

Rhabdoviridae

Taxonomy

Realm- Ribovira

Kingdom- Orthornavirae

Phylum- Negarnaviricota

Subphylum-Haploviricotina

Class- Monjiviricetes

Order- Mononegaviriales

Family- Rhabdoviridae

Genus- Lyssavirus

Genus-Ephemerovirus

Rhabdoviridae: The family

Derivation of names

Rhabdoviridae: from rhabdos (Greek) meaning rod, referring to virion morphology.

Member taxa

Vertebrate host

- *Lyssavirus*
- *Novirhabdovirus*
- *Perhabdovirus*
- *Sprivivirus*
- *Tupavirus*

Vertebrate host, arthropod vector

- *Curiovirus*
- *Ephemerovirus*
- *Hapavirus*
- *Ledantevirus*
- *Sripuvirus*
- *Tibrovirus*
- *Vesiculovirus*

Invertebrate host

- *Almendravirus*
- *Alphanemrhavirus*
- *Caligrhavirus*
- *Sigmavirus*

Plant host

- *Cytorhabdovirus*
- *Dichorhavirus*
- *Nucleorhabdovirus*
- *Varicosavirus*

The family *Rhabdoviridae* includes 20 genera and 144 species of viruses with negative-sense, single-stranded RNA genomes of approximately 10–16 kb. Virions are typically enveloped with bullet-shaped or bacilliform morphology but non-enveloped filamentous virions have also been reported. The genomes are usually (but not always) single RNA molecules with partially complementary termini. Almost all rhabdovirus genomes have 5 genes encoding the structural proteins (N, P, M, G and L); however, many rhabdovirus genomes encode other proteins in additional genes or in alternative open reading frames (ORFs) within the structural protein genes. The family is ecologically diverse with members infecting plants or animals including mammals, birds, reptiles or fish. Rhabdoviruses are also detected in invertebrates, including arthropods some of which may serve as unique hosts or may act as biological vectors for transmission to other animals or plants. Rhabdoviruses include important pathogens of humans, livestock, fish or agricultural crops.

Table 1. *Rhabdoviridae*. Characteristics of members of the family *Rhabdoviridae*.

Characteristic	Description
Typical member	vesicular stomatitis Indiana virus (AF473864), species <i>Indiana vesiculovirus</i> , genus <i>Vesiculovirus</i>
Virion	Bullet-shaped or bacilliform particle 100–430 nm in length and 45–100 nm in diameter comprised of a helical nucleocapsid surrounded by a matrix layer and a lipid envelope. Some rhabdoviruses have non-enveloped filamentous virions.
Genome	Negative-sense, single-stranded RNA of 10.8-16.1 kb (unsegmented or bi-segmented).
Replication	Ribonucleoprotein (RNP) complexes containing anti-genomic RNA are generated and serve as templates for synthesis of nascent RNP complexes containing genomic RNA.
Translation	From capped and polyadenylated mRNAs transcribed processively from each gene (3' to 5'), sometimes containing multiple ORFs.
Host Range	Vertebrates, arthropods and plants; many vertebrate and plant rhabdoviruses are arthropod-borne.
Taxonomy	20 genera containing 143 species and one unassigned species (<i>Moussa virus</i>). Many rhabdoviruses remain unclassified.

Genus *Lyssavirus*. Lyssaviruses infect a wide range of mammals including humans in which they can cause fatal encephalitis (rabies). Natural transmission is via saliva, usually through a bite by an infected animal. The genome is relatively simple, containing the genes which encode five structural protein but feature a long 3'-untranslated region (ψ) in the *G* gene; additional proteins may be expressed from alternative initiation codons in the *P* gene.

Virion

Morphology

Enveloped virions have been reported to be in the range of 100–460 nm in length and 45–100 nm in diameter (Figure 1. *Rhabdoviridae*). The longer forms may represent virions fused end-to-end.

Defective-interfering (DI) virus particles are proportionally shorter. Viruses infecting vertebrates are typically bullet-shaped or cone-shaped; however, some rhabdoviruses infecting animals and most plant rhabdoviruses appear bacilliform when fixed prior to staining. In unfixed preparations, they may appear bullet-shaped or pleomorphic. The outer surface of virions (except for the quasi-planar end of bullet-shaped viruses) is covered with projections (peplomers) which are 5–10 nm long and about 3 nm in diameter. They consist of trimers of the viral envelope glycoprotein (G). A honeycomb pattern of peplomers is observed on the surface of some viruses. Internally, the nucleocapsid (30–70 nm in diameter) has helical symmetry and appears to have cross-striations. The nucleocapsid consists of a ribonucleoprotein (RNP) complex comprising the genomic RNA and tightly bound nucleoprotein (N) together with an RNA-dependent RNA polymerase (L) and polymerase-associated phosphoprotein (P). The RNP complex is active for transcription and replication: the N-RNA template is processed by L, which contains various enzymatic activities, and its cofactor P. In the cytoplasm, the RNP complex is uncoiled and filamentous, about 700 nm in length and 20 nm in diameter. In the virion, the lipid envelope containing G interacts with the coiled RNP complex via the matrix protein (M).

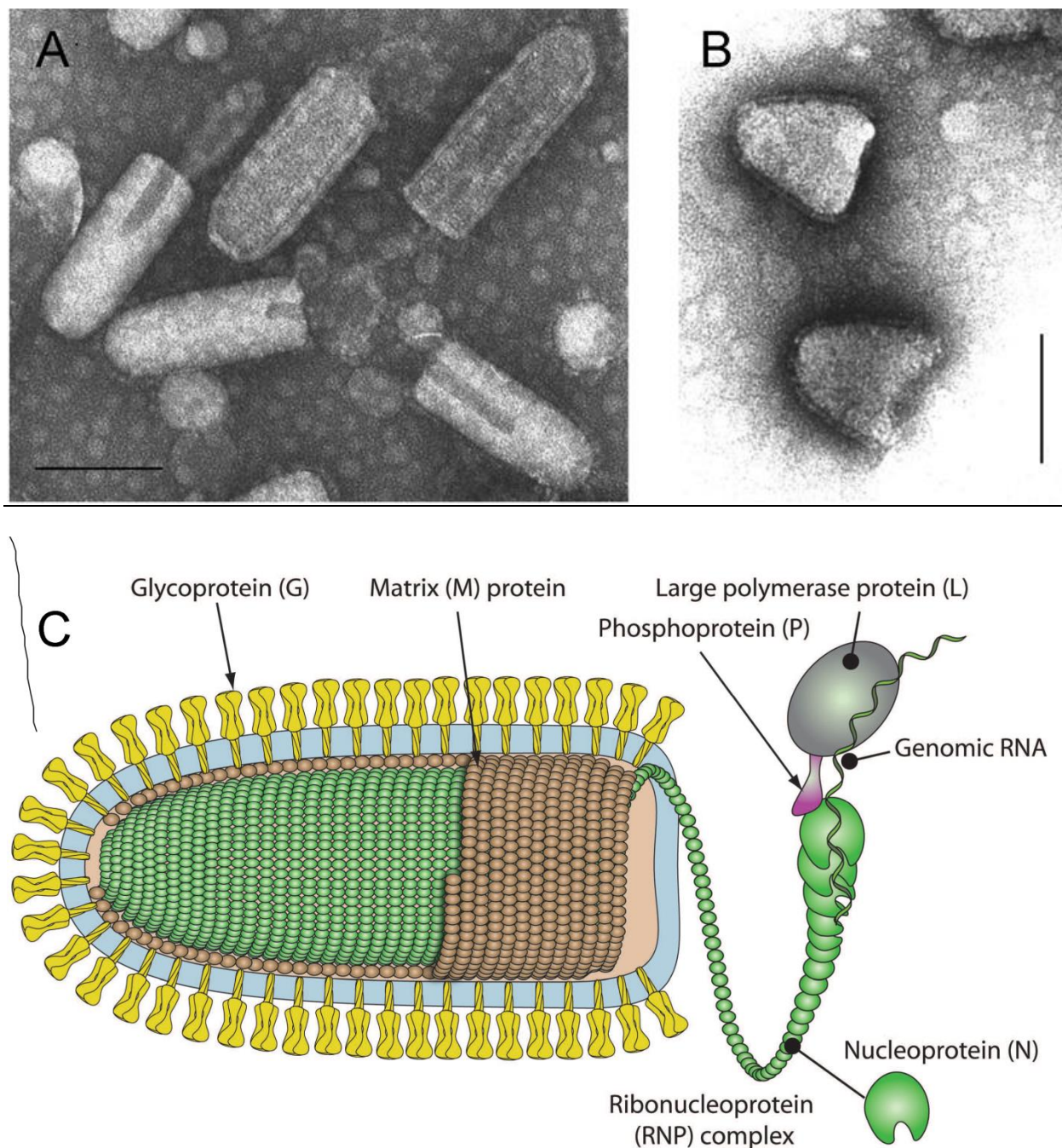


Figure 1. *Rhabdoviridae*. (A) Negative-contrast electron micrograph of vesicular stomatitis Indiana virus particles. The bar represents 100 nm (Courtesy of P. Perrin). (B) Negative-contrast electron micrograph of RABV defective-interfering (DI) particles. (Courtesy of P. Perrin). (C) Schematic illustration of a rhabdovirus virion and ribonucleocapsid structure. Unravelling of the RNP is illustrative only to show more clearly its association with the L and P proteins (Courtesy of P. Le Mercier).

Physicochemical and physical properties

Reported virion M_r ranges from $0.3\text{--}1.0 \times 10^9$. Virion buoyant density is $1.19\text{--}1.20 \text{ g cm}^{-3}$ in CsCl and $1.16\text{--}1.19 \text{ g cm}^{-3}$ in sucrose. Virus infectivity is rapidly inactivated at $56 \text{ }^\circ\text{C}$, or following UV-, gamma- or X-irradiation, or exposure to formalin or to lipid solvents such as detergent.

Nucleic acid

Virions typically contain a single molecule of linear, negative-sense single-stranded RNA (M_r 3.4×10^6 to 5.4×10^6 ; approximately 10–16 kb); rhabdoviruses with segmented genomes also occur with each RNA segment encapsidated independently ([Kormelink et al., 2011](#)). The RNA typically represents about 1–3% of virion weight. The RNA has a 3'-terminal free hydroxyl group and a 5'-triphosphate and is not polyadenylated. The ends have inverted complementary sequences encoding transcription and replication initiation signal. Defective-interfering RNAs, usually substantially shorter than full-length RNA (less than half length), may be identified in RNA recovered from virus populations. They are usually negative-sense; however, hairpin RNA forms are also found. Defective-interfering RNAs replicate only in the presence of homologous and, occasionally, certain heterologous helper rhabdoviruses which provide the functional genes. Full-length positive-sense RNA, which is an intermediate during the replication process, may constitute a significant proportion of a viral RNA population.

Proteins

Virions generally have five structural proteins (designated N, P, M, G and L; see Table 2. *Rhabdoviridae* for a summary of their locations, masses and functions). The structural proteins represent 65–75% of dry weight of the virion.

Table 2. *Rhabdoviridae*. Location and functions of rhabdovirus structural proteins.

Protein	Location, mass and function
L	A component of the viral nucleocapsid (ca. 220–240 kDa) responsible for most of the functions required for transcription and replication: RdRP, mRNA 5'-capping, 3'-poly(A) synthesis and protein kinase activities. Observed masses by SDS-PAGE are 150–240 kDa.
G	Associates into trimers to form the virus surface peplomers (monomer ca. 65–90 kDa). Binds to host cell receptor(s), induces virus endocytosis then mediates fusion of viral and endosomal membranes. G is variously N-glycosylated and palmitoylated; it lacks O-linked glycans and may have hemagglutinin activity. Induces and binds virus-neutralizing antibodies and elicits cell-mediated immune responses. In some cases, G is involved in tropism and pathogenicity.
N	Major component of the viral nucleocapsid (ca. 47–62 kDa). It associates with full-length negative- and positive-sense genomic RNAs, or defective-interfering RNAs, but not mRNAs. N is an active element of the template, presenting the bases to the polymerase. Newly synthesised N probably modulates the balance between genome transcription and replication by influencing the recognition of the transcription signals. N elicits cell-mediated immune responses and humoral antibodies. In plant nucleorhabdoviruses, N translocates to a sub-nuclear compartment when co-expressed with the cognate P.
P	A cofactor of the viral polymerase (ca. 20–30 kDa). It is variously phosphorylated and generally migrates by SDS-PAGE as a protein of about 40–50 kDa; nucleorhabdovirus P migrates faster. P is essential for at least two fundamental functions: (i) it mediates the physical link and the correct positioning of L on the N-RNA template; and (ii) it acts as a chaperone during the synthesis of N, by forming N-P complexes that prevent N from self-aggregation and binding to cellular RNA. During the genome replication process, N is then transferred from these N-P complexes to the nascent viral RNA to ensure its specific encapsidation into new RNPs. P elicits cell-mediated immune responses. In several rhabdoviruses P also plays a fundamental role in evading the host innate anti-viral response.
M	A basic protein that is an inner component of the virion (ca. 20–30 kDa). It is believed to regulate genome RNA transcription. M binds to nucleocapsids and the cytoplasmic domain of G, thereby facilitating the process of budding. It is sometimes phosphorylated or palmitoylated. M is found in the nucleus and inhibits host cell transcription. It also mediates other pathological effects (cell rounding for VSIV, apoptosis for RABV, intracellular accumulation of the inner nuclear

membrane for potato yellow dwarf virus (PYDV).

Lipids

Virions are composed of about 15–25% lipid, with their composition reflecting that of the host cell membrane where virions bud. Generally, phospholipids represent about 55–60%, and sterols and glycolipids about 35–40% of the total lipids. G may have covalently associated fatty acids proximal to the lipid envelope.

Genome organisation

Rhabdovirus genomes contain at least five ORFs in the negative-sense genome in the order 3'-N-P-M-G-L-5'. The genes are flanked by conserved transcription initiation and termination/polyadenylation signals, about 10 nt in length. For many rhabdoviruses, additional genes are interposed between the structural protein genes and alternative, overlapping or consecutive ORFs may occur within the structural protein genes or in the additional genes. Some rhabdovirus genomes are segmented. Consequently, genomes of viruses assigned to different genera may vary greatly in length and organisation (Figure 2. *Rhabdoviridae*).

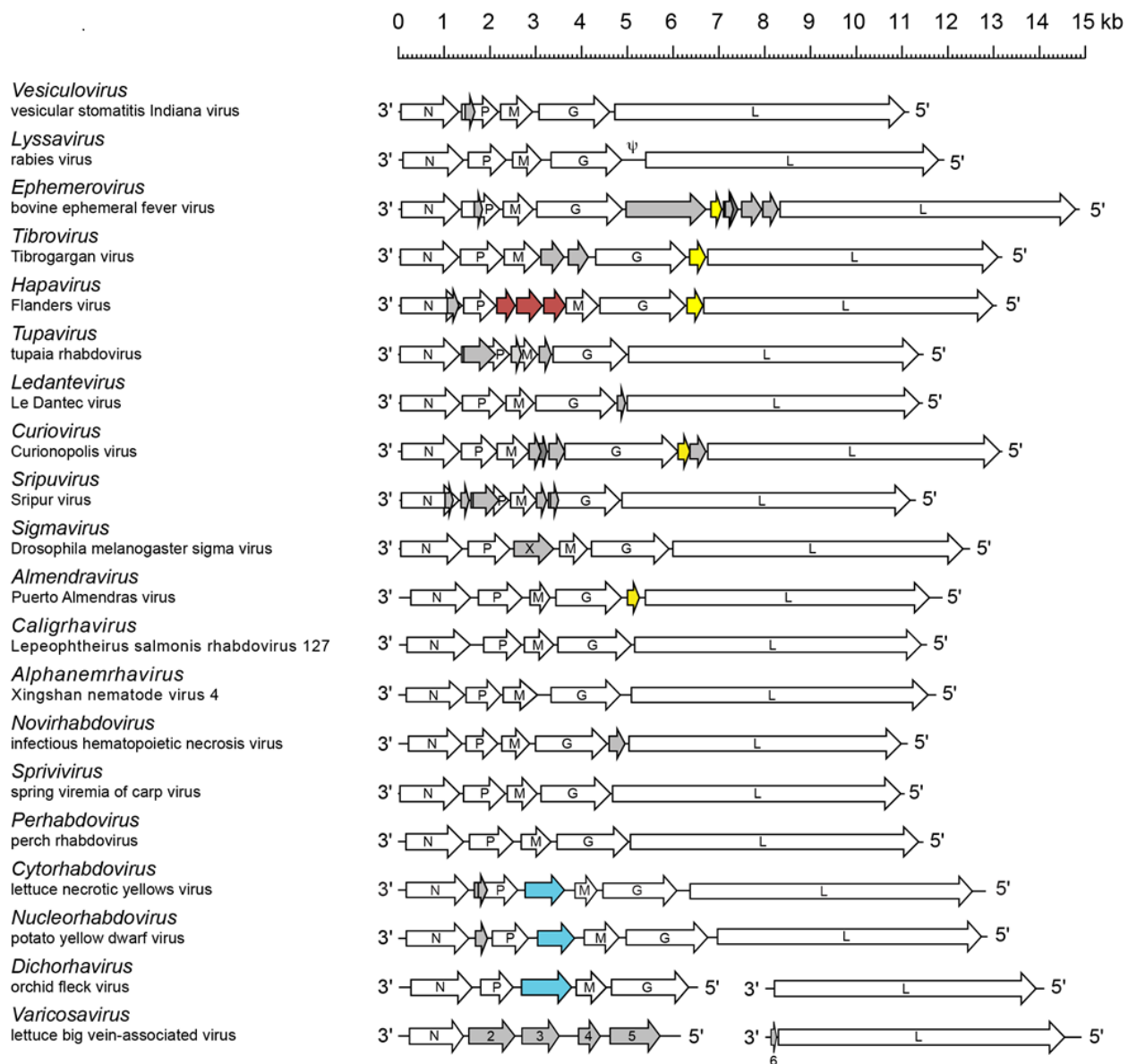


Figure 2. Rhabdoviridae. Schematic representation of rhabdovirus genome organizations exemplifying variations in the number and location of accessory genes. A typical member of each genus is represented. Each arrow indicates the position of a long open reading frame (ORF). Other alternative ORFs occur in some genes; only ORFs (≥ 180 nt) that appear likely to be expressed are shown

Figure 3.*Rhabdoviridae*. Genome organization, transcription and replication of vesicular stomatitis Indiana virus. Top: genome structure Middle: process of consecutive transcription of leader RNA and messenger RNAs. The role of N (green circles), P (red blob) and L (grey oval) is indicated. Bottom: replication of the negative-sense genome (light green N) via a positive-sense anti-genome intermediate (dark green N). The switch from transcription to replication is regulated by N. The genome and anti-genome strands are not generated in equimolar amounts.

Antigenicity

G induces virus-neutralising antibodies which define viruses as serotypes and can provide protective immunity. Antigenic cross-reactions in complement-fixation or indirect immunofluorescence tests occur primarily between rhabdoviruses within a genus and involve antigenic determinants located on the N protein. Cross-reactions in indirect immunofluorescence tests have also been detected between some animal rhabdoviruses that are now assigned to different genera.

Rhabdoviridae

G.R. Carter¹ and D.J. Wise²

¹Professor Emeritus of the Department of Medical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA. ²Department of Biology, Concord University, Athens, West Virginia, USA.

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This is a large family of negative-sense, single-stranded RNA viruses comprising six genera with species that infect vertebrates, invertebrates and plants.

Viral Characteristics

- These are cylindrical or bullet-shaped viruses (180 nm x 80 nm) with a helical nucleocapsid (see [Fig. 19.1](#)) containing a single stranded, negative sense RNA molecule 13 to 16 kb in length (see [Table 1.1](#)).
- The helical capsid is surrounded by a lipoprotein envelope with glycoprotein spikes. The space between the nucleocapsid and the envelope is filled out with the matrix protein.
- Replication takes place in the cytoplasm, using the negative sense RNA as a template for transcription of mRNA.

- After entry into the cell the virus RNA polymerase synthesizes five mRNAs that code for the viral proteins.
- Like other enveloped virions with negative-sense genome the [synthetic phase](#) is initiated by a virus-associated polymerase that transcribes the negative-sense into positive sense.
- Progeny RNA molecules are assembled with the viral proteins to form the nucleocapsid.
- The envelope is acquired as the virion buds through the plasma membrane.
- They are stable through a wide pH range, sensitive to UV light and to heat at 56°C.

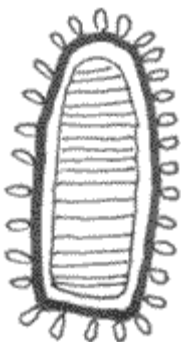


Figure 19-1. Rhabdoviridae (180 nm x 80 nm). Cylindrical or bullet-shaped viruses with a helical nucleocapsid surrounded by a lipoprotein envelope with glycoprotein spikes. To view click on figure

Classification

The Rhabdoviridae is divided into of six genera based on virion properties and serological relationships:

- **Vesiculovirus**
 - Vesicular stomatitis viruses.
 - Other Vesicular stomatitis viruses: These are discussed below under vesicular stomatitis.
- **Lyssavirus**
 - Nucleotide sequencing of genomic RNA and antigenic studies have identified genotypes and serotypes of lyssaviruses; they are all related and distinguishable from the common rabies virus. The principal lyssaviruses are:
 - - Rabies virus, the type species, causes rabies throughout the world excepting some island countries; it is designated genotype 1 and serotype 1.
 - - Lagos bat virus. Recovered from African fruit bats; not recovered from disease in humans.
 - - Australian bat lyssavirus. Recovered from species of flying-fox and insectivorous bats in Australia. Caused rabies-like encephalitis in a human.
 - - European bat lyssavirus 1 and 2. Two serologically distinct species occurring in bats in a number of European countries; potentially pathogenic.
 - - Mokola virus. Isolated from shrews and children with CNS disease in Nigeria.
 - - Duvenhage virus. Recovered from bats in Africa and Europe; has caused fatal human infections.
- **Ephemerovirus**
 - Bovine ephemeral fever virus
- **Novirhabdovirus** (Fish)
- **Cytorhabdoviruses** (Plant viruses)
- **Nucleorhabdovirus** (Plant viruses)

Vesiculovirus

Vesicular Stomatitis

Cause

Vesicular stomatitis virus (VSV). The two most important serotypes of VS virus are the Indiana and New Jersey variants. These and other serologic varieties are mentioned below.

Occurrence

Probably all VS serotypes and subtypes have the potential to infect cattle, swine, horses, humans and a variety of wild animals, including deer and raccoons.

The serologic varieties are as follows:

- The Indiana and New Jersey serotypes occur in North and South America and are responsible for most outbreaks.
- The VS Alagoas virus (subtype 1 of Indiana virus) has been identified in Brazil.
- Argentina or cocal virus (subtype 2 of Indiana virus) occurs in South America.
The disease is endemic in cattle, swine, and horses in some regions of Central and South America and Mexico. VS occurs occasionally in the southern United States; there were a small number of cases in horses in 1998 and again in 2004.

Transmission

The mechanism of infection is uncertain; possibly via oral wounds and abrasions. Spread is by direct and indirect contact (fomites) and arthropod vectors such as biting black and sand flies.

Clinical & Pathologic Features

The disease, which may occur as an epidemic or involving a few animals, clinically resembles foot-and-mouth (FMD) disease in cattle but is considerably milder.

Lesions most commonly involve the mouth and teats. These vesicular lesions occur on the lips, tongue, and oral mucosa, and affected cattle display signs of hypersalivation, depression, and anorexia. Teat lesions often lead to decreased milk production and mastitis in lactating cows. The disease usually runs a benign course with complete recovery within 2 - 3 weeks.

Mouth lesions are the most common clinical manifestation of VS in horses, whereas lesions of the feet are most often seen in affected swine.

Diagnosis

- Clinical specimens: Vesicular fluid, saliva, and affected mucous membranes collected early in the disease.
- Humans are susceptible to infection. Precautions should be taken including wearing gloves when examining animals for evidence of VS.
- Because of the possibility of FMD a prompt diagnosis is important.
- FMD does not affect horses; this may be used as a differential diagnosis.
- A rapid presumptive diagnosis can be achieved by the electron microscopic demonstration of rhabdovirus in distilled H₂O lysates of lesion material.
- Viral antigen is detected with ELISA and complement fixation.
- The virus is easily isolated in cell cultures derived from various animal species. Identification is accomplished using serologic procedures, such as ELISA, complement fixation and virus neutralization.
- In former years VS was differentiated from other vesicular diseases by the inoculation of the animals listed in [Table 19.1](#).

Table 19.1. Differentiation of Vesicular Diseases by Animal Inoculation				
	Cattle	Swine **	Horse *	Guinea Pig †
Foot-and-mouth disease	+	+	-	+

Table 19.1. Differentiation of Vesicular Diseases by Animal Inoculation				
	Cattle	Swine **	Horse *	Guinea Pig †
Vesicular stomatitis	+	+	+	+
Vesicular exanthema	-	+	+	-
Swine vesicular disease	-	+	-	-

* Intradermal-tongue; ** Intramuscular; † Intradermal.

Prevention

- Vesicular stomatitis is a reportable disease in United States, Canada and South American countries. Affected animals and herds are quarantined until recovered.
- Vaccination is not practiced.

Public Health Significance

Most human cases have occurred in laboratory workers, although infections can be acquired in the field as well. Human infections resemble influenza and are mainly characterized by sore throat and fever.

Lyssavirus

Rabies

Cause

Rabies virus. Rabies viruses are identified by genotype (nucleotide sequencing) and serotyping. Most rabies cases are caused by genotype 1 / serotype 1 strains.

Occurrence

The natural hosts are terrestrial carnivores and bats. Most mammals can be experimentally infected.

The disease is widely distributed, although some countries including the British Isles, Australia, and the West Indian Islands, are free. Rabies is frequently endemic in wild mammals, including particularly bats, skunk, fox, and raccoon. Asymptomatic salivary gland infections occur in bats, resulting in a prolonged viremia. The rabies virus has been recovered from the air of caves harboring bats.

There have been fewer than 40 cases of human rabies in the USA in the period 1990 - 2004.

Transmission

Spread to humans and animals is almost always by bites. Vampire bats, insectivorous, and fruit bats also transmit the virus by bites. There are rare instances of human infection by inhalation, e.g., in bat-infected caves and laboratories and by infected tissues used as transplants.

Pathogenesis

The virus ordinarily enters the animal or human body via the bite of a rabid animal. The virus multiplies at the bite site and infects the sensory neurons. It then moved by axons to CNS. During transport within the nerve the virus is protected from the immune system. The virus multiplies in the CNS and subsequently travels down the peripheral nerves to the salivary glands and thus infectious saliva. There doesn't appear to be a viremic stage.

Encephalitis develops with demyelination and death of neurons. Infected neurons contain eosinophilic cytoplasmic inclusions, termed Negri bodies.

Clinical & Pathologic Features

The incubation period in dogs is a minimum of about 10 days with an average of 20 - 60 days, and rarely as long as six months to a year or longer. The length of the incubation period is dependent on the amount of virus introduced by the bite and the location of the bite. The nearer the bite to the brain, the shorter the incubation period.

The disease begins with the prodromal phase then proceeds to mainly the excitive phase or paralytic phase. There may be overlapping of the latter phases.

- *The prodromal phase*: Involves change in behavior and lasts 2 - 3 days. Anxiety, irritability and unease are characteristic. Some are more alert, restless and sensitive to light and noise.
- *The excitive or furious phase*: Signs include restlessness, depraved appetite, hiding, wandering, aggressive biting, excessive salivation, dysphagia, muscle tremors, incoordination and staggering.
- *The paralytic or dumb phase*: This develops in several days with seizures, paralysis, coma and death in 3 - 4 days. In horses and cattle the paralytic phase appears to be predominant.

It is of interest that affected bats can sometimes be seen flying in the daytime and even attacking animals, people and other bats. Skunks, raccoons, foxes are often aggressive toward humans and domestic animals. The most significant histopathologic finding is the presence of eosinophilic cytoplasmic inclusions, called Negri bodies, in affected neurons. Negri bodies are of great diagnostic significance and are most readily found in the hippocampus major.

Diagnosis

- Clinical specimens: Brain.
- The fluorescent antibody procedure (FA) is widely used and is the preferred method for rabies diagnosis. It is used on animals that have died, and is recommended for the immediate examination of wild animals that cannot be readily held for observation. Smears of the hippocampus major are usually employed. The FA test is highly accurate and provides for a rapid diagnosis.
 - If the FA test is negative it is recommended that suckling mice be inoculated intracerebrally; they will die within 7 - 21 days if rabies virus is present.
- The FA test is used occasionally on formalin fixed brain tissue (when fresh tissue is unavailable) to confirm a rabies diagnosis based on the microscopic finding of Negri bodies.
- Rabies virus can be propagated in cell cultures and in suckling mice inoculated intracerebrally. The FA test is used to confirm the presence of the virus in the brain of mice.

Prevention

- This is best accomplished by insuring that dogs are properly vaccinated with either modified live or killed vaccines. Only inactivated vaccines are used in the United States. Modified live vaccines are usually administered to puppies at three months of age, when they are one year old, and thereafter every three years. Some killed vaccines require yearly boosters.
- Vaccinated dogs bitten by a known rabid animal should be given a booster vaccination and confined for 90 days; unvaccinated dogs should be euthanized.
- A healthy dog or cat that bites a human being should be confined and observed for 10 days. If clinical signs do not develop during this period it can be assumed that virus was not present in the saliva.
- Any wild animal that bites (unprovoked) a human being should be destroyed immediately and tested for rabies.
- Individuals at high risk of exposure should be vaccinated with the currently recommended human diploid cell culture vaccine. This vaccine and human rabies immunoglobulin are used for post-exposure treatment.
- To control rabies in wildlife populations oral vaccines in baits (e.g., fish meal) are being placed in rural areas and also in more densely populated areas in the USA and Europe. In large areas they are dropped from low-flying planes. Surveys indicate that they have been very effective. Both live attenuated virus vaccines and recombinant vaccines have been used in baits. A vaccinia vector vaccine expressing rabies glycoprotein has been effective.

- Rabies virus remains viable in brain tissue for more than a week at room temperature and for several weeks at refrigerator temperature. The virus is killed by an adequate concentration of formaldehyde or sodium hypochlorite.

Ephemerovirus

Bovine Ephemeral Fever

Cause

Bovine ephemeral fever virus.

Occurrence

Bovine ephemeral fever (BEF) occurs in cattle and water buffalos in Africa, Asia and Australia. Many other ruminants are subject to subclinical infections.

Transmission

The virus is thought to be transmitted by bites of insect vectors and particularly *Culicoides* species. The reservoir of the virus is thought to be water buffaloes, water buck and arthropod vectors in which the virus multiplies.

Clinical & Pathologic Features

Bovine ephemeral fever is generally a mild disease characterized by a biphasic febrile response, depression, anorexia, salivation, muscle twitching, and generalized stiffness. The respiratory rate may be increased and some infected animals may have ocular and nasal discharges. Recovery is usually rapid and uneventful.

Diagnosis

- Clinical specimens: Blood.
- Diagnosis is often based on clinical signs and history. The finding of polyserositis with fibrin-rich fluid in the pleural and peritoneal cavities is supportive.
- Confirmation is accomplished with ELISA or virus neutralization for antibodies using paired sera.
- Virus isolation is not usually attempted.

Prevention

- Modified-live virus and killed vaccines are available. Trials are being carried out with subunit and recombinant vaccines.
- Insect control is not usually practicable.

Glossary

Synthetic Phase:

Refers to the initiation of transcription and translation, resulting in the production of new virion particles.