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**Arteriviridae and Roniviridae**

Viruses within the families *Arteriviridae* and *Roniviridae* are included in the order *Nidovirales*, along with those in the family *Coronaviridae*. Viruses in these families have very different virion morphology, but the grouping reflects their common and distinctive replication strategy that utilizes a nested set of 3' co-terminal subgenomic messenger RNAs (mRNAs). The family *Roniviridae* contains viruses that have been detected only in crustaceans, specifically, several genotypes of gill-associated and yellow head viruses. The name of the family *Arteriviridae* is derived from the disease caused by its type species, equine arteritis virus. Other arteriviruses include porcine reproductive and respiratory syndrome, lactate dehydrogenase-elevating, and simian hemorrhagic fever viruses (Table 25.1). The host range of arteriviruses is highly restricted, and all arteriviruses share the capacity to establish asymptomatic prolonged or persistent infections in their respective natural hosts; most can cause severe disease in certain circumstances.

**PROPERTIES OF ARTERIVIRUSES AND RONIVIRUSES**

**Classification**

Despite marked differences in their virion morphology, viruses in the families *Arteriviridae* and *Roniviridae* have a similar genome organization and replication strategy. The family *Arteriviridae* comprises a single genus, *Arterivirus*, which contains all member viruses, and the family *Roniviridae* contains a single genus, *Okavirus*.

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**Table 25.1 Arteriviruses of Animals**

| Virus                                      | Host    | Disease                                                                 |
|--------------------------------------------|---------|-------------------------------------------------------------------------|
| Equine arteritis virus                     | Horse   | Systemic influenza-like disease, arthritis, abortion, pneumonia in foals |
| Porcine reproductive and respiratory       | Swine   | Porcine reproductive and respiratory syndrome, systemic disease; abortion of sows or birth of stillborn or mummified fetuses; respiratory disease |
| Porcine reproductive and respiratory       | Mice    | Usually none, but the presence of the virus may confound research using infected mice |
| Simian hemorrhagic fever virus             | Macaques| Systemic hemorrhagic disease, death                                      |
Virion Properties

Arterivirus virions are enveloped, spherical, and 45–60 nm in diameter, which is about half the size of those of coronaviruses (Figure 25.1). In contrast to the nucleocapsids of coronaviruses and roniviruses, which are helical, arterivirus nucleocapsids are isometric, 25–35 nm in diameter. Whereas envelope glycoprotein spikes are prominent on coronaviruses and roniviruses, they are small and indistinct on arterivirus virions. The genome of arteriviruses consists of a single molecule of linear positive-sense, single-stranded RNA, approximately 12.7–15.7 kb in size that includes 9–12 open reading frames (Figure 25.2). There are untranslated regions of 156–224 nt and 59–177 nt at the 5′ and 3′ ends of the genome respectively, and a 3′-poly(A) terminal sequence. Arterivirus virions include a single nucleocapsid protein, N, and six envelope proteins, designated E, GP2, GP3, GP4, GP5, and M. Of these, three minor envelope proteins (GP2, GP3, and GP4) form a heterotrimer, and the non-glycosylated triple-membrane spanning integral membrane protein, M, and the large envelope glycoprotein, GP5, form a heterodimer. The major neutralization determinants are expressed on GP5, although M protein exerts a conformational influence on GP5. Simian hemorrhagic fever virus is not as well characterized as the other arteriviruses, and its genome includes three additional open reading frames that may represent re-duplications of genes encoding structural viral proteins.

Ronivirus virions are approximately 150–200 nm × 40–60 nm, bacilliform, with rounded ends and prominent glycoprotein envelope spikes (Figure 25.3). Nucleocapsids have helical symmetry and a diameter of 20–30 nm. The genome consists of a single molecule of linear, positive-sense, single-stranded RNA approximately 26.2 kb in size that includes five long open reading frames, 5′- and 3′-untranslated regions, and a 3′-terminal poly(A) sequence. Virions consist of at least three structural proteins, and the envelope glycoproteins are cleavage products of a larger polyprotein precursor.

Virus Replication

Arteriviruses replicate in macrophages and a very limited number of other cell types within their respective hosts. The host range of arteriviruses is highly restricted, and the viruses typically grow in vitro only in cultured macrophages, macrophage cell lines, and a few other cell lines. Some arteriviruses effectively can subvert protective host innate immune responses, including apoptosis of infected macrophages and interferon signaling pathways.

The heterotrimer of envelope proteins GP2, GP3, and GP4 is responsible for cell tropism and receptor binding of equine arteritis virus, and arteriviruses appear to enter susceptible cells by a low-pH-dependent endocytic pathway. The receptors for most arteriviruses are uncharacterized; however, potential receptors involved in the attachment and internalization of porcine reproductive and respiratory syndrome virus include CD163 (a cellular protein in the scavenger receptor cysteine-rich superfamily), sialoadhesin (a macrophage-restricted surface molecule), and heparan sulfate glycosaminoglycans.

The two large open reading frames at the 5′ end of the arterivirus genome encode two replicate polyproteins that

![Figure 25.1](image-url)

**FIGURE 25.1** Family *Arteriviridae*, genus *Arterivirus*. (A) Schematic representation of an arterivirus particle. Seven virion-associated proteins have been identified in equine arteritis virus: N, nucleocapsid; M, membrane protein; GP5, major glycoprotein; GP2, GP3, GP4, minor glycoproteins; E, small integral envelope protein. MEM, lipid membrane; ICS, inner capsid space; AAAA, 3′ poly(A). [A: From Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses (C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, L. A. Ball, eds.), p. 965. Copyright © Elsevier (2005), with permission.] (B) Lactate dehydrogenase-elevating virus and (C) equine arteritis virus virions; negative stain electron microscopy.
FIGURE 25.2  Overview of arterivirus genome organization and replicase polyproteins. (A) General genome organization. ORFs are represented by boxes. The proteins encoded by the equine arteritis virus (EAV) open reading frames (ORFs) are indicated. The 5' leader sequence is depicted by a small black box; 3' poly(A) tails are not shown. The arrow between ORF1a and ORF1b represents the ribosomal frameshift site. The grey boxes represent regions where porcine reproductive and respiratory syndrome virus (PRRSV), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever (SHFV) contain major insertions compared to EAV. (B) Overview of proteolytic processing and domain organization of the EAV replicase polyproteins, pp1a and pp1ab, with differences in PRRSV and LDV indicated. Polyprotein cleavage sites are depicted with arrowheads matching the color of the protease involved. Abbreviations: PCP, papain-like cysteine protease; CP, nsp2 cysteine protease; SP, nsp4 chymotrypsin-like serine protease; h, hydrophobic domain; RdRp, RNA-dependent RNA polymerase; ZF, zinc finger; HEL, NTPase/helicase; EN, putative endoribonuclease. N, nucleocapsid; M, membrane protein; GP5 major glycoprotein; GP2, GP3, GP4, minor glycoproteins; E, small integral envelope protein. [From Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses (C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, L. A. Ball, eds.), p. 966. Copyright © Elsevier (2005), with permission.]

FIGURE 25.3  (Top left) Schematic illustration of an okavirus virion. (Top right) Transmission electron micrograph of negative-stained particles of gill-associated virus (GAV). (Bottom left) Transmission electron micrograph of partially disrupted yellow head virus (YHV) virion displaying the internal nucleocapsid and a ring-like structure which appears to be a disrupted virion in cross-section. (Bottom right) Transmission electron micrograph of cytoplasmic unenveloped nucleocapsids in a thin section of GAV-infected lymphoid organ cells. The bars represent 100 nm. (Courtesy of K. Spann, P. Loh, J. Cowley and R.J. McCulloch and reproduced with permission). p20, nucleocapsid; gp64, small spike glycoprotein; gp116, large spike glycoprotein; ssRNA, positive single-strand RNA. [From Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses (C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, L. A. Ball, eds.), p. 975. Copyright © Elsevier (2005), with permission.]
are expressed directly from viral genomic RNA through a ribosomal frameshifting mechanism. These replicase polyproteins are co- and post-translationally modified by viral proteinases into at least 12 non-structural proteins that mediate replication. The genes that encode the viral structural proteins are overlapping, and located in the 3’ end of the genome; they are expressed from a nested set of 3’ co-terminal subgenomic RNAs. These subgenomic RNAs all include a common 5’ leader sequence derived from the 5’-untranslated region of viral genomic RNA, at least one unique open reading frame encoding one or more structural virion proteins, and a common 3’-poly(A) tail. The individual open reading frames that are included in these subgenomic mRNAs reflect overlapping reading frames contained in the 3’ end of the viral genome. It is believed that the subgenomic mRNAs are generated by discontinuous transcription that links non-contiguous portions of the viral genome, to produce negative-strand templates that are transcribed into positive-strand subgenomic mRNAs that are then translated into the individual virion proteins.

Arterivirus replication occurs in the cytoplasm of infected cells, although individual non-structural (nsp1) and structural (N) proteins selectively translocate to the nucleus. Viral RNA replication complexes localized in double membrane vesicles derived from the endoplasmic reticulum produce the genomic and subgenomic mRNAs (Figure 25.4). Viral nucleocapsids bud into the lumen of the endoplasmic reticulum and/or Golgi complex of infected cells, and from there move to the surface of the cell in vesicles and are released by exocytosis (Table 25.2).

The overall strategy for replication of roniviruses is similar to that of arterviruses, but the structural proteins are expressed from a nested set of just two subgenomic mRNAs that each encode several proteins. The subgenomic RNAs also lack a common 5’ leader sequence derived from genomic viral RNA.

MEMBERS OF THE FAMILY ARTERIVIRIDAE, GENUS ARTERIVIRUS

EQUINE ARTERITIS VIRUS

Descriptions of a disease that very probably was equine viral arteritis were first published in the late 18th and
early 19th centuries, with colloquial names such as “pink-eye,” “infectious or epizootic cellulites,” “influenza erysipelatosa,” and “Pferdestaufe.” Early investigators also recognized that apparently healthy stallions transmitted this disease to susceptible mares at breeding. The causative agent, equine arteritis virus, was first isolated in 1953 from lung tissue of aborted fetuses during an epizootic of abortion and respiratory disease on a breeding farm near Bucyrus, Ohio. Although serologic studies indicate that infection occurs worldwide, the incidence of both infection and overt disease varies markedly among countries and among horses of different breeds.

Clinical Features and Epidemiology

Most natural infections with equine arteritis virus are asymptomatic, and descriptions of fatal disease are based on experimental infections with highly horse-adapted laboratory strains of the virus. Nevertheless, relatively virulent field strains of equine arteritis virus periodically cause natural outbreaks of equine viral arteritis in horses. After an incubation period of 3–14 days, the onset of disease is marked by fever (greater than 41°C), leukopenia, depression, excessive laceration, anorexia, conjunctivitis, rhinitis and nasal discharge, urticaria of the head, neck, and trunk, and edema, which is most pronounced over the eyes (supraorbital), the abdomen, including the prepuce, scrotum, and mammary glands, and the hind limbs (often resulting in a stiff gait). Although naturally infected horses usually recover uneventfully, death as a result of rapidly progressive bronchointerstitial pneumonia occurs sporadically in young foals. Abortion is characteristic of infections of pregnant mares with particular strains of the virus, and infection of large numbers of susceptible (unvaccinated) pregnant mares can lead to “abortion storms.” Abortion generally occurs 10–30 days after infection and at any time between 3 and 10 months of gestation; it is linked closely with the late febrile or early convalescent phase of infection, but can occur even if no clinical signs are noticed.

Equine arteritis virus is spread by both the respiratory and venereal routes, respectively by aerosol from acutely infected horses or in the semen of persistently infected carrier stallions (Figure 25.5). The latter are the essential natural virus reservoir, and persistent infection occurs in some 30–70% of postpubertal colts and stallions. Carrier stallions are otherwise normal, and infection is confined to the reproductive tract during persistence. Virus is spread from carrier stallions exclusively via the venereal route; semen collected from persistently infected stallions and used in artificial insemination has been responsible for outbreaks of disease. Furthermore, genetic diversity is generated in equine arteritis virus during persistent infection of stallions. Persistent infection is maintained in the reproductive tract of individual stallions for variable intervals, from several weeks to life-long.

Pathogenesis and Pathology

Initial replication of equine arteritis virus takes place in alveolar macrophages and endothelial cells after aerosol respiratory infection of susceptible horses, and virus then rapidly spreads to the draining bronchial lymph nodes; subsequently it is disseminated via the blood stream. Although macrophages and endothelial cells are the principal sites of virus replication, the virus also productively infects selected epithelia, mesothelium, and smooth muscle of the media of arteries and the uterine wall. The clinical manifestations of equine arterial arteritis reflect vascular injury. However, in
the pathogenesis of vascular injury, the relative roles and importance of the direct involvement of viruses, as opposed to the involvement of virus-induced cytokines derived from macrophages and endothelial cells, are not clear. Strains of equine arteritis virus clearly differ in their virulence, including their potential to cause abortion, and in their ability to induce pro-inflammatory cytokine mediators.

The characteristic gross lesions of severe cases of equine viral arteritis in adult horses are edema, congestion, and hemorrhage. Pleural and pericardial effusion are characteristic of the fulminant disease caused by the highly pathogenic horse-adapted laboratory strain of equine arteritis virus, as is terminal disseminated intravascular coagulation, which leads to necrosis and hemorrhage in several organs. Foals with bronchointerstitial pneumonia develop marked pulmonary edema, with accumulation of protein-rich fluid in airspaces and lesions typical of acute respiratory distress syndrome. They also may develop pleural and pericardial effusion, and intestinal hemorrhage and necrosis. Aborted fetuses are usually expelled together with the placenta (fetal membranes) and without premonitory clinical signs. Aborted fetuses are typically autolyzed, and seldom exhibit characteristic gross or histologic lesions. Some may have excess fluid in the peritoneal and pleural cavities, and petechial hemorrhages in peritoneal and pleural mucosal surfaces.

The pathogenesis of the carrier state in stallions is poorly characterized. Virus concentrations are greatest in the accessory sex glands and in the vas deferens. The carrier state is testosterone dependent, as persistent virus shedding does not occur in either geldings or mares. Furthermore, persistently infected stallions that are castrated cease shedding virus in semen, whereas those supplemented with testosterone after castration continue to shed virus.

**Diagnosis**

Detection of equine arteritis virus in tissues samples and fluids can be achieved by either virus isolation or reverse-transcriptase-polymerase chain reaction (RT-PCR) assay. Virus isolation is routinely carried out in rabbit kidney cells. There can be marked variation in the amount of virus shed in the semen of individual stallions over time, and the highest quantities of virus are associated with the sperm-rich fraction of the ejaculate. Serum antibodies to equine arteritis virus are usually detected by virus neutralization assay, although several enzyme-linked immunosorbent assays (ELISAs) have been developed and partially characterized.

**Immunity, Prevention, and Control**

Despite its worldwide distribution, equine arteritis virus causes disease outbreaks only occasionally, with instances or outbreaks of abortion being especially devastating. Epizootics occur where horses are congregated from several sources, such as at sales and shows, and on breeding farms. The virus readily is transmitted by horizontal aerosol spread during outbreaks, and likely sources of initial infection include susceptible mares that were recently bred to a carrier stallion. A high percentage of seronegative stallions and postpubertal colts infected during an outbreak will subsequently become persistently infected carriers of the virus. Identification of carrier stallions is central to control strategies, as only immune mares should be bred to these animals. Semen used for artificial insemination should be tested for the presence of virus, so that the use of contaminated semen can be restricted to immune mares. Furthermore, mares should be isolated after being bred to a carrier stallion, to prevent transmission to susceptible cohorts.

Virus neutralizing antibodies appear in serum within approximately 1 week after infection, coinciding with virus elimination from the circulation. There is only one known serotype of equine arteritis virus, and neutralizing antibodies prevent reinfection. Neutralizing antibodies persist for years after natural infection, and protection is long-lasting, if not life-long. Colostrum from immune mares moderates or prevents equine viral arteritis in young foals.

Immunization of horses with attenuated or inactivated virus vaccines can induce immunity, thus immunization of valuable breeding animals is justified. To prevent the establishment of persistent infections in stallions that will be used for breeding, vaccination of colts may be done at 6–8 months of age. This timing is important, because vaccination should be done after maternal antibody has waned but before puberty, to preclude any possibility of inducing persistent infection. The carrier state does not occur in colts exposed to equine arteritis virus before puberty. To prevent abortions, mares may be vaccinated before becoming pregnant.

During outbreaks, the spread of virus is best controlled by: (1) movement restrictions; (2) isolation of infected horses, followed by a quarantine period after recovery; (3) good hygiene, including assignment of separate personnel to work with infected and uninfected animals; (4) laboratory-supported surveillance.

**Porchine Reproductive and Respiratory Syndrome Virus**

A previously unrecognized disease—initially designated as “mystery swine disease” in North America—appeared in pigs in North America in the 1980s, and subsequently in Europe. A virus identified as “Lelystad virus” was first isolated in the Netherlands and proven to reproduce the disease in 1991. Porcine reproductive and respiratory syndrome virus has since become a major pathogen in swine
populations worldwide, and retrospective serological studies indicate the causative virus first appeared in the United States in 1979, Asia in 1985, and Europe in 1987. It has been speculated, but not proven, that this virus arose by the “species jumping,” to swine, of the closely related lactate dehydrogenase-elevating virus from its natural host, the house mouse, *Mus musculus*. Field strains of porcine reproductive and respiratory syndrome virus are genetically heterogeneous, and there are distinct European and North American genotype lineages that share only some 60% sequence identity.

**Clinical Features and Epidemiology**

Porcine reproductive and respiratory syndrome virus infects only domestic and wild pigs. The disease is initially characterized by anorexia, fever, and lethargy. Clinically affected animals are hyperpneic or dyspneic, and exhibit transient hyperemia or cyanosis of the extremities. Nursery pigs have roughened hair coats and reduced growth rates. Infection of sows in early to mid gestation may have little adverse consequence, whereas infection of sows in late gestation frequently results in reproductive failure characterized by abortion, premature births, stillbirths, and mummified fetuses. Piglets that are born alive after *in-utero* infection are often weak and die quickly, typically with respiratory distress. Mortality in infected sows reflects the virulence of the infecting virus strain, but it can be high. Other types of infectious diseases are more common in herds with enzootic porcine reproductive and respiratory syndrome virus infection.

The virus is spread by direct contact, including pugilism, and the virus is shed from infected pigs in all secretions and excretions. Transplacental transmission also occurs commonly in fully susceptible sows.

**Pathogenesis and Pathology**

Porcine reproductive and respiratory syndrome virus replicates primarily in macrophages in the lungs and lymphoid tissues of infected pigs, although there also may be infection of endothelial cells, respiratory epithelium, and fibroblasts. Viremia begins within 24 hours of infection, and persists in some animals for several weeks in the presence of antibodies. The characteristic lesions of acute infection include lymph node enlargement and interstitial pneumonia, the severity of which reflects the virulence of the infecting virus strain.

Porcine reproductive and respiratory syndrome virus appears to utilize several novel mechanisms to subvert protective host immune responses to facilitate its replication, including: (1) inhibition of caspase-dependent apoptosis of infected macrophages; (2) suppression of the type I interferon response through blockade of the retinoic-acid-inducible gene 1 (*RIG-I*) and interferon regulatory factor 3 (*IRF3*) signaling pathways; (3) use of decoy epitopes and extensive glycosylation of the N-terminal portion of the GP5 protein, both of which limit the impact of the neutralizing antibody response.

**Diagnosis**

Porcine reproductive and respiratory syndrome can be provisionally diagnosed on the basis of clinical signs and lesions in affected animals. The virus can be detected by RT-PCR assay, and, in the tissues of infected pigs, by immunohistochemical staining. The virus grows in swine lung macrophages and some, but certainly not all, virus strains grow well in an African green monkey kidney cell line (MA-104) and cotton rat lung cells. Serological diagnosis can be made using commercial ELISA.

**Immunity, Prevention, and Control**

Infected pigs develop a variable, but frequently weak, immune response to porcine reproductive and respiratory syndrome virus; however, recovered animals typically are immune to reinfection, indicating that immunity is effective and vaccination is feasible. Neutralizing antibodies are directed against epitopes on the N-terminal portion of GP5, and there is marked variation in the glycosylation of this region amongst field strains of the virus. The extent of glycosylation in this area probably affects the ability of antibodies to neutralize the virus, and the neutralizing antibody response of many infected pigs is both weak and slow to develop. Pigs also develop a cellular immune response to porcine reproductive and respiratory syndrome virus, but, despite these responses, virus clearance is delayed, leading to prolonged infection in some animals. It has been proposed that innate immune responses and the availability of susceptible populations of macrophages are major determinants of the outcome of primary infections of swine with the virus.

Control of porcine reproductive and respiratory syndrome virus in free herds is by exclusion, as the virus is spread between herds by the movement of infected swine or infective semen used in artificial insemination. It also is spread mechanically by fomites, and perhaps by long-distance aerosol. Once introduced, the virus spreads quickly in naïve swine populations; thus spread within herds is principally as a result of direct contact, and separation of pens markedly reduces the rate of transmission. Once established in a herd, enzootic infection is perpetuated by a cycle of transmission from sows to piglets *in utero* or through colostrum or milk, and by the regular introduction of new animals into the sow herd and the co-mingling of susceptible and infected pigs. Control in herds with enzootic infection is difficult, and usually achieved through a combination of vaccination and management strategies. Both live-attenuated and inactivated vaccines are commercially available, but
vaccines are not infallible—perhaps because of the remarkable genetic variation amongst strains of the virus, and because of the uncertain nature of what constitutes a protective immune response. Furthermore, there is controversy regarding the potential transmission, circulation, and revers to virulence of live-attenuated vaccine viruses.

**LACTATE DEHYDROGENASE-ELEVATING VIRUS**

Lactate dehydrogenase-elevating virus initially was identified in several laboratories in the early 1960s, during experiments using transplantable tumors in mice. The virus generally causes persistent infections that reveal themselves only by increased concentrations of numerous plasma enzymes, including lactate dehydrogenase. Presence of the virus in laboratory mice may confound experiments, as the infection can alter the immune response and thereby distort the results of immunological experiments.

**Clinical Features and Epidemiology**

Infected mice usually exhibit no clinical evidence of infection and live a normal lifespan, despite persistent life-long infection with the virus. The virus is spread between mice by direct contact, and especially by pugilism, through bite wounds. The virus also is contained in the secretions and excretions from infected mice, and may be disseminated by aerosol or ingestion to susceptible cohorts. The most likely source of infection in mouse colonies is by inoculation of mice with contaminated biological material such as transplantable tumors or cell lines.

**Pathogenesis and Pathology**

Lactate dehydrogenase-elevating virus replicates selectively in differentiated tissue macrophages in all strains of inbred laboratory mice. The virus rapidly achieves an extremely high-titered viremia by cytolytic infection of target macrophages in many tissues, including peritoneum, bone marrow, thymus, spleen, lymph nodes, liver, pancreas, kidneys, gonads, and so on, which quickly depletes this cell population. Persistent infection then follows in infected mice by selective infection of a renewable and continually generated subpopulation of macrophages. Virus-induced cytolysis of tissue macrophages delays the clearance of plasma enzymes such as lactate dehydrogenase, causing the characteristic increase in the concentrations of these enzymes in plasma.

Although infected mice develop antibodies to lactate dehydrogenase-elevating virus, they are ineffective in mediating virus clearance. Extensive glycosylation of the N-terminal portion of GP5, which expresses the neutralization determinants of lactate dehydrogenase-elevating virus, reduces the immunogenicity of this region, apparently by blocking access of neutralizing antibodies to neutralization sites. Strains of the virus that lack some or all of these glycosylation sites are highly susceptible to antibody-mediated neutralization, and have altered tissue tropism; specifically, viruses lacking these glycosylation sites do not establish persistent infection, but are neurovirulent in immunosuppressed C58 and AKR mice. Interestingly, age-dependent poliomyelitis that occurs in these mice occurs because they express an endogenous retrovirus in several tissues, and co-infection of spinal cord ventral horn motor neuron cells with both lactate dehydrogenase-elevating virus and the endogenous retrovirus results in poliomyelitis and paralysis. These events do not occur under natural conditions, as they are unique to the nature of selected inbred strains of mice and their corresponding complement of endogenous retroviruses.

**Diagnosis**

Virus is most readily detected in tissues or biological products by RT-PCR, or by the mouse antibody production test. Plasma concentrations of lactate dehydrogenase are substantially increased in mice infected with this virus, with an 8–11-fold increase typically reached at 3–4 days after infection. Antibodies can be detected 1–3 weeks after infection, by either ELISA or immunofluorescence assays.

**Immunity, Prevention, and Control**

Mice infected with lactate dehydrogenase-elevating virus develop both cellular and humoral immune responses, neither of which are effective in mediating clearance of virus strains that have heterogeneous glycosylation of the N-terminal portion of the GP5 ectodomain. Destruction and subsequent loss of the target macrophage population is the major factor in reducing viremia in early infection. Cytotoxic T lymphocyte responses disappear in the course of persistent infection, as a result of clonal exhaustion. Although antibodies are ineffective in preventing persistent infection, polyclonal B cell activation occurs during persistence, with formation of immune complexes. The combination of viral infection of macrophages, polyclonal B cell activation with immune complex formation, and clonal exhaustion of cytotoxic T cells modulates the immune capability of infected mice, which is the major concern regarding adventitious lactate dehydrogenase-elevating virus infection of laboratory mice.

Vaccines are not available, neither are they indicated, as control of lactate dehydrogenase-elevating virus infection in laboratory mice is by exclusion. Prevention of infection in mouse colonies can be accomplished by: (1) preventing entry of infected laboratory and wild mice or biological products; (2) use of barrier-specific, pathogen-free breeding and housing systems; (3) surveillance based on laboratory
testing. The virus can be eliminated from contaminated cell lines or tumors by in-vitro culture or by passage through athymic nude rats, as either approach eliminates the source of susceptible mouse macrophages that the virus requires for its continued replication.

**SIMIAN HEMORRHAGIC FEVER VIRUS**

Simian hemorrhagic fever was first recognized in 1964, in both the United States and the former Soviet Union, in macaques imported from India. Nearly all infected animals died in these initial outbreaks. There have been remarkably few documented occurrences of this devastating disease since then, although, in the United States in 1989, there were epizootics at three primate colonies, resulting in the death of more than 600 cynomolgus macaques (*Macaca fascicularis*).

Serological studies indicate that subclinical simian hemorrhagic fever virus infection occurs in African cercopithecine monkeys, including Patas monkeys (*Erythrocebus patas*), African green monkeys (*Cercopithecus aethiops*) and baboons (*Papio anubis* and *P. cynocephalus*). Similarly, serological studies indicate subclinical or asymptomatic infection of Asian macaques in China, the Philippines, and Southeast Asia, probably with attenuated virus strains. In contrast, transmission of simian hemorrhagic fever virus from persistently infected African monkeys to Asian macaques (*Macaca mulatta*, *Macaca arctoides*, and *Macaca fascicularis*) results in acute, typically fatal hemorrhagic disease. Transmission occurs by direct contact, aerosol, and fomites, including contaminated needles. Epizootics in macaque colonies originate from accidental introduction of the virus from other primate species that are infected persistently without showing clinical signs.

The onset of disease in macaques is rapid, with early fever, facial edema, anorexia, dehydration, skin petechiae, diarrhea, and hemorrhages. Death occurs at between 5 and 25 days; mortality approaches 100%. Within a colony, infection spreads rapidly, probably via contact and aerosol. Lesions include hemorrhages in the dermis, nasal mucosa, lungs, intestines, and other visceral organs. Shock is suspected as the underlying cause of death. Like other arteriviruses, simian hemorrhagic fever virus replicates in macrophages, although there is much variation in the cellular tropism, immunogenicity, and virulence of individual virus strains in different species of monkey. Virus strains derived from African monkeys are highly infectious and fatal in macaques, whereas baboons and Patas and African green monkeys are persistently infected carriers of these viruses.

Vaccines are not available for simian hemorrhagic fever, and control is based on management practices, including species segregation to prevent transmission of the virus from persistently infected African monkeys, such as Patas monkeys, to macaques.

**MEMBERS OF THE FAMILY RONIVIRIDAE, GENUS OKAVIRUS**

The penaeid shrimp, *Penaeus monodon*, which occurs in Asia, Australia, and East Africa, is the principal aquatic invertebrate host for at least six genotypes of roniviruses. Two genotypes of the virus—designated yellow head virus (genotype 1) and gill-associated virus (genotype 2)—cause significant disease and mortality in cultured shrimp populations. The remaining genotypes have been identified in shrimp without specific disease signs.

**YELLOW HEAD AND GILL-ASSOCIATED VIRUSES**

Yellow head virus disease occurs in postlarval and subsequent stages of *P. monodon* as well as a wider range of juvenile penaeid and palemonid shrimp and krill. Infected shrimp cease feeding and congregate near the surface or corners of the pond. The disease is named because of the characteristic pale appearance of the cephalothorax as a result of yellowing of the underlying hepatopancreas, which is normally brown. Mortality up to 90% can occur after appearance of the disease. Shrimp with gill-associated virus infections also undergo an abrupt cessation of feeding and swimming near the surface, develop a reddened body, and may exhibit pink to yellow coloration of the gills. A wide range of tissues of both ectodermal and mesodermal origin are targets of yellow head virus, including the organ of Oka, which gives rise to the genus designation *Okavirus* used for the virus. Diagnosis of infections caused by roniviruses is best made from moribund shrimp from the pond borders. Stained preparations of gill filaments or hemolymph directly in the field may provide presumptive diagnoses, but standard fixation and processing for hemotoxylin and eosin staining are used to identify characteristic 2-μm spherical basophilic inclusions in the cytoplasm of ectoderm- and mesoderm-derived tissues (e.g., lymphoid organ, stomach subcuticulum, and gills). RT-PCR, immunoblot, in-situ hybridization tests, and electron microscopy may be used to confirm presumptive diagnoses of these infections. The frequent presence of subclinical infections requires establishing that disseminated virus is associated with characteristic lesions in target tissues.

Vaccination or chemotherapeutic approaches to control are not available. Disinfection procedures, use of specific-pathogen-free seed stocks as demonstrated by RT-PCR screen, and use of water supplies confirmed to be free of virus are the major control methods that are used.

**UNCLASSIFIED NIDOVIRUSES OF FISH**

Additional unclassified nidoviruses have been identified among cyprinid fishes in Germany and the United States.
For example, an agent isolated from farmed juvenile fathead minnows (*Pimephales promelas*) in the United States was associated with disease. This virus induces up to 90% mortality among experimentally infected fathead minnows, but not in several other commercially important freshwater fish species, including channel catfish, goldfish, golden shiners, and rainbow trout. The agent is bacilliform (130–180 nm in length and 31–47 nm in diameter) as is characteristic of other nidoviruses, and the virus can be isolated using cell cultures of cyprinid fish origin. RT-PCR assays with generic and specific primer sets and sequencing of the amplified products confirms their relationship with other nidoviruses, and provides confirmation of the infection in fathead minnows.