not been seen but may form transiently during replication. It is possible that these complementary sequences exist to promote cyclization of the RNA, which may be required for replication of the genome or synthesis of mRNAs. It has been shown for influenza A virus that the viral RNA replicase interacts with both ends of the RNA during synthesis of RNA, similar to the story for alphaviruses and flaviviruses described in Chapter 3. Another possible explanation for the complementary sequences is that the promoter at the 3′ end of the genomic RNA that is recognized by the viral RNA synthetase for the production of antigenomes is the same, at least in part, as the promoter at the 3′ end of the antigenomic RNA that is used to initiate the production of genomic RNA.

In this event, the sequences at the two ends of the genome or antigenome that encompass these promoters would be complementary.

Host Range of the (−)RNA Viruses

All seven families contain members that infect higher vertebrates, including humans. For five of the families, only vertebrate hosts are known. The rhabdoviruses and bunyaviruses, however, have a broader host range. Some are arboviruses that replicate in an arthropod vector as well as in a vertebrate host, and others infect only insects. In addition, some genera of rhabdoviruses and bunyaviruses consist of plant viruses. Some of these are transmitted to the plants by insect vectors in which the viruses also replicate.

FAMILY *RHABDOVIRIDAE*

The genome organization of the rhabdoviruses is the simplest of the (−)RNA viruses and it is useful to begin our coverage with this group. The genome is a single piece of minus-strand RNA 11–15 kb in size. The genomes of all rhabdoviruses contain five core genes, called N, P, M, G, and L in that order in the genome reading 3′ to 5′, which result in the production of five to seven proteins, five of which are present in the virion. Some rhabdoviruses contain only these five genes, but others contain one to five extra genes inserted in various regions of the genome. The animal rhabdoviruses are bullet shaped, approximately 200 nm long and 75 nm in diameter (Fig. 2.23), whereas some of the plant viruses are bacilliform, being rounded at both ends. The rhabdoviruses infect mammals, birds, fish, insects, and plants, and are presently divided into six genera. A listing of these genera and a representative sample of the viruses in each genus, together with several characteristics of each virus, are shown in Table 4.2. Members of three genera infect mammals, namely, the vesiculoviruses (type virus: vesicular stomatitis Indiana virus), lyssaviruses (type virus: rabies virus), and ephemeroviruses (type virus: bovine ephemeral fever virus). The novirhabdoviruses infect fish, and the cytorhabdoviruses and nucleorhabdoviruses infect plants. Some or all of the members of four genera are transmitted by arthropods (Table 4.2). In addition, a large number of the more than 175 currently known rhabdoviruses have not been assigned to a genus. The animal rhabdoviruses replicate in the cytoplasm, but certain of the plant rhabdoviruses may replicate in the nucleus.

Genus *Vesiculovirus*

Vesicular stomatitis virus (VSV) has been extensively studied and serves as a model for the replication of (−)RNA viruses in general and rhabdoviruses in particular. Three serotypes have been recognized, Indiana (VSIV), New Jersey (VSNJV), and Alagoas. VSIV is the prototype virus of the genus and has a genome size of 11,161 nt. The genome is neither capped nor polyadenylated, consistent with the fact that it is minus-strand RNA.

Synthesis of mRNAs

The VSV nucleocapsid has about 1250 copies of N protein as its major structural component, leading to the conclusion that each N protein interacts with 9 nucleotides of RNA. The nucleocapsid also contains about 470 molecules of P and 50 copies of L. It can synthesize RNA, and P, L, and N are all required for this activity. The organization of the genome and the production of five mRNAs from it are illustrated in Fig. 4.2. There is a single polymerase entry site at the 3′ end of the genome, and production of mRNAs is obligatorily sequential. Synthesis begins at the exact 3′ end of the genome and a leader RNA of 48 nucleotides is first synthesized. The leader is released and synthesis of the first mRNA, that for N, is initiated. The RNA polymerase complex has capping activity, and the mRNA is capped during or shortly after initiation. At the end of the gene for N, the transcriptase reaches a conserved sequence AUACUUUUUUU, where it begins to stutter and produces a poly(A) tract at the 3′ end of the mRNA. The polymerase complex will not terminate or stutter unless the conserved AUAC is present immediately upstream of the U_7 tract, and the sequence $AUACU_7$ is therefore a consensus termination-polyadenylation signal. The capped and polyadenylated mRNA for N is terminated and released, the transcriptase skips the next two nucleotides, which are referred to as the intergenic sequence, and initiates synthesis of the second mRNA, that for P, at the conserved gene start signal UUGUC. Following synthesis of this mRNA, the polymerase again stutters at the oligo(U) tract in the $AUACU_7$ signal to produce a poly(A) tract, releases the capped and polyadenylated mRNA, skips the next two nucleotides, and begins synthesis of the third gene, that for M. The process continues in this way through the fourth gene (the G protein) and the fifth gene (the L protein, L for large because it comprises about 60% of the genome). In this way, five capped and polyadenylated mRNAs are produced. In VSV, the intergenic sequence is always two nucleotides. After releasing the L mRNA, the polymerase complex terminates synthesis some 50 nucleotides before the 5′ end of the genome is reached.

As described earlier, synthesis of the mRNAs proceeds in strict sequential order and the attenuation that occurs at each initiation step results in a gradient in the amounts of mRNAs produced. This attenuation appears to be important for regulation of the virus life cycle, so that the mRNAs for proteins needed in most abundance are produced in most abundance. Reorganization of the genome to change the order of genes gives rise to viable virus, but the yield of such virus during an infection cycle in cultured cells, and thus the fitness of the virus, is reduced.

TABLE 4.2 *Rhabdoviridae*

Genus/members[a]	Virus name abbreviation	Usual host(s)	Transmission/ vector?	Disease	World distribution
Vesiculovirus					
Vesicular stomatitis Indiana	VSIV	Humans, horses, ruminants, swine	Airborne, Insects?	Vesicles on tongue and lips	Americas
Chandipura Virus	CHPV	Mammals, including humans	Sandflies	Febrile illness	India, Asia?
Piry	PIRYV	Mice, humans	Sandflies	Febrile illness	Brazil
Lyssavirus					
Rabies	RABV	Humans, dogs, skunks, foxes, raccoons	Infectious saliva	Malaise, then delirium, then coma and death	Worldwide except some islands, and Australia
Bat lyssaviruses	ABLV,[b] EBLV, LBV	Bats, humans	Infectious saliva	Like rabies	Europe, Africa, Australia
Mokola	?	Humans, dogs, cats, shrews	?	Like rabies	Africa
Ephemerovirus					
Bovine ephemeral fever	BEFV	Cattle, water buffalo	Hematophagous arthropods	Fever, anorexia	Africa, Asia, Australia
Adelaide River	ARV	Cattle			
Berrimah	BRMV	Cattle			
Novirhabdovirus					
Infectious hematopoietic necrosis (and other fish viruses)	IHNV	Salmonid fish	Waterborne, contaminated eggs	Hemorrhage	Pacific Northwest of North America
Cytorhabdovirus					
Lettuce necrotic yellows		Plants	Aphids	?	? ?
Northern cereal mosaic		Plants	Leafhoppers		
Strawberry crinkle		Plants	Aphids		
Nucleorhabdovirus					
Potato yellow dwarf		Plants	Leafhoppers		
Maize mosaic		Plants	Leafhoppers		
Sonchus yellow net		Plants	Aphids		

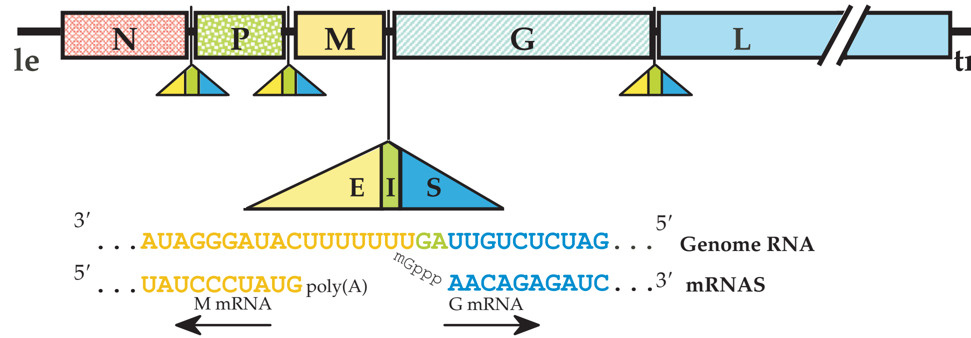
[a] Representative members of each genus are shown, and the first virus listed is the type species.
[b] Virus name abbreviations: ABLV, Australian bat lyssavirus; EBLV, European bat lyssaviruses -1 and -2; LBV, Lagos bat virus.

The mRNAs for N, M, G, and L are each translated into a single protein. That for P is translated into three proteins. The major translation product of this mRNA is P, which is produced using an initiation codon near the 5′ end of the mRNA. Initiation of translation also occurs at two downstream AUGs. These two downstream AUGs are in frame with one another but in a different reading frame from P. Use of these alternative AUGs leads to the synthesis of short proteins of 55 and 65 amino acids (of which the shorter protein is a truncated version of the longer one). The functions of these small proteins have not been established for VSV, but extra products translated from the P gene of the paramyxoviruses are known to interfere with host defense mechanisms, as described later.

Replication of the Genome

Synthesis of viral proteins, in particular of the N protein, allows the enzymatic activity present in the genomic nucleocapsid to switch from synthesis of messengers to replication of the genome. Replication requires producing a full-length antigenomic template, and the immediate encapsidation of the newly synthesized (+)RNA into plus-strand RNP containing N, P, and L during synthesis is required. In the absence of N for encapsidation, the system defaults to synthesis of mRNAs. The M protein also appears to regulate RNA synthesis. In the replication mode, the polymerase complex ignores all of the initiation, termination, and polyadenylation signals utilized to produce mRNAs, and instead produces a perfect

A Location of intergenic sequences of VSV (a rhabdovirus), and detailed view of the M/G intergenic region



B Genomic sequences at other intergenic regions in the VSV genome

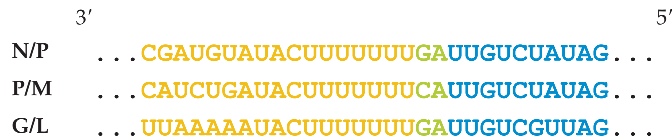


FIGURE 4.2 (A) Schematic diagram of the VSV genome. le is the leader sequence; tr is the trailer sequence. The 5 genes N, P, M, G, and L were defined in the legend to Fig. 4.1 and are described in more detail in the text. The positions of the conserved regulatory sequences at the gene boundaries are shown by the triangles. Each of these intergenic sequences is composed of E (end), I (intergenic), and S (start) domains. (B) Sequences in VSV at the other three gene boundaries. Data for this figure came from Rose and Schubert (1987).

complementary copy of the genome. The antigenomic RNA can be copied by the polymerase activity in the (+)RNP to produce more genomic RNA. This also requires that the RNA be immediately encapsidated. The new genomic RNP can be used to amplify the replication of viral RNA or, later in infection, can bud to produce progeny virions.

Maturation of Virus

The G protein has a 16-residue N-terminal signal sequence that leads to its insertion into the endoplasmic reticulum during translation. The signal is removed by cellular signalase. The resulting 495-residue protein is anchored near the C terminus by a 20-residue transmembrane anchor, with the 29 C-terminal residues forming a cytoplasmic domain (i.e., it is a type 1 integral membrane protein). G is glycosylated on two asparagine residues and transported to the plasma membrane, where progeny viruses are formed by budding (Fig. 2.23D). The M protein appears to form an adaptor between the glycoprotein present in the plasma membrane and the nucleocapsids assembled inside the cell. M also acts to repress RNA synthesis by the viral nucleocapsid. The G protein contains the fusion activity and receptor recognition activities of the virus, and it is the only protein present on the surface of the virion. The assembled virion contains about 1200 molecules of G, present as trimers that form spikes visible in the electron microscope, and about 1800 molecules of M.

Vesiculovirus Diseases

VSV causes nonfatal but economically important and debilitating disease in cattle, pigs, and horses. The name of the virus comes from the vesicles that it induces on the tongue and lips. These symptoms resemble those caused by foot-and-mouth disease virus and epidemics of VSV disease in domestic animals result in disruptive quarantines as well as complications in efforts for control of FMDV. Human infection is common in rural areas where VSV is endemic in domestic animals; 25–90% of farmers in such areas may have anti-VSV antibodies, showing past infection by the virus. Human infection is largely asymptomatic or associated with a mild febrile illness, sometimes accompanied by herpes-like lesions in the mouth or on the lips or nose. Serological surverys also show that the virus infects bats, deer, and monkeys in endemic areas. The virus also replicates in numerous arthropods and has been isolated from mosquitoes, sand flies, black flies, culicoides, houseflies, and eye gnats. The natural cycle of VSV in nature is not understood and the epidemiological importance of mosquitoes or other hematophagous arthropods in transmission of the virus is not clear.

VSV is endemic in Latin America from Mexico to northern South America where outbreaks of disease occur every year. VSNJV accounts for the majority of the clinical cases in this region. Sporadic outbreaks occur both north and south of this endemic area. In the United States, sporadic outbreaks occur in the Southwest at intervals of about 10 years, caused by both VSIV and VSNJV. In the Southeast, VSNJV was endemic until the 1970s. After this, VSNJV remained endemic only on Ossabaw Island off Georgia, where it is transmitted to feral pigs by sand flies. In the rest of the Southeast, no clinical disease caused by VSV has been reported since 1976 and there have been only occasional findings of seropositive wild animals.

Chandipura virus, another member of the genus *Vesiculovirus* (Table 4.2), is widespread in India, where it infects humans and domestic animals. It is also present in Senegal. It has been isolated from sand flies, which are believed to serve as vectors of the virus. Until recently it was thought to cause no disease or only mild febrile illness in humans. However, recent epidemics of encephalitis in children in India in 2003 and 2004 have been traced to the virus, showing that it has the potential to be a significant human pathogen. In the 2003 epidemic in Andhra Pradesh, for example, 183 of 329 affected children died.

More than 20 other vesiculoviruses are known. As one example, Isafahan virus has been isolated from sand flies in Iran. There is serological evidence of human infection in several central Asian countries but no definite evidence for human illness caused by it.

Genus *Lyssavirus*

The rhabdovirus of greatest medical interest is rabies virus, which belongs to the genus *Lyssavirus*. Seven genotypes or species of lyssavirus are currently recognized and two additional genotypes have been proposed. Genotype 1 is classical rabies virus and is virtually worldwide in distribution. It is the only lyssavirus found in the United States where it infects a wide range of hosts, notably raccoons, wolves, skunks, and bats. Mokola virus is an African virus that is known to infect dogs and cats as well as shrews and humans. The remaining five genotypes are bat-associated lyssaviruses. Lagos bat virus and Duvenhage virus are African, there are two European bat lyssaviruses called type 1 and type 2, and Australian bat lyssavirus is Australian as its name implies. Two additional genotypes of bat viruses present in central Asia have been proposed.

The genome of rabies closely resembles that of the VSV, although very little sequence identity exists between the genomes of the viruses belonging to the two genera. One difference is the lack of a second protein encoded in the P gene of lyssaviruses.

Rabies Virus

Most lyssaviruses cause the disease called rabies in humans and other mammals. It is a uniformly fatal disease of man and of other mammals, and has been known since the twenty-third century B.C. Rabies virus is present in the saliva of a rabid animal and is transmitted by its bite. Infection begins in tissues surrounding the site of the bite. Without treatment the virus may be transmitted to the brain, where replication of the virus leads to the disease called rabies. It is believed that the virus enters neurons by using acetylcholine receptors as a receptor, followed by transport up the axon until it reaches the cell body. The probability that rabies will develop following the bite of a rabid animal depends on the location of the bite, the species doing the biting, and the virus strain. In the absence of treatment, bites on the face and head result in rabies in 40–80% of cases, whereas bites on the legs result in rabies in 0–10% of cases. The incubation period to development of symptomatic rabies can vary from less than a week to several years. Once the virus reaches the brain, it spreads from there to a variety of organs. To be transmitted, it must spread to the salivary glands. Infection of neurons in the brain may result in behavioral changes that cause the animal to become belligerent and bite other animals, so that the virus present in salivary fluid is transmitted. In humans, the disease may be paralytic or may result in nonspecific neurological symptoms including anxiety, agitation, and delirium. Biting behavior is not a consequence of rabies-induced neurological disease in humans, and human-to-human transmission does not occur. Two to 7 days after symptoms of rabies appear, coma and death ensue. Only three cases of humans recovering from symptomatic rabies have been recorded.

For centuries, the saliva of a rabid dog was thought to be the source of rabies infection, but it was only in 1804 that Zinke succeeded in transmitting rabies from it. In the late 1800s, Pasteur adapted rabies virus to laboratory animals and developed the concept of protective vaccination against rabies. The dessicated spinal cords from rabies-infected rabbits became the first rabies vaccine. On July 6, 1885, this vaccine was used to immunize Joseph Miester, who had been bitten 14 times by a rabid dog. Because of the multiplicity of bites, he would almost surely have died, but the Pasteur vaccine saved him. A vaccine grown in nervous system tissue and inactivated by phenol rather than drying was the accepted rabies vaccine for decades. In the 1960s, a safer inactivated virus vaccine derived from virus grown in cultured human cells was introduced. The rabies vaccine is unique in that it is normally given after exposure to the virus, in conjunction with anti-rabies antiserum. This is possible because there is a window of time following the bite of a rabid animal before rabies develops, during which a protective immune response can be induced. Veterinarians and wildlife workers who are potentially exposed to rabid animals, as well as biologists who work with rabies virus in the

laboratory, are immunized prophylatically, but the protective immune response can be of short duration and immunity must be tested at regular intervals.

In the United States, Canada, and Western Europe, where vaccination of domestic dogs is widely practiced, wild animals such as raccoons and skunks maintain the virus and transmit it to humans or their domestic animals. Fig. 4.3 shows the decline in number of cases of rabies in dogs and humans in the United States since the 1940s, the result of compulsory vaccination of pet dogs. Fig. 4.3 also shows the increase in rabies in wild animals since 1940. Fig. 4.4 illustrates the explosive spread of rabies in raccoons on the eastern seaboard in the last 20 years. The focus of this spread in Virginia arose from the import of 3500 raccoons from Florida into the Washington, D.C., area by members of the cabinet of President Carter, for the purpose of raccoon hunting. These imported animals ignited an epidemic of rabies in raccoons that has slowly spread up and down the Atlantic seaboard, as shown in Fig. 4.4. In other parts of the United States, foxes and skunks are important hosts for rabies and disease also occurs at times in wolves and coyotes. Bats are also an important carrier of rabies. In other parts of the world, where licensing and immunization of pets is not required, domestic dogs continue to be the principal vectors that transmit rabies to humans. Rabies remains a significant global health problem. More than one million people annually undergo antirabies treatment following exposure to the virus, and 50,000 people die of rabies each year.

Efforts to control rabies in wildlife in the United States and Western Europe have met with some success. These efforts involve vaccinating wildlife with attenuated rabies virus or with recombinant vaccinia viruses that express the rabies G protein, using bait containing one of these viruses that is dispersed by hand or by airplane. In the eastern United States, spread of baits has been used to slow or prevent the further spread of rabies up the eastern seaboard. In Europe, baits have been used to set up barriers to halt the spread of rabies in foxes. The European efforts have been more successful than those in the United States.

The perpetuation of rabies in nature is somewhat of a mystery because of the fact that it can be maintained only in rabid animals who die quickly of the infection. How is it that the virus manages to persist? One possibility arises from recent findings that rabies virus can establish a latent infection in humans. Five cases have been documented in which people did not develop symptoms for 7 or more years after infection with the virus. In at least some of these cases, progression to rabies appeared to be triggered by hormonal changes during puberty. If the virus can establish a latent infection in other animals that is later followed by reemergence of the virus and its transmission to new susceptibles, this could serve as a reservoir of the virus.

Bats may also be an important reservoir of (classical) rabies virus (bat lyssaviruses are discussed separately later). Rabies virus infection of bats seems to take longer to kill the animal, during which time the virus may be transmissible through the bite of an infected bat or through aerosols from infected bat feces or saliva spray. However, rabies virus in bats is distinguishable from rabies virus strains in other wildlife by nucleotide sequence analysis. Thus, mixing of bat rabies and rabies in other wildlife is infrequent. Bats can transmit rabies to humans, and cases of human rabies transmitted by bats in the United States have been documented. In fact, in the United States in recent years, cases of human

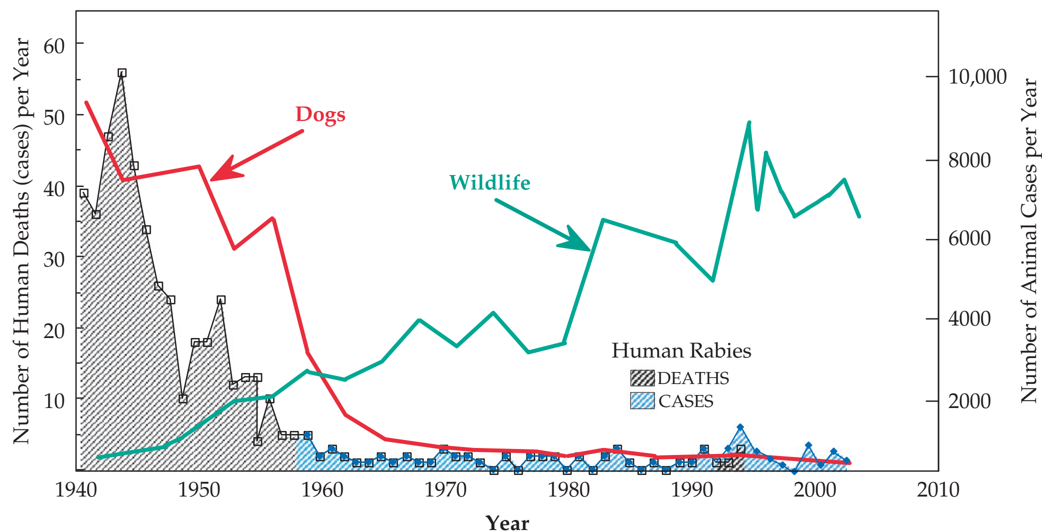


FIGURE 4.3 Rabies in domestic dogs and wild animals (right scale) versus human cases (left scale) in the United States 1940–2003. Note that untreated rabies in humans is uniformly fatal. Data from Smith *et al*. (1995), and *MMWR Summaries of Notifiable Diseases*, *1996* and *2003*.

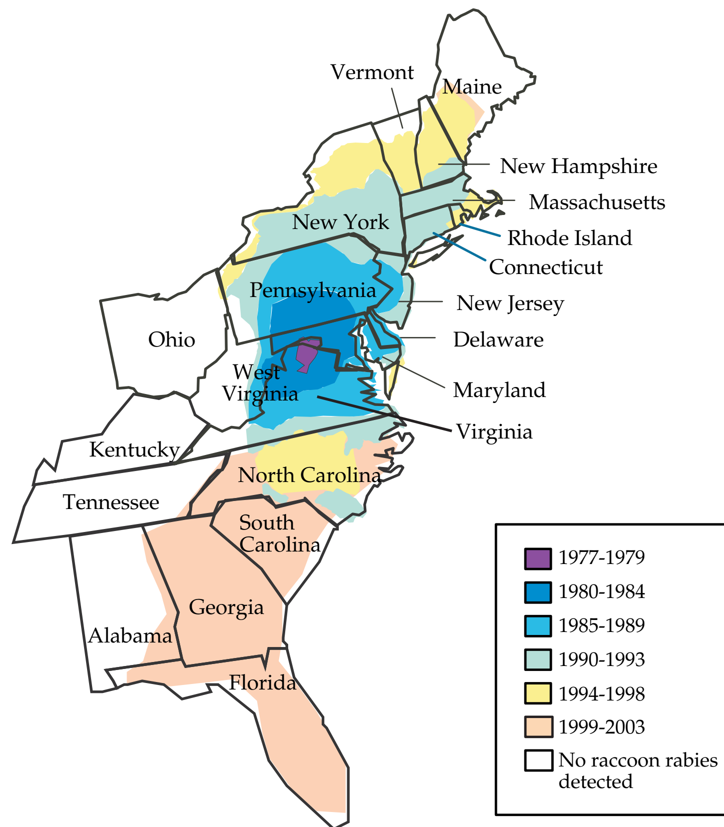


FIGURE 4.4 Spread of raccoon rabies over 3- to 5-year increments in the states of the Atlantic seaboard. Over 25 years the virus has spread from a small focal area in northern Virginia to encompass much of the entire region from southern Maine to Florida. From *Morbidity and Mortality Weekly Report* (*MMWR*) (1997) Vol. 45, p. 1119, updated with data from CDC found on www.rabavert.com/caserc.html.

rabies resulting from infection with bat-associated rabies virus have been more numerous than cases resulting from infection by bites of other rabid wildlife. In many cases of bat-associated rabies, the mechanism by which the virus was transmitted to the human is not known, because no exposure to bats, rabid or otherwise, could be shown.

Bat Lyssaviruses

Australia was long believed to be completely free of rabies. However, it has recently been found that many Australian bats carry a virus known as Australian bat lyssavirus (ABLV). Two cases of fatal human rabies that were caused by infection with this bat virus have occurred in the last few years. In one case, a woman caring for injured bats was bitten by a bat in her care. In the second incident, a woman was bitten while trying to remove a bat that had landed on a child. No rabies has been found in other animals, presumably because there is no efficient mechanism for transmission of the virus among other mammals present in the continent. However, the disease could potentially spread to dogs and cats that have been introduced into Australia over the years.

There are two major types of bats. Bats belonging to the suborder *Megachiroptera*, of which 40 genera are recognized, are large and feed on fruit and nectar in flowers. Members belonging to the genus *Pteropus* are often called flying foxes and are found from Australia across India to Madagascar. There are four species of flying foxes in Australia. Bats belonging to the suborder *Microchiroptera* are smaller, feed on insects, and have developed echolocation to find their prey in the dark. Both types of bats carry ABLV in Australia, and of the two cases of human infection that have resulted, one was from a flying fox and the second was from an insectivorous bat. The strains of virus in the two types of bats are distinguishable, differing by about 20% in nucleotide sequence.

Two different European bat lyssaviruses exist. Four human deaths resulting from infection by these viruses have occurred, and there is concern that more cases might occur. Mokola virus has also infected humans in Africa.

Other Genera of Rhabdoviruses

Bovine ephemeral fever virus, genus *Ephemerovirus*, is an arbovirus that causes economically important disease in

domestic cattle and water buffalo in tropical areas of the Old World. This virus, as well as other members of the genus, are not known to be human disease agents. The genome encodes five additional genes located between G and L. One of these encodes an additional glycoprotein whose significance is unknown. The other four genes encode five small proteins of unknown function.

The novirhabdoviruses infect salmon and other fish and are responsible for economic losses in fish farming operations. One additional gene is present in infectious hematopoietic necrosis virus, located between G and L. The nucleorhabdoviruses and cytorhabdoviruses are plant viruses that are transmitted by arthropods. They replicate in both the arthropod vectors as well as in plants and are the plant equivalent of arboviruses. Two (in maize fine streak virus, a nucleorhabdovirus) or four (in northern cereal mosaic virus, a cytorhabdovirus) additional genes are positioned between P and M. There are in addition many plant rhabdoviruses that have not been assigned to genus.

FAMILY *PARAMYXOVIRIDAE*

The family *Paramyxoviridae* has seven genera, listed in Table 4.3 together with representative viruses in each genus. The relationships among the genera are illustrated in the tree shown in Fig. 4.5. Each genus represents a distinct lineage. Furthermore, *Respirovirus*, *Morbillivirus*, *Henipavirus*, and *Rubulavirus* are more closely related to one another than to *Pneumovirus* and *Metapneumovirus*, and the family is divided into two subfamilies, *Paramyxovirinae* and *Pneumovirinae*. Many of the viruses belonging to this family are very important human pathogens. Some, such as measles virus and mumps virus, have been known for a long time—the infectious diseases caused by these viruses were known to the ancients. At the other extreme, Hendra virus, first described as an "equine morbillivirus of Australia," and the related Nipah virus of Southeast Asia have been known for less than a decade. These two new viruses have been classified into a new genus, *Henipavirus*.

Replication of the *Paramyxoviridae*

The genome organizations of five viruses representing five genera of the paramyxoviruses are shown in Fig. 4.6. The paramyxovirus genome is larger than that of the rhabdoviruses, 15–20 kb, and encodes more proteins, 8–11 or more. A core of six genes is present in paramyxoviruses, N, P, M, F, H, L (different names are used for some of the genes as shown in the figure). The P gene uses more than one reading frame to encode multiple proteins in most of these viruses. Rubulaviruses possess a seventh gene, encoding a protein called SH, and pneumoviruses possess an even larger constellation of genes, 10 in number. It is possible that still other genes are hidden within some of these large genomes. For example, the SH gene, encoding a very small protein, was discovered only recently.

The N or NP (= N in rhabdoviruses), P, M, and L proteins serve the same functions as their counterparts in rhabdoviruses. G of rhabdoviruses is replaced by two glycoproteins in paramyxoviruses, one called F and the other H or HN or G, depending on the virus. The order of genes in the paramyxoviruses is the same as in the rhabdoviruses, and the genome of the ancestral paramyxoviruses could have arisen from that of a rhabdovirus by insertion of extra genes (or vice versa by deletion of genes). Of interest is the fact that the rhabdovirus N protein binds nine nucleotides whereas the paramyxovirus N protein binds six nucleotides, and paramyxoviral RNAs, where studied, contain a number of nucleotides divisible by six.

Virus replication occurs in the cytoplasm. Like the rhabdoviruses, paramyxovirus mRNAs are transcribed sequentially beginning at the 3 end of the genome and the mechanisms to produce these mRNAs are similar to those employed by rhabdoviruses. A leader is first transcribed, poly(A) tracts are added by stuttering at oligo(U) stretches at the end of each gene, intergenic nucleotides are skipped by the polymerase during synthesis of mRNAs, and attenuation of mRNA synthesis occurs at each junction. The intergenic nucleotides are variable among paramyxoviruses, however. They are GAA or GGG for some viruses, but are variable in sequence and in length, from 1 to 60 nucleotides, for others. The mechanisms by which the virus switches from synthesis of mRNAs to replication of the genome are the same as those used by the rhabdoviruses.

The Viral Glycoproteins

Paramyxovirus virions are 150–350 nm in diameter and contain a helical nucleocapsid that is 8–12 nm in diameter. Virions are usually round but pleomorphic (Fig. 2.22C), and filamentous forms are also produced by some viruses that are more common in clinical isolates (Fig. 2.25E). Virions are produced by budding from the plasma membrane (Figs. 2.25E and F). The virion size differs even within a single species and the composition of the virion is not as well defined as for some enveloped viruses. There are two glycoproteins on the surface. One is a fusion protein that is required for the fusion of the viral membrane with the cell plasma membrane. Paramyxoviruses fuse with the plasma membrane, not with endosomal membranes, and fusion does not require exposure to low pH. The fusion protein is produced as a precursor called F_0. When first synthesized, F_0 has an N-terminal signal sequence that results in its insertion into the endoplasmic reticulum during translation. The signal sequence is removed by signal peptidase and the resulting type 1 integral membrane protein is anchored by a membrane-spanning region near the C terminus. F_0 is cleaved either by cellular furin (many paramyxoviruses)

TABLE 4.3 *Paramyxoviridae*

Genus/members[a]	Virus name abbreviation	Usual host(s)	Transmission	Disease	World distribution
Paramyxovirinae					
Respirovirus					
Human parainfluenza 1,3	HPIV-1,3	Humans	Airborne	Respiratory disease	Worldwide
Bovine parainfluenza 3	BPIV-3	Cattle, sheep	Airborne	Respiratory disease	Worldwide
Sendai	SeV	Mice	Airborne	Respiratory disease	Worldwide
Rubulavirus					
Mumps	MuV	Humans	Airborne	Parotitis, orchitis, meningitis	Worldwide
Human parainfluenza 2, 4a,4b	HPIV-2,4	Humans	Airborne	Respiratory disease	Worldwide
Simian virus 5	SV-5	Monkeys, canines	Airborne	Respiratory disease	Worldwide
Menangle	?	Bats/swine	???	Reproductive abnormalities	Australia
Morbillivirus					
Measles	MeV	Humans, monkeys	Airborne	Fever, rash, SSPE[b], immune suppression	Worldwide
Rinderpest	RPV	Cattle, swine	Airborne	Gastroenteritis	Worldwide
Distemper	CDV, PDV	Dogs, marine mammals	Airborne	Immune suppression, gastroenteritis, CNS disease	Worldwide
Henipavirus					
Hendra (equine morbillivirus)	HeV	Humans, equines, *Pteropus* fruit bats	Body fluids?	Respiratory disease, encephalitis	Australia
Nipah	NiV	Humans, swine, cats, dogs	Body fluids?	Respiratory disease, encephalitis	Malaysia, Singapore
Avulavirus					
Newcastle disease, avian paramyxoviruses 2–9	NDV	Gallinaceous birds	Airborne	Respiratory distress, diarrhea	Worldwide
Pneumovirinae					
Pneumovirus					
Human respiratory syncytial	HRSV	Humans	Airborne	Respiratory disease	Worldwide
Bovine respiratory syncytial	BRSV	Cattle	Airborne	Respiratory disease	Worldwide
Pneumonia virus of mice	PVM	Mice	Airborne	Respiratory disease	Worldwide
Metapneumovirus					
Turkey rhinotracheitis	TRTV	Turkeys	Airborne	Respiratory disease	Worldwide

[a] Representative members of each genus are shown, and the first virus listed is the type species.
[b] Abbreviations: SSPE, subacute sclerosing panencephalitis; CNS, central nervous system; CDV, canine distemper virus; PDV, phocine distemper virus.

or cathepsin L (henipaviruses) within the cell or by other cellular enzymes after release of the virion from the cell, depending on its sequence. Cleavage is required for the virus to be infectious and the cleavage products, F_1 (the N-terminal part of the precursor) and F_2 (the C-terminal part which is anchored in the membrane), remain covalently linked through a disulfide bond. The fusion domain consists of the N-terminal 20 amino acids of F_2, but this domain is not fusogenic until cleavage of F_0 has occurred. Those strains whose F_0 can be cleaved intracellularly by furin (which recognizes the sequence RXRR or RXKR) or cathepsin L are able to spread systemically and in general cause serious disease. In contrast, viruses that require cleavage of F_0 by proteases after the release of (noninfectious) virions from the cell, usually at a single basic residue by trypsin-like enzymes such as Clara or miniplasmin that are limited in their distribution in the animal, cannot spread systemically and are usually restricted to the respiratory tract. F oligomerizes to form trimers that are visible as spikes on the surface of the virion.

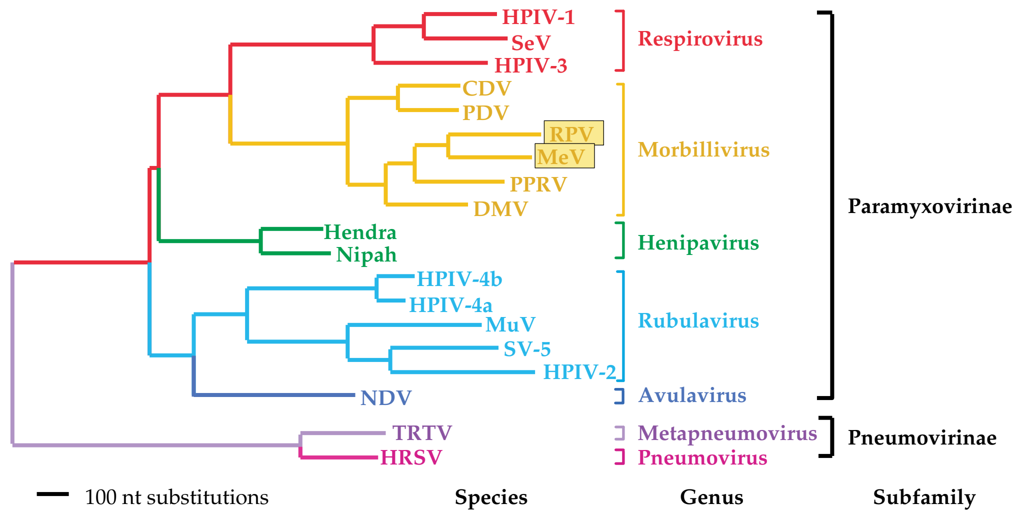


FIGURE 4.5 Phylogenetic tree of the *Paramyxoviridae* derived from the nucleotide sequences of the N ORF. Most of the virus abbreviations are found in Table 4.3. CDV, canine distemper; PDV, phocine distemper; PRRV, peste-des-petits-ruminants; DMV, dolphin morbillivirus. Notice that the closest relative of measles (MeV, boxed), a human virus, is rinderpest (RPV, boxed), a virus of cattle and pigs. Adapted from Chua *et al*. (2000).

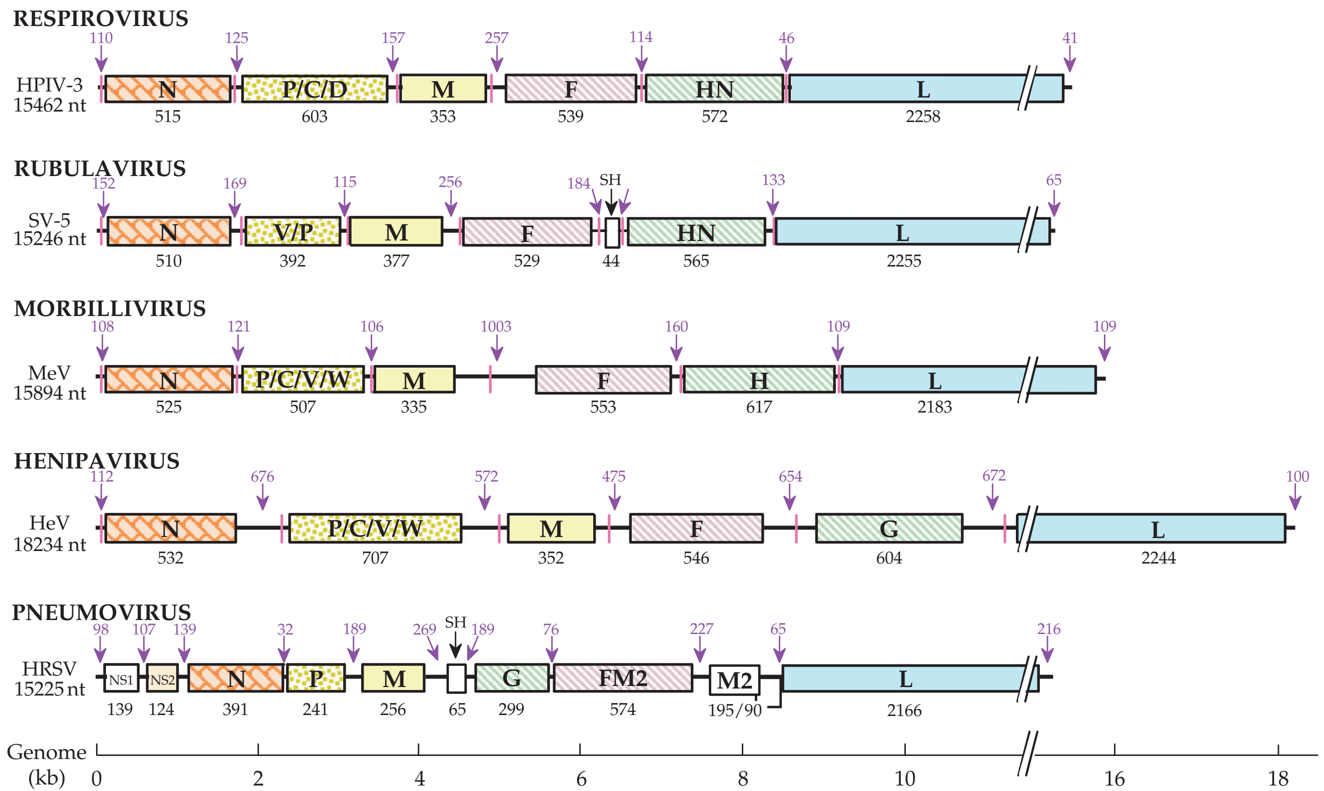


FIGURE 4.6 Genome organizations of the five genera of the *Paramyxoviridae* that infect mammals. The genome is shown 3′ to 5′ for the minus-strand RNA. For the top four genera, each gene begins with the vertical pink bar marking the intergenic sequence. The untranscribed intergenic sequences of respiroviruses, morbilliviruses, and henipaviruses are 3 nt in length. Those of rubulaviruses and pneumoviruses vary in length from 1 to more than 60 nucleotides. The boxes are the ORFs encoding the nucleocapsid (N), the P (V, C, W, D) complex, the matrix protein (M), the fusion protein (F), the glycoprotein (G, HN, or H), and the polymerase (L). Numbers above the arrows are the total number of nucleotides between the ORFs; numbers below the boxes are the number of amino acids in the protein. HPIV-3, human parainfluenza virus 3; SV5, simian virus 5; MeV, measles virus; HeV, Hendra virus; and HRSV, human respiratory syncytial virus.

The second glycoprotein is called the hemagglutinin-neuraminidase (HN), the hemagglutinin (H), or simply G (for glycoprotein), depending on the virus. This protein is a type 2 integral membrane protein. The signal sequence at the N terminus is not removed but instead serves as the transmembrane anchor for the protein, so that it has its N terminus inside and its C terminus outside. This protein contains the receptor-binding activity of the virus.

Many paramyxoviruses belonging to the genera *Respirovirus* and *Rubulavirus* use sialic acid (*N*-acetylneuraminic acid) bound to protein or lipids as a receptor. Because this receptor is also present on red blood cells, these viruses can cause red blood cells to clump or agglutinate, a process called hemagglutination (*heme* = the red compound in red blood cells that binds oxygen). In paramyxoviruses that use sialic acid as a receptor, this second glycoprotein is also a neuraminidase, in which case it is called HN. Neuraminidase removes sialic acid from potential receptors and from virus glycoproteins. By removing sialic acid from the virus glycoproteins and from the cell surface, released virus is prevented from aggregating with itself or sticking to infected cells. It also increases the probability that the virus will successfully initiate infection of a suitable animal. Mucus, which lines the respiratory tract where the viruses begins infection, contains sialic acid and might otherwise bind virus, preventing its entry into cells, if the virus could not release from mucous by destroying these receptors.

Other receptors used by paramyxoviruses include, among others, CD46 (measles virus), Ephrin B2 (henipaviruses), and glycosaminoglycans (respiratory syncytial virus). If the receptor used by the virus is found on red blood cells, the viruses will hemagglutinate, but if the receptor is not sialic acid, the virus will not contain a neuraminidase. In this case, the second glycoprotein is called H. If the viruses are not known to hemagglutinate, the second glycoprotein is simply called G, for glycoprotein. In any event, this second glycoprotein of paramyxoviruses, best studied in the case of the HN of some paramyxoviruses, oligomerizes to form tetrameric spikes on the surface of the virion.

Some paramyxoviruses belonging to the genera *Rubulavirus* and *Pneumovirus* encode a third integral membrane protein. This small (44–64 residues) protein is called SH or 1A and is glycosylated in the pneumovirus respiratory syncytial virus but not in the rubulaviruses SV5 and mumps. In the rubulaviruses the protein is a type 1 integral membrane protein whose gene is positioned between F and H (or HN). In SV5 and, probably, in mumps virus also, the protein interferes with the TNF-α mediated apoptosis pathway. Mutants lacking this protein will replicate in cell culture but cause extensive apoptosis, and are attenuated in animals.

The P Gene

Expression of the P gene of paramyxoviruses belonging to the subfamily *Paramyxovirinae*, sometimes called the P/V or P/C/V gene, is remarkable, as illustrated in Fig. 4.7. The P gene, or its equivalent, of most (−)RNA viruses is used to make more than one protein, as was described earlier for the vesiculoviruses and as will be described later for other viruses, but the translation strategies used by some paramyxoviruses result in maximal use of the potential information contained within this gene. In some paramyxoviruses, alternative AUG start codons are used to produce two different proteins translated from different reading frames, similar to what occurs in the vesiculoviruses. A second strategy used by paramyxoviruses is to add nontemplated nucleotides to the mRNA during synthesis in order to shift the reading frame downstream of the added nucleotides. The ultimate use of the paramyxovirus P gene occurs in some viruses in which all three reading frames are translated by using one or both of these strategies to produce four or more proteins.

In respiroviruses, morbilliviruses, and henipaviruses, translation of P mRNA can start at one of two different AUGs that are in different reading frames. One of the two proteins produced is called C and the other P (Fig. 4.7). In addition, during transcription of P mRNA in most members of the *Paramyxovirinae*, nontemplated G residues are added at a specific site in the gene. In measles or Sendai viruses, addition of one G shifts the reading frame after this point to produce a new protein called V, which is rich in cysteine residues. Thus P and V share their N-terminal sequence but diverge after the site where the extra G is added. In the case of parainfluenza virus 3, addition of two G's leads to mRNA translated into a protein called D. Another respirovirus, HPIV-1, lacks editing in the P gene (Fig. 4.7). In the rubulaviruses, the V protein is translated from the unmodified transcript, and production of mRNA for P requires addition of two nontemplated G residues. In mumps virus, addition of 4 G residues also occurs to produce a third protein.

During translation of these P mRNAs, the situation becomes even more complicated. In some viruses, multiple in-frame start codons are used to initiate translation of C. Thus, different forms of C are produced that are variously truncated at their N terminus. For example, Sendai virus expresses four C proteins, called C′, C, Y1, and Y2, of which C′ starts at an ACG codon. The many different protein products produced from the P gene have not been fully characterized, and perhaps not yet fully enumerated, and the various functions of this wealth of proteins are only partially unraveled.

Addition of the nontemplated G residues is thought to involve a mechanism similar to the stuttering that produces a poly(A) tract opposite a string of U's in the template. The nontemplated G's are always added at a specific, unique place in the genome characterized by a string of C's. There must be some signal within the genome that is recognized by the viral polymerase for the addition of the extra G's, similar to the case for the addition of the poly(A) tract at the end of mRNAs.

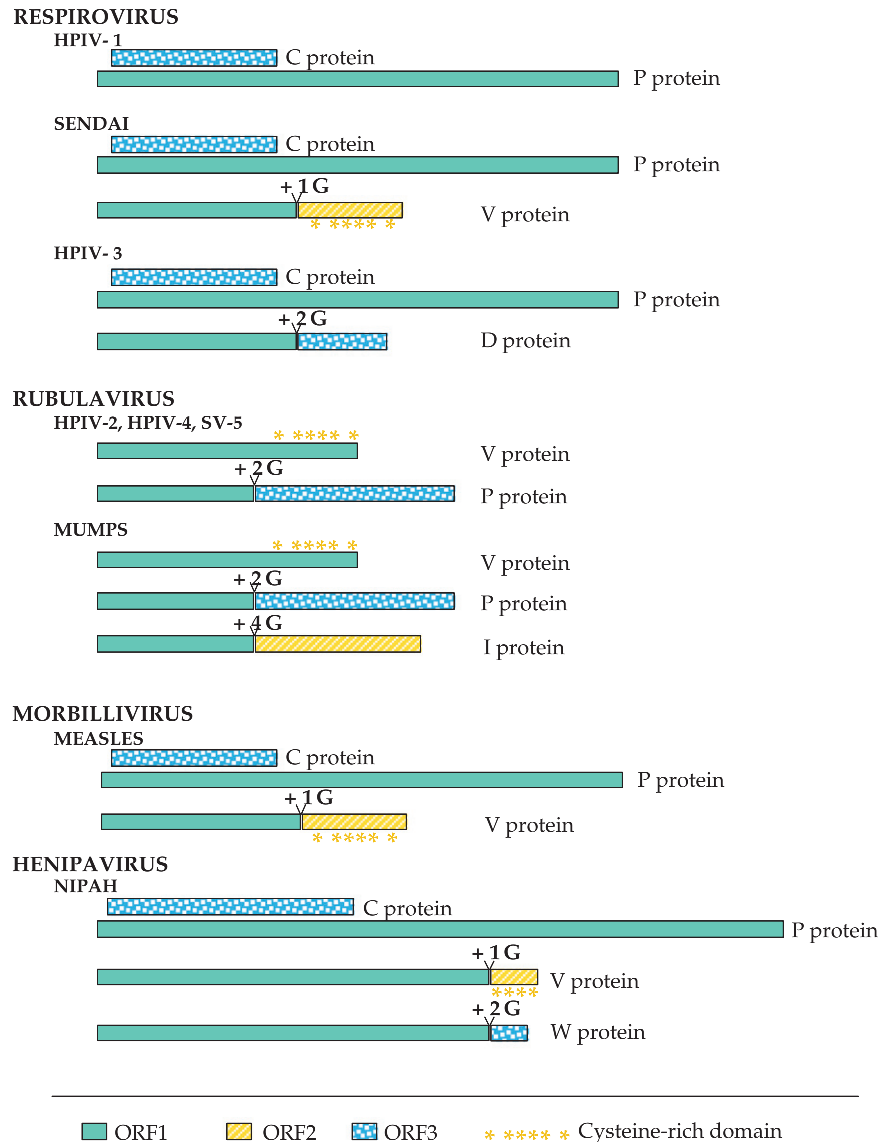


FIGURE 4.7 Translation strategy of the P gene of paramyxoviruses. In most paramyxoviruses, nontemplated nucleotides are inserted during transcription of P to shift the translation frame. Alternative translation start codons are also used. The result is the production of up to four proteins from this one gene. Adapted from Strauss and Strauss (1991) and Chua *et al*. (2000).

An important function of the V and C proteins, perhaps the primary function, is to block the action of the hosts interferon (IFN) system. Such an activity is called a luxury function because it is not needed for virus replication in cultured cells but is needed for a successful infection of an animal. Production of IFN is the first line of defense of birds and mammals against virus infection and the system is described in detail in Chapter 10. The importance of IFN in controlling viral infection is shown by the fact that most if not all viruses interfere with its action in some way. In paramyxoviruses, the V and C proteins act in different ways to block IFN production or its activity once induced. A potent inducer of IFN production is double-strand RNA (dsRNA), and there are at least two cellular sensors that detect dsRNA and induce the production of IFN (described in more detail in Chapter 10). Intracellular dsRNA can be sensed by a helicase called RIG-1. Through a complicated pathway involving caspase-recruitment domains (CARD), two transcription factors, IRF-3 and NFκB, are activated and transported to the nucleus. These form a complex that leads to the transcription of the mRNA for IFN-β. An overlapping pathway can start from extracellular dsRNA, which is bound by a cellular receptor called Toll-like receptor 3. The resulting signal cascade results in the production of the same two activated

transcription factors. The V proteins of several paramyxoviruses interfere with either activation pathway, preventing the induction of IFN. In addition, the W protein of Nipah virus blocks the activity of IRF-3 in the nucleus. The cysteine-rich C-terminal domains of the V proteins are highly conserved and presumably work in the same way in this pathway.

The V proteins, and in some cases the P proteins, also block the activity of IFN-β once it is produced. IFN-β is exported from the cell where it can be bound by Type I IFN receptors at the surface of the same cell or other cells. Once IFN-β is bound, the receptor, which consists of two different subunits, heterodimerizes and associated adaptor proteins phosphorylate one another and phosphorylate transcription factors called STAT1 and STAT2. Once phosphorylated, the STATs heterodimerize and are transported to the nucleus where they form part of a transcription complex that transcribes mRNA from hundreds of IFN-responsive genes and the products of these genes establish an antiviral state. The V protein and P protein of Nipah virus cause the STATs to aggregate into large, inactive complexes. The V proteins of several other paramyxoviruses cause STAT1 or STAT2 to be degraded by proteasomes. The W proteins of Nipah virus also blocks the activity of the STATs in the nucleus by causing the proteins to aggregate. The net result of these activities is that the genes responsive to activation by IFN-β are not transcribed and IFN activity is aborted (see also Chapter 10).

The V proteins are also involved in the regulation of RNA synthesis after infection. Where studied, they downregulate the production of viral RNA.

Genus *Respirovirus*

The genus *Respirovirus* contains several parainfluenza viruses (abbreviated PIVs) and Sendai virus (from Sendai, Japan, where it was isolated; also called mouse PIV-1) (Table 4.3). The two human respiroviruses, HPIV-1 and HPIV-3, cause a respiratory illness similar to that caused by influenza virus and utilize sialic acid as a receptor, as does influenza. They were once grouped with influenza virus as myxoviruses (*myxo* from mucus because the viruses attach to mucus, which contains sialic acid). When they were separated from influenza virus into a distinct family, they were called parainfluenza viruses and the family was named *Paramyxoviridae*.

The respiratory tract infections caused by HPIV-1 and HPIV-3 may be limited to the upper respiratory tract, causing colds, or may also involve the lower respiratory tract, causing bronchopneumonia, bronchiolitis, or bronchitis. These viruses are widespread around the world and are an important cause of lower respiratory tract disease in young children. Serological studies have shown that most children are infected by HPIV-3 by 2–4 years of age, and that the incidence of infection can be as high as 67 out of 100 children per year during the first 2 years of life (i.e., reinfections are common). Thus, immunity is incomplete and the viruses continue to reinfect older children and adults. However, subsequent infections are normally less severe and there is a reduction in the incidence of lower respiratory tract disease (which is more serious than infection of the upper respiratory tract). The viruses, as is common for respiratory tract infections, are spread by respiratory droplets.

Attempts to develop vaccines against the HPIVs have not met with success. Because of incomplete immunity produced by natural infections, the primary purpose of a vaccine would be to decrease the severity of natural infection by the virus. Even so, results to date have been disappointing. Inactivated virus vaccines developed for HPIV-1 and -3, as well as for HPIV-2, a rubulavirus, were antigenic but failed to induce resistance to the viruses. This could have resulted from failure to develop IgA following a parenterally administered vaccine (Chapter 10), and attempts to develop effective vaccines are continuing.

Genus *Rubulavirus*

Mumps Virus

The genus *Rubulavirus* gets its name from an old name for mumps, which is the disease produced in humans by mumps virus. The only natural hosts for mumps virus are humans and the virus is transmitted from person to person by contact. The disease has been known (at least) from the fifth century B.C. The incubation period, that is, the period of time between infection by the virus and the development of symptoms, is about 18 days. During the last 7 days of the incubation period, a person sheds virus and is capable of infecting others. Infection of children is usually not serious, but mumps virus infection can cause serious illness, particularly in adults. Infection begins in the upper respiratory tract but becomes systemic with the virus infecting many organs, where it replicates in epithelial cells. It is best known for infection of the parotid salivary glands leading to painful swelling of these glands. More serious disease can result from the replication of the virus in other organs, however. The central nervous system (CNS) is a common target for the virus and 0.5–2.3% cases of mumps encephalitis are fatal. Infection of the pancreas can occur, and it has been suggested that mumps may be associated with sudden onset insulin-dependent diabetes. The heart is sometimes infected, resulting in myocarditis. Infection of the testes in adult males can lead to orchitis and, in rare cases, to sterility. Infection of the fetus can result in spontaneous abortion.

At one time, mumps was one of the common childhood diseases that was contracted by almost everyone. It is now controlled in developed countries by an effective attenuated virus vaccine that was selected by passage of the virus in embryonated eggs. This mumps vaccine is given as part of the MMR (measles–mumps–rubella) combination vaccine.

The dramatic decline in cases of mumps in the United States after introduction of this vaccine is shown in Fig. 4.8. Because mumps is exclusively a human virus that induces effective immunity following infection, and infection of an individual requires direct contact with a person actively shedding the virus, the virus requires a population of at least 200,000 people to sustain it. Such a population density was first attained 4000 or 5000 years ago, before which mumps could not have existed, at least in its current form.

Other Rubulaviruses

Other human rubulaviruses include HPIV-2 and HPIV-4. They are named human parainfluenza viruses because the disease they cause is similar to that caused by HPIV-1 and HPIV-3. However, they are genetically related to the rubulaviruses rather than to the respiroviruses (Fig. 4.5). Other members of the rubulavirus genus infect many mammals and birds. One of the most intensively studied rubulaviruses (studied as a model system for replication of members of the family) has been SV-5 (simian virus 5). During the development of the polio vaccine, rhesus monkey kidney cells were used for replication of poliovirus in culture, and these cultures were often contaminated with monkey viruses. These simian viruses (SVs) were simply numbered as they were isolated, and any particular SV may be totally unrelated to any other. Two of the most widely studied are SV-5 and SV-40, which are not related to one another: SV-5 is an RNA-containing paramyxovirus and SV-40 is a DNA-containing polyomavirus (Chapter 7).

The avian viruses include nine serologically distinct paramyxoviruses. These viruses form a distinct lineage, but clearly group with the rubulaviruses (Fig. 4.5). APMV-1 is also known as Newcastle disease virus (NDV), which causes a highly contagious and fatal disease of birds. NDV has serious economic consequences because it infects chickens, among other avian hosts. When epidemics break out, and they do with some regularity, many birds die, causing economic losses. Quarantines are placed on the movement of birds during epidemics in an effort to curtail the spread of the virus, which has further economic consequences.

Menangle virus is a newly described virus that emerged in Australia in 1997 as the cause of severe reproductive disease in pigs. Menangle virus is a bat virus that was present in flying foxes that formed a large colony near the pig farm where the virus emerged. How the virus was transmitted to pigs is not known with certainty. Two farm workers appeared to have been infected during the outbreak and suffered influenza-like symptoms. Menangle is an example of emerging viral diseases, a topic covered in more detail in Chapter 8.

Genus *Morbillivirus*

The genus *Morbillivirus* contains measles virus as well as a number of nonhuman pathogens that include rinderpest virus, which infects cattle and pigs, and distemper viruses of dogs, dolphins, and porpoises. The relationships among these viruses were illustrated in Fig. 4.5.

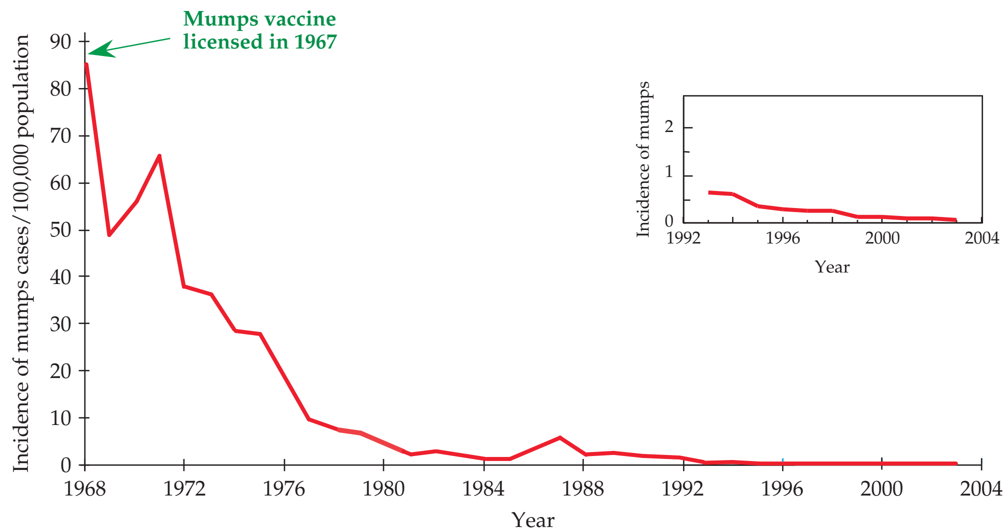


FIGURE 4.8 Incidence of mumps (cases per 100,000 population) in the United States. The minor resurgence of mumps cases in the late 1980s is thought to be due to a pool of susceptible teenagers and young adults who were not aggressively immunized during the first decade after the introduction of the vaccine. In 2003 there were a total of 231 cases of mumps in the United States, the lowest number ever reported for one year (< 0.08 cases per 100,000). The last deaths in the United States from mumps occurred in 2000. However, in 2006 there was a major outbreak of mumps in the Midwest, resulting in 5783 cases. From *MMWR, Summary of Notifiable Diseases-1996*, Vol. 45, p. 45; the comparable Summaries for 2001 and 2003; and *MMWR* (2006) Vol. 55, p. 1152.

Measles

Measles virus causes serious illness in man. Infection begins in the upper respiratory tract but becomes systemic, and many organs become infected. Lymphoid organs and tissues are prominent sites of viral replication, and one consequence of virus infection is immune suppression that lasts for some weeks, apparently due to suppression of T-cell responses. Immune suppression can result in secondary infections that may be serious, even life threatening, and interference with immune function is a major cause of measles mortality. Measles also has uncommon neurological complications, including encephalomyelitis and subacute sclerosing panencephalitis (SSPE). In SSPE, the virus sets up a persistent but modified infection in the brain in which M protein is produced in only low amounts; downregulation of production of M protein appears to be necessary to establish the disease syndrome. Symptoms of SSPE appear several years after measles infection, and the disease progresses slowly but inexorably. Serious complications caused by viral infection of other organs can also occur.

Natural History of Measles Virus

Like mumps, measles is a disease of civilization. The virus is a human virus. Although subhuman primates can be infected by the virus and suffer the same disease as humans, humans are the only reservoir of the virus in nature. Infection requires direct contact with an infected person and recovery from infection results in solid lifelong immunity to the virus. Thus, a minimum size population is required to maintain the virus, in which the continuing birth of new susceptibles occurs at a rate sufficient to maintain continuous virus infection within the community. The requirement for a minimum sized human population to sustain the virus is illustrated in Fig. 4.9. In this figure, data from 1949–1964 (before tourism became as popular as it is today) are plotted that show the duration of measles epidemics on various islands. In Fig. 4.9A, we see that an island must have a population sufficient to produce about 16,000 surviving newborns a year (population about 500,000) in order to maintain the virus continuously in the population. If the population is smaller, the epidemic burns itself out when all susceptibles have been infected. The island is then free of measles until sufficient new susceptibles have been born and measles is once again introduced into the island from outside. The smaller the population, the longer this takes. Note that Guam and Bermuda, with their heavy tourist influx, had measles present more than expected from the curve because the virus is introduced more often, that is, the island population is not truly isolated. Figure 4.9B illustrates that the more densely packed the population, the more readily the virus spreads and therefore the sooner the epidemic burns itself out. The islands shown in this panel all have about the same population, but when the population is compressed into a smaller area, such as in Tonga, person-to-person spread is more efficient and epidemics do not last as long as when the population is dispersed over a larger area, such as in Iceland.

The study of measles epidemics on islands first demonstrated that lifelong immunity arises following infection by measles. After an epidemic of measles in the Faeroe Islands in 1781, the islands were free of measles until the virus was again introduced in 1846 by a Danish visitor. In the 1846 epidemic, 77% of the population of the islands contracted measles, but no one over 65 years of age came down with the disease.

The requirement for a minimum sized population to maintain the virus means that even though measles virus is extraordinarily infectious, the virus could not have existed until perhaps 5000 years ago when human population density became sufficient to support it. At about this time, large population centers arose in the Fertile Crescent, a region of the Middle East encompassing parts of modern Iraq, Syria, Jordan, Israel, Lebanon, and Turkey, which included the upper Tigris and Euphrates rivers and whose climate was conducive to primitive agriculture. These population centers were associated with the cultivation of food plants and the domestication of animals, including bovines. Measles virus is most closely related to rinderpest virus (Fig. 4.5), which infects cattle and swine. An obvious hypothesis is that the close contact between humans and their domesticated animals allowed rinderpest virus, or perhaps another virus of domestic animals, to jump to humans and evolve to become specific for humans. Subsequent coexistence of the virus with its human host led to the present situation where infection results in significant but relatively low morbidity and mortality.

Introduction of Measles into the Americas and Island Populations

Epidemics of measles were undoubtedly widespread in the Old World following the appearance of measles, although it is difficult now to ascertain the causes of epidemics that occurred thousands of years ago. However, it is clear that measles was widespread in Europe at the time the Europeans began their explorations of the Americas and of the many isolated island communities around the world, and Europeans carried measles with them as they traveled. Introduction of measles virus into virgin populations resulted in very high mortality. Mortality was 26% in Fiji islanders when measles was introduced in 1875, for example. It has been estimated that 56 million people in the Americas died of Old World diseases following European exploration of the New World, and measles and smallpox (Chapter 7) were significant contributors to these deaths. The introduction of measles and smallpox by the Spaniards facilitated the conquest of the Americas by them, and the subsequent depopulation of Central and South America allowed the Spaniards to remain dominant.

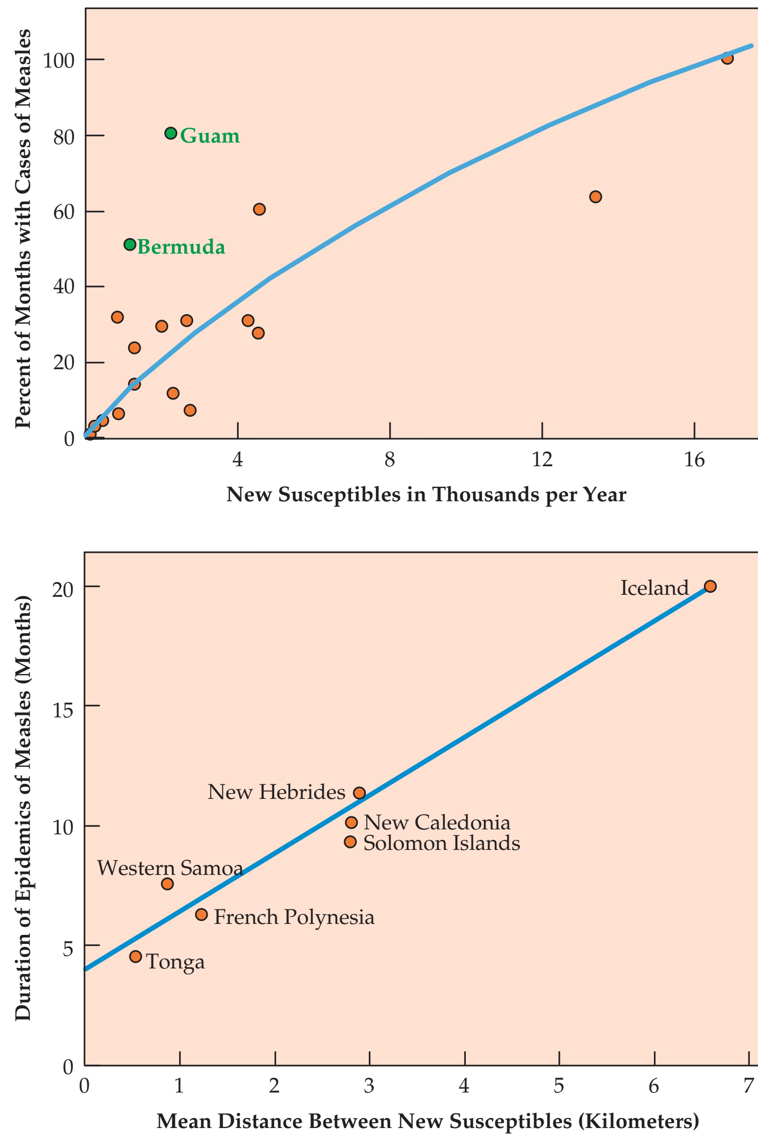


FIGURE 4.9 Effect of population size and density on the epidemiology of measles. Upper panel: percent of months with measles (true endemicity = 100%) in island populations as a function of number of new susceptibles per year. Measles periodically fades out in isolated populations of less that 500,000 (= approximately 15,000 new susceptibles per year). Each dot represents a different island population. Note that Guam (with a transient military population) and Bermuda (with a steady influx of tourists) do not fall on the curve, as they are not truly isolated populations. Vaccination for measles can reduce the number of new susceptibles, even in large urban populations, below the number needed to sustain transmission. Data from Black (1966). Lower panel: relation between the average duration of measles epidemics and the dispersion of populations in isolated islands. All of the islands shown have about the same population, sufficient to introduce 2000–4000 new susceptible children per year. The abscissa is

$$\frac{1}{\sqrt{\dfrac{\text{Population input}}{(\text{Land area of the archipelago in kilometers})^2}}}$$

which represents the average distance between infants added to the population each year. Population input is defined as births minus infant mortality.

It has been suggested that the depopulation of the Americas caused by these diseases led the Europeans to introduce Africans as slaves to replace Native Americans being used as slaves.

The very high mortality caused by the virus in naive populations, which contrasts with the low mortality in Europeans, was probably due to two causes. Europeans and other Old World peoples have been continuously exposed to

measles for millennia and have been selected for resistance to measles. The people in the Americas had never experienced measles infection, however. A second factor that led to high mortality rates was the introduction of measles into a virgin population, in which not only young children but also all of the adults were susceptible, meaning that the entire population became seriously ill simultaneously. This surely disrupted the ability of the society to maintain itself because there was no one healthy enough to care for the sick.

Vaccination against Measles

At one time, measles virus was epidemic throughout the world and caused one of the childhood illnesses contracted by almost everyone. Because of the extraordinary infectiousness of the virus, very few people escaped infection by it. In the United States, there were about 4 million cases of measles a year, of which about 50,000 required hospitalization and 500 were fatal. There were 4000 cases of measles encephalitis each year, with many patients suffering permanent sequelae. In addition, some fraction of children infected as infants went on to develop SSPE, which is a progressive neurological disease that results in death within about 3 years of the appearance of symptoms. Throughout the world, an estimated 2.5 million children died annually of measles.

Because measles was a widespread and serious disease, attempts to develop a vaccine began at about the same time as attempts to develop a poliovirus vaccine. One vaccine used in the United States from 1963 to 1967 consisted of inactivated measles virus. The vaccine was poorly protective and recipients of this vaccine exposed to measles sometimes developed a more serious form of measles, called "atypical measles," characterized by higher and more prolonged fever, severe skin lesions, and pneumonitis (inflammation of the lungs, from *pneumon* = lung and *itis* = inflammation). The increased severity may have resulted from an unbalanced immune response primed by the formalin inactivated virus or to a lack of local immunity in the respiratory tract (see Chapter 10). Similar problems occurred following vaccination with inactivated respiratory syncytial virus, a paramyxovirus described later.

An attentuated measles virus vaccine, now given as part of the MMR combination vaccine, produced much more satisfactory results. The live virus vaccine gives solid protection from disease caused by the virulent virus and has largely controlled the virus within the United States (Fig. 4.10). Following introduction of the vaccine, the number of cases dropped dramatically. The virus continued to circulate among nonimmunized individuals, however, and thousands of cases per year still occurred, sometimes associated with epidemics of more than 50,000 cases. As vaccine coverage became more effective, cases dropped to new lows, but another epidemic in 1989–1991 caused about 50,000 cases. This epidemic occurred in young immunized adults as well as in young children who had not been immunized. Some of the cases in young adults were due to vaccine failures (about 5% of humans immunized with a single dose of the measles vaccine fail to develop immunity to measles), but other cases appear to have been due to waning immunity. Thus, immunity induced by the vaccine is probably not lifelong, in contrast to natural infection by wild-type measles virus. The guidelines now call for a second immunization on entry to elementary or middle school. This not only boosts immunity in individuals whose immunity is waning, but also usually leads to immunity in those who did not develop immunity after the first dose. In addition, some colleges require immunization on entry. With these changes, the number of cases of measles in the United States was only 100 in 1998.

Molecular genotyping has increased our understanding of the few cases of measles that occur annually in the United States today. In 1988–1992, all the reported isolates of measles virus were subgroup 2, the indigenous North American strain. However, by 1994–1995, all outbreaks were caused by one of four other subgroups that are endemic in other parts of the world. Thus, these outbreaks were initiated by viremic visitors from Asia and Europe. One notable outbreak is thought to have been initiated by a single visitor to Las Vegas and resulted in small epidemics in five states.

After control of measles in the United States and other developed countries, the virus remained epidemic in many parts of the developing world. Control has recently been established throughout most of the Americas, but measles remains a serious pathogen in other parts of the developing world. In fact, measles is the leading cause of vaccine preventable deaths in the world. The World Health Organization has initiated a campaign to eradicate measles but the campaign has met a number of stumbling blocks. Because the virus infects only humans in nature, it should be possible to eradicate it using the same techniques that were used for smallpox and that are being used for poliovirus. A major problem with measles, however, has been the inability to effectively immunize young infants against the disease before they become naturally infected by the virus. Newborns are protected from infection for 6–12 months by maternal antibodies, and a live vaccine does not take while they are thus protected. In many societies, measles is so pervasive that very shortly after the infant becomes susceptible to infection, infection by wild-type virus occurs, thus keeping wild-type virus in circulation. As described in Chapter 10, attempts to overcome maternal immunity by increasing the dose of the vaccine virus have not led to satisfactory results. New guidelines being developed recommend multiple immunizations against measles in such circumstances, starting at an earlier age, in order to catch the child with the vaccine as soon as it becomes susceptible to the virus.

Measles Neuraminidase

The receptor for measles virus is a protein called CD46 that is expressed on the surface of human and monkey cells (Chapter 1), which is bound by the measles H pro-

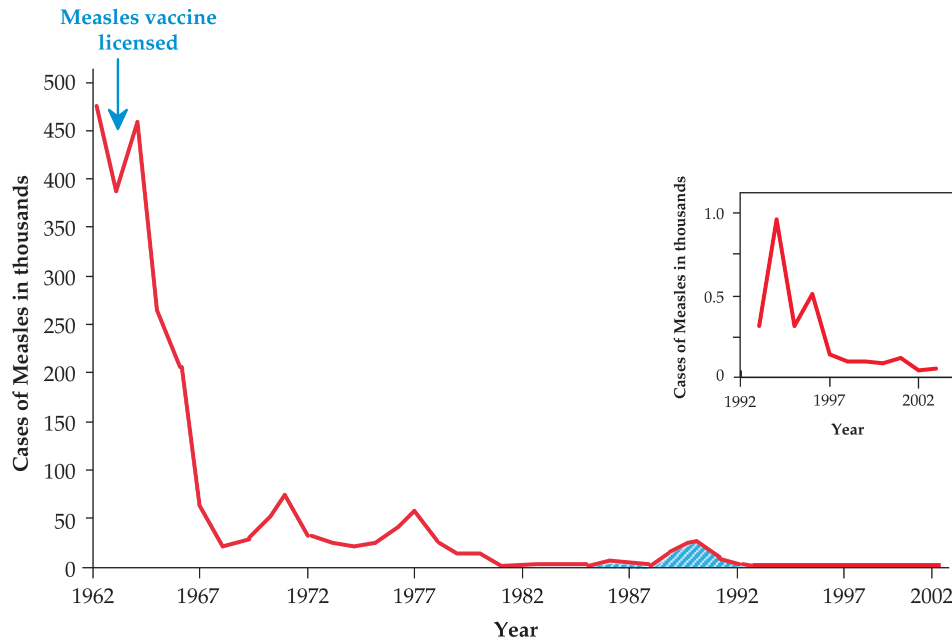


FIGURE 4.10 Cases of measles in the United States by year. Note the difference in the scales of the two graphs. The most recent epidemic of measles, in 1989–1991, with a total of 45,622 cases, was probably due to vaccine failure and waning immunity in children immunized earlier with only a single dose of MMR vaccine. In 2003 a total of 56 cases of measles was reported, of which 24 were internationally imported, and 19 were of persons exposed to the imported infections. In 2005, a total of 66 cases of measles were reported of which 24 were imported and an additional 33 import-linked cases were part of a single outbreak in Indiana initiated by exposure in Romania. From *MMWR, Summary of Notifiable Diseases-1998*; the comparable summary for 2004; and *MMWR* (2006), Vol. 55, p. 1348.

tein. In view of the fact that sialic acid was not the measles receptor, the apparent lack of neuraminidase activity in the H protein was not surprising. Recent studies of the morbillivirus H protein have shown that it is related in structure to the HN protein of paramyxoviruses, however, and that the related rinderpest and peste-des-petits-ruminants viruses possess neuraminidase activity. This neuraminidase activity differs in its specificity from that exhibited by the respiroviruses and the orthomyxoviruses, explaining why it had not been observed previously. Presumably this activity is also found in the measles H protein, and the function of this enzyme in the measles life cycle remains to be determined.

Genus *Henipavirus*

Two species of henipaviruses are currently recognized, Hendra virus and Nipah virus. Hendra virus first emerged in 1994 when an outbreak of severe respiratory illness with a 75% fatality rate occurred in horses near Brisbane, Australia. Two humans also contracted the disease, of whom one died. Hendra virus was quickly isolated and shown to be responsible for the disease. Subsequent studies established that the reservoir of the virus was fruit bats called flying foxes. Small outbreaks of Hendra also occurred in northern Australia in 1994, 1999, and 2004. Hendra virus is widespread and antibodies to it have been found in all four species of flying foxes in Australia, but from no other animal except the horses infected in these outbreaks.

Nipah virus shares 83% amino acid sequence identity with Hendra virus. It is widely distributed in Southeast Asia and has been isolated from fruit bats in Malaysia, Cambodia, and Bangladesh. It first emerged in 1998–1999 when an outbreak of 258 cases of human encephalitis occurred in Malaysia and Singapore that had a 40% mortality rate. The epidemic was associated with an outbreak of respiratory disease in pigs, and humans infected with the disease were pig farmers or others closely associated with pig farming. There was no evidence for human-to-human transmission in this outbreak.

Recent epidemics of Nipah virus encephalitis have occurred in Bangladesh in 2001, 2003, 2004, and 2005. In these epidemics there was no evidence for the infection of an intermediate animal such as occurred in the Malasian epidemic. Furthermore, in the 2004 epidemic evidence was obtained that person-to-person transmission of the virus had occurred and it is possible that the disease was transmitted directly from bats to humans, possibly by human consumption of partially eaten fruit followed by person-to-person transmission. The fatality rate in these epidemics has been as high as 75%. In nearby India, an epidemic of Nipah occurred in 2001.

Hendra virus and Nipah virus represent emerging pathogens. They are previously unknown viruses that are causing serious disease over widely separated geographic areas.

A more detailed discussion of emerging viral diseases is found in Chapter 8.

Genus *Pneumovirus*

The genus *Pneumovirus*, subfamily *Pneumovirinae*, contains the respiratory syncytial viruses (RSVs). RSVs are known for cattle, mice, sheep, goats, and turkeys, as well as humans. The genome of RSV is more complex than other *Paramyxoviridae*, having more genes (Fig. 4.6). The polymerase gene of RSV is more closely related to those of the filoviruses than to those of the *Paramyxovirinae*, making classification of these viruses problematical.

Human RSV is the most important cause of pneumonia in infants and children worldwide. Half of hospital admissions in the United States in January and February of infants less than 2 years old are due to infection by RSV. Infants are normally infected at 6 weeks to 9 months of age. Infection begins as an upper respiratory tract infection that progresses to the lower respiratory tract in 25–40% of primary infections. Immunity following infection is incomplete and reinfection is common in children and adults, but reinfection tends to produce less severe disease. Symptoms can include bronchitis and pharyngitis (*itis* = inflammation, so inflammation of the mucous membranes of the bronchi or pharynx), rhinorrhea (runny nose), cough, headache, fatigue, and fever. Pneumonia (inflammation of the lungs in which the air sacs become filled with exudate) can result, particularly in infants or the elderly. It is estimated that 17,000 people in the United States die annually from RSV infection, and the great majority of these are people over 65 years old. RSV infection is particularly serious in the immunocompromised. As one example, individuals of any age undergoing bone marrow transplantation have a 90% mortality rate if infected by RSV.

No vaccine is available at the current time for RSV. Because of the widespread prevalence of infection by the virus and the severity of the disease it causes, especially in infants, efforts are ongoing to develop a vaccine that would provide protection against disease or that would at least protect against severe disease. A clinical trial with an inactivated virus vaccine in a group of children some years ago gave disastrous results, however. Not only did the inoculation with the candidate vaccine fail to protect the children against subsequent infection by RSV, but it was found that when infected the vaccinated group suffered a much higher proportion of serious illnesses such as viral pneumonia than did the control group. Thus, immunization potentiated illness, possibly because of an unbalanced immune response. This result has impeded efforts to develop a vaccine and made it clear that a better understanding of the interaction of the virus with the immune system is important.

Many viruses belonging to several different families have now been described that cause respiratory disease, and more viruses belonging to other families will be described later. For comparative purposes, an overview of viruses that cause respiratory disease is shown in Table 4.4. This table is not meant to be comprehensive and includes only a sampling of viruses. Furthermore, some of the viruses in the table, such as measles virus, are better known for disease other than respiratory disease. However, the table makes clear that a large number of diverse viruses can infect the respiratory tract and cause illness.

FAMILY *FILOVIRIDAE*

Table 4.5 lists the known filoviruses, which are classified into two genera, *Marburgvirus* and *Ebolavirus*. Four species of *Ebolavirus* are known, three from Africa (Zaire, Sudan, and Ivory Coast ebolaviruses) and one from the Philippines and/or Southeast Asia (Reston ebolavirus). The filovirus genome is 19 kb in size and contains seven genes, which result in the production of seven or eight proteins following infection (Fig. 4.1). The biology of filoviruses has been very difficult to study. Most filoviruses are severe human pathogens that must be handled under biosafety level 4 conditions (BSL-4), which restricts the number of laboratories that can work with the virus and the number of experiments that can be done. Furthermore, until recently the reservoir of the virus in nature was unknown, limiting studies of the ecology of the virus. A dendrogram showing the relationships among the filoviruses is shown in Fig. 4.11.

The viruses have a genome organization similar to that of other members of the *Mononegavirales*. Their sequences suggest that they are most closely related to the pneumoviruses, and they are assumed to replicate in a manner similar to that for the rhabdoviruses and paramyxoviruses. There are four structural protein genes, encoding the nucleoprotein NP, the glycoprotein GP, and two matrix proteins VP24 (M2) and VP40 (M1). There are three nonstructural protein genes, encoding the viral polymerase and two proteins called VP30 and VP35. The GP gene gives rise to one protein, GP, in Marburg virus. In the Ebola viruses, however, a second glycoprotein, called sGP, is also produced from an edited version of the mRNA for GP. sGP is a soluble, truncated version of GP whose function is unknown, but it is speculated that sGP interferes with the host immune system in some way.

The filovirus virion is enveloped, as is the case for all minus-strand viruses, but rather than being spherical, the virion is long and thread-like (whence the name *filo* as in filament). The infectious virion is 800–1000 nm in length and 80 nm in diameter (Fig. 2.23E), but preparations examined in the electron microscope are pleomorphic and oddly shaped, often appearing as circles or the number 6 but sometimes branched (Figs. 2.23F and G). There is one glycoprotein (GP) in the envelope, present as homotrimers, that is both *N*- and *O*-glycosylated and has a molecular weight of 120–170 kDa.

TABLE 4.4 Viruses Causing Respiratory Disease

Family	Virus[a]	Nucleic acid	Host range	Disease(s)
Orthomyxoviridae	Influenza	(−)RNA	Humans, birds, horses, swine	Rhinitis, pharyngitis, croup, bronchitis, pneumonia
Paramyxoviridae	RSV	(−) RNA	Humans, cattle	Rhinitis, pharyngitis, croup, bronchitis, pneumonia
	Canine distemper		Dogs	Bronchitis, pneumonia
	NDV		Birds	Respiratory distress
	Human parainfluenza		Humans	Rhinitis, pharyngitis, croup, bronchitis, pneumonia
	Measles		Humans	Pneumonia
Bunyaviridae	Sin Nombre	(−) RNA	Humans, rodents	Repiratory distress, pneumonia
Picornaviridae	Rhinoviruses	(+) RNA	Humans	Common cold (rhinitis), pharyngitis
	Coxsackie A		Humans	Rhinitis, pharyngitis
Caliciviridae	Feline calicivirus	(+) RNA	Cats	Rhinitis, tracheitis, pneumonia
Coronaviridae	HCoV	(+) RNA	Humans	Rhinitis
	IBV		Fowl	Bronchitis
	SARS		Humans	Severe acute repiratory disease
Adenoviridae	Human Ad40,41	ds DNA	Humans	Rhinitis, pharyngitis, pneumonia
	CLTV		Dogs	Pharyngitis, tracheitis, bronchitis, and bronchopneumonia
Herpesviridae	Cytomegalovirus	ds DNA	Humans	Pharyngitis, pneumonia
	Herpes simplex, EBV, varicella		Humans	Pharyngitis, pneumonia
	Various alphaherpesvirinae		Cattle, cats, horses, fowl	Rhinotracheitis

[a] Virus name abbreviations: RSV, respiratory syncytial virus; NDV, Newcastle disease virus; HCoV, human coronavirus; IBV, infectious bronchitis virus; CLTV, canine laryngotracheitis; EBV, Epstein-Barr virus.
Adapted from Granoff and Webster (1999), pp. 1493, 1494.

TABLE 4.5 *Filoviridae*

Genus/members	Virus name abbreviation	Usual host(s)[a]	Transmission	Disease	World distribution
Marburgvirus					
Lake Victoria marburgvirus	MARV	Humans	Contact with blood or other body fluids	Severe hemorrhagic disease	Africa
Ebolavirus					
Zaire ebolavirus	ZEBOV	Humans	Contact with blood or other body fluids	Severe hemorrhagic disease	Africa
Sudan ebolavirus	SEBOV				
Cote d'Ivoire ebolavirus	CIEBOV				
Reston ebolavirus	REBOV	Cynomolgus monkeys	?	Severe hemorrhagic disease in monkeys, attenuated in man	Philippines

[a] Natural reservoirs unknown for many years, but were recently found to be bats.

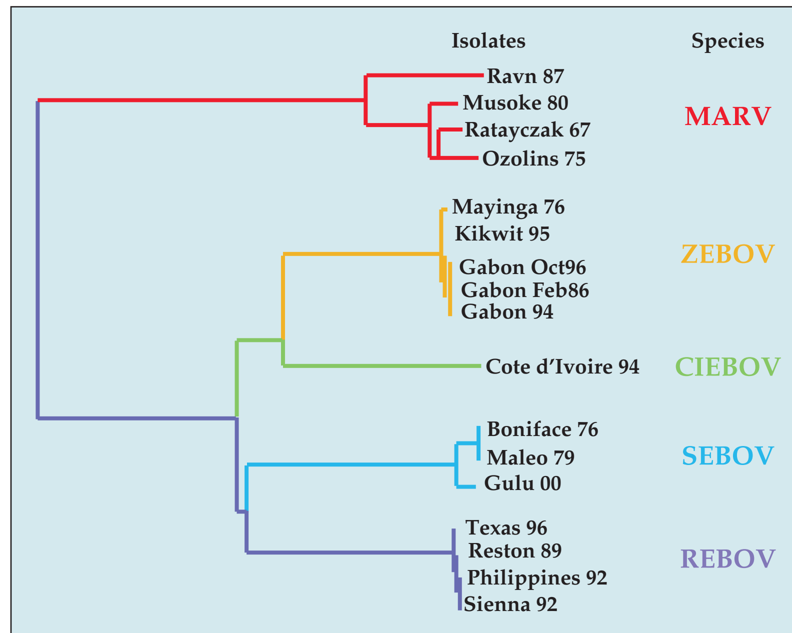


FIGURE 4.11 Phylogenetic tree of the *Filoviridae,* derived from nucleotide sequences for the entire coding region of the glycoprotein genes, using the neighbor-joining method. Adapted from Fauquet *et al*. (2005), Figure 3 on p. 652. Isolates are named by the location and year of isolation. Abbreviations are defined in Table 4.5.

Most of the known filoviruses cause severe hemorrhagic fever in humans with a mortality rate as high as 90%. Because of the dramatic symptoms of the disease involving bleeding from multiple orifices, and the high fatality rate following infection, these viruses, especially Zaire ebolavirus, have been the subject of much discussion in the popular press and have appeared in a number of works of nonfiction as well as fiction. To date, ebolaviruses have caused only a limited number of human cases, but there is always the fear that if the virus were to adapt to humans in a way that allowed for easier transmission, it could become a very big problem.

The filoviruses are examples of emerging viruses and a more detailed discussion of their emergence and the epidemics of disease caused by them are found in Chapter 8. Marburg virus was first isolated in 1967 when it caused an outbreak of hemorrhagic fever in Germany and Yugoslavia, originating from African green monkeys imported from Uganda. The virus is native to central Africa and there have subsequently been epidemics in Kenya, Zaire (the Democratic Republic of Congo), Angola, and Zimbabwe involving from one or a few cases to as many as 374 cases, with an overall mortality rate of 80–90%.

Ebola virus was first isolated during a 1976 epidemic of severe hemorrhagic fever in Zaire and Sudan and named for a river in the region. Subsequent epidemics in these countries and in Gabon and Uganda have occurred at regular intervals (see Chapter 8). The number of cases in an epidemic has been as high as 600 and the case fatality rate has varied from 50 to 90%. Asymptomatic infection during these epidemics appears to be rare. A milder strain of Ebola virus was isolated from the Ivory Coast in 1994 and three strains or species of African ebolaviruses are now recognized which differ in their virulence (Table 4.5 and Fig. 4.11). Zaire ebolavirus is the most virulent with a case fatality rate approaching 90%, Sudan ebolavirus is less virulent, and Ivory Coast ebolavirus is the least virulent. Human-to-human transmission of the virus requires close contact with the tissues, blood, or other exudates from an infected person and barrier nursing is sufficient to contain the spread of the disease. No vaccine exists for the viruses, but candidate vaccines are in an advanced stage of study.

The natural reservoir of Ebola virus in Africa has recently been shown to be bats. It is clear that monkeys can be infected by the virus and spread it to humans, often when humans use monkeys for food. How the monkeys contract the virus or how human epidemics get started when monkeys are not implicated is not known.

A fourth strain of Ebola virus called Reston ebolavirus first appeared in 1989 in a primate colony in Reston, Virginia, as the causative agent of an epidemic of hemorrhagic fever in monkeys imported from the Philippines. Although highly lethal in monkeys, the virus is not known to cause human disease. Nucleotide sequencing has shown that Reston ebolavirus is closely related to the African Ebola viruses, and the reason it is attenuated in humans is not known.

FAMILY *BORNAVIRIDAE*

Borna disease virus is the sole representative of the family *Bornaviridae* in the order *Mononegavirales* (Table 4.6). It has a nonsegmented, minus-sense genome of 8.9 kb, containing, by one definition, five genes. The general organization of the genome and the suite of genes contained in the genome resembles that of other members of the *Mononegavirales* (Fig. 4.1). However, the virus replicates in the nucleus, not in the cytoplasm. Splicing of mRNAs occurs to form an incompletely characterized set of alternatively spliced mRNAs from the five genes. Furthermore, alternative start codons are used during translation of some of the mRNAs, a trait shared with other (−)RNA viruses, so that overlapping open reading frames are present in some of the genes. Overall, the number of protein products produced and the complexity of the readout of the genome exceed that of other (−)RNA viruses. The genome organization and transcriptional map of Borna disease virus, as currently understood, is shown in Fig. 4.12.

TABLE 4.6 *Bornaviridae*

Genus/members	Virus name abbreviation	Usual host(s)[a]	Transmission	Disease	World distribution
Bornavirus					
Borna disease	BDV	Horses, sheep other mammals	??	Encephalopathy, fatal paralysis	Europe, possibly worldwide

[a] It was recently reported that the bicolored white-toothed shrew *Crocidura leucodon* is the reservoir host in Switzerland where the disease is endemic. These animals live on the ground and do not climb. Presumably horses are infected when grazing on forage contaminated by excretions from infected shrews; horses fed exclusively from feeding troughs in modern barns are seldom affected (Hilbe *et al.*, 2006).

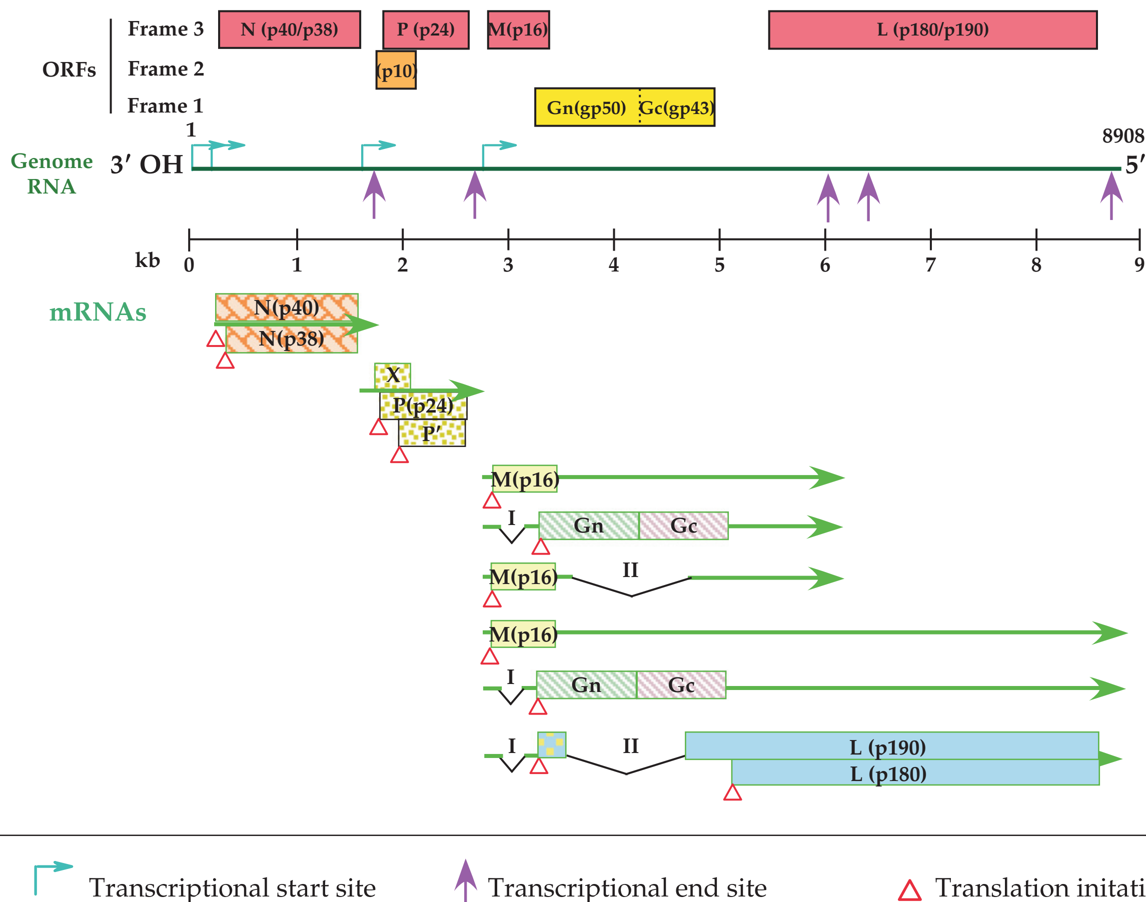


FIGURE 4.12 Genome organization and transcriptional map of an isolate of Borna disease virus. ORFs are represented by the boxes at the top, color coded by reading frame. Messenger RNAs are shown below, with the boxes color coded according to the function of the product as in Figs. 4.1 and 4.6. The positions of two introns, I (nucleotides 1932 to 2025) and II (nucleotides 2410 to 3703), are indicated. Adapted from Fauquet *et al*. (2005), Figure 2 on p. 617.

Borna disease virus is neurotropic and establishes a chronic or persistent infection despite an immune response to viral infection. The chronic infection results at least in part because the virus downregulates its replication, resulting in very low production of infectious virus. Downregulation to establish a persistent infection uses a mechanism different from those described in Chapter 3 for alphaviruses (shut-off of minus-strand RNA) and for pestiviruses (titration of a cellular component required for RNA replication). Borna disease virus, like other (−)RNA viruses, has an inverted terminal repeat at the ends of the genomic RNA that contains promoters for RNA replication. During replication, the four terminal nucleotides at the 5′ ends of both the genomic and antigenomic RNA are often trimmed so that the majority of RNAs are missing these four nucleotides. The truncated RNA can be transcribed to produce mRNA but cannot replicate, thus resulting in downregulation of RNA replication.

Borna disease virus appears to have a very wide host range. It was originally described as a pathogen of sheep and horses in Germany, but is now known to infect a wide variety of warm-blooded vertebrates, birds as well as mammals. The reservoir host in Switzerland has recently been reported to be a shrew (*Crocidura leucodon*). These rodents are exclusively terrestrial and it is thought that horses become infected by grazing on forage contaminated by excretions from infected shrews.

As described, the virus establishes a chronic infection characterized by neurotropism and low production of virus. Infection may be asymptomatic or may result in disease characterized by movement and behavioral abnormalities. Naturally infected horses exhibiting such abnormalities usually recover, but the disease may progress to paralysis and death. Experimentally infected rats and primates also exhibit behavioral abnormalities. Because of these effects on other animals, several recent studies have tried to determine if the virus is associated with neurological disease in man, in particular with schizophrenia. Serological surveys have found that psychiatric patients are more likely to have antibodies to bornavirus than normal controls. Surveys which assay for the presence of viral RNA in peripheral blood mononuclear cells (PMBCs) are even more suggestive: in some surverys up to 66% of psychiatric patients, including schizophrenics, are positive for bornaviral RNA, compared to <5% of normal controls. Furthermore, very small amounts of virus-specific RNA have been isolated from postmortem brain samples from patients suffering from schizophrenia and bipolar disorder, but not from normal individuals or patients suffering from other neurological disorders. Interestingly, a recent study found that two patients hospitalized for severe depression exhibited a rise in bornavirus antigen in PMBCs during the course of the disease, which fell to very low levels on recovery. Whether these different associations are indicative of causality remains to be determined, but it is conceivable that the virus causes recurrent episodes of depression on reactivation of a latent infection.

FAMILY *ORTHOMYXOVIRIDAE*

The family *Orthomyxoviridae* (*ortho* = true or correct) contains three genera of influenza viruses: *Influenzavirus A*, which contains influenza virus A; *Influenzavirus B*, which contains influenza virus B; and *Influenzavirus C*, which contains influenza virus C (Table 4.7). Thogoto virus, a tick-borne virus of mammals, forms a fourth genus, *Thogotovirus* and infectious salmon anemia virus belongs to a fifth genus,

TABLE 4.7 *Orthomyxoviridae*

Genus/members	Virus name abbreviation	Usual host(s)	Transmission	Disease	World distribution
Influenzavirus A					
Influenza A	FLUAV	Humans, birds, swine	Airborne	Respiratory disease	Worldwide
Influenzavirus B					
Influenza B	FLUBV	Humans	Airborne	Respiratory disease	Worldwide
Influenzavirus C					
Influenza C	FLUCV	Humans	Airborne	Respiratory disease	Worldwide
Thogotovirus					
Thogoto virus	THOV	Mammals	Tick-borne	Respiratory disease	Southern Europe, Africa
Isavirus					
Infectious salmon anemia	ISAV	Fish	Waterborne	Anemia, hemorrhagic liver necrosis	North Atlantic, North America

Isavirus. Influenza viruses A and B are closely related, but influenza A infects a wide spectrum of birds and mammals including humans, with birds being the reservoir, whereas influenza B infects primarily humans and humans are the reservoir. Influenza C is more divergent. Eight segments of (−)RNA, totaling about 14 kb, comprise the genomes of influenza A and B viruses (Fig. 4.1) whereas influenza C has only seven segments. Influenza viruses use sialic acid as a receptor, but the form used by influenza A and B viruses differs from that used by influenza C virus, and the enzymes encoded by the viruses to destroy receptors are correspondingly different. All three influenza viruses infect humans and cause disease, but influenza A represents the most serious human pathogen because it causes very large, recurrent epidemics with significant mortality. Influenza A has therefore been the most intensively studied and has been the focus of efforts to control influenza in humans.

Proteins Encoded by the Influenza Viruses

The proteins encoded in the different gene segments of influenza A and influenza C viruses are described in Table 4.8. Influenza A produces 10 proteins from its eight genome segments, and most of these proteins have analogues in other (−)RNA viruses (Fig. 4.1). The matrix protein, M1, and the nucleocapsid protein, NP, perform functions similar to those of M (when present) and N of other (−)RNA viruses, respectively. The three proteins encoded in the three largest segments of influenza, called PB2, PB1, and PA (B or A refers to a basic or acidic pK), possess the RNA polymerase activities encoded in the L protein and the P protein of other (−)RNA viruses. Influenza A and B have two surface glycoproteins, called HA and NA, but influenza C has only one, called HEF. These glycoproteins have the receptor-binding, fusion, and receptor-destroying activities present in surface glycoproteins of (−)RNA viruses.

Two proteins, called NS1 and NS2 (NS for nonstructural), are produced from RNA segment 8. NS1 is produced from the unspliced mRNA (replication occurs in the nucleus). It binds to RNAs in the nucleus, including cellular pre-mRNAs, cellular snRNAs which are involved in splicing, and dsRNA. Its activities inhibit the transport of cellular mRNAs from the nucleus and promote the synthesis of influenza mRNA. NS1 also regulates splicing of influenza mRNAs and their transport from the nucleus to the cytosol. Another function of NS1 is to interfere with the interferon pathway (Chapter 10), in part by binding dsRNA, which is a major inducer of interferon and a cofactor for some proteins in the interferon response to viruses, and in part by interacting with cellular proteins involved in the interferon response. Influenza virus lacking NS1 is very sensitive to interferon and is replication defective in cells or hosts capable of synthesizing interferon,

TABLE 4.8 Genome Segments of Influenza Viruses

Influenza A					Influenza C			
RNA segment	Length (nt)	Encoded Protein Name	Encoded Protein (aa)	Function[a]	RNA segment	Length (nt)	Encoded Protein Name	Encoded Protein (aa)
1	2341	**PB2**	759	Cap recognition, RNA synthesis	**1**	2365	**PB2**	774
2	2341	**PB1**	757	RNA synthesis	**2**	2363	**PB1**	754
3	2233	**PA**	716	RNA synthesis	**3**	2183	**PA**	709
4	2073	**HA**	566	Hemagglutinin, fusion, major surface antigen, sialic acid binding. HEF of FLUCV also has esterase activity	**4**	2073	**HEF**	655
5	1565	**NP**	498	Nucleocapsid protein	**5**	1809	**NP**	565
6	1413	**NA**	454	Neuraminidase				
7	1027	**M1**	252	Matrix protein	**6**	Spliced	**M1**	242
						1180	p42	374
							⇓Signalase	
	Spliced	**M2**	97	See footnote[b]			M1′(p31) + **CM2**	259 + 115
8	934	**NS1**	230	Nonstructural protein	**7**	934	**NS1**	286
	Spliced	**NS2**	121	See footnote[b]		Spliced	**NS2**	122

[a] All functions other than those in footnote "b" apply to both influenza A and influenza C.
[b] M2 of Flu A forms an ion channel, and NS2 of FluA is a nuclear export protein; the functions of the comparable moieties of Flu C are unknown.
Source: Adapted from Fields *et al*. (1996) Table 2 on p. 1355 and data in Fauquet *et al*. (2005) p 683.

whereas the wild-type virus is resistant to the interferon pathway. NS2 is produced from a spliced mRNA. It interacts with M1 attached to influenza RNP and promotes the transport of the RNP to the cytoplasm. It is present in small quantities in the virion and so is not truly nonstructural.

Protein M2 is produced from a spliced mRNA from segment 7. It forms ion channels in membranes, probably as a tetramer, that allow passage of H^+ ions. During transport of HA to the cell surface, the presence of M2 in the membrane of the transport vesicle causes the pH within the vesicle to equilibrate with that in the cytosol. This prevents low pH activation of the fusion activity of HA during transport, because transport vesicles are otherwise acidic. M2 is also present in virions and is required for the disassembly of the virus and for the activation of the RNA polymerase activity. To become active, the polymerase in the interior of the virus must be exposed to low pH. Influenza virus enters the cell in endosomes, which are progressively acidified. The acidic pH not only triggers a conformational change in HA that results in fusion of the viral membrane with the endosomal membrane, but it also activates the RNA polymerase of the virion through the activity of M2. M2 is the target of the drug amantadine, one of the relatively few drugs that are effective against a viral disease. Amantadine binds M2 of most influenza strains and prevents it from acting as an ion channel, which prevents the activation of the polymerase. When taken early during infection, amantidine ameliorates the symptoms of influenza. A worrisome trend is the appearance of amantidine-resistant variants of influenza, in particular the H5N1 strain referred to as "bird flu."

In most, but not all, influenza A viruses an 11th protein (PB1-F2) is made. This protein is translated from an alternative reading frame from the mRNA of PB1. It is present in mitochrondria in infected cells and may serve to regulate apoptosis by the cell.

Influenza Glycoproteins

Comparison of the glycoproteins of influenza A virus and the paramyxovirus SV-5 is of interest. In both influenza A virus and SV-5, one of the glycoproteins is type 1 (N terminus out) and one is type 2 (C terminus out). In both cases, the type 1 glycoprotein is produced as a precursor that must be cleaved to activate the fusion activity required for entry into cells. The type 1 glycoprotein of influenza A has fusion and receptor-binding (hemagglutinating) activities and is called the hemagglutinin or HA. The precursor is called HA_0 and the cleaved products are called HA_1 and HA_2 (which remain covalently linked by a disulfide bond after cleavage of the peptide bond) (Fig. 1.6). Cleavage is required to activate the fusion activity of the virus and the nature of the cleavage site influences the virulence of the virus. If the cleavage site consists of a single basic amino acid, cleavage is extracellular and influenza replication is restricted to the respiratory tract, and in the case of birds the gut as well, where there are enzymes that can cleave this site. If the cleavage site consists of multiple basic residues that can be recognized by the intracellular enzyme furin, the virus can replicate systemically in at least some hosts. The SV-5 type 1 glycoprotein has only fusion activity and is called F. As described before, it is produced as a precursor F_0 which is cleaved to F_1 and F_2, and the nature of the cleavage site affects the virulence of the virus (see Viral Glycoproteins under *Paramyxoviridae* earlier in this chapter).

The receptor bound by both influenza A virus and by SV-5 for entry into cells is sialic acid. The type 2 glycoprotein of influenza has neuraminidase activity and is called the neuraminidase or NA. It removes sialic acid from glycoproteins for the same reasons as described for the paramyxoviruses that use sialic acid as a receptor. The type 2 glycoprotein of SV-5 has both neuraminidase activity and receptor-binding (hemagglutinating) activities and is called HN.

Influenza HA is present as a trimer on the surface of the virus (as is F of SV-5). The trimeric spike has a long stalk and a head containing the sialic acid binding sites. As shown in Fig. 1.6, exposure to acid pH in endosomes produces a dramatic rearrangement of the spike in which the fusion peptide, which forms the N terminus of HA_2, is moved over a distance of more than 10 nm to the tip of the spike. Here it inserts into the target membrane and promotes fusion of the viral membrane with the target membrane. NA is present as a tetramer (as is HN of SV-5), and forms a spike that is distinguishable in the electron microscope from the HA spike.

There is only one surface glycoprotein in influenza C, the hemagglutinin-esterase-fusion protein (HEF). Influenza C virus has, therefore, one fewer gene segments than influenza A. HEF has receptor-binding (hemagglutination), fusion, and receptor-destroying activities. The receptor is sialic acid, but the activity that destroys the receptor is an esterase activity. The esterase does not remove sialic acid from proteins as does NA of influenza A. Instead it removes the 9-*O*-acetyl group from 9-*O*-acetyl-*N*-acetylneuraminic acid, the receptor used by influenza C, and the virus does not bind to the deacylated sialic acid.

Replication of Influenza RNA and Synthesis of mRNAs

Synthesis of influenza virus RNAs occurs in the nucleus, rather than in the cytoplasm as for most RNA viruses. This makes possible the differential splicing observed for two of the mRNAs. Following infection by the virus, the viral RNPs are transported to the nucleus and mRNA synthesis begins. During synthesis of mRNA, influenza engages in a process called "cap-snatching." Capped cellular pre-mRNAs present in the nucleus are bound by NS1, and the 5′-terminal 10–13 nucleotides, containing the 5′ cap, are removed by PB2. This oligonucleotide is used to prime synthesis of mRNA from the influenza genome segments, as illustrated in Fig. 4.13. Once initiated, other aspects of mRNA synthesis resemble

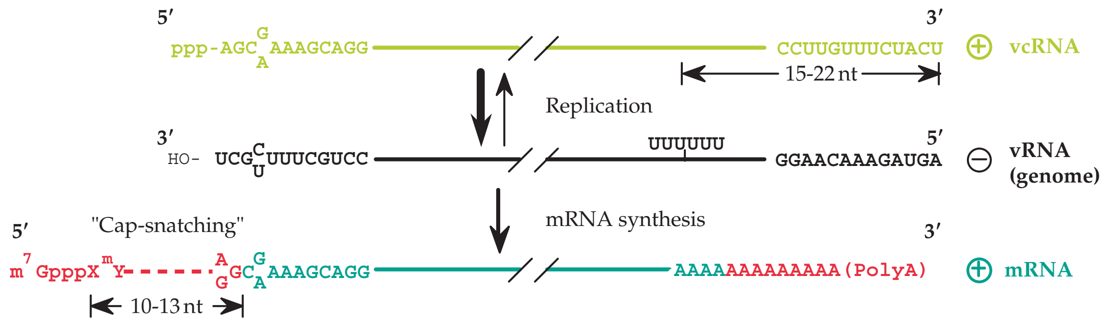


FIGURE 4.13 Relationship between genome RNAs, mRNAs, and vcRNAs of influenza virus. Transcription of mRNAs in the cell nucleus requires a primer of 10–13 nucleotides derived from cellular pre-mRNAs by "cap-snatching," and mRNAs terminate with a poly(A) tail. Those portions of the mRNA which are not complementary to the genome RNA are shown in red. In contrast, vcRNAs are exact complements of the genomic minus strands. Adapted from Strauss and Strauss (1997).

those that occur in rhabdo- and paramyxoviruses. Synthesis continues to near the end of the genome segment, where an oligo(U) stretch is encountered. Here the enzyme stutters to produce a poly(A) tail on the messenger and then releases it. In addition to its role as a primer, using a cap derived from cellular mRNA relieves the virus of the necessity of encoding enzymes required for capping and ensures that the virus mRNA has a cap suitable for the cell in which it is replicating. This mechanism also results in interference with the synthesis and transport of host mRNAs. Furthermore, because the mRNAs have a different 5′ end and lack the 3′ end of the antigenomic RNA, they lack promoters required for replication and packaging and are therefore dedicated mRNAs.

Each genome segment gives rise to one primary mRNA species. However, two of these can be spliced, and both the unspliced and spliced RNAs serve as messengers. Thus, two mRNAs are formed from each of two of the segments, and in total, 10 mRNAs are formed and 10 proteins are produced (11 in the case of viruses that also produce PB1–F2 described earlier). The formation of the two mRNAs from segment 7 and their translation into proteins is illustrated schematically in Fig. 4.14.

When sufficient amounts of viral proteins have been synthesized and transported to the nucleus, viral RNA replication begins. Replication requires encapsidation of progeny genomic and antigenomic RNAs as described for other (−)RNA viruses, and the mechanisms that lead to a switch between synthesis of mRNAs and replication are thought to be similar to those that occur in rhabdoviruses and paramyxoviruses. During replication, the viral genome is copied into a faithful antigenomic RNA (vcRNA) (Fig. 4.13), which is a perfect complement of the genome and serves as a template for production of genomic RNA.

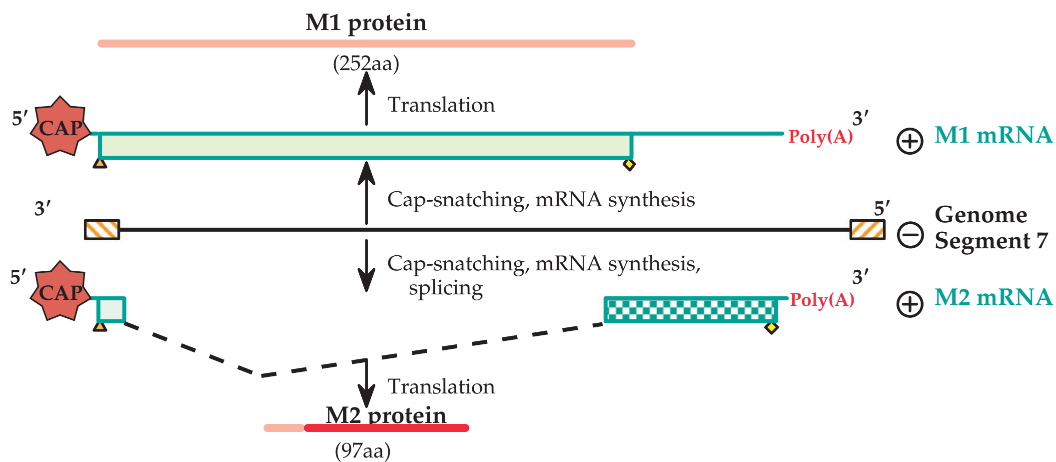


FIGURE 4.14 Synthesis of two mRNAs for the M1 and M2 proteins from gene segment 7 of influenza A. M1 RNA is translated from ORF 1 (open box). M2 RNA starts identically, but after the splice it is translated in ORF2 (checkered box). Both proteins are found in infected cells. The AUG initation codon is shown as a triangle; termination codons are shown as filled diamonds. Patterned boxes at the end of the genome RNA are self-complementary sequences that could form panhandles.

Synthesis of viral RNA, whether plus strand or minus strand, requires that the synthetase interact with both ends of the RNA, whether vRNA or vcRNA; that is, the promoter for synthesis of RNA is composed of elements from both ends of the RNA. This is analogous to what has been found for alphaviruses and flaviviruses, described in Chapter 3, and may be a general mechanism used by many or all RNA viruses. Thirteen nucleotides at the 5′ end of the vRNA and 12 nucleotides at the 3′ end are highly conserved in influenza A viruses and these seem to contain the entire promoter element. These sequences form an inverted terminal repeat and are capable of forming a panhandle structure (Fig 4.15A), bringing the two ends together where they might interact with the RNA synthetic machinery. An alternative structure, called the corkscrew structure, is thought to be the structure recognized by the synthetase for initiation of RNA synthesis (Fig. 4.15B). Cyclization is also hypothesized to play a role in addition of poly(A) to mRNAs, by causing the polymerase to stutter at the oligo(U) tract located just before the double-strand stem of the circular structure. Figure 4.15C illustrates an experiment to examine the sequence requirements within the panhandle or corkscrew structure.

Assembly of Progeny Virions

Influenza virus matures by budding of nucleocapsids through the cell plasma membrane. Virions are pleomorphic but clinical specimens are primarily filamentous and can be up to a micrometer or more in length. Upon passage in cell culture, most strains eventually give rise to virions that are primarily spherical, averaging 100 nm in diameter. The form that the virions assume is genetically determined. Studies of a strain of influenza A that remained filamentous after passage in cell culture could be induced to form spherical particles by changes in the M1 protein. The significance of filamentous versus spherical particles is unknown, but filamentous forms must have a selective advantage in the infected animal, whereas spherical forms seem to be selected upon passage in cell culture.

During assembly, the eight genome segments are reassorted in progeny virions if the cell is infected with more than one strain of influenza. Reassortment to produce viruses with mixed genomes is efficient—the segments are almost randomly reassorted to give all possible combinations of genome segments in the progeny virions. This

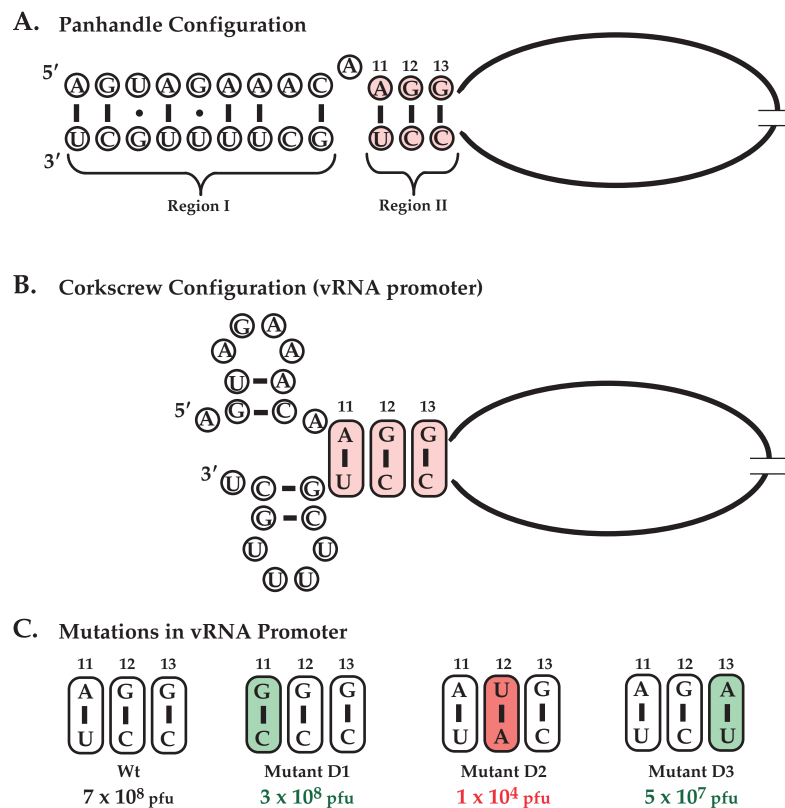


FIGURE 4.15 Models for the influenza A virus promoter. (A) The Panhandle model, with a partially double-stranded structure for the 5′ and 3′ terminal sequences. (B) The Corkscrew model predicting base pairing within the ends. After Neumann *et al*. (2004). (C) Alternative base pairs introduced into the vRNA promoter and the effect of these changes on viral yield in MDBK cells. Adapted from Catchpole *et al*. (2003), Figure 1.

process is analogous to the reassortment of chromosomes that takes place during sexual reproduction in diploid organisms.

Budding must result in the packaging of the 8 different genomic segments that constitute the viral genome into one virus particle if it is to be infectious. Reoviruses (Chapter 5) have an assembly mechanism whereby the 10–12 different segments are recognized and assorted so that each virus particle has one each of the different segments. The case for influenza virus is not completely clear. Evidence has been presented that the virus appears to package more than 8 segments, possibly about 10, that are randomly chosen from the intracellular pool. Random packaging of 10 segments would result by chance in about 3% of the virions having at least 1 each of the 8 different genome segments. However, more recent data argue that the virions package exactly 8 segments, one each of the 8 different segments. For this to occur, the packaging machinery has to recognize internal sequences in each of the segments and not just a packaging signal in the conserved ends of the viral RNAs.

Influenza A Virus

Natural History of Influenza Virus

Influenza A virus infects a wide variety of birds and mammals. A phylogenic tree that shows the relationships of the NP genes of viruses isolated from humans, pigs, and birds is shown in Fig. 4.16. The human isolates and the pig isolates are closely related; as described later the pig viruses probably originated from a human virus. The human–pig clade is distinct from the avian clade, however.

Influenza A viruses are characterized by their two major surface antigens, HA and NA. There are 16 different HA subtypes (numbered H1 to H16). HAs in different subtypes differ by 30% in sequence and are not immunologically cross protective. There are also 9 different NA subtypes (numbered N1 to N9). The major reservoirs of influenza A in nature are wild ducks and other waterfowl such as gulls, terns, and shearwaters, and viruses containing all 16 subtypes of HA and all 9 subtypes of NA have been isolated from waterfowl. Influenza replicates in the lung and in the gut of birds and the infection is normally asymptomatic (but epidemics of fatal influenza have occurred in turkeys and chickens, and the emerging H5N1 virus has caused fatal infection in a number of different bird species). Ducks can excrete virus in feces for weeks, infecting other ducks via contaminated water, and a significant fraction of ducks may become infected by the virus in this process. Migratory ducks then spread the virus around the world, normally in a north–south direction. The viruses in birds are in stasis. Almost no differences in amino acid sequences of the various proteins are present in viruses separated by many decades, although the nucleic acid sequences encoding these proteins do drift. This together with the fact that the viruses seldom cause disease in their avian reservoirs show that influenza in birds is ancient and the virus has adapted to its primary host.

The gene segments of influenza A virus reassort readily during mixed infection, and viruses with new combinations of genes arise frequently. Newly arising reassortants can cause major epidemics of influenza when introduced into humans, a process called antigenic shift. Not all combinations of genes give rise to viruses that are capable of epidemic spread in humans. Only three subtypes of HA (H1, H2, and H3) and two or three subtypes of NA (N1, N2, and possibly N8) have been found to date in epidemic strains of human influenza virus. The first influenza virus isolated, in 1933, was called H1N1. This virus first appeared as the cause of the great influenza epidemic of 1918 (see later). The virus isolated in the epidemic of 1957 had a different subtype of both HA and NA and was called H2N2. The H2N2 virus replaced the H1N1 virus as the cause of influenza epidemics (Fig. 4.17). The H2N2 virus was itself replaced by H3N2 virus beginning with the epidemic of 1968. Serological surveys suggest that prior to 1918 the virus that circulated was an H3N8 virus that first appeared as the cause of an epidemic in 1890. The reason that only a subset of HAs appear to be capable of causing epidemics in humans is, at least in part, the fact that the receptors for the virus are somewhat different in birds and humans. Sialic acid is linked to galactose predominantly by α2,6 linkages in humans but by α2,3 linkages in birds.

Similarly, only certain types of the other segments are compatible with infection of and epidemic spread in humans. For example, the nucleocapsid gene has diverged into five lineages, but only one of these lineages is present in viruses isolated from humans (see, e.g., Fig. 4.16). NS1 is also at least partially host specific and thus only certain NS1s are compatible with human infection. Other proteins also differ somewhat for optimal replication in birds versus mammals. It is thought that reassortment can result in the introduction of a new HA or NA gene into a human virus, that is, a virus whose other gene segments are optimized for human infection. The HA and NA proteins are the most important antigens of the virus, and change of one or both of these antigens gives rise to a virus for which the majority of the human population has no immunity and which is therefore capable of causing a global pandemic. One possible scenario is that pigs serve as intermediates (“mixing vessels”) in the recombination process, because pigs can be infected by both avian and human viruses (they contain sialic acid in both α2,3 and α2,6 linkage) and reassortment could occur in this host.

Influenza A virus is an example of a zoonotic disease in humans. The reservoir of the virus is ducks and other birds, and human infection is irrelevant for the maintenance of the virus in nature.

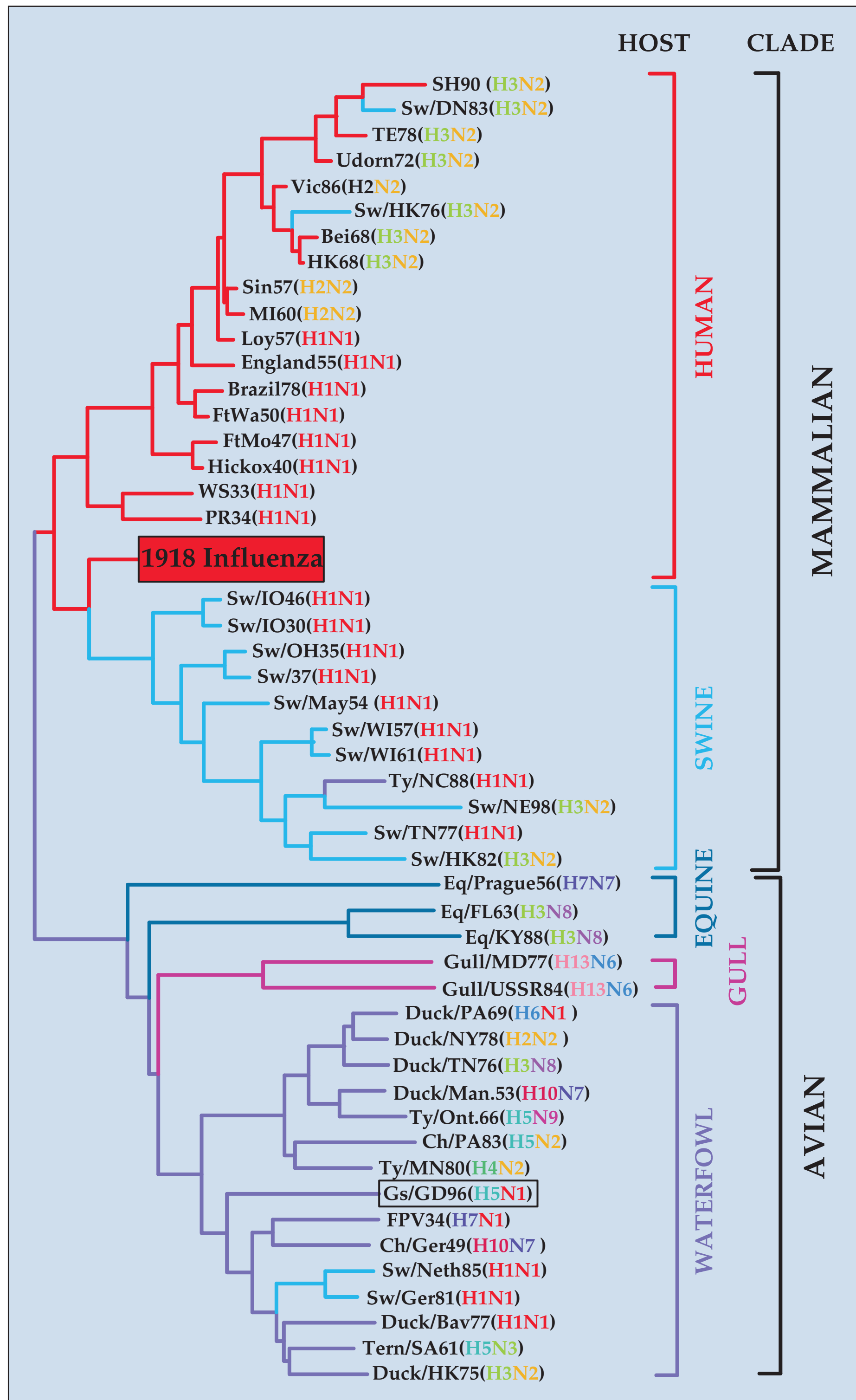


FIGURE 4.16 Phylogenetic tree of the nucleotide sequences of the influenza A virus NP gene sequences, constructed with a neighbor-joining algorithm. Each isolate name includes the location and year of isolation, preceded for non-human viruses by a species designation and a diagonal slash. Species abbreviations: Sw, swine; Ty, turkey; Ch, chicken; Gs, goose. Standard two letter abbreviations for states in the United States are used. Other location abbreviations: SH, Shanghai; DA, Dandong; Vic, Victoria; HK, Hong Kong; Sin, Singapore; Loy, Loygang; Ft Wa, Fort Warren; Ft Mo, Fort Monmouth; Man, Manitoba; Ont, Ontario; GD, Guangdong; Ger, Germany; Neth, Netherlands; Bav, Bavaria; SA, South Africa. The boxed isolate is the probable source of the H5 hemagglutinin in the currently worrisome "bird flu" spreading from China. Adapted from Reid *et al*. (2004), Figure 2.

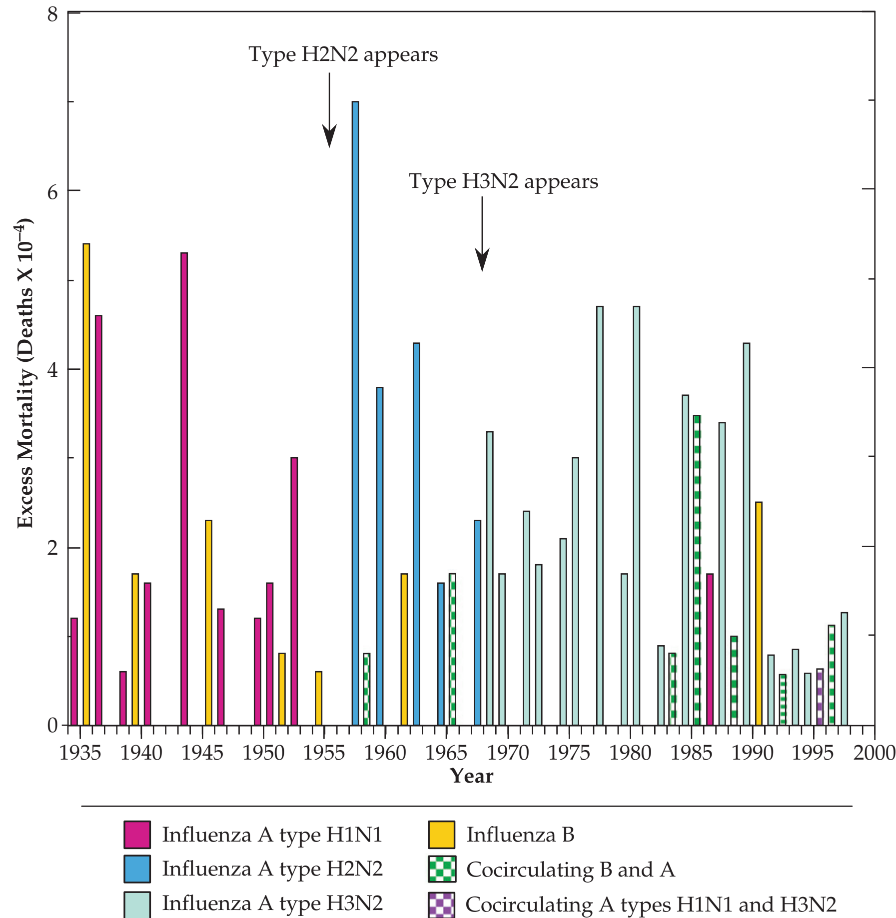


FIGURE 4.17 Excess mortality caused by influenza A and B virus in the United States between 1934 and 1998. "1935" refers to the winter of 1934–1935. Excess mortality due to the three dominant subtypes of influenza A and influenza B are indicated by the colors shown in the key. Green cross-hatched bars are excess mortality in years when both A and B viruses circulated. In 1955 and 1965, type H2N2 circulated with B, in 1983 and 1988 type H1N1 circulated with B, and in 1985, 1992, 1996, and 1998 H3N2 circulated with B. In 1995 H1N1 and H3N2 types of influenza A both circulated (hatched purple bar). Redrawn from Fields *et al*. (1996) p. 1421, with additional data from Thompson *et al*. (2003).

Epidemics of Influenza

Influenza A virus causes a serious human illness, influenza. It is perhaps confusing and unfortunate that the term *flu* is often used to describe any respiratory tract infection (and at times even infections of the gastrointestinal tract), even those that are fairly mild. The symptoms of true influenza are usually more severe than those resulting from other respiratory tract infections and include fever, headache, prostration, and significant muscle aches and pains (myalgias) that last for 3–6 days. Weakness and cough can last 1–2 weeks more. The fever can be high (39–40°C is not uncommon in adults and can be higher, especially in children). The morbidity that accompanies the disease can cause the patient to remain bedridden for a week or longer. In young children, the high fever can result in Reye's syndrome, an encephalopathy that may be fatal. The probability of contracting Reye's syndrome is higher if aspirin is administered to control the fever.

Lower respiratory tract infection can also occur following influenza infection and result in primary viral pneumonia. Invasion of the damaged lungs by pathogenic bacteria may follow and result in secondary bacterial pneumonia. Influenza can be fatal, usually because of pneumonia resulting from viral infection, whether the pneumonia is due to primary viral infection or, more commonly, due to secondary bacterial infection. Fatal infection is more common in the very young (whose immune system is not fully developed) and in the elderly (whose immune system may be waning). Before the advent of antibiotics, bacterial pneumonia killed many following severe bouts of influenza, but even today influenza remains a serious killer. It has been estimated that influenza virus infects 10–20% of the world's population every year causing five million cases of severe illness and 250,000 to 500,000 deaths. In the United States alone the estimated death rate from influenza in an average year is 20,000–30,000 and can be significantly higher in epidemic

years. People over 65 are at particular risk from influenza. The annual death rate in the United States from influenza A in people over 65 is 1 per 2200, and in an epidemic year the death rate may be 1 in 300 (i.e., 1 of every 300 people over the age of 65 die of influenza during the epidemic). The excess mortality caused by influenza is illustrated in Fig. 4.17, in which the different strains of influenza A or B responsible for the epidemics are indicated. Although influenza A is usually the most serious cause of mortality, in some years influenza B is more of a problem than influenza A.

The 1918 Influenza Epidemic

A pandemic of influenza erupted in 1918 due to the emergence of a virulent H1N1 strain. This extremely virulent virus swept around the world over a period of about a year and infected an estimated 30% of the world's population, causing 20–100 million deaths. Although the very young and the elderly are normally at the most risk from influenza, this influenza pandemic of 1918–1919 was unusual in that mortality was highest in healthy young adults. The age distributions of people dying of influenza and the related pneumonia are compared for the years 1917 and 1918 in Fig. 4.18. The much higher death rates in the young and the elderly in 1917, the normal pattern, is apparent. The dramatic increase in the death rate in the 20- to 29-year-old group in 1918, in which people of this age were more likely to die than the old and the young, is striking. Death rates in young adults 15–34 years of age were more than 20-fold higher in the 1918–1919 pandemic than in the preceding years, and the death toll in young adults in the United States was high enough that overall life expectancy dropped sharply, as illustrated in Fig. 4.19.

The overall mortality was perhaps 2% of the world population but in some regions of the world, for example, regions of Central America and certain islands in the Pacific, 10–20% of the entire population died in the epidemic. In some remote Alaskan villages, more than 70% of all adults died, usually as a result of the simultaneous incapacitation of the entire population so that supportive care was not available. The final death toll can never be known with certainty and estimates vary widely, from 20 to 100 million. The death toll exceeded that produced by World War I, which was ongoing at the time. In fact, 80% of deaths in the U.S. Army during World War I resulted from influenza, and it is thought that the final collapse of the German army in 1918 may have been precipitated by widespread influenza in the troops. The surgeon general of the United States had expressed the hope

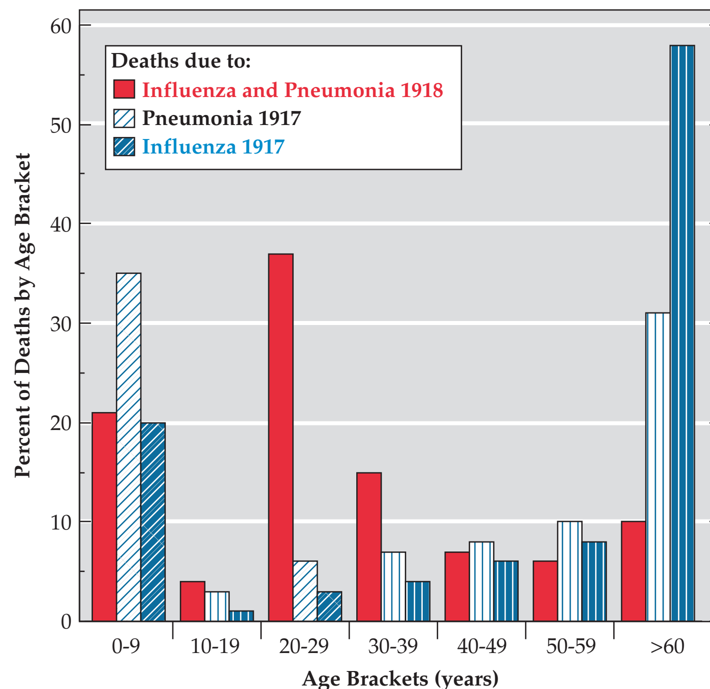


FIGURE 4.18 Age distribution of deaths due to pneumonia and influenza in the United States in 1917 and 1918. Age at death of patients has been divided into 7 intervals of 10 years each. The percent of deaths due to pneumonia in 1917, due to influenza in 1917, and due to the combined effects of pneumonia and influenza during the great epidemic year 1918 which fall into each age bracket are shown. The epidemic shows the atypical preponderance of deaths in the 20–29 and 30–39 year old brackets during the 1918 epidemic. Data from Crosby (1989). For comparison, from 1990 to 1998 only 3.8% of deaths due to influenza and pneumonia occurred in persons <49, 4.75% in persons 50–64, and 91% in persons over 65 years old (updated information from Thompson *et al*. 2003).

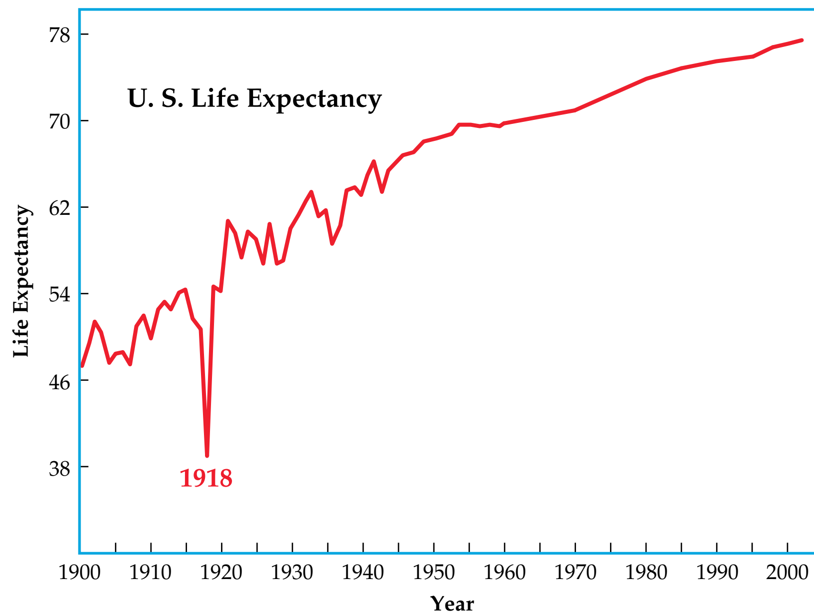


FIGURE 4.19 Life expectancy in the United States, showing the precipitous drop in 1918 because of deaths due to the "Spanish flu." This drop interrupted an otherwise fairly uniform increase in life expectancy that resulted from better health care, sanitation, and living conditions. Note also the leveling off in the late 1980s and 1990s due to AIDS. Adapted from *ASM News*, July 1999, and more recent data from the National Center for Health Statistics.

that WWI would be the first war in which more U.S. soldiers died of war injuries than died of disease, but this hope was shattered by the influenza epidemic. Descriptions of the epidemic with a focus on its effects on U.S. society are found in the books *Flu*, by G. Kolata, *America's Forgotten Pandemic*, by A. W. Crosby, and, quite recently *The Great Influenza*, by John M. Barry.

The reasons for the extreme virulence of the 1918 virus, and why healthy young people were more likely to die, a topic made even more important by the appearance of H5N1 "bird flu" (see Chapter 8) have been addressed recently using the power of modern molecular biology. The pandemic of 1918 occurred before influenza virus could be isolated. However, the sequences of all eight gene segments of the 1918 influenza genes have been obtained starting from a number of tissue isolates. Samples of preserved lung tissue taken at autopsy from two U.S. soldiers who died of influenza in September 1918 in New York and South Carolina were found to contain detectable influenza RNA, albeit in fragmented condition. A third source of influenza RNA came from an Alaskan Inuit victim who died in November 1918 and was buried in permafrost, and whose body was sufficiently well preserved that lung samples containing (fragmented) viral RNA were obtained. Two additional sources of influenza sequences come from two victims of influenza who died of pneumonia in November 1918 and February 1919 at the Royal London Hospital. Reverse transcriptase–polymerase chain reaction technology was used to obtain sequences from influenza RNA in these tissue samples that could be used to reconstruct the complete sequences of genome segments. The sequences from these five victims are almost identical and showed that the virus belonged to strain H1N1. The HA genes from these five humans differ by only one to three nucleotides despite the fact that they came from five humans whose deaths were separated by over 7500 miles and several months in time. The sequence of this gene places it in the human–swine lineage, not in the avian lineage, and at the root of the tree leading to later isolates of human or swine influenza (Fig. 4.20). Thus, the HA of the virus does not appear to have come directly from an avian source.

It is now possible to use reverse genetics to take a cloned DNA copy of an influenza gene and rescue a virus containing this gene. To do this, cells are transfected with up to 17 plasmids that express the 8 genome segments of influenza as well as the RNA polymerase proteins PB1, PB2, and PA, and the NP protein, and in some cases the other influenza proteins as well. Infectious influenza virus is produced and buds from the cell. Using this system influenza virus has been produced that contains various combinations of the 1918 HA and NA genes with other cloned genes from the 1918 virus or from recent isolates, including virus that contains the complete complement of the 1918 genes and thus is a complete reconstruction of the 1918 virus. Various constructs have been tested in mice. Whereas recent isolates of influenza virus cause only mild disease in mice, the 1918 virus causes severe, often fatal disease. In mice, a virus

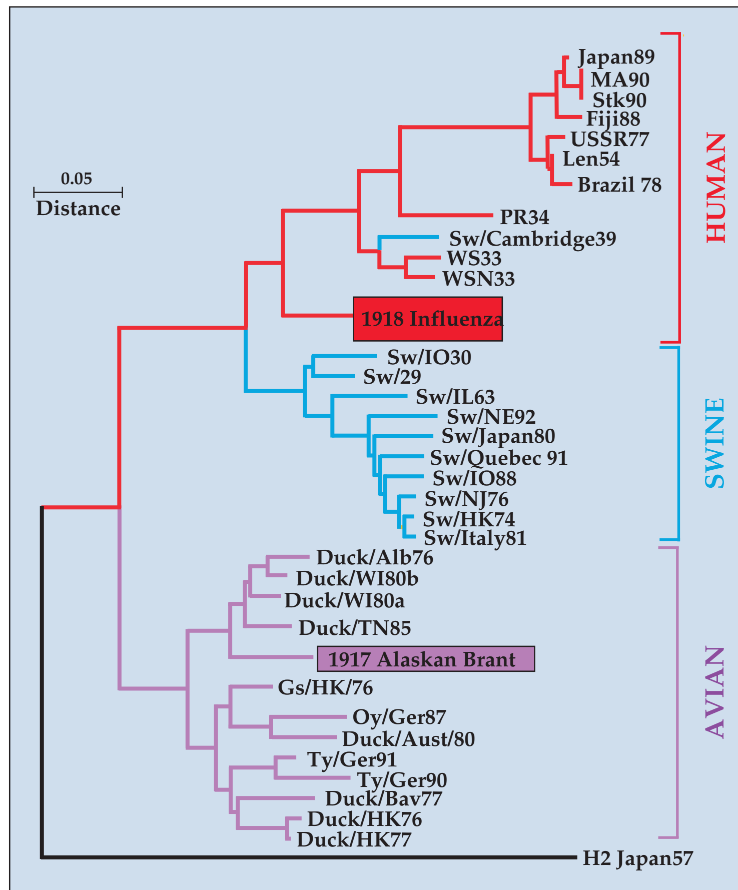


FIGURE 4.20 Phylogeny of the H1 hemagglutinin genes (bases 494–659 aligned to the comparable sequence of PR34). Viral names include species of isolation followed by location and year of isolation. Species include: Sw, swine; Gs, goose; Ty, turkey; Oy, oystercatcher. In the United States the standard two letter abbreviation for the state is used; outside the United States the following abbreviations are used: Len, Leningrad; Ger, Germany; HK, Hong Kong; Bav, Bavaria; Aust, Australia; Stk, Stockholm; Alb, Alberta. The sequence of the 1918 pandemic strain and the avian strain most closely related chronologically are boxed. A distance bar, where a distance of 0.05 = 11.2 synonymous differences, is shown above and beside the tree, and the H2 hemagglutinin of Japan 57 virus is used as an outgroup. Adapted from Fanning *et al*. (2002).

containing only the H1 and N1 of the 1918 virus was found to be highly virulent and caused fatal infection in mice. Virus grew to high titer in the lungs of the mice and was associated with an influx of neutrophils and macrophages into the infected lung.

The complete 1918 virus has also been tested, under BSL-4 high containment conditions, in monkeys as well as mice. The virus caused severe, usually fatal, disease in monkeys that was marked by much higher replication rates and more extensive spread in the lungs. It was also marked by an abnormal innate immune response (see Chapter 10). Certain elements of the innate response were attenuated, perhaps because of the activity of the NS1 gene which is known to interfere with the immune response. In contrast, other immune responses, in particular inflammatory cytokines, were enhanced, resulting in a "cytokine storm." The results are consistent with the hypothesis that in humans the 1918 virus provoked an extreme but unbalanced reaction by the immune system, and that healthy young people, who have the strongest immune systems, suffered from more extensive release of potent cytokines that resulted in more extensive tissue destruction.

The devastation caused by the 1918 virus raises continuing concern that a strain of influenza of equal virulence might appear and again cause immense suffering worldwide. New pandemic strains of influenza appear three or four times a century. If a pandemic strain emerged from a virus such as the H5N1 strain of bird flu (see Chapter 10), which has a very high mortality rate in humans, the resulting epidemic could indeed be devastating.

Antigenic Shift and Drift

Immunity to influenza A virus following infection is long lived but may not be complete and is subtype specific and even strain specific. The continuing appearance of new

strains that arise from antigenic drift and of new subtypes that arise from antigenic shift lead to continuing epidemics. Normally, two or three strains of influenza A circulate in the human population at any one time. Spread from person to person is by respiratory droplets, requiring close proximity, but people travel extensively and new strains of the virus speed around the globe as they arise. Antigenic drift is the process by which mutations accumulate in the virus genome, usually because of immune selection, that result in the development of new strains of the virus. These new strains are partially resistant to the immunity induced by infection with previous strains of virus. After several years of drift, the strain may be sufficiently distinct to cause disease in a person previously infected, but the illness is usually less severe because of partial immunity to the new strain. However, new strains capable of causing serious illness can arise by antigenic shift whereby reassortment results in change of the surface glycoproteins of the virus. The reassortants that cause the biggest problems are those belonging to a new subtype (as illustrated by Fig. 4.17). As described, such a new subtype may cause a pandemic in the human population because there is little immunity to the virus carrying these new surface antigens, as happened in 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2).

H1N1 virus, which had disappeared with the appearance of the H2N2 epidemic strain in 1957, suddenly reappeared in 1977. This H1N1 virus, which first appeared in northern China in May 1977 and was called the Russian flu, was virtually identical to influenza virus isolated from an epidemic in humans in 1950. It circulated in young people who had not been exposed to H1N1 virus. Because it was virtually unchanged despite 27 years having elapsed, it seems unlikely that it arose again *de novo*. Presumably this virus had been preserved in a frozen state, probably in a laboratory freezer. In 1976, in response to reports that investigators outside Western Europe planned to develop and test vaccines against H1N1 influenza, a WHO meeting report urged extreme caution in developing live vaccines from epidemic H1N1 strains because of the possibility of spread of the virus. One year later the virus reappeared.

Vaccination against Influenza A Virus

Because of the seriousness of influenza disease, especially in the elderly, attempts are made each year to vaccinate the population at risk. Because of drift and shift, the vaccine must be reformulated every year to reflect the viruses currently circulating in the human population. There are three strains of virus included in the most common vaccine, an inactivated virus vaccine produced from viruses grown in eggs. These are two influenza A viruses and one influenza B virus. These viruses are chosen from those that are circulating in late spring, because these viruses are usually those that will cause epidemics the following winter. The choice must be made by late spring in order to allow time for the pharmaceutical companies to prepare the vaccines, and an element of risk is involved that the right choices will not be made. The World Health Organization publishes choices and supplies seed virus based upon the recommendation of an international group of scientists, but the final selections are made by individual health agencies and the choices are usually, but not always, correct. The number of vaccine manufacturers has declined dramatically in the United States over the last 2 decades because of legal liability problems, and what limited capacity that exists for manufacturing flu vaccine is mostly present in Europe. Production problems by one of the manufacturers has resulted in recent shortages of vaccine.

The necessity to grow the virus in fertilized eggs also limits the amount of vaccine that can be produced. There are efforts to develop a cell culture system for virus production for vaccine use, which could then be produced in larger amounts. Efforts are also being made to develop better adjuvents for use with the vaccine, which could reduce the amount of antigen required per inoculation. In addition, obtaining the reassortants required for vaccine production is a time-consuming endeavor using classical methods of coinfecting cells with two different viruses and searching through the progeny for the wanted reassortants. If reverse genetics described earlier can be developed in a way that satisfies the regulatory agencies concerned with vaccine safety, the desired reassortants could be obtained much more quickly, allowing quicker responses to new strains of virus.

In addition to the inactivated virus vaccine that is very widely used, a new live virus vaccine based on a cold attenuated virus has been licensed recently. Reassortment is used to introduce the HA and NA of the predicted epidemic strains into this attenuated virus. Because the attenuation of the virus results from changes in other genome segments, the recombinant strain is also attenuated. The vaccine is administered by nasal spray rather than by injection as is the inactivated virus vaccine. To date this vaccine is only licensed for use in people between the ages of 5 and 49, and thus it cannot be used for the populations most at risk for serious illness, but clinical trials are continuing. It remains to be seen how well accepted this vaccine will be.

The necessity of reformulating the vaccine every year is inconvenient for a number of reasons including the fact that the vaccine cannot be stored for use in the following years. In addition, the vaccine is not always effective because wrong predictions were made about which strains of virus would be the biggest problems. There is an effort being made to develop universal vaccines that would target all strains of influenza A and B, and that would therefore provide protection against all influenza strains and that could be used year after year. One possibility that is being pursued is to use influenza A M2 protein as an antigen. This protein is highly conserved among all A strains but is

not normally seen by the immune system for some reason. Preliminary studies have shown that this protein linked to hepatitis B core protein is highly immunogenic in mice and provides protection against influenza A infection in mice, regardless of strain. For influenza B, a subunit vaccine based upon the sequence surrounding the cleavage site of the HA precursor, which includes a highly immunogenic part of the fusion peptide, shows promise in early animal trials.

Swine Flu Virus

Continuing surveillance of influenza strains in nature is required in order to reformulate the vaccines each year. This surveillance also serves to watch for the possible appearance of another killer strain of influenza. An episode that occurred during the Ford administration, however, illustrates the potential difficulties of identifying such a strain and reacting in time. In February of 1976, a young soldier at Fort Dix died of influenza and others became seriously ill. Tests showed that most of the soldiers were suffering from the A/Victoria strain of influenza that was epidemic in the United States at the time or from adenovirus infection. However, the soldier who died and three other soldiers who were ill were infected with an influenza strain that was epidemic in pigs, referred to as swine flu. Serology studies indicated that 200 or more other soldiers had been infected by this virus as well, showing that the virus was being transmitted from person to person. The swine flu virus was closely related to the 1918 pandemic virus, and is thought to have been introduced into pigs in 1918 from humans and to have continued to circulate in pigs after it had died out in humans. Could it be possible that the 1918 virus had reappeared as an epidemic virus in humans? The decision was made by President Ford, in consulation with leading scientists, to begin a crash program to develop a vaccine against swine flu and to begin to immunize the American population. It was thought, with some justification, that to wait for an epidemic to begin before an immunization program was undertaken would mean that it would be too late to be effective, given the speed with which influenza epidemics spread. Further, influenza is usually epidemic in winter, and the early detection of this virus made possible the preparation of a vaccine before the (next) winter flu season set in. Forty million Americans were immunized against swine flu. No epidemic of swine flu developed, however, and litigation began. The pharmaceutical companies had been reluctant to participate in the program, pointing out that at any one time a certain fraction of Americans would develop encephalitis or rheumatoid arthritis or any one of hundreds of other diseases. If disease developed in proximity to receiving a new and relatively untested vaccine, a lawsuit would certainly follow and the potential damages were enormous. The program could only advance when Congress agreed to indemnify the pharmaceutical houses. The vaccine was never conclusively shown to cause disease, although there seemed to be a slight increase of Guillain-Barré syndrome following inoculation. Litigation went on for years and substantial damages were paid out. In retrospect it is easy to criticize the program as an overreaction, but what would have been the reaction if nothing had been done and an influenza epidemic developed that resulted in 50–100 million Americans becoming seriously ill with 1–2 million deaths? Given the state of knowledge at the time, many leaders felt there was no choice. Further, the decision to vaccinate was not so different from current policy, where strains of influenza A circulating in the spring are incorporated into a vaccine to be given in the fall. A quote from the U.S. Surgeon General at a meeting of the Association of State and Territorial Health Officiers in 1957 is worth thinking about: "I am sure that what any of us do, we will be criticized either for doing too much or for doing too little."

Bird Flu

A recent scare began when 18 people in Hong Kong became seriously ill from influenza in 1997 and 6 died. The culprit was an avian influenza (H5N1) that was epidemic in birds being sold in the markets for food. Avian viruses do not normally infect people, and there was fear that an avian virus had made the jump to humans and might cause an epidemic of lethal influenza. The Hong Kong authorities destroyed 1.6 million domestic birds in order to eradicate the epidemic in birds. No human-to-human transmission took place and the virus disappeared. In 2002, however, H5N1 virus reappeared and by 2006 it has spread throughout Asia and into Africa and Europe. This virus has a mortality rate of about 50% in humans and more than 140 people have died of H5N1 infection as of this date. There is no person-to-person transmission to date, but there is concern that the virus might mutate and cause a wide and devastating pandemic of influenza. This subject is considered at more length in Chapter 8.

Influenza B and C Viruses

Humans are the reservoir of influenza B virus. It causes influenza in humans but there exists only one subtype and antigenic shift does not occur. Antigenic drift does occur, and the virus can cause epidemics of serious illness that result in increased mortality, particularly among the elderly, as shown in Fig. 4.17. For this reason, the current strain of circulating influenza B is included in the annual flu vaccine. However, wide-ranging pandemics do not occur and the virus is therefore not as much of a problem as influenza A. Less attention has accordingly been given to the study of this virus. Influenza C is not a serious human pathogen and has been even less well studied.

Other Orthomyxoviruses

Thogoto virus is present in regions of Africa, southern Europe, and Asia. It is a tick-borne virus that is primarily known from infection of livestock such as cattle, camels, and sheep. There is significant amino acid sequence identity between some of the Thogoto proteins and their counterparts in influenza so these viruses are fairly closely related. It has only six genomic segments, however. Human infection is known to occur in endemic areas.

Infectious salmon anemia virus infects salmonid fish. Atlantic salmon are particularly susceptible to the virus and the virus is a particular problem for fish farming. Farm populations can suffer 100% mortality in outbreaks. The virus has eight gene segments.

FAMILY *BUNYAVIRIDAE*

The family *Bunyaviridae* contains more than 300 viruses grouped into five genera. A representative sampling of these viruses is shown in Table 4.9. Members of four genera, *Orthobunyavirus*, *Nairovirus*, *Phlebovirus*, and *Hantavirus*, infect vertebrates and contain important human pathogens, whereas viruses belonging to the genus *Tospovirus* infect plants. The human pathogens in the family variously cause hemorrhagic fever, hantavirus pulmonary syndrome which can be fatal, encephalitis, or milder febrile illnesses, as shown in the table. Some of these pathogens were listed in Table 3.11, which contains a partial listing of arboviruses that cause disease in humans. All members of the *Bunyaviridae*

TABLE 4.9 *Bunyaviridae*

Genus/members[a]	Virus name abbreviation	Usual host(s)	Transmission/ vector	Disease in humans	World distribution
***Orthobunyavirus* (~48 viruses)**					
Bunyamwera	BUNV	Rodents, rabbits	*Aedes* mosquitoes	Febrile illness	Worldwide
La Crosse	LACV	Humans, rodents	*Aedes triseriatis*	Encephalitis	Midwest United States
Snowshoe hare	SSHV	Lagomorphs	Mosquitoes (*Culiseta* and *Aedes*)	Rarely infects humans	Northern United States
California encephalitis	CEV	Rodents, rabbits	*Aedes melanimon, Ae. dorsalis*	Encephalitis (rare)	Western United States, Canada
Jamestown Canyon	JCV	White-tailed deer	*Aedes* species, *C. inornata*	Increasing	North America
***Hantavirus* (~22 viruses)**					
Hantaan	HTNV	*Apodemus agrarius*	Feces, urine, saliva	Hemorrhagic fever	Eastern Asia, Eastern Europe
Seoul	SEOV	*Rattus* species	Feces, urine, saliva	Hemorrhagic fever	Worldwide
Prospect Hill	PHV	*Microtus pennsylvanicus*	?	None?	United States
Sin Nombre	SNV	*Peromyscus maniculatus*	Feces, urine, saliva	Pulmonary syndrome	Western United States and Canada
***Nairovirus* (~7 viruses)**					
Dugbe	DUGV	Sheep, goats	Tick-borne	?	Africa
Crimean-Congo hemorrhagic fever	C-CHFV	Humans, cattle, sheep, goats	Tick-borne	Hemorrhagic fever	Africa, Eurasia
***Phlebovirus* (~9 viruses)**					
Rift Valley fever	RVFV	Sheep, humans, cattle, goats	Mosquitoes, also contact, aerosols	Hemorrhagic fever	Africa
Sandfly fever Sicilian	SFSV	Humans	Phlebotomous flies	Nonfatal febrile illness	Mediterranean
Uukuniemi	UUKV	Birds	Tick-borne	??	Finland
***Tospovirus* (~8 viruses)**					
Tomato spotted wilt	TSWV	Plants	Thrips	None	Australia, Northern hemisphere

[a] Representative members of each genus are shown; the first virus listed is the type species.

except the hantaviruses are transmitted to their vertebrate or plant hosts by arthropods, and transovarial transmission is important in the maintenance of many of the arboviruses in nature. The hantaviruses, in contrast, are associated with rodents and are transmitted to humans by aerosolized excreta from infected rodents. Thus, their epidemiology resembles that of the arenaviruses considered later, rather than that of other bunyaviruses. In the following discussion, the term bunyavirus refers to any member of the family unless indicated otherwise.

Replication of the *Bunyaviridae*

Genome Organization

The genomes of representative viruses belonging to the five genera of the *Bunyaviridae* are illustrated in Fig. 4.21. All bunyavirus genomes consist of three segments of RNA, referred to as S(mall), M(edium), and L(arge), that together total from 11 to 19 kb, depending on the virus (Table 4.10). The S segment encodes the nucleocapsid protein, M the two surface glycoproteins, and L the polymerase protein. In addition, viruses belonging to three of the genera encode two nonstructural proteins, NS_s in segment S and NS_m in seg-ment M.

Replication of bunyavirus genomes and the synthesis of mRNAs take place in the cytoplasm. The L protein and N protein are required components of the RNA synthesis machinery. Like influenza viruses, these viruses engage in cap-snatching in order to prime mRNA synthesis. In bunyaviruses, however, the caps are captured from cytoplasmic mRNAs rather than from nuclear pre-mRNAs. The promoter for mRNA synthesis and for RNA replication involves nucleotides located at both ends of the genomic template, which

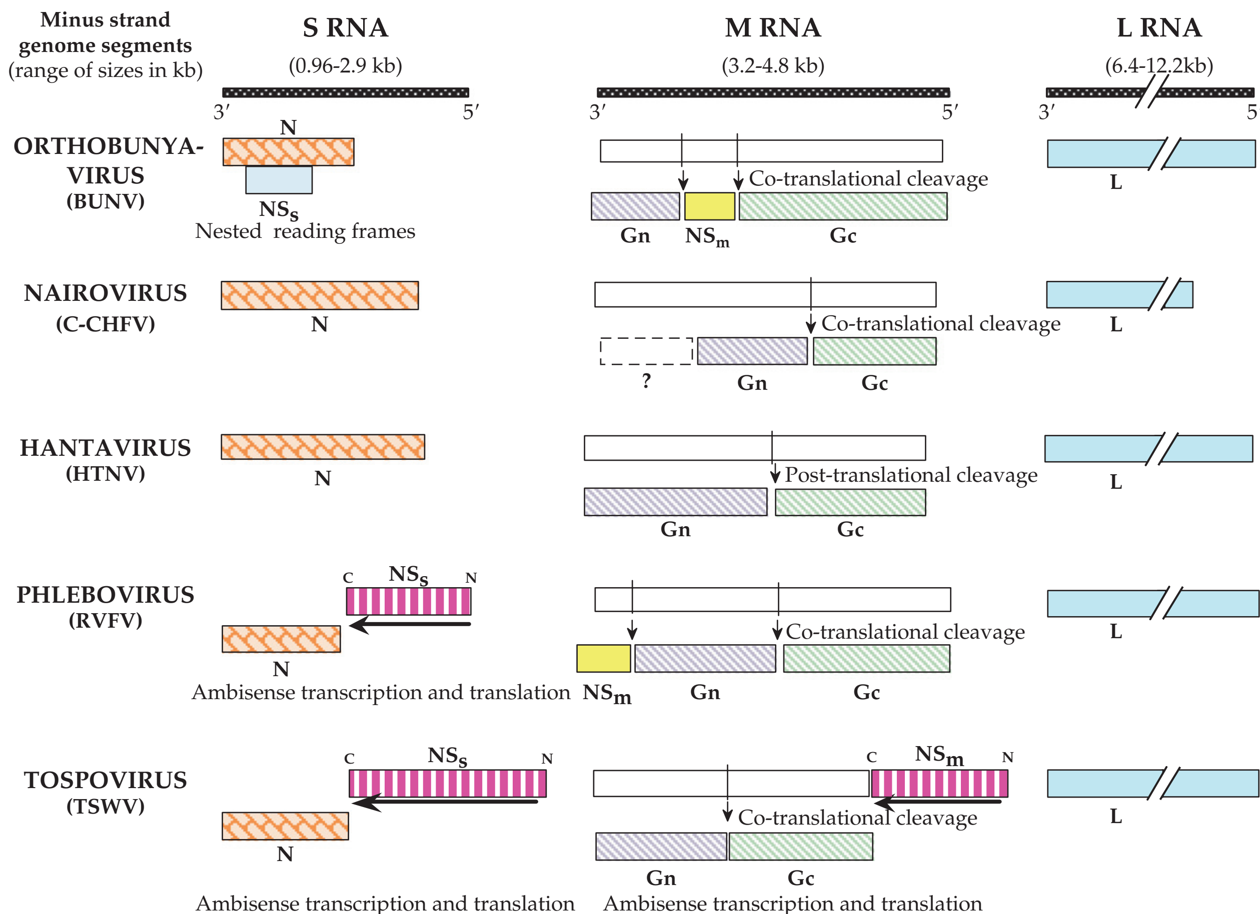


FIGURE 4.21 Genome organization of five genera of *Bunyaviridae*. Protein products encoded in each of the three genome segments and the various strategies used to produce these proteins are shown. Unless otherwise noted the mRNA (not shown) would extend 5' to 3' from left to right, and the protein product is shown N terminal to C terminal in the same direction. The products are illustrated roughly to scale. Structural proteins are N (the nucleocapsid protein) and the two glycoproteins, named Gn and Gc according to their proximity to the N or C termini of the precursor polyprotein; white boxes are precursor proteins. Arrows indicate the direction of synthesis and translation of ambisense mRNAs (magenta hatch). Note that not all phleboviruses encode an NS_m protein on the M segment. Virus abbreviations are as follows: BUNV, Bunyamwera; RVFV, Rift Valley fever; TSWV, tomato spotted wilt; C-CHFV, Crimean-Congo hemorrhagic fever; HTNV, Hantaan.

TABLE 4.10 Deduced Sizes (kD) of Proteins Encoded by *Bunyaviruses*

RNA (nts) Protein (kD)	Genus				
	Orthobunyavirus	Hantavirus	Nairovirus	Phlebovirus	Tospovirus
L Segment	6875–6980	6550–6562	12,255	6404–6423	8776–8897
L protein (RNA Polymerase)	259–263	246–247	459	238–241	330–332
M Segment	4458–4526	3616–3696	4888	3231–4215	4821–4972
Glycoprotein Gn	29–41	68–76	30–45	50–72	46–58
Glycoprotein Gc	108–120	52–58	72–84	55–75	72–78
Precursor preG	—	—	78–85, 92–115	—	—
Nonstructural NS_m	15–18	None	None	None or 78	34–37
S Segment	961–980	1696–2059	1712	1690–1869	2916–2992
Nucleoprotein N	10–26	48–54	48–54	24–30	29
Nonstructural NS_s	10–13	None	None	29–32	52

Sizes of precursor proteins are shown in blue, nonstructural proteins are shown in green, and those translated from ambisense transcripts are in red.

are complementary and form hairpin circles, but with some unpaired nucleotides that are thought to be important recognition signals (Table 4.11). Thus, as with many other RNA viruses, the viral RNA polymerase must interact with both ends of the RNA template in order to initiate synthesis, and complementarity between nucleotides at the 5′ and 3′ ends is required for promoter recognition. The terminal complementary sequences are highly conserved within each genus of bunyaviruses but differ between genera (Table 4.11). Perhaps because of this, reassortment occurs only between viruses belonging to the same genus.

During initiation of mRNA, as studied in hantaviruses, the L protein cleaves the 5′-terminal 7–18 nucleotides from a cellular mRNA. Cleavage is after a G residue, which pairs with the C residue at position 3. The primer is elongated by a few residues, and there is then a backward shift of 3 nucleotides so that the mRNA attached to the primer begins precisely at the 3′ end of the template. This "prime and realign" strategy (Fig. 4.22) works because of the repeat triplets at the 3′ end of the RNA template (Table 4.11). Transcription continues to near the end of the template, but the termination of an mRNA does not appear to be precise and the exact mechanism used for termination of the mRNAs is not known. No poly(A) is added to the 3′ end of the mRNA upon its release and, thus, the mRNAs are capped but not polyadenylated.

During replication of the genome, an exact complementary copy, called cRNA or vcRNA, is produced. This RNA serves as a template for producing genomic RNA and, in the case of ambisense segments, for producing the mRNA for producing the ambisense-encoded protein. The switch to replication is assumed to use the same mechanisms as used by other (−)RNA viruses.

TABLE 4.11 Terminal Sequences of the Genome Segments of the Five Genera of *Bunyaviridae*

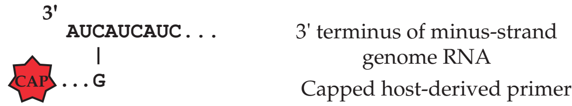
Genus	Nucleotide sequences of the L, M, and S segments
Hantavirus	5′ UAGUAGUA …
	3′ AUCAUCAUCUG …
Orthobunyavirus	5′ UCAUCACAUGA …
	3′ AGUAGUGUGCU …
Nairovirus	5′ AGAGUUUCU …
	3′ UCUCAAAGA …
Phlebovirus	5′ UGUGUUUC …
	3′ ACACAAAG …
Tospovirus	5′ UCUCGUUA …
	3′ AGAGCAAU …

Repeated sequences are underlined.

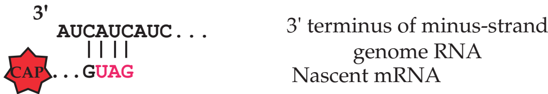
Expression of Proteins Encoded in S

The S segment of bunyaviruses encodes one or two proteins (Fig. 4.21). In the *Hantavirus* and *Nairovirus* genera, S encodes only N. In the other genera, S encodes both N and NS_s, using one of two different mechanisms. In the genus *Orthobunyavirus*, the two proteins are translated from a single mRNA using two different start codons in different reading frames. The coding region for NS_s is completely contained within that for N. In the phleboviruses and tospoviruses, however, an ambisense coding strategy is used for the two proteins (*ambi* = both). In this strategy, the two genes encoded in a genomic segment are linked tail to tail so that they are in different polarities, as illustrated in Fig. 4.23. The gene for N is present at the 3′ end of the genomic S segment in the minus-sense orientation, and synthesis of the mRNA for N occurs from the genome segment. Expression of this gene occurs early because its mRNA is

Step 1 - Priming



Step 2 - Initial Elongation



Step 3 - Realigning

Step 3 - Final Elongation

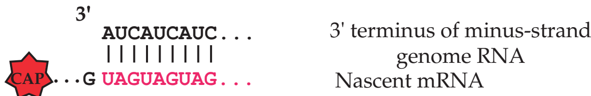


FIGURE 4.22 Steps in the prime–align mechanism of transcription of mRNAs by hantaviruses. This mechanism is made possible by the nucleotide repeats in the 3′ and 5′ termini of bunyaviruses (see Table 4.11). Adapted from Kukkonen *et al*. (2005).

synthesized from the entering genome by the polymerase activity present in viral nucleocapsids. The gene for NS_s is plus sense within the genome, but the genomic RNA does not serve as mRNA. Instead, an mRNA for NS_s is synthesized from the antigenomic RNA. Thus, NS_s is expressed late because its mRNA can only be made after replication of the incoming genomic RNA to produce the antigenomic RNA. Termination of either mRNA occurs at a secondary structure between the genes for N and NS, which appears to cause the polymerase to fall off and release the mRNA.

N has a number of functions in viral infection. It encapsidates the viral RNA, interacts with L to synthesize viral RNAs, and is believed to interact with one of the glycoproteins during virus assembly. In at least some viruses the protein also modifies cellular metabolism, presumably antagonizing antiviral defenses of the cell or otherwise subverting cellular processes to support viral replication. The N protein of hantaviruses, which is larger than those of other bunyaviruses except for that of the nairoviruses, interacts with a number of cellular proteins. The best studied of these are proteins in the small ubiquitin-like protein (SUMO) pathways such as Ubc-9 (which conjugates SUMOs to proteins), SUMO-1 itself, and Daxx (to which SUMOs are conjugated). Sumolation of proteins is an important regulatory process in cellular metabolism.

The NSs protein of Rift Valley fever virus, and presumably of other viruses as well, inhibits host mRNA synthesis, including the mRNA for interferon-α and -β. Thus it suppresses the host immune response and is a major virulence factor.

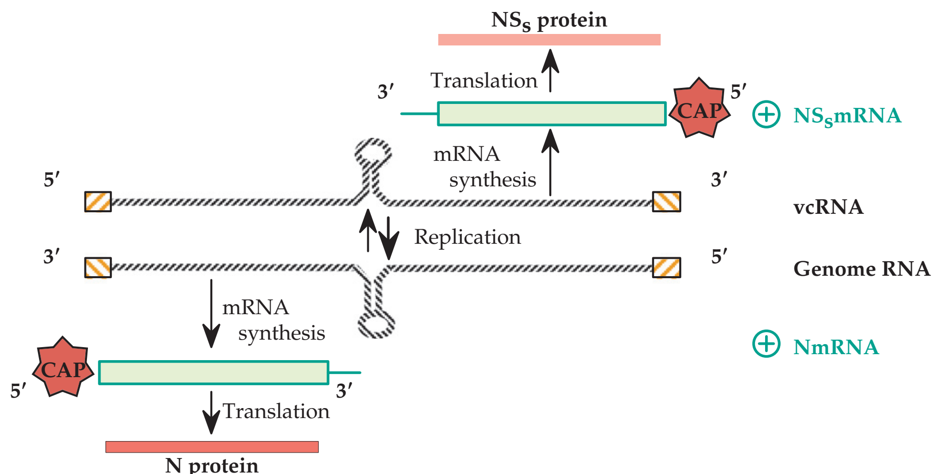


FIGURE 4.23 Ambisense coding strategy of the S RNA of a phlebovirus, family *Bunyaviridae*. The mRNA for the N protein is synthesized from the S genome segment using primers derived by cap-snatching (similar to the mechanism for influenza mRNA priming in Fig. 4.13) from cytoplasmic host mRNAs. The mRNA for the NS_s protein is formed in the same way, but with vcRNA as the template. Diagonally striped boxes are the self-complementary termini. The loops in the middle of the viral genomic and antigenomic RNAs indicate a secondary structure in the RNAs which terminates synthesis of the mRNAs. No poly(A) is added to the 3′ terminus of the mRNAs.

Expression of Proteins Encoded in M

Two glycoproteins, at one time usually called G1 and G2 but now called G_N and G_C, are translated from mRNA made from M (Fig. 4.21). They are produced as a polyprotein that is cleaved by a cellular protease to separate the two glycoproteins, analogous to what happens in some of the (+)RNA viruses that have envelopes (e.g., coronaviruses and flaviviruses). G_N is N terminal in the polyprotein and G_C is C terminal. Where studied, the enzyme responsible for the cleavage is a subtilase, SKI-1/S1P or a related enzyme. G_N and G_C form a heterodimer that is transported to the Golgi apparatus. Virus budding occurs at the Golgi membrane. Heterodimerization recalls the processes that occur in the assembly of alphaviruses and flaviviruses, and like E1 or E of these viruses, respectively, the G_C protein is a class II fusion protein.

The M segments of hantaviruses and nairoviruses encode only the two glycoproteins, but in the other three genera M encodes a third protein called NS_m (Fig. 4.21 and Table 4.10). In phleboviruses and members of the genus *Bunyavirus*, NS_m forms part of the polyprotein translated from the single mRNA produced from M. NS_m is formed during posttranslational processing of the polyprotein. In tospoviruses, an ambisense strategy is used to encode NS_m and the translation strategy is the same as that shown in Fig. 4.23. The function of NS_m is not known.

There is no matrix protein in bunyaviruses. Budding at the Golgi membrane is assumed to involve a direct interaction between the glycoproteins and the nucleocapsid protein. The virion is spherical, 80–120 nm in diameter. The three nucleocapsids are circular when isolated from the virion.

Genus *Orthobunyavirus*

There are about 50 currently recognized species in the genus *Orthobunyavirus*, of which the majority have several distinct strains that are often given separate names. Together, these viruses have a worldwide distribution. Most of these viruses are mosquito-borne, although some are tick-borne and some may be transmitted by culicoid flies or phlebotomines. They are true arboviruses, replicating in the arthropod vector as well as in vertebrates.

Bunyamwera virus, the prototype member of the genus, was first isolated in Uganda in 1943. It causes a febrile illness accompanied by headache, arthralgia, rash, and occasional nervous system involvement. There are 24 named subtypes in the bunyamwera serogroup. Of interest is a reassortant virus isolated during an epidemic of hemorrhagic fever in Kenya and Somalia in 1998. Rift Valley fever virus was responsible for some of the cases, but many were caused by a bunyavirus whose L and S segments were derived from a Bunyamwera virus but whose M segment came from a different bunyavirus. This reassortant virus, named Garissa virus, thus caused a disease different from Bunyamwera virus, perhaps due to the different properties of the M segment.

Viruses belonging to the California encephalitis group, of which La Crosse virus is the best known, are also of medical interest. La Crosse virus was named for the town of La Crosse, Wisconsin, where it was first identified as the causative agent of encephalitis, primarily in children. About 100 cases per year of encephalitis are caused by La Crosse virus, concentrated in the Midwest. Mortality is low (0.3%) but 10% of patients suffer neurological sequelae. No vaccine exists for the virus and control measures have involved control of the mosquito vector. The principal vector of La Crosse is *Aedes triseriatus*. This mosquito breeds in tree holes, but abandoned tires filled with rainwater constitute an important breeding area for it close to human habitation. Such abandoned tires serve as a beautiful incubator for the development of mosquito larvae, and efforts to eliminate this source of mosquitoes, as well as the institution of other mosquito control measures, has resulted in a reduction in the number of cases of disease.

Abandoned tires are important in the transmission of other arboviruses as well. Old tires are abundant in Puerto Rico, for example, and contribute to the endemic transmission of dengue virus, all four serotypes of which are present on the island. Old tires have also been responsible for the introduction into the United States of *Aedes albopictus*, the so-called Asian tiger mosquito that is the vector of dengue virus in Asia. Loads of old tires that were brought from Asia to Houston for recycling contained eggs or larvae of the mosquito. After its introduction into the Houston area, this mosquito spread over large areas of the United States and there is fear that it might become an efficient vector of arboviral disease in this country.

Genus *Phlebovirus*

The ICTV currently recognizes 9 species of phleboviruses but there are an additional 16 tentative species. Many of the species have a number of strains that are given their own names. All are arboviruses transmitted by mosquitoes, phlebotomine flies, or ticks. The most important of these is Rift Valley fever virus, an African virus that was first isolated in 1930 in the Rift Valley of East Africa. The virus is transmitted by mosquitoes and causes hemorrhagic fever in humans. It also causes disease in domestic animals, and many widespread epidemics in cattle, sheep, and humans have occurred over the years in Africa. In 1977–1978, for example, an epizootic in Egypt infected 25–50% of cattle and sheep in some areas, and 200,000 human cases resulted in at least 600 deaths. A more recent large epidemic in East Africa in 1997–1998 was associated with the heavi-

est rainfall in 35 years, 60–100 times normal in some areas. As described before, the epidemic was caused by Rift Valley fever virus and by Garissa virus. Losses of 70% of sheep and goats and 20–30% of cattle and camels were reported, and there were hundreds of cases of human hemorrhagic fever. Contact with livestock was statistically associated with acute infection with Rift Valley fever virus, indicating that during epidemics contact transmission becomes important as a means of spread to humans. Laboratory-acquired cases contracted through aerosols are also known.

Sand fly fever virus is transmitted by phlebotomine flies and causes an acute, nonfatal influenza-like disease in man. It is found in the Mediterranean area, North Africa, and Southwest Asia. Related viruses are found in South America.

Genus *Nairovirus*

The nairoviruses have a much larger genome than members of the other genera, primarily because the L segment is twice the size of those of the other genera of animal viruses (Table 4.10). They are named for Nairobi sheep disease virus, now considered a strain of Dugbe virus. There are seven species recognized, all of which consist of multiple strains with distinct names. The viruses are tick-borne although a few can also be transmitted by culicoid flies or mosquitoes. Nairobi sheep disease virus causes acute gastroenteritis with hemorrhagic symptoms in sheep and goats, with mortality rates over 90% in some populations. It was first identified as the causative agent of the disease in 1917 and is transmitted by the tick *Rhipicephalus appendiculatus*. Humans can be infected by the virus but suffer only mild illness. There is a close relative of the virus called Ganjam virus present in India which also causes disease in sheep and goats; it is transmitted by the tick *Haemaphysalis intermedia*.

Crimean-Congo hemorrhagic fever virus (CCHF) is the most important nairovirus in terms of human disease. It was first identified in the 1940s in the Crimean region of the former USSR and in the Democratic Republic of Congo. The virus is now known from at least 30 countries. It is found from southern Africa through Eastern Europe and the Middle East to western China. The principal vector is *Hyalomma* ticks, but *Dermacentor* and *Rhipicephalus* ticks can also transmit the virus. Sheep, goats, cattle, ostriches, wild herbivores, and hares become infected by CCHF but most infections result in subclinical disease. In contrast, infection of humans results in severe hemorrhagic fever with a 30% mortality rate. Humans are infected by the bite of a tick or by contact with blood or tissues of infected livestock. Transmission to hospital personnel treating infected patients has occurred.

Genus *Hantavirus*

There are 22 species of hantaviruses currently recognized, and, as with other genera of the bunyaviruses, many of the species have a number of named strains. Many hantaviruses cause serious human disease, including hemorrhagic fevers and hantavirus pulmonary syndrome. Unlike other members of the *Bunyaviridae*, they are not arboviruses. The hantaviruses are associated with rodents, which form their natural reservoir, and are transmitted to humans through contact with aerosolized urine or feces from infected rodents. Each hantavirus establishes persistent infections in one particular species of rodent and is maintained in nature in this way. Humans are not an important host for the virus and do not contribute to its maintenance in nature. Related to this is the fact that the viruses do not cause serious disease in their rodent hosts, but many cause quite serious illness in humans.

An evolutionary tree of hantaviruses is shown in Fig. 4.24. The rodent hosts for the viruses are also indicated. The viruses assort by host rather than by geographical proximity. All of the viruses whose hosts belong to the order *Murinae* group together, as do those that use rodents in the order *Arvicolinae* and those that use rodents in the order *Sigmodontinae*. As one example, consider Prospect Hill virus and New York virus, both found in the northeastern United States. Prospect Hill virus is associated with rodents of the genus *Microtus*, order *Arvicolinae*, and is more closely related to Puumala virus of Europe, which uses *Clethrionomys glareolus*, order *Arvicolinae*, than it is to New York virus. New York virus is associated with rodents in the genus *Peromyscus*, order *Sigmodontinae* and is closely related to Sin Nombre virus of the southwestern United States, which is associated with *Peromyscus maniculatus*. The fact that the evolutionary tree of the hantaviruses resembles that of their rodent hosts rather than being based on geographical proximity is evidence that they have coevolved with their rodent hosts over a very long period of time.

The first of the hantaviruses to be identified was the causative agent of more than 3000 cases of hemorrhagic fever with renal syndrome, now called Korean hemorrhagic fever, that occurred in U.S. troops during the Korean war. The virus was called Hantaan virus after a river in the area where it was isolated. In Korea, Hantaan virus is associated with the field mouse *Apodemus agrarius*. The virus also occurs in Eastern Europe and China, where it is associated with *Apodemus flavicollis* and causes a disease similar to Korean hemorrhagic fever (Fig. 4.25).

Viruses related to Hantaan virus have now been isolated from all over the world, including the Americas. Many Old World viruses cause hemorrhagic fever in humans, and more than 100,000 cases occur worldwide with a case fatality rate between 0.1 and 10%, depending on the virus. Puumula virus occurs in Western Europe (Fig. 4.25) and causes a disease characterized by acute fever with renal involvement. Seoul virus, first identified in Seoul, Korea, is associated with wild urban rats (*Rattus norvegicus*) and has been found all over the world because wild urban rats have been inadvertently

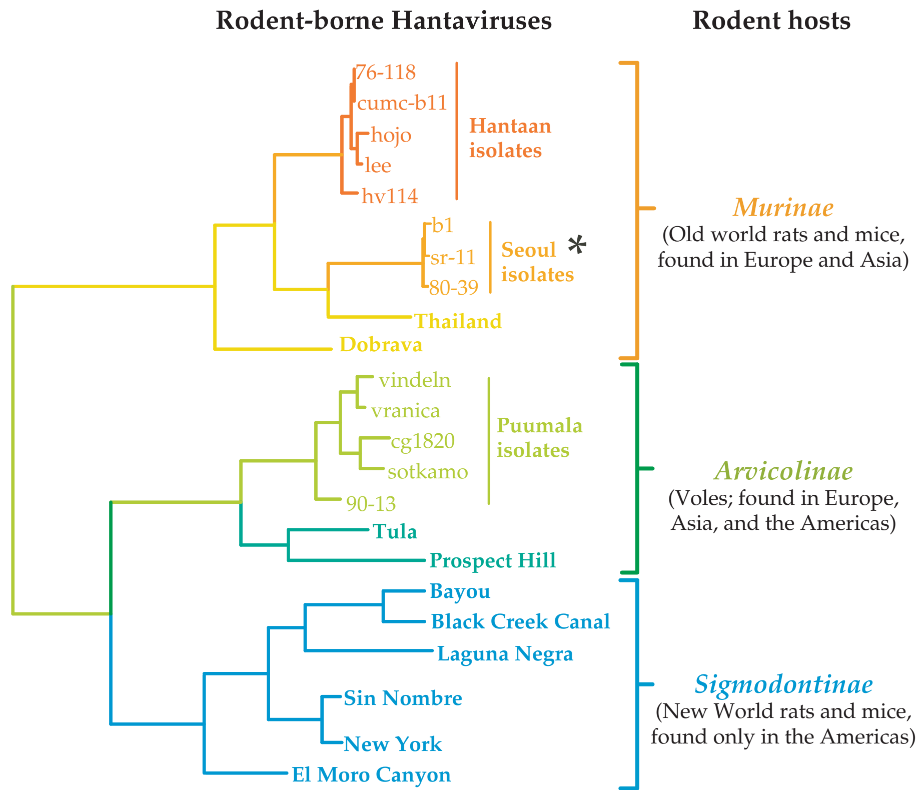


FIGURE 4.24 Phylogenetic tree of rodent-borne hantaviruses derived from the nucleotide sequence of the M RNA segment. This tree illustrates that hantaviruses have coevolved with their rodent hosts for millions of years. However, note (*) that in contrast to other members of this group, Seoul virus, which infects *Rattus norvegicus,* is found worldwide, due to the widespread distribution of these rats. Adapted from Peters (1998a), Figure 2.

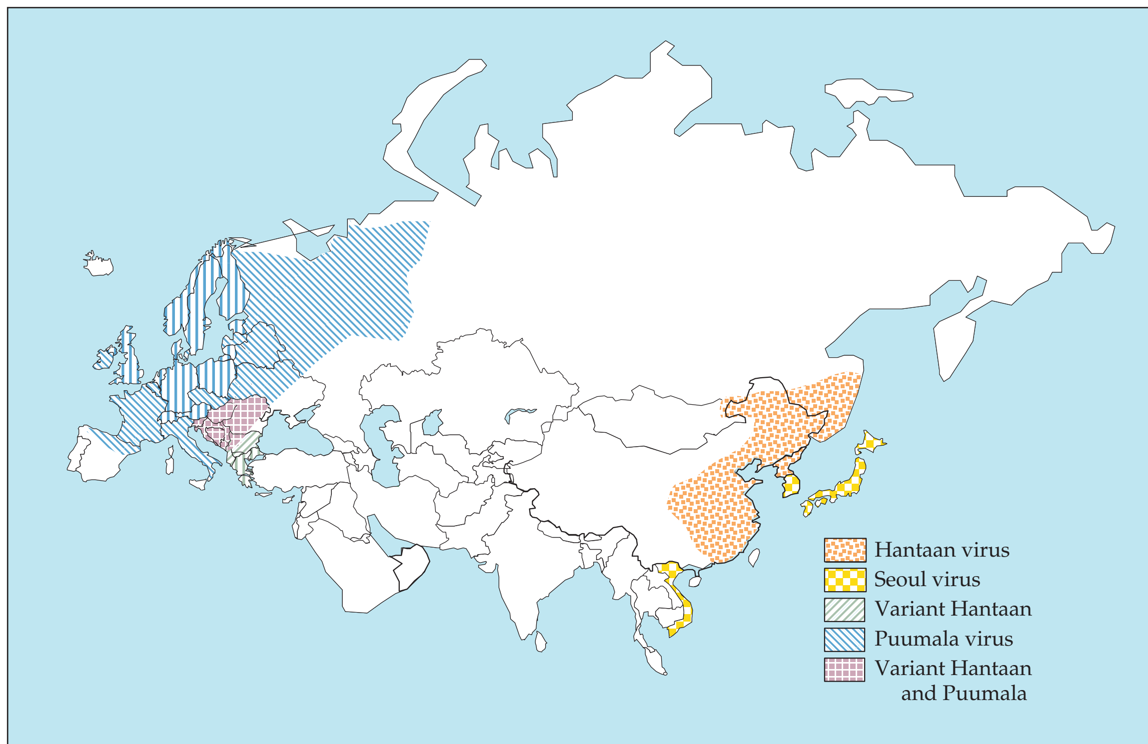


FIGURE 4.25 Map of Eurasia showing the disjunct distribution of different hantaviruses. Adapted from Porterfield (1995) p. 276 and data from Lee (1996).

introduced almost everywhere. It causes a mild form of Korean hemorrhagic fever in Seoul but does not cause apparent illness in most other areas where it has been found. The discovery of Seoul virus led to an intensive study of rats in central Baltimore, where it was found that a high percentage of them were infected with Seoul virus and, furthermore, that a substantial fraction of the people living in the slums of downtown Baltimore showed evidence of infection by hantavirus. No disease is known to be associated with this virus, but statistical studies suggest that infection may lead to high blood pressure and, possibly, renal failure.

The New World hantaviruses that cause disease in humans cause a syndrome called hantavirus pulmonary syndrome or HPS, which has a fatality rate of 20–40%. The first such virus to be identified was Sin Nombre virus, which caused an epidemic of HPS in the Four Corners area of the United States in 1993 that resulted in about 25 deaths. The virus is associated with the deer mouse *Peromyscus maniculatus*. Sin Nombre virus or related viruses have now been identified in virtually all states within the United States and into Latin America, and fatalities due to infection by the virus have occurred in many states. One of the cases in California is of interest because the person died more than a year before the Four Corners epidemic; retrospective studies of serum collected from the patient at the time of his hospitalization showed that he was infected with a hantavirus. The number of cases of HPS in the Americas from 1993 to 1998, totaled by country, and the names of the viruses responsible in various areas are shown in Fig. 4.26. Of interest is Andes virus, which has the potential for human-to-human transmission.

The mortality rate following infection with Sin Nombre virus or its close relatives is close to 50%. The mortality in the earliest cases was even higher because the pulmonary syndrome results from the rapid extravasation of fluids into

FIGURE 4.26 Cases of hantavirus pulmonary syndrome (HPS) in the Americas, with locations and names of the viruses responsible. Case numbers are cumulative totals from the time that HPS was recognized in 1993 in the Four Corners region of Arizona and New Mexico through 2004. Although several other hantaviruses have been isolated in this region, only those which have been identified as human pathogens are shown. To the current time, cases of HPS have been diagnosed in the United States in 30 states, predominantly in the Western United States, with a few cases as far East as Rhode Island. The total number of cases represented on this map is 1910. Adapted from Peters (1998a) Figure 1 and Table 3, and updated with data from Yates *et al*. (2002), and the Pan American Health Organization at http://www.paho.org/.

the lungs, which can result in respiratory death. This loss of fluids from the intravascular compartment also leads to an increase in the hematocrit (the percentage of blood volume occupied by red blood cells). Early attempts to decrease the hematocrit by supplying fluid intraveneously simply exacerbated the pulmonary edema. Even with the best treatment today, however, the mortality rate is still very high.

It is clear that hantaviruses are widely distributed around the world and have been present in their rodent hosts for a very long time. Although many are capable of causing serious illness in man, the number of human cases is fortunately small. However, there is always the fear that one of these viruses might acquire the ability to spread more readily from human to human and thereby become a more serious problem.

FAMILY *ARENAVIRIDAE*

A listing of the 22 currently recognized arenaviruses is found in Table 4.12. They can be grouped on the basis of sequence alignments and serological cross-reactions into four clades. The Old World viruses form a single clade, whereas the New World viruses group into three different clades, called A, B, C. The genomes consist of two segments of (−)RNA which together total about 11 kb. As for other (−)RNA viruses, the genomic RNA is present in helical nucleocapsids. Budding to acquire the viral envelope is from the plasma membrane (Fig. 2.25B). Virions are spherical but variable in size, with diameters ranging from 50 to 300 nm. It is believed that the number of RNA segments incorporated into a virus particle is not fixed. Multiple copies of the genome segments may be present in virions and this may account, in part if not entirely, for the variation in the size of virions. Also incorporated into the budding virions are variable numbers of ribosomes. The name for the family comes from the Latin word for sand (arena) because the ribosomes in the virions give them a grainy appearance. Why ribosomes are incorporated into virions is not known, as they do not appear to serve a useful function for viral assembly or replication.

The arenaviruses share many features with the hantaviruses. They are associated with rodents and have coevolved with them, as have the hantaviruses. They are transmitted to humans by contact with aerosolized rodent urine or feces; many cause very serious illness, often hemorrhagic fever, with a high mortality rate. Their genome organization and mode of replication has much in common with the hantaviruses, as described later.

Genome Organization and Expression

The genome organization of an arenavirus is illustrated in Fig. 4.27. Arenavirus genomes consist of two segments of RNA, naturally called L(arge) and S(mall). Both genomic RNAs are ambisense in character. The S segment corresponds to the bunyavirus S and M segments linked tail to tail in an ambisense arrangement (Fig. 4.1). The L segment corresponds to the L segments of bunyaviruses but with the addition of a second gene, encoding a protein called Z, in an ambisense orientation. Expression of the encoded genes follows an ambisense strategy as described for some of the bunyaviruses. The mRNA for one gene is synthesized from the genomic RNA and is expressed early, whereas the mRNA for the second gene is synthesized from the antigenomic or vcRNA and is expressed late (Fig. 4.27). As in the bunyaviruses, synthesis of arenavirus mRNA occurs in the cytoplasm using a primer that is snatched from cellular mRNAs, there is a secondary structure in the RNA between the two ambisense genes that causes termination of transcription, and the mRNAs are not polyadenylated.

The genomic S RNA is the template for synthesis of the mRNA for N, and N is therefore expressed early after for the synthesis of infection. Because N is required for the replication of the viral RNA, as is the case for all (−)RNA viruses, this arrangement is necessary if the virus is to replicate. The mRNA for the glycoproteins G1 and G2 is transcribed from the antigenomic copy of S and is therefore expressed late. The glycoproteins are produced as a polyprotein that is cleaved in a process that is similar to what happens in the bunyaviruses. There is an N-terminal signal sequence that leads to the insertion of the precursor called GPC into the endoplasmic reticulum. The signal sequence is removed by cellular signalase. The resulting precursor is cleaved by the cellular subtilase SKI-1/S1P, the same enzyme that processes the hantavirus glycoprotein precursor, into the N-terminal G_N (sometimes called G1 or GP-1) and the C-terminal G_C (sometimes called G2 or GP-2). G_N and G_C remain associated as a heterodimer. Only G_C has a transmembrane anchor, and the process thus resembles what happens in HA of influenza or F of paramyxoviruses where a type I glycoprotein is cleaved into N-terminal and C-terminal subunits that remain associated by noncovalent bonds.

Producing the glycoproteins late has the effect of delaying virus assembly. This allows RNA amplification to proceed for an extended period of time before it is attenuated by the incorporation of nucleocapsids into virions. Attenuation of RNA synthesis is also effected by the Z protein.

In the case of the L segment, the mRNA for protein L is produced early by synthesis from the genomic RNA. Proteins L and N are necessary and sufficient for RNA replication, and this orientation of the genes is necessary for virus replication. The mRNA for protein Z mRNA is transcribed from the antigenomic and thus Z is expressed late, after replication of the RNA begins. Z is a small protein of about 11 kDa that has multiple functions in viral replication. It has a RING finger motif and binds zinc. It downregulates RNA replication and the synthesis of mRNAs. It is also required for budding of virions. In fact, expression of Z in the absence

TABLE 4.12 *Arenaviridae*

Genus/[a] members	Virus name abbreviation	Natural rodent host(s)[b]	Transmission	Disease in humans	World distribution
Old World Arenaviruses					
Lymphocytic choriomeningitis	LCMV	*Mus musculus*	Urine, saliva[c]	Aseptic meningitis	Worldwide
Lassa	LASV	*Mastomys* sp.	Urine, saliva	Hemorrhagic fever (HF)	West Africa
Mopeia	MOPV	*Mastomys natalensis*	Urine, saliva	Nonpathogenic?	Mozambique, Zimbabwe
Mobala	MOBV	*Praomys* sp.	??	??	Central African Republic
Ippy	IPPYV	*Arvicanthis* sp.	??	Nonpathogenic?	Central African Republic
New World Arenaviruses					
Group A[d]					
Tamiami	TAMV	*Sigmodon hispidus*	Urine, saliva	Nonpathogenic?	Florida (U.S.)
Whitewater Arroyo	WWAV	*Neotoma albigula*	Urine, saliva	Three fatal cases of ARDS in California[e]	Western United States
Bear Canyon	BCNV	*Peromyscus californicus*	Urine, saliva	??	Western United States
Paraná	PARV	*Oryzomys buccinatus*	Urine, saliva	Nonpathogenic?	Paraguay
Flexal	FLEV	*Oryzomys* ssp.	Urine, saliva	Nonpathogenic?	Brazil
Pichinde	PICV	*Oryzomys albigularis*	Urine, saliva	Nonpathogenic?	Colombia
Pirital	PIRV	*Sigmodon alstoni*	Urine, saliva	Nonpathogenic?	Venezuela
Allpahuayo	ALLV	*Oecomys bicolor and O. paricola*	Urine, saliva	Nonpathogenic?	Northeastern Peru
Group B					
Guanarito	GTOV	*Zygodontomys brevicauda*	Urine, saliva	Venezuelan HF	Venezuela
Junin	JUNV	*Calomys musculinus*	Urine, saliva	Argentine HF	Argentina
Machupo	MACV	*Calomys callosus*	Urine, saliva	Bolivian HF	Bolivia
Sabiá	SABV	Unknown	???	Isolated from a fatal case, and has caused two severe laboratory infections	Brazil
Amapari	AMAV	*Oryzomys capito, Neacomys guianae*	Urine, saliva	Nonpathogenic	Brazil
Tacaribe	TCRV	*Artibeus* spp. bats[f]	Has been isolated from mosquito	Nonpathogenic	Trinidad
Cupixi	TCRV	*Oryzomys capito*		Nonpathogenic	Brazil
Group C					
Latino	LATV	*Calomys callosus*	??	??	Bolivia
Oliveros	OLVV	*Bolomys obscurus*	??	??	Argentina

[a] LCMV is the type virus of the family.
[b] Most of these viruses cause chronic infections in their natural rodent hosts.
[c] At least one case is known where several recipients contracted LCMV after organ transplants from an asymptomatic donor.
[d] White Water Arroyo, Tamiami, and Bear Canyon viruses have nucleoprotein genes related to those of Pichinde and Pirital in Group A, but glycoprotein genes related to Tacaribe, Junin, and Sabiá in Group B.
[e] ARDS, acute respiratory distress syndrome. Until these cases in 1999–2000, WWAV was not known to cause human illness.
[f] Originally isolated from fruit-eating bats, but subsequent isolation attempts from bats have failed.
Source: Fields *et al*. (1996), Table 1 on p. 1522; Porterfield (1995), Table 11.1 on p. 228; and recent information from Fauquet *et al*. (2005).

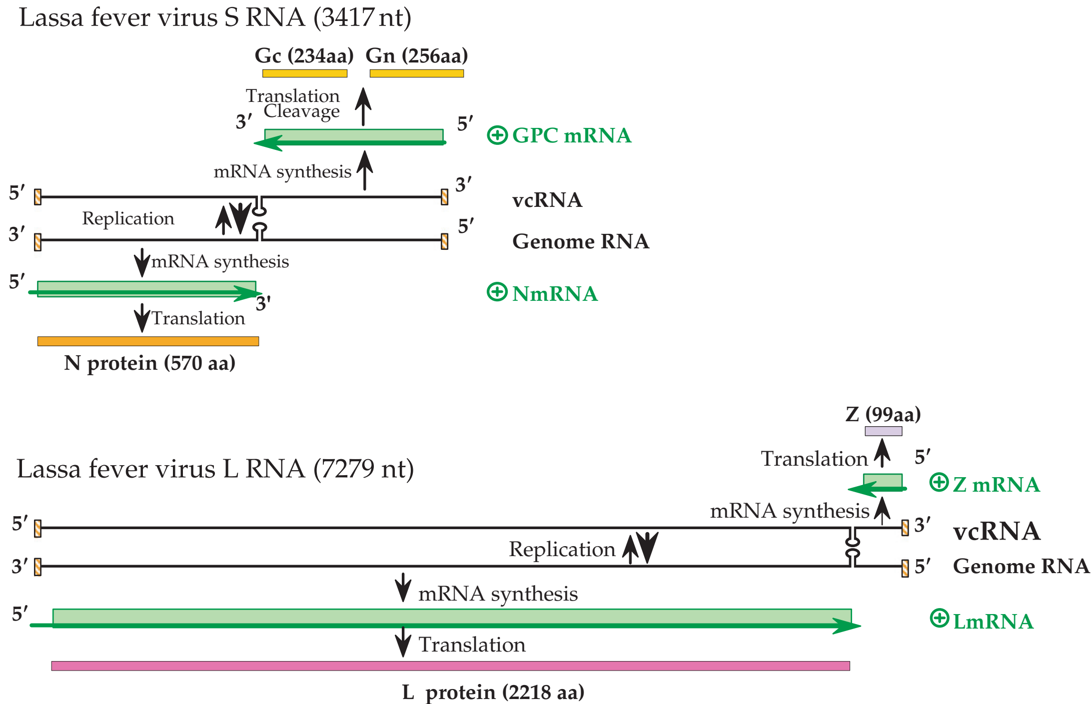


FIGURE 4.27 Genome organization and replication strategy of an arenavirus, Lassa fever virus. The genome consists of two segments of RNA, L and S. Both segments are expressed using an ambisense strategy. The nucleocapsid protein mRNA is synthesized from the 3′ end of the genomic S RNA, while the GPC mRNA is synthesized from the vc S RNA. A similar strategy occurs with the L segment. In this case, however, more than 95% of the coding capacity is used for the L protein, the RNA dependent RNA polymerase. Also note that the 5′ nontranslated region of L mRNA is 157 nt, which is unusually long for an arenavirus. The Z protein is a so-called "ring finger protein" that is involved in regulation of transcription and replication. Drawn from data in Lukashevich *et al*. (1997) and Clegg *et al*. (1990).

of other viral proteins results in the formation of virus-like particles, and Z has a role in budding analogous to the role played by the M proteins of other (−)RNA viruses or the Gag protein of retroviruses. It has been found that Z recruits a cellular protein called Tsg101 to the site of budding. Tsg101 has been shown to be required for budding of (at least) two arenaviruses, of HIV, and of Ebola virus. Tsg is a component of the vacuolar protein sorting machinery of the cell and is therefore active in promoting cellular budding pathways.

Z was originally thought to be a nonstructural protein and was called NS. It is now known to be present in the virion. The stoichiometry of proteins in virions of Lassa virus was found to be 1:160:60:60:20 for L:N:G_N:G_C:Z.

Natural History and Diseases

The natural history of the arenaviruses is very similar to that of the hantaviruses (Table 4.12). They establish a persistent infection in a single rodent host. Many cause hemorrhagic fever in humans following infection by aerosolized virus excreted in urine or feces. They appear to have coevolved with their hosts: An evolutionary tree of arenaviruses resembles the tree that describes their rodent hosts, as was true of the hantaviruses. The many similarities in genome organization and expression, the association with a single rodent species, and the nature of the disease caused in humans all suggest that the arenaviruses are closely related to the hantaviruses. A reasonable hypothesis is that the arenaviruses arose from the hantaviruses by fusion of the S and M segments to form one segment, which allowed finer control of the virus life cycle.

The cellular receptor for entry of many arenaviruses is α-dystroglycan, which plays a critical role in cell-mediated assembly of basement membranes. This protein is widely distributed in animals and many arenaviruses have a broad tissue tropism. For example, Lassa infection of humans results in high virus titers in spleen, lung, liver, kidney, heart, placenta, and mammary gland. Viruses that have a high binding affinity for α-dystroglycan replicate preferentially in the white pulp of the spleen and infect large numbers of lymphocytes that are important in the immune response to viral infection. The ability of these lymphocytes to act as antigen-presenting cells results in impairment of immune responses resulting in a generalized immunosuppression. Such viruses are more virulent than those that bind less avidly to α-dystroglycan. Immunosuppression may be important for the establishment of persistent infections in the rodent host, in which the virus does not cause disease.

In humans, however, immunosuppression may lead to much more serious illness.

The arenaviruses can be divided into Old World viruses and New World viruses (Table 4.12). Because of their association with a single rodent species, their geographic range is restricted to that of their host, and rodents have a restricted range. The exceptions are rodents that have been distributed widely by humans such as the house mouse and the urban rat. Many arenaviruses cause hemorrhagic fever in man with significant mortality rates (Table 4.12).

Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis virus (LCMV), the prototype virus of the family, is associated with the house mouse *Mus domesticus* and *Mus musculus*. This virus is widespread in Europe, along with its host, and spread to the Americas with the (inadvertent) introduction of the house mouse by European travelers. LCMV has been intensively studied in the laboratory as a model for the arenaviruses, in part because it is less virulent for humans than many arenaviruses, and in part because its natural host is widely used as a laboratory model for animal work. Mice are small, reproduce rapidly, and there is a great deal of experience in maintaining this animal in the laboratory. LCMV is widespread, often being present in colonies of laboratory mice even without overt introduction. It is also present in wild mice and may be present in pets such as hamsters.

LCMV infection of humans usually results in mild or even inapparent illness, although serious illness can result with occasional mortality. In a recent incident, a woman had been infected with LCMV from a pet hamster. She suffered no apparent illness from the viral infection but died of an unrelated cause, a stroke. Her liver, lungs, and kidneys were harvested for transplantation. Transplantation of liver, lungs, and kidney requires immunosuppression so that the transplanted organs are not rejected. Three patients receiving the liver, lungs, and a kidney developed overwhelming infection by LCMV and died. A fourth patient who received a kidney also became quite ill from LCMV infection but survived, aided by reduction in the immunosuppressive drugs being given.

Lassa Virus

The rodent reservoir of Lassa virus is *Mastomys natalensis*. Lassa virus causes outbreaks in West Africa of an often fatal illness in humans called Lassa fever. The mortality rate averages 10–15% but may be as high as 60% in some outbreaks. The virus has a broad tissue tropism and symptoms include fever, myalgia, and severe prostration, often accompanied by hemorrhagic or neurological symptoms. Development of hemorrhagic symptoms indicates a poor prognosis and death often follows. Fatal infection is also characterized by higher viral loads. Survivors of severe infection often suffer nerve damage and may be deaf because of such damage. The full extent of Lassa disease is not known because most Africans infected by the virus do not seek help and there is little monitoring of the disease. However, estimates range from 100,000 to 300,000 cases per year.

Lassa virus was first isolated in 1969 when a nurse in a rural mission hospital in Nigeria became infected. She was transported to Jos, Nigeria where several health care workers became infected. Serum samples were sent to the United States and a well-known virologist at the Yale Arbovirus Research Unit, Dr. Jordi Casals, became infected with the virus while working with it and became very seriously ill. He eventually recovered but later that same year a technician in another laboratory at Yale became infected with Lassa fever virus and died, whereupon Yale ceased to work with the virus. The containment facilities in 1969 were not of the quality of those in current use and virologists in those days literally took their lives in their hands when working with dangerous agents. The study of virology owes a great deal to the courage exhibited by these earlier workers.

Lassa has been imported to the United States on at least one occasion in the form of a viremic individual. A resident of Chicago attended the funerals of relatives in Nigeria who had died of Lassa fever and became infected there. On return to Chicago he began suffering symptoms of Lassa fever but the local hospitals were unable to diagnose the cause of his disease, being unfamiliar with it. He eventually died of Lassa fever, but fortunately there were no secondary cases.

New World Group B Viruses

Several South American arenaviruses belonging to Group B are very important disease agents because they cause large outbreaks of hemorrhagic fever with high mortality rates. The names of a number of these viruses and the places where they are found are shown in Fig. 4.28. They include Junín virus (causative agent of Argentine hemorrhagic fever), Machupo virus (Bolivian hemorrhagic fever), Guanarito virus (Venezuelan hemorrhagic fever), and Sabiá virus (cause of an unnamed disease in Brazil). The diseases caused by these viruses are often referred to as emerging diseases because the number of human cases has increased with development and expanding populations. The increasing number of cases results from development of the pampas or other areas for farming, bringing humans in closer association with the rodent reservoirs. Furthermore, the storage of grain near human habitation results in an increase in the local rodent population, and plowing of the fields leads to the production of aerosols which may transmit the disease to humans. An attenuated virus vaccine against Junín virus has been developed and is widely used in populations at risk. The vaccine is effective and has reduced dramatically the number of cases of Argentine hemorrhagic fever. No vaccines are in use for the other viruses, however.

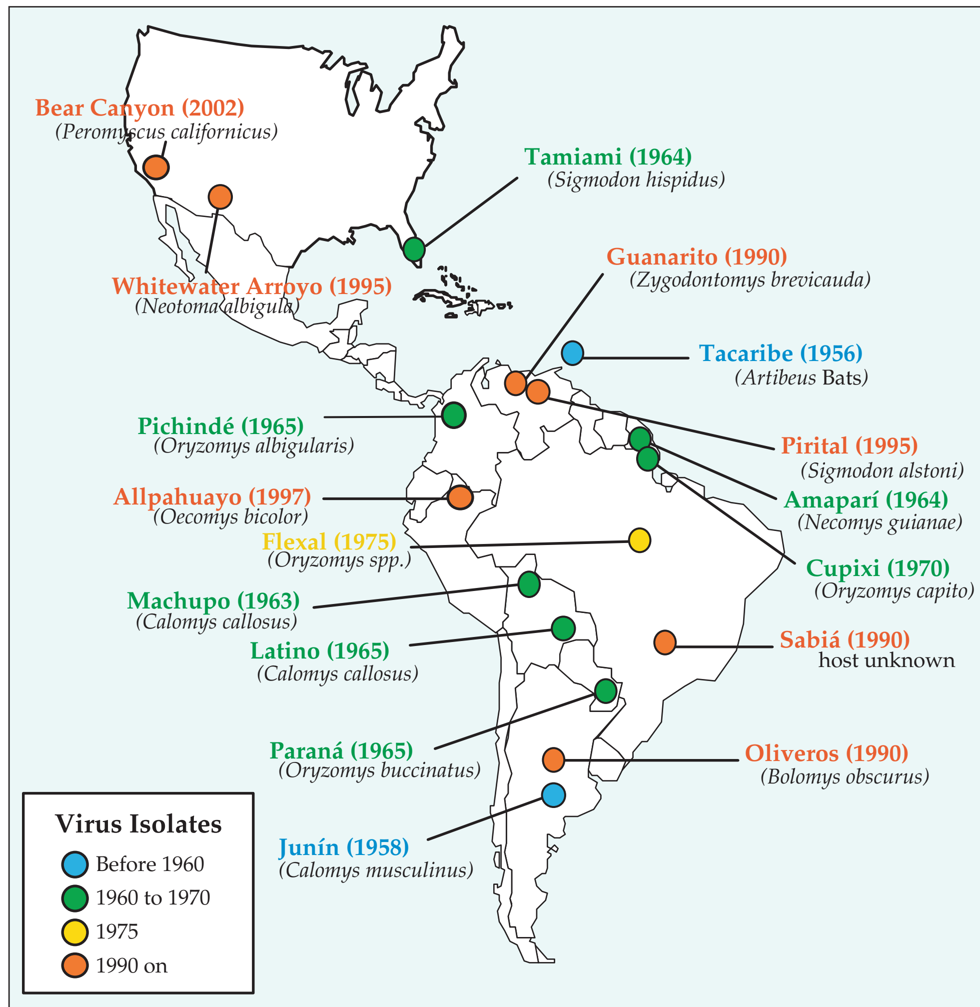


FIGURE 4.28 Arenavirus isolates in the New World. Also shown are the year of first isolation, and the rodent host of each virus where known. Adapted from Peters (1998b), Figure 1.

New World Group A Viruses

Three arenaviruses have been isolated in the United States, Whitewater Arroyo virus, present in the Southwest, Bear Canyon virus in California, and Tamiami virus, present in Florida (Fig. 4.28). None of these viruses, all of which belong to Group A, had been known to cause illness in humans until very recently. In 1999–2000, three Californians died following infection by Whitewater Arroyo virus. The disease these three suffered was ARDS (acute respiratory disease syndrome), although two also had hemorrhagic manifestations. Thus, like the hantaviruses, the U.S. arenaviruses may cause isolated cases of serious illness. There are also a number of Group B viruses in South America (Table 4.12), but these are not known to cause disease in humans.

Agents Causing Hemorrhagic Fevers in Humans

Many viruses, belonging to several different families, have been described that cause hemorrhagic fever in humans. Table 4.13 contains a listing of many of these viruses. These viruses include members of the *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, and *Flaviviridae*. Many cause severe disease with high mortality, but although the disease is severe, with the exception of some arenaviruses, survivors have few sequelae. The dramatic symptom of profuse bleeding has excited the purple prose of many lay authors, best illustrated by recent discussions of Ebola virus, and struck terror in native populations. With the exceptions of yellow fever virus and Junín virus, there are no vaccines, and treatments are primarily supportive, although ribavirin therapy holds some

TABLE 4.13 Viruses That Cause Hemorrhagic Fevers in Humans

Virus	Disease[a]	Geographic range	Vector transmission	%Case mortality[b]	Treatment (prevention)
Arenaviridae					
Junin	Argentine HF	Argentine pampas	Infected field rodents, *Calomys musculinus*		Antibody effective, ribavirin probably effective; preventive vaccine exists
Machupo	Bolivian HF	Beni province, Bolivia	Infected field rodents, *Calomys callosus*	15–30	Ribavirin probably effective
Guanarito	Venezuelan HF	Venezuela	Infected field rodents, *Zygodontomys brevicauda*		No data for humans, ribavirin probably effective
Sabiá	HF	Rural areas near Salo, Brazil	Unidentified infected rodents		Intravenous ribavirin effective in one case
Lassa	Lassa fever	West Africa	Infected *Mastomys* rodents	15	Ribavirin effective
Bunyaviridae					
Rift Valley fever	Rift Valley fever	Sub-Saharan Africa	*Aedes* mosquito	50	Rapid course; ribavirin or antibody might be effective
Crimean-Congo HF	Crimean-Congo HF	Africa, Middle East, Balkans, Russia, W. China	Tick-borne	15–30	Ribavirin used and probably effective
Hantaan, Seoul, Puumala, and others	HFRS	Worldwide (See Fig. 4.25)	Each virus maintained in a single species of infected rodents	Variable[c]	Ribavirin useful; supportive therapy is mainstay
Sin Nombre and others	HPS, also rare HF	Americas (See Fig. 4.26)	As for viruses causing HFRS	40–50	Rapid course makes specific therapy difficult
Filoviridae					
Marburg Ebola	Filovirus HF	Africa	Unknown	Marburg 25 EbolaZ 30–90	No effective therapy, barrier nursing prevents spread in epidemics
Flaviviridae					
Yellow fever	Yellow fever	Africa, South America	*Aedes* mosquito	20	Very effective vaccine
Dengue	DHF, DSS	Tropics and subtropics worldwide	*Aedes* mosquito	<1	Supportive therapy useful; vector control
Kyasanur forest disease	KFD	Mysore State, India	Tick-borne	0.5– 9	???
Omsk hemorrhagic fever	OHF	Western Siberia	Poorly understood cycle involves ticks, voles, muskrats??	?	Needs further study

[a] Abbreviations: HF, hemorrhagic fever; HFRS, hemorrhagic fever with renal syndrome; HPS, hantavirus pulmonary syndrome; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; KFD, Kyasanur Forest disease; OHF, Omsk hemorrhagic fever.
[b] In humans.
[c] Hantaan is 5–15% fatal, while Puumala is <1% fatal.
Source: This table includes data from Nathanson *et al*. (1996) Table 32.1 on p. 780.

promise for arenavirus disease. Human-to-human transmission is uncommon. Where limited transmission has occurred, it has been by exposure to contaminated blood, or possibly exposure to other bodily fluids, and resulted in limited epidemics for such viruses as Ebola and Machupo virus.

EVOLUTION OF MINUS-STRAND RNA VIRUSES

As has been described, all (−)RNA viruses share a number of features. These include virion structure (enveloped viruses with helical nucleocapsids); mechanisms for

replicating the genomic RNA (replication within RNP which requires ongoing protein synthesis; self-complementarity of the ends of the RNA (with its implications for promoter elements involved in replication); mechanisms for synthesis of mRNA (synthesis of leaders or the use of primers for synthesis of mRNA, the presence of intergenic sequences); and the suite of proteins encoded. These similarities make it seem likely that all (−)RNA viruses have diverged from a common ancestor fairly recently on a geological timescale, certainly more recently than the divergence of the extant plus-strand RNA viruses from a common ancestor. The (+)RNA viruses are much more divergent in structure and in the strategies used for replication and expression of the genome, suggesting that they have had a much longer period in which to diverge from one another. Although the suite of proteins encoded is very similar in all (−)RNA viruses, the rate of evolution of RNA viruses is so fast that little sequence identity can be demonstrated between different groups. However, where studies have been performed, evidence for common origin of at least some of these proteins has been shown. As an example, the M proteins of VSV and influenza virus are related and have diverged from a common ancestor. It seems likely that most of the various proteins are related in this way, although it is clear that some viruses have genes that are not represented in all viruses and which presumably arose by recombination events that led to the insertion of new functions, or to deletion events that resulted in a virus with fewer genes, or, probably, to both.

Because the (−)RNA viruses appear to be more recent than the (+)RNA viruses, it is reasonable to postulate that they arose from the (+)RNA viruses. If so, one obvious candidate for the ancestor is a coronavirus. Like the (−)RNA viruses, coronaviruses are enveloped viruses with a helical nucleocapsid that synthesizes RNA leaders and use primers to prime mRNA synthesis, traits in which the coronaviruses differ from other (+)RNA viruses.

If the (−)RNA viruses did arise from the (+)RNA viruses, what traits might account for their success once they arose? One obvious possibility is the ability to synthesize individual mRNAs for each protein needed. This trait also carries with it the necessity to include the RNA synthesis machinery in the virion, but the ability to control the order of synthesis and the translation frequency of the different proteins has obvious advantages for control of the replication cycle. The (−)RNA viruses with segmented genomes also have the ability to undergo ready reassortment, which is clearly advantageous in the *Orthomyxoviridae* and probably important for all viruses with segmented genomes. (+)RNA viruses that infect animals do not have segmented genomes, except for a few insect viruses with bipartite genomes, for reasons that are not clear. (+)RNA viruses of plants with segmented genomes are common, however. It is perhaps suggestive that the (−)RNA viruses have not been as successful in plants as they have been in animals, and the (−)RNA plant viruses that do exist also replicate in arthropods, which serve as vectors for transmitting the virus to plants.

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