**Chapter Outline**

**Properties of ARTERIVIRUSES and RONIVIRUSES**

- Classification
- Virion Properties
- Virus Replication

**MEMBERS OF THE FAMILY ARTERIVIRIDAE, GENUS ARTERIVIRUS**

- Equine Arteritis Virus
- Lactate Dehydrogenase-Elevating Virus

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

- Yellow Head and Gill-Associated Viruses

Viruses within the families **Arteriviridae** and **Roniviridae** are included in the order **Nidovirales**, along with those viruses in the families **Coronaviridae** and **Mesoniviridae** (see Chapter 24: **Coronaviridae**). The **Arteriviridae** and **Coronaviridae** include a large group of viruses that infect vertebrates (principally mammalian viruses), whereas the **Roniviridae** and **Mesoniviridae** include viruses that infect invertebrates—crustaceans and insects, respectively. 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' coterminus subgenomic messenger RNAs (mRNAs).

The name of the family **Arteriviridae** is derived from the disease caused by its prototype species, equine arteritis virus. The family **Roniviridae** contains several genotypes of gill-associated and yellow head viruses.

**PROPERTIES OF ARTERIVIRUSES AND RONIVIRUSES**

**Classification**

The family **Arteriviridae** currently comprises a single genus, **Arterivirus**, which contains all member viruses: equine arteritis virus, porcine reproductive and respiratory syndrome virus, lactate dehydrogenase-elevating virus, simian hemorrhagic fever virus, and provisionally, wobbly possum disease virus, a novel nidovirus that was identified recently in Australian brushtail possums (**Trichosurus vulpecula**) in New Zealand (Table 25.1). It has been proposed that the family **Arteriviridae** be further subdivided taxonomically to accommodate the recently identified, highly divergent arteriviruses of African nonhuman primates and rodents. Five genera are included in this proposed classification, based on sequence and phylogenetic analysis of the open reading frame 1b. The family **Roniviridae** currently contains a group of related viruses causing disease in crustaceans that are members of a single genus, **Okavirus**.

**Virion Properties**

Arterivirus virions are enveloped, spherical, and 45–60 nm in diameter, which is only about half the size of those of coronaviruses (Fig. 25.1; also see Fig. 24.1). 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 (Fig. 25.2). There are untranslated regions at the 5' and 3' ends of the genome (156–224 and 59–177 nt, respectively), and a 3'-poly(A) terminal sequence. Arterivirus virions include a single nucleocapsid protein (N) and seven envelope proteins, designated E, GP2, GP3, GP4,
TABLE 25.1 Animal Arteriviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host</th>
<th>Disease</th>
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<tbody>
<tr>
<td>Equine arteritis virus</td>
<td>Horse</td>
<td>Systemic influenza-like disease, arthritis, abortion, pneumonia in foals</td>
</tr>
<tr>
<td>Porcine reproductive and respiratory syndrome virus</td>
<td>Swine</td>
<td>Porcine reproductive and respiratory syndrome, systemic disease; abortion of sows or birth of stillborn or mummified fetuses; respiratory disease</td>
</tr>
<tr>
<td>Lactate dehydrogenase-elevating virus</td>
<td>Mice</td>
<td>Usually none, but the presence of the virus may confound research using infected mice</td>
</tr>
<tr>
<td>Simian hemorrhagic fever virus</td>
<td>Macaques</td>
<td>Systemic hemorrhagic disease, death</td>
</tr>
<tr>
<td>Wobbly possum disease virus</td>
<td>Australian brushtail possum</td>
<td>Neurologic disease</td>
</tr>
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ORF5a protein, GP5, and M. Of these, three minor envelope proteins (GP2, GP3, and GP4) form a heterotrimer, and the nonglycosylated triple-membrane spanning integral membrane protein, M, and the large envelope glycoprotein, GP5, form a heterodimer. The major neutralization determinants of arteriviruses are expressed on GP5, although the M protein exerts a conformational influence on GP5. The structural proteins of simian hemorrhagic fever virus are not as well characterized as that of the other arteriviruses, and its genome includes four additional open reading frames that may represent reduplications of genes encoding minor structural viral proteins (the reduplicated proteins being designated as E’, GP2’, GP3’, and GP4’).

Ronivirus virions are bacilliform, 40–60 nm × 150–200 nm, with rounded ends and prominent glycoprotein envelope spikes (Fig. 25.3; also see Fig. 24.1). The nucleocapsid has helical symmetry and is comprised of a coiled filament 16–30 nm in diameter. The nucleocapsid is surrounded by the envelope, which has diffuse projections (approximately 8 nm thick and 11 nm in length) extending from the surface. Like other nidoviruses, the ronivirus genome is a positive-sense, single strand RNA molecule of 26.2–26.6 kb that contains a 5’ cap structure, a 3’-poly(A) tail, and includes five open reading frames (Fig. 25.2; also see Fig. 24.2). Virions consist of at least three envelope proteins (gp116, gp64, and an N-terminal triple-membrane spanning protein of unknown function). The envelope proteins are cleavage products of a larger polyprotein precursor.

Virus Replication

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. Arteriviruses replicate in macrophages and a very limited number of other cell types within their respective hosts. Equine arteritis virus is less fastidious than other arteriviruses as it replicates in vitro in a variety of primary cell cultures, including equine pulmonary artery endothelial, horse kidney, rabbit kidney, and hamster kidney cells, and in a wide variety of cell lines such as baby hamster kidney (BHK-21) and rabbit kidney (RK-13). Other arteriviruses (eg, porcine reproductive and respiratory syndrome, lactate dehydrogenase elevating, and simian hemorrhagic fever viruses) typically replicate in only cultured macrophages or a very limited number of other cell types and/or lines. Some arteriviruses effectively can subvert protective host innate immune responses, including apoptosis of infected macrophages and interferon signaling pathways.

Similar to other enveloped viruses, arteriviruses bind to cell surface receptor(s) using their envelope proteins to mediate the process of cell attachment and fusion with the host cell membrane. 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 (or sialic acid-binding immunoglobulin (Ig)-like lectin 1 (CD169)), and heparan sulfate glycaminoglycans. Recent studies using recombinant chimeric viruses confirm that the ectodomains of the major envelop proteins GP5 and M are not the essential determinants of cellular tropism of equine arteritis virus, rather the heterotrimer of minor envelope proteins GP2, GP3, and GP4 is responsible for cell tropism and receptor binding. Arteriviruses appear to enter susceptible cells by a low-pH-dependent endocytic pathway.

Similar to other nidoviruses, replication and transcription of arteriviruses are processed through different minus-strand intermediates: a full-length minus-strand RNA template (or antigenome) is used for replication, while subgenome-sized minus strands produced during a process of discontinuous RNA synthesis are used to synthesize subgenomic mRNAs (Fig. 25.4). The two large open reading frames at the 5’ end of the arterivirus genome encode two replicase polyproteins that 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 13 (equine arteritis virus) or 16 (porcine reproductive and respiratory syndrome virus) nonstructural proteins (nsps) 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′ coterminal subgenomic RNAs (Fig. 25.2). 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 noncontiguous 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.
In arterivirus-infected cells, newly synthesized nonstructural proteins are incorporated into cellular organelles, particularly the endoplasmic reticulum, which results in membrane pairing and formation of double-membrane vesicles. Immunoelectron microscopic studies have confirmed that viral nonstructural proteins that are part of the replication/transcription complexes and nascent viral RNA are associated with these double-membrane vesicles suggesting that these vesicles carry the enzyme complex responsible for virus replication and subgenomic mRNA synthesis. Arterivirus replication occurs in the cytoplasm of
infected cells, although nonstructural (nsp1) and structural (N) proteins selectively translocate to the nucleus. During the early stages of infection, nsp1 is primarily located in the nucleus while its perinuclear cytoplasmic localization becomes evident later in infection. The newly synthesized genome is encapsidated into the N protein to form the nucleocapsid, which becomes enveloped by budding through the endoplasmic reticulum—Golgi intermediate compartment that contains membranes that include the viral envelope proteins. Newly formed virions mature in the Golgi complex during their movement through the exocytic pathway and are ultimately released from infected cells (Table 25.2).

The overall strategy for replication of roniviruses is similar to that of arteriviruses, but the structural proteins are expressed from a nested set of just two subgenomic mRNAs that each encode several proteins. The subgenomic mRNAs 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 “pinkeye,” “infectious or epizootic cellulites,” “influenza erysipelas,” and “Pferdestaupe.” 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 an aborted fetus during an epizootic of abortion and respiratory disease on a breeding farm near Bucyrus, Ohio. After isolation of the causative virus and description of characteristic vascular lesions, equine viral arteritis was identified as an etiologically distinct disease of the
horse. Equids are the only known natural host of equine arteritis virus.

Although serologic studies indicate that infection occurs worldwide, the incidence of both infection and overt disease varies markedly between countries and among horses of different breeds. Serologic surveys confirm equine arteritis infection of horses in North and South America, Europe, Australia, Africa, and Asia, whereas other countries such as Iceland and Japan are apparently free of the virus. New Zealand recently eradicated equine arteritis virus infection.

**Clinical Features and Epidemiology**

Most natural infections with equine arteritis virus are subclinical or mild, 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 (typically 6–8 days following venereal exposure), the onset of disease is marked by fever (\(\geq 41^\circ\text{C}\)), leukopenia, depression, excessive lacrimation, 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 (neonatal) foals, and a progressive “pneumo-enteric” syndrome is described in 1–3 month-old 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.

**TABLE 25.2 Properties of Arteriviruses**

<table>
<thead>
<tr>
<th>Property</th>
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<tbody>
<tr>
<td>Virions are spherical, 50–70 nm in diameter, with an isometric nucleocapsid and a closely adherent smooth-surfaced envelope with ring-like structures.</td>
</tr>
<tr>
<td>The genome consists of a single molecule of linear, positive-sense, single-stranded RNA, 13–15 kb in size. Virion RNA has a 5’ cap and its 3’ end is polyadenylated; the genomic RNA is infectious.</td>
</tr>
<tr>
<td>Replication takes place in the cytoplasm; the genome is transcribed to form a full-length negative-sense RNA, from which is transcribed a 3’ coterminnal nested set of mRNAs; only the unique sequences at the 5’ end of each mRNA are translated.</td>
</tr>
<tr>
<td>Virions are formed by budding into the endoplasmic reticulum; from where they are released by exocytosis.</td>
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</tbody>
</table>
A variable proportion of acutely infected stallions (10–70%) become persistently infected and shed the virus exclusively in their semen. Persistent infection is maintained in the reproductive tract of individual stallions for variable intervals, from several weeks to lifelong. There is no compelling evidence of equine arteritis virus causing persistent infection in mares, geldings, or foals.

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 (Fig. 25.5). The latter are the essential natural virus reservoir, and play an important role in maintenance and spread of the virus in the equine population. 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.

**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 bloodstream. 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 viral arteritis reflect vascular injury but the role and importance of the virus itself in the pathogenesis of vascular injury—as compared to the involvement of virus-induced cytokines derived from macrophages and endothelial cells—is not yet 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.

Host genetic factors influence the clinical outcome of equine arteritis virus infection, as reflected by the production of pro-inflammatory and immunomodulatory cytokines in clinically affected versus unaffected horses. Specifically, horses segregate into susceptible and resistant phenotypic groups based on the in vitro susceptibility of their CD3⁺ T lymphocytes to virus infection. Similarly, stallions that display the in vitro CD3⁺ T-cell susceptible phenotype are at a higher risk of becoming persistently infected carriers.
of equine arteritis virus as compared to stallions that lack this phenotype. The pathogenesis of the carrier state in stallions is poorly characterized. The virus persists in the ampulla, and to a lesser extent other accessory sex glands of the stallion’s reproductive tract. The establishment and maintenance of the carrier state in the stallion is testosterone dependent. Furthermore, persistently infected stallions that are castrated cease shedding virus in semen, whereas those supplemented with testosterone after castration continue to shed virus.

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 highly pathogenic horse-adapted laboratory strains of equine arteritis virus, as is terminal disseminated intravascular coagulation that 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.

Diagnosis

Equine arteritis virus can be detected by either virus isolation or RT-PCR in nasal or conjunctival swabs and whole blood (either EDTA or sodium citrate anticoagulant) of acutely infected horses, fetal tissues (placenta, fetal fluids, lung, spleen, or lymph nodes) following abortion, and the semen (sperm rich fraction) of carrier stallions. Virus can also be detected in the lungs, spleen, and lymphoid tissues (eg, thymus, mesenteric, and bronchial lymph nodes) of foals that die of “pneumoeenteric syndrome.” Whole blood collected in heparin is not suitable for laboratory testing for the presence of equine arteritis virus. Virus isolation is usually done in rabbit kidney-13 cells, but RT-PCR has significant advantages over virus isolation in terms of reproducibility between laboratories, ease and speed of completion, and cost.

The diagnosis of equine viral arteritis also can be confirmed by serological demonstration of rising neutralizing antibody titers (fourfold or greater) in paired serum samples. Serum antibodies to equine arteritis virus are usually detected by virus neutralization assay, although more convenient and rapid competitive ELISA (cELISA) has been developed recently.

Immunity, Prevention, and Control

The innate response of the mucosal lining of the respiratory and genital tracts constitutes the first line of defense to equine arteritis virus infection in susceptible equids (see Chapter 4: Antiviral Immunity and Virus Vaccines). Natural infection results in long-lasting humoral immunity that likely provides lifelong protection against reinfection with most if not all strains of the virus. The humoral immune response to equine arteritis virus is characterized by production of both complement-fixing and virus-specific neutralizing antibodies that develop 1–2 weeks after infection. The first appearance of neutralizing antibodies coincides with the disappearance of virus from the blood and tissues of infected horses. However, virus persists in the reproductive tract of the carrier stallion for a variable period despite the presence of high titers of virus neutralizing antibodies in serum. Foals born to immune mares are protected against clinical equine viral arteritis by passive transfer of virus neutralizing antibodies in colostrum. Virus neutralizing antibodies appear in foal serum a few hours after colostrum feeding, peak at 1 week of age, and gradually decline to extinction between 2 to 6, and rarely 7, months of age. The cell-mediated immune response to equine arteritis virus is poorly characterized.

Outbreaks of equine viral arteritis occur when 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; acutely infected mares that were recently bred to a carrier stallion are often the initial source of virus. Thus, appropriate biosecurity and control programs are critical to preventing the introduction and/or spread of the virus on farms, racetracks, horse shows and veterinary clinics and hospitals. Rapid identification and isolation of index cases is especially critical, along with any contact horses, as equine arteritis virus is readily spread by secretions from infected horses as well as by fomites. Identification of carrier stallions is also central to effective control, thus 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 potential virus transmission to susceptible cohorts. There is no specific therapy or treatment for horses infected with equine arteritis virus. The carrier state in persistently infected stallions is testosterone dependent but surgical castration remains the only reliable method for eliminating this infection. Transient suppression of testosterone production in carrier stallions using antagonists of GnRH can temporarily limit the shedding of virus in the semen.
Immunization of horses with attenuated or inactivated virus vaccines induces immunity against infection, thus immunization of valuable breeding animals is justified. Vaccination of colts at 6–8 months of age is done to prevent the establishment of persistent infections in breeding stallions. 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, susceptible mares should be vaccinated before breeding.

During outbreaks, the spread of equine arteritis virus is best controlled by: (1) animal 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 horses; (4) implementation of guidelines for breeding stallions and mares that incorporate appropriate screening and annual vaccination; (5) laboratory-supported surveillance.

**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 (*Mus musculus domesticus*). 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. Attempts to infect *Peromyscus* mice, rats, guinea pigs and rabbits with lactate dehydrogenase-elevating virus have been unsuccessful.

**Clinical Features and Epidemiology**

Despite lifelong persistent infection, lactate dehydrogenase elevating virus-infected mice usually have a normal life expectancy and exhibit no clinical evidence of infection other than the elevated level of plasma enzymes and subtle changes in their immune status. Infection of laboratory mice now is very uncommon. 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 cytolysis of permissive macrophages in many tissues, including peritoneum, bone marrow, thymus, spleen, lymph nodes, liver, pancreas, kidneys, and gonads, 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 from apparently virus-nonpermissive precursor cells. 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. The replication of lactate dehydrogenase elevating virus in macrophages allows it to avoid host defense mechanisms, although the precise mechanism of immune evasion and persistent infection is still unclear.

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 coinfection 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 heterogenous 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.

PORCINE 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 subsequently to reproduce the disease. 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 isolates of porcine reproductive and respiratory syndrome virus are genetically heterogeneous and broadly classified into two distinct genotypes, European (type 1 porcine reproductive and respiratory syndrome virus; prototype Lelystad virus) and North American (type 2 PRRSV; prototype VR-2332 virus). Viruses belonging to both genotypes have been associated with outbreaks of clinically similar reproductive and respiratory disease in pigs, despite the fact that there is only 55–70% nucleotide identity in the various genes of viruses of the two genotypes. Phylogenetic analyses of field strains of virus from around the world have identified several different subtypes and lineages within these two virus genotypes. The global distribution of individual virus subtypes varies considerably, and the international movement of pigs and their products (including germplasm) is likely responsible for the dissemination of different virus genotypes and subtypes around the world.

Clinical Features and Epidemiology

Porcine reproductive and respiratory syndrome virus infects only domestic and wild pigs. Pigs of all ages are susceptible in immunologically naive herds. Clinical signs are variable and influenced by the virulence of the virus strain and by the immune status and management practices of the individual herd. Low-virulence strains of porcine reproductive and respiratory syndrome virus may result in widespread infection of swine (high morbidity) with minimal occurrence of disease (low mortality), whereas highly virulent virus strains can cause severe outbreaks of disease in susceptible herds. 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, reduced growth rates, and increased mortality. In 2006, an especially virulent strain of porcine reproductive and respiratory syndrome virus emerged in China (also known as “porcine high fever disease”) and spread regionally in Asia. Pigs infected with this highly virulent virus developed prolonged high fever (41–42°C), severe respiratory signs, and red discoloration of the skin and cyanosis of the ears. Mortality was especially high (20–50%) amongst affected pigs.

Porcine reproductive and respiratory syndrome virus infection of sows in early to mid-gestation may have little adverse consequence, whereas infection of sows in late gestation (usually after 100 days of gestation) frequently results in reproductive failure. Affected litters typically contain a variable mixture of normal pigs, weak small pigs, stillborn pigs, and partially or completely mummified fetuses (so-called SMEDI; stillbirth, mummification, embryonic death, and infertility). 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. Infected sows may also exhibit neurological signs such as ataxia and circling. Infected boars may continue to shed virus in their semen for prolonged periods of time.

Porcine reproductive and respiratory syndrome virus is spread by direct contact between infected and uninfected swine, including pugilism. The virus is shed from infected pigs in all secretions and excretions, including respiratory
Pathogenesis and Pathology

Porcine reproductive and respiratory syndrome virus replicates primarily in macrophages in the lungs and lymphoid tissues of infected pigs. Infection of endothelial cells, respiratory epithelium, and fibroblasts also occurs. Viremia begins within 24 hours of infection, and can last for 1–2 weeks in mature pigs and as long as 8 weeks in young pigs. Characteristic lesions of acute porcine reproductive and respiratory syndrome virus infection of susceptible pigs include lymph node enlargement and interstitial pneumonia, the severity of which reflects the virulence of the infecting virus strain. Microscopic lesions include diffuse interstitial pneumonia, myocarditis, vasculitis, and encephalitis. Lymphoid tissues exhibit lymphoid hyperplasia and follicular necrosis with mixed inflammatory cell infiltration.

The clinical signs and lesions that result from porcine reproductive and respiratory syndrome virus infection are caused by several mechanisms, including apoptosis of virus-infected macrophages and surrounding cells (indirect apoptosis), production of pro-inflammatory and immunomodulatory cytokines, polyclonal B cell activation, and reduced phagocytosis and killing of bacteria by macrophages leading to pneumonia and/or septicemia. Pigs of different breeds may vary in their inherent resistance/susceptibility to disease expression following porcine reproductive and respiratory syndrome virus infection as the virus modulates the innate response of infected cells, notably macrophages. Virus-mediated inhibition of innate immune responses by infected swine also compromises their subsequent adaptive immune response, leading to weak cell-mediated immunity, delayed appearance of the neutralizing antibodies, and, potentially, prolonged infection and viremia. The virus appears to utilize multiple mechanisms to subvert protective host immune responses to facilitate its replication, including: (1) inhibition of caspase-dependent apoptosis of infected macrophages; (2) interference with antigen presentation by dendritic cells and macrophages; (3) reduction in natural killer cell activity; (4) suppression of the type I interferon response through blockade of the retinoic-acid-inducible gene 1 (RIG-1) and interferon regulatory factor 3 (IRF3) signaling pathways; (5) induction or suppression of interleukin production (IL-10 and IL-12); (6) 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; (7) genetic and antigenic variation; (8) glycan shielding of neutralizing epitopes; (9) interference with virus-specific T-cell responses.

Porcine reproductive and respiratory syndrome virus apparently can establish prolonged (up to approximately 5 months) infections within lymph nodes (eg, inguinal and sternal), tonsils, and the male reproductive tract, perhaps leading to prolonged shedding of virus in oropharyngeal secretions and semen. Infectious virus, however, has only been detected in boar semen for up to 42 days after virus infection.

Diagnosis

Provisional diagnosis of porcine reproductive and respiratory syndrome is based on the occurrence of characteristic clinical signs and lesions in affected swine. The presence of porcine reproductive and respiratory syndrome virus in bronchoalveolar lavage fluid or oral secretions, blood, semen, or tissues (lung, lymph node) can be detected by either RT-PCR assay or by virus isolation in porcine alveolar macrophages or the MA104 and related cell lines. Strains of the virus vary in their ability to be isolated and propagated in individual cell types thus RT-PCR assay is the preferred method of virus detection. Virus-specific antibodies may be detected using one of a variety of serological assays, including ELISA, microsphere immunoassay, immunofluorescence or immunoperoxidase monolayer assays, or virus neutralization assay.

Immunity, Prevention, and Control

Pigs develop a variable, frequently weak, immune response following porcine reproductive and respiratory syndrome virus infection. Recovered animals typically are immune to reinfection, indicating that immunity is effective and vaccination is feasible. Furthermore, passive transfer of neutralizing antibodies can protect pregnant sows against infection with virulent virus, and piglets that ingest colostrum from immune sows acquire protective antibodies that persist for 6–8 weeks. However, the humoral immune response to porcine reproductive and respiratory syndrome virus varies significantly between pigs, and there are suggestions that protective immunity might be virus strain-specific with varying degrees of heterologous protection against other strains of the virus. Infected pigs produce virus-specific
ELISA antibodies within 5–7 days, and ELISA antibody titers peak by 5–6 weeks after infection and persist thereafter. Virus neutralizing antibodies appear more slowly, usually appearing at only 4–5 weeks after infection and these do not peak until approximately 10 weeks after infection. The appearance of neutralizing antibodies coincides with virus clearance from the lungs of infected pigs. The neutralization determinants (epitopes) of porcine reproductive and respiratory syndrome virus have not been fully characterized. The GP2, GP3, GP4, GP5, and M envelope proteins have all been identified, using different techniques, as inducers of neutralizing antibodies. However, specific neutralization epitopes have been identified only in the GP3, GP4, and GP5 proteins of European strains of the virus, and only the GP5 protein of North American virus strains. Marked variation in glycosylation of the GP3, GP4, and GP5 proteins amongst field strains of the virus may affect the ability of antibodies to neutralize the virus, and may also be responsible for the weak and delayed neutralizing antibody response of many infected pigs. Pigs also develop a cellular immune response to PRRSV, but, despite these responses, virus clearance is delayed, leading to prolonged infection in some animals. The porcine T-cell response appears to be directed against GP2, GP3, GP4, GP5, M, and N proteins of the virus, and the M protein may also express important T-cell epitopes.

It is also 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 porcine reproductive and respiratory syndrome virus. Several viral nonstructural proteins, including nsp1, nsp2, nsp 4, and nsp 11, can inhibit production of type I interferon in infected cells, potentially modulating the course of infection in pigs.

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 comingling of susceptible and infected pigs. Control in herds with enzootic infection is difficult, and usually achieved through a combination of vaccination and management strategies as there are no specific treatments for affected swine. 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. However, modified live virus vaccines are used widely to reduce disease occurrence and severity, as well as duration of viremia and virus shedding.

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 that resulted 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 anubius and Papio 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. Genetically divergent variants of simian hemorrhagic fever virus were recently identified from colobus (Procolobus rufomitratus tephrosceles) and African red-tailed (guenon) (Cercopithecus ascanius) monkeys.

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.

**WOBBLY POSSUM DISEASE VIRUS**

Wobbly possum disease virus was first identified in 1995 in an Australian brushtail possum (*T. vulpecula*) with fatal neurological disease, and infection with this virus has subsequently been confirmed amongst free-living possums in New Zealand. Partial genomic sequence and phylogenetic analyses indicate that wobbly possum disease virus is mostly closely related to viruses of the family *Arteriviridae*. The neurologic disease has been reproduced experimentally using a variety of inocula from affected possums and administered to healthy possums. The disease is characterized by inappetance and decreased responsiveness to environmental stimuli, followed by fine head tremors, ataxia, apparent blindness, and cachexia. Histologically, the disease is characterized by perivascular mononuclear infiltrates in a variety of tissues including brain, liver, spleen, and kidney. Viral nucleic acid can be detected in tissues by RT-PCR. To date, there is no suitable cell culture system with which to isolate and propagate the virus.

**OTHER ARTERIVIRUSES**

Genetically novel arteriviruses have been identified in a variety of African nonhuman primates (*Procolobus* [*Piliocolobus*] *rajomitratus* *tephrosceles*; *Cercopithecus asca*; *Papio cynocephalus*; *Papio anubis*; *Chlorocebus pygerythrus*; *Papio kindae*; *Cercopithecus neglectus*), an African forest giant pouched rat (*Cricetomys emini*), and in cattle. Although some of the primate viruses were identified in Asian macaques with simian hemorrhagic fever, the pathogenic significance of many of these viruses remains to be established.

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

**YELLOW HEAD AND GILL-ASSOCIATED VIRUSES**

The rapid expansion of shrimp aquaculture in Asia and the Americas has been accompanied by several episodes in which emerging diseases have devastated large portions of the industry. Among the most severe, is yellow head disease, caused by a rod-shaped nidovirus (yellow head virus). Yellow head disease was first detected in 1990 in black tiger shrimp (*Penaeus monodon*) farmed in Central Thailand and has since spread into most shrimp farming areas of the world including Southeast Asia, the Indo-Pacific region, and the Americas. Yellow head viruses infect a wide range of tissues of both ectodermal and mesodermal origin, including the organ of Oka, which gives rise to the genus designation as *Okavirus*. Subsequently, a similar virus was detected in the gills of moribund farmed *P. monodon*. This gill-associated virus was determined to be a second genotype of yellow head virus and serves as the type species of the genus. It is now known that *P. monodon*, which occurs in Asia, Australia, and East Africa, is the principal aquatic invertebrate host for at least six genotypes of closely related roniviruses. This “yellow head complex” includes yellow head virus (genotype 1) that is the cause of yellow head disease and gill-associated virus (genotype 2) that occasionally causes disease and “mid-crop mortality syndrome” in cultured shrimp populations. The remaining genotypes (3–6) of viruses in the yellow head complex are highly prevalent and occur mainly in shrimp without specific disease signs. Evidence of recombination among viruses of the various genotypes has been reported.

Yellow head disease occurs in postlarval and all subsequent stages of *P. monodon*, as well as a wide range of juvenile penaeid and palemonid shrimp and krill species. Infected shrimp abruptly 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. Explosive mortality of up to 100% can occur within 3–5 days after appearance of the disease. Affected tissues include those of the lymphoid organ, gills, subcutis, gut, antennal gland, gonads, and nerve ganglia, as well as hemocytes and hematopoietic tissues. Microscopically, inclusion bodies are observed in the cytoplasm of infected cells. Shrimp with gill-associated virus infections also cease feeding, swim near the surface, develop a reddened body, and may exhibit pink to yellow coloration of the gills. Yellow head virus is efficiently transmitted horizontally via several routes, including exposure to water-borne virus during cohabitation and via cannibalism of infected carcasses. Yellow head virus is stable in seawater for up to 72 hours. There is no direct evidence of transmission of yellow head virus vertically, but there is substantial evidence of vertical transmission of gill-associated virus in wild and farmed *P. monodon*. Shrimp surviving infections with roniviruses may become long-term carriers and high levels of persistent infections are found among wild shrimp, providing an important reservoir of infection. Diagnosis of yellow head disease 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 histologic evaluation is used to identify
characteristic 2-µm spherical basophilic inclusions in the cytoplasm of cells in affected tissues (e.g., lymphoid organ, stomach subcuticulum, and gills). Subclinical infections are common, thus diagnosis requires establishing that virus is associated with characteristic lesions in target tissues. Confirmatory tests include RT-PCR, immunoblot, \textit{in situ} hybridization tests, and electron microscopy. Several RT-PCR assays, some genotype-specific, can be used for screening broodstock or for surveillance of wild stocks of shrimp. Sequencing of PCR amplicons can be used to identify specific genotypes.

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