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## Transmission Cycles

Human and livestock encroachment into natural transmission cycles is the most common means of exposure to arboviral infections. This virus transmission scenario is sometimes termed 'spillover' because the principal enzootic vector(s) has a wide host feeding range not limited to the natural, amplifying, vertebrate hosts. Arboviruses in this category of transmission include, but are not limited to, West Nile, sylvan Yellow fever, Western equine encephalitis and La Crosse viruses. African swine fever virus is maintained in sub-Saharan Africa in a cycle involving *Ornithodoros* ticks and warthogs. Infected ticks infesting warthog burrows can also transmit the virus to their progeny by transovarial transmission. Infected warthogs do not show signs of the disease; however, domestic swine are severely affected with mortality rates approaching 100%. In addition to tick bite, transmission between domestic pigs can occur through direct contact and the ingestion of infected offal.

Domestication of animals for human use has provided arboviruses with new and abundant hosts. For example, Rift Valley fever virus circulates in extensive areas of Africa and is transmitted by certain species of *Aedes* and *Culex* mosquitoes to various native vertebrates. The virus is thus maintained by horizontal transmission in the vertebrates and by transovarial and/or vertical transmission of the virus in mosquito eggs during periods of drought. El Nino events can lead to sustained rainfall and flooding, resulting in the production of massive numbers of other species of *Aedes* and *Culex* mosquitoes that enter into the existing, endemic transmission cycle. Infected vectors disperse and bloodfeed on livestock, initiating a secondary amplification cycle. Viremic livestock become the source for a dramatic increase in the numbers of infected vectors, which in turn engenders human epidemics and livestock epizootics.

The third pattern of arboviral transmission is one in which humans serve as both the reservoir and amplification hosts. Arboviruses maintained in human→vector→human cycles are generally transmitted by domestic mosquito vectors, most importantly, *Aedes aegypti*. The dengue viruses, the most important arboviruses in terms of human disease, are the classic example of an arbovirus that has adapted to the human host. Other examples include Chikungunya and Yellow fever viruses which can cause spectacular outbreaks in areas infested with *Aedes aegypti* vectors. These latter viruses generally disappear after a sufficient numbers of humans become immune, but persist in sylvan transmission cycles until nonimmune human hosts become available or when human herd immunity wanes.

See also: Akabane Virus; Crimean-Congo Hemorrhagic Fever Virus and Other Nairoviruses.

## Further Reading

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## Arteriviruses

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## History

Equine arteritis virus (EAV) was first isolated in 1953 in Bucyrus, Ohio from the lung tissues of an aborted fetus during an epidemic of abortions and arteritis in pregnant mares. However, an equine disease similar to that caused by EAV was first observed in the late 1800s. At the time of its discovery, EAV was distinguished from equine (abortion) influenza virus.

Lactate dehydrogenase-elevating virus (LDV) was discovered by accident in 1960 during a study to find methods for early detection of tumors in mice. A five- to tenfold increase in lactate dehydrogenase (LDH) levels in serum 4 days after inoculation of mice with either Ehrlich carcinoma cells or cell-free extracts suggested that an infectious agent was responsible for the observed LDH elevation.

Porcine respiratory and reproductive syndrome was first observed in North America in 1987 and in Europe

in 1990. This disease has also been referred to as porcine epidemic abortion and respiratory syndrome (PEARS), swine infertility and respiratory syndrome (SIRS), and mystery swine disease (MSD). The causative agent of the disease is now referred to as porcine respiratory and reproductive syndrome virus (PRRSV).

Simian hemorrhagic fever virus (SHFV) was isolated in 1964 during outbreaks of a fatal hemorrhagic fever disease in macaque colonies in the US, Russia, and Europe. A number of additional SHFV outbreaks in macaque colonies have occurred since the 1960s. The most 'famous' of these was the one in Reston, Virginia, which occurred in conjunction with an Ebola virus outbreak in the same facility.

## Taxonomy and Classification

On the basis of virion size and morphology as well as the positive polarity of the RNA genome, LDV and EAV were originally classified within the family *Togaviridae*. In 1996, following the sequence analysis of their genomes, EAV, LDV, SHFV, and PRRSV were classified as species within a new family, *Arteriviridae*, genus *Arterivirus*. EAV was designated the prototype of this family. At the same time, the family *Arteriviridae* was classified together with the family *Coronaviridae* in the order *Nidovirales*. This order also includes two additional virus groups, the toroviruses (a genus in the *Coronaviridae* family) and the family *Roniviridae*. The arterivirus genome shares similar general organizational features and conserved replicase motifs with corona-, toro-, and ronivirus genomes, but is only about half their size. In addition, arterivirus particles are smaller than those of other nidoviruses, differ from them morphologically, and are the only ones to have an isometric nucleocapsid structure.

## Geographic Distribution

Viruses with biological properties identical to those of LDV have been isolated from small groups of wild mice (*Mus musculus*) in Australia, Germany, the US, and UK. Natural infections with EAV and EAV-induced disease in horses and donkeys have been documented in North America, Europe, and Japan and anti-EAV antibodies have been detected in horse sera from Africa and South America, indicating that EAV infection is geographically widespread. Natural PRRSV infections in pigs have been reported in North America, Europe, and Asia. SHFV infection in captive patas monkeys has been documented and this virus has also been detected in the blood of wild-caught patas and African green monkey as well as baboons, suggesting that these African primates are the natural hosts for SHFV.

## Host Range and Virus Propagation

Natural infections with EAV occur only in horses and donkeys. Field isolates of EAV can be readily obtained from field samples (semen, fetal tissues, and buffy coats) using RK-13 cells. Laboratory strains of EAV have been successfully grown in primary cultures of horse macrophages and kidney cells, rabbit kidney cells, and hamster kidney cells and also in cell lines, such as BHK-21, RK-13, MA-104, and Vero.

LDV replicates efficiently in all strains of laboratory and wild *Mus musculus* and somewhat less efficiently in the Asian mouse *Mus caroli*. Numerous attempts to infect other rodents such as rats, hamsters, guinea pigs, rabbits, deer mice (*Peromyscus maniculatus*), and dwarf hamsters (*Phodopus sungorus*) with LDV have not been successful. LDV replicates only in primary murine cell cultures that contain macrophages, such as spleen, bone marrow, embryo fibroblast, and peritoneal exudate cell cultures. Although peritoneal cultures prepared from starch-stimulated adult mice contain 95% phagocytic cells, only 6–20% of these cells support LDV replication as demonstrated by autoradiographic, *in situ* hybridization, immunofluorescence and electron microscopic techniques, suggesting that LDV infects an as yet uncharacterized subpopulation of macrophages. A much higher percentage of cells in peritoneal exudate cells obtained from infant mice are susceptible to virus infection.

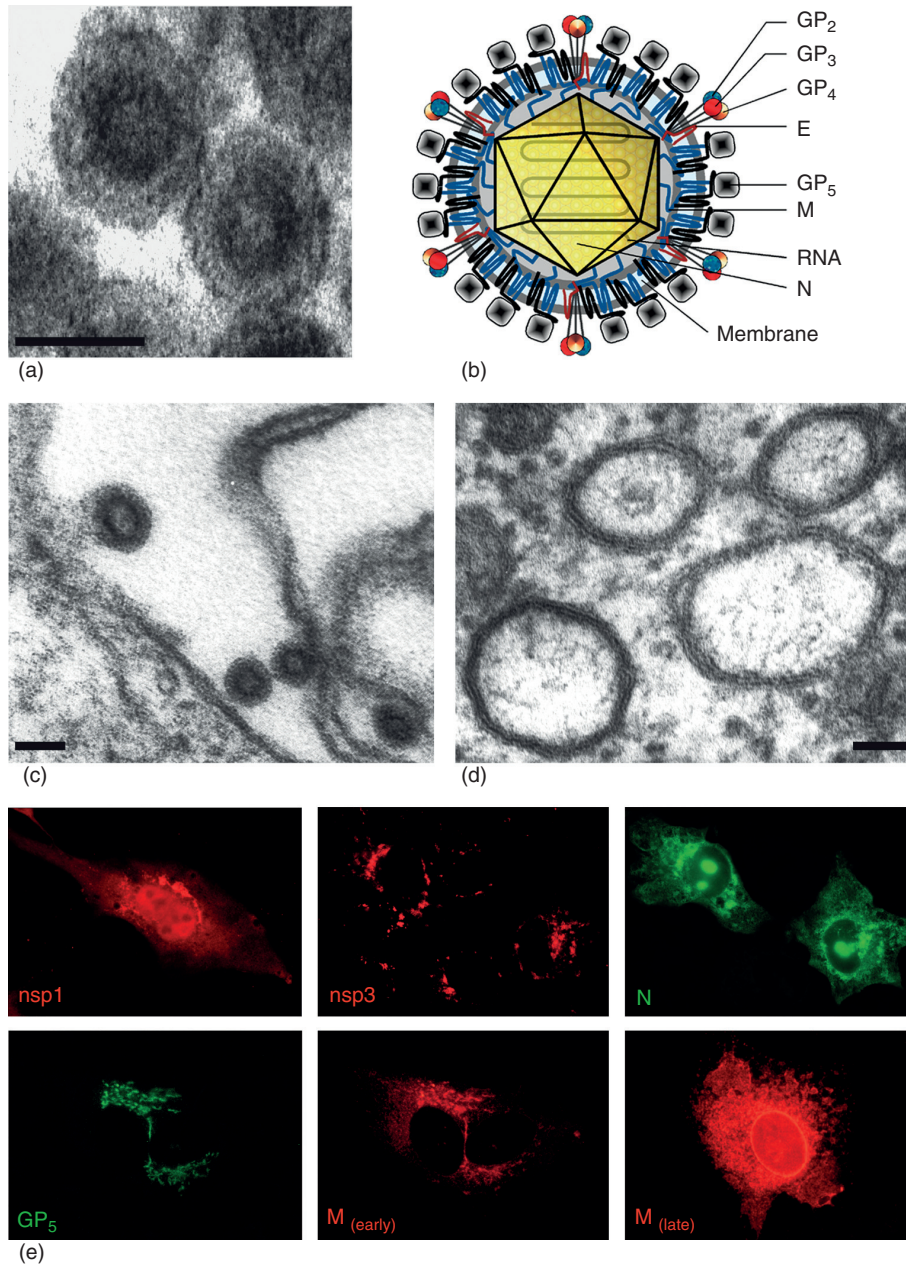
Natural infections with PRRSV were thought to be restricted to pigs. However, one report suggested that chickens and mallard ducks may be susceptible to the virus. PRRSV can replicate in primary cultures of porcine alveolar macrophages and macrophages from other tissues. Some, but not all, isolates of PRRSV can be adapted to replicate in a subclone of the MA-104 cell line.

Natural infections with SHFV occur in several species of African primates, namely *Erythrocebus patas*, *Cercopithecus aethiops*, *Papio anuibus* and *Papio cynocephalus*. SHFV infection of members of the genus *Macaca* has occurred in primate facilities in a number of countries and was associated with a fatal hemorrhagic fever. Isolates of SHFV can replicate in primary cultures of rhesus alveolar lung macrophages or peripheral macrophages and some isolates can replicate efficiently in the MA-104 cell line.

Maximum arterivirus yields after infection of susceptible cell cultures are observed by 10–15 h after infection. The maximum titers obtained for LDV and PRRSV are  $10^6$ – $10^7$  ID<sub>50</sub> ml<sup>-1</sup> and can exceed  $10^8$  PFU ml<sup>-1</sup> for EAV and SHFV.

## Properties of the Virion

Arterivirus particles are spherical, enveloped, and 40–60 nm in diameter (Figures 1(a) and 1(b)). Unfixed



**Figure 1** (a) An electron micrograph of extracellular PRRSV particles. (b) Schematic representation of an arterivirus particle and its seven structural proteins. (c) An electron micrograph of EAV particles budding from smooth membranes in (or close to) the Golgi region of infected baby hamster kidney cells (BHK-21) cells. (d) An electron micrograph of typical double-membrane vesicles found in the cytoplasm of arterivirus-infected cells that have been implicated in replication complex formation and viral RNA synthesis. (e) Localization of selected EAV nonstructural and structural proteins in infected BHK-21 cells by immunofluorescence microscopy. In contrast to all other nsps (e.g., nsp3), the N-terminal replicase subunit nsp1 only partially localizes to the perinuclear region and is partially targeted to the nucleus. The double-labeling for nsp3 and the N protein shows that a considerable part of the latter co-localizes with the viral replication complex, whereas another fraction of the N protein is targeted to the nucleus. Early in infection, double-labeling for the major glycoprotein GP<sub>5</sub> and the M protein showed almost complete co-localization of the two proteins in the Golgi complex, in the form of the heterodimer that has been found to be critical for virus assembly. Later in infection, the M protein accumulates in the endoplasmic reticulum. Scale = 50 nm (a, c–d). (a) Reprinted from Snijder EJ and Meulenber JM (1998) The molecular biology of arteriviruses. *Journal of General Virology* 79: 961–979. (b) Reprinted from Snijder EJ, Siddell SG, and Gorbalenya AE (2005) The order Nidovirales. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections, Vol. 1: Virology*, 10th edn., pp. 390–404. London: Hodder Arnold. (c, d) Reprinted from Snijder EJ and Meulenber JM (1998) The molecular biology of arteriviruses. *Journal of General Virology* 79: 961–979. (e) Images courtesy of Yvonne van der Meer and Jessika Zevenhoven, Leiden University Medical Center, The Netherlands.

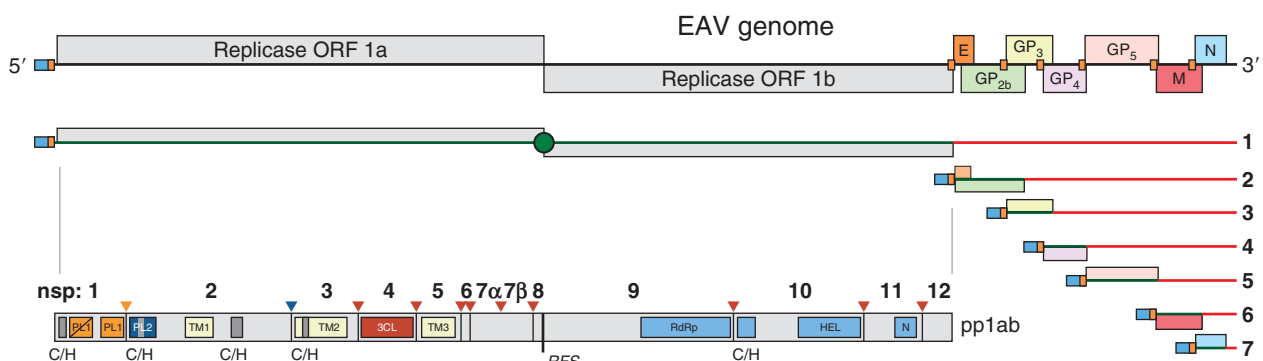
virions undergo distortion and disintegration during standard negative staining procedures. The virion surface appears rather smooth. The virion capsid is icosahedral and about 25–35 nm in diameter. Buoyant densities of 1.13–1.17 g cm<sup>-3</sup> and sedimentation coefficients of 214S to 230S have been reported for arteriviruses. Virions can be stored indefinitely at -70 °C but are heat labile. For instance, the infectivity of LDV samples in plasma decreased by half after 4 weeks at -20 °C and by about 3.5 logs after storage for 32 days at 4 °C. Virus in media supplemented with 10% serum is stable for 24 h at room temperature, but completely inactivated by heating at 58 °C for 1 h. Virions are fairly stable between pH 6 and pH 7.5, but are rapidly inactivated by high or low pH. Virus is efficiently disrupted by low concentrations of nonionic detergent.

The locations of the seven structural proteins in an EAV virion are indicated schematically in **Figure 1(b)**. The icosahedral capsid is composed of the nucleocapsid (N) protein. The major envelope glycoproteins, GP<sub>5</sub> and M, form a disulfide-linked heterodimer. The minor glycoproteins GP<sub>2</sub>, GP<sub>3</sub>, and GP<sub>4</sub> form a disulfide-linked heterotrimer. GP<sub>2</sub>-GP<sub>4</sub> heterodimers have also been detected in EAV virions. Although all six of these proteins were shown to be required for EAV and PRRSV infectivity, not all of the minor structural proteins have been identified so far in the other arteriviruses and the nomenclature for the SHFV structural proteins differs due to an insertion in the 3' region of the SHFV genome. Virions bud into the lumen of cytoplasmic vesicles (**Figure 1(c)**).

## Properties of the Genome

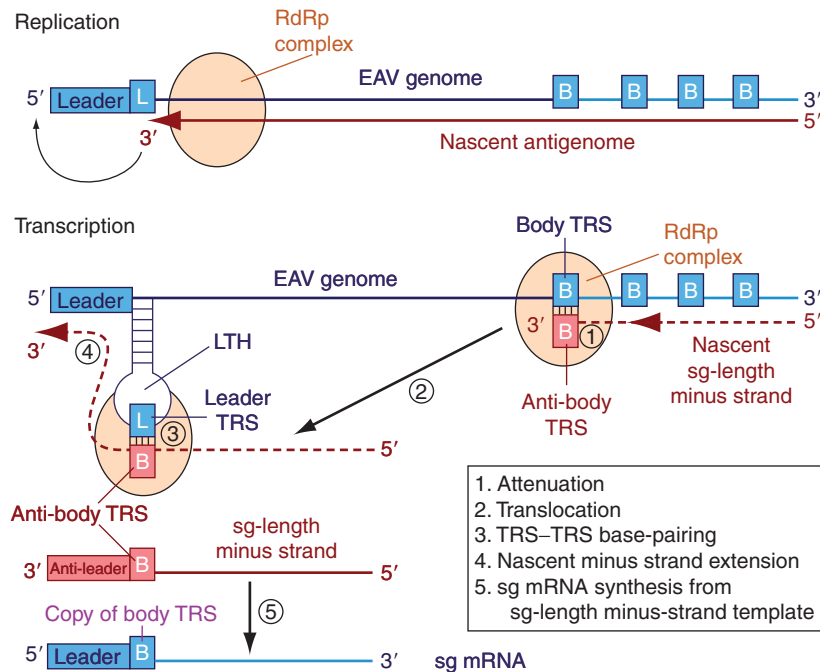
Arterivirus genomes are single-stranded RNAs of positive polarity that contain a 3' poly(A) tract of approximately 50 nucleotides in length and a type I cap at the 5' end. The genome lengths are 12.7 kb for EAV, 14.1 kb for LDV, 15.1 kb for PRRSV, and 15.7 kb for SHFV.

The large nonstructural or 'replicase' polyproteins are encoded by open reading frames (ORFs) 1a and 1b and occupy the 5' three-fourths of the genome. ORF 1b is translated only when a -1 ribosomal frameshift occurs in the short ORF 1a/ORF 1b overlap region. A 'slippery sequence' upstream of a pseudoknot directs frameshifting and for EAV, a frameshifting efficiency of 15–20% has been reported. ORF 1a encodes three or four proteases that post-translationally cleave the pp1a and pp1ab polyproteins at multiple sites into the mature viral nonstructural proteins (**Figure 2**). The lengths of the ORF 1a regions of the different arteriviruses vary. ORF 1b encodes major conserved domains, in particular an RNA-dependent RNA polymerase, a putative zinc-binding domain, an RNA helicase, and a nidovirus uridylylate-specific endoribonuclease. The multiple 3'-proximal ORFs (**Figure 2**) encode the structural proteins. There are six such ORFs in the genomes of EAV, PRRSV, and LDV, while SHFV contains nine ORFs downstream of ORF 1b. Limited sequence homology suggests that the SHFV ORFs 2a, 2b, and 3 may be duplications of ORFs 4, 5, and 6, respectively. In most cases, adjacent structural protein genes of arteriviruses are in different reading frames and overlap. Conserved



**Figure 2** Arterivirus genome organization and expression are illustrated using the family prototype EAV as an example. The genomic open reading frames are indicated and the names of the corresponding proteins are given. Below the genome, the nested set of mRNAs found in infected cells is depicted, with RNA1 being identical to the viral genome and subgenomic RNAs 2–7 being used to express the structural protein genes located in the 3'-proximal quarter of the genome. With the exception of the bicistronic mRNA2, the subgenomic mRNAs are functionally monocistronic. The EAV replicase gene organization is depicted in the polyprotein pp1ab from the replicase (pp1a is identical to the nsP1–8 region of pp1ab). Ribosomal frameshift (RFS) delineates the boundary between amino acids encoded in ORF 1a and ORF 1b and arrows represent sites in pp1ab that are cleaved by papain-like proteases (yellow and blue) or the main (3CL) protease (red). The proteolytic cleavage products (nsPs) are numbered and the locations of various conserved domains are highlighted. These include domains with conserved Cys and His residues (C/H), putative transmembrane domains (TM), protease domains (PL1, PL2, and 3CL), the RNA-dependent RNA polymerase domain (RdRp), helicase (HEL), and uridylylate-specific endoribonuclease (N). Adapted from Siddell SG, Ziebuhr J, and Snijder EJ (2005) Coronaviruses, toroviruses, and arteriviruses. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections, Vol. 1: Virology*, 10th edn., pp. 823–856. London: Hodder Arnold.





**Figure 3** Arterivirus RNA synthesis (using a hypothetical virus that produces four subgenomic (sg) mRNAs). Both replication (top panel) and transcription (bottom panel) are depicted. In the ‘replication mode’, the RdRp produced a full-length minus-strand RNA (antigenome) that serves as the template for synthesis of new genomic RNA. In the ‘transcription mode’, minus-strand RNA synthesis is thought to be discontinuous and regulated by transcription-regulating sequences (TRSs). Body TRSs (B) in the genome would act as attenuators of minus-strand RNA synthesis. Subsequently, the nascent minus strand, with an anti-body TRS at its 3′ end, would be redirected to the 5′-proximal region of the genomic template by a base-pairing interaction with the leader TRS (L) that (for EAV) has been shown to reside in a leader TRS hairpin (LTH) structure. Following the addition of the anti-leader to the nascent minus strands, the sg-length minus strands would then serve as templates for sg mRNA synthesis.

transcription-regulating sequences (TRSs; **Figure 3**) are located at the 3′ end of the genomic leader sequence (leader TRS) and upstream of each of the 3′-proximal ORFs (body TRSs). RNA hairpin structures are located near the 5′ end of the genome (including a leader TRS-presenting hairpin) and also in the 3′ NTR.

### Properties of the Viral Proteins

Arterivirus proteins that are encoded at the 5′ end of the genome are translated directly from the genomic RNA as polyproteins (pp1a and pp1ab; **Figure 2**). The proteins generated from these ORFs contain all functions required for viral RNA synthesis. Both pp1a/pp1ab contain multiple papain-like cysteine proteases and a chymotrypsin-like (or ‘3C-like’) serine protease (**Figure 2**). The EAV papain-like cysteine proteases each cleave at a single site. The nsp4 serine protease, or main protease, cleaves at six sites in the pp1a region and at three additional sites in the ORF 1b-encoded part of pp1ab. Due to the existence of two alternative processing cascades (minor and major pathways) a variety of processing intermediates and mature proteins are generated from the C-terminal half of pp1a. In total, 13 (EAV) or 14 (PRRSV/LDV) mature proteins are (predicted to be) generated from the

arterivirus replicase polyproteins. Three hydrophobic regions in pp1a are thought to be important for membrane association of the viral replication–transcription complexes. With the exception of nsp1, which is partly found in the nucleus, the rest of the nonstructural proteins localize to endoplasmic reticulum-derived double-membrane structures (**Figure 1(d)**) in the perinuclear region (**Figure 1(e)**). The mature ORF 1b-encoded proteins (nsp9–nsp12) are thought to be the primary enzymes of the viral replication–transcription complexes that direct viral RNA synthesis.

The proteins encoded in the 3′-proximal quarter of the genome are expressed from six (nine for SHFV) overlapping subgenomic mRNAs (a 3′ co-terminal nested set; **Figure 2**). Although the subgenomic mRNAs, with the exception of the smallest one, are structurally polycistronic, in general only the 5′ terminal ORF is translated. An exception is mRNA 2, which is bicistronic encoding GP<sub>2b</sub> and E. The nucleocapsid (N) protein is encoded by ORF 7 (ORF 9 in SHFV). Analysis of the crystal structure of the C-terminal domain of the PRRSV N protein suggests that arteriviruses have a unique capsid-forming domain. The M protein which is encoded by ORF 6 (ORF 8 in SHFV), is a triple-membrane-spanning protein and the major nonglycosylated envelope protein. The major envelope glycoprotein is encoded by ORF 5 (ORF 7 in SHFV). GP<sub>2b</sub>, GP<sub>3</sub>, and GP<sub>4</sub> are each about

20 kDa in size and are minor envelope glycoproteins (EAV, **Figure 2**). GP<sub>2b</sub> and GP<sub>4</sub> (GP<sub>4b</sub> and GP<sub>6</sub> in SHFV) are class I integral membrane proteins. A soluble, non-virion-associated form of PRRSV GP<sub>3</sub> is released from infected cells. E is an unglycosylated small hydrophobic minor envelope protein. The PRRSV E protein has been shown to possess ion channel protein-like properties.

The nonstructural protein nsp1 and the N protein have been detected in the nucleus as well as the cytoplasm (**Figure 1(e)**). However, the biological significance of the nuclear localization of these two viral proteins is currently not known.

## Replication

Cell tropism is determined in part at the level of a receptor on the cell surface, since in some cases cells that are nonpermissive for an arterivirus have been reported to be productively infected after transfection of viral genomic RNA. Evidence for a specific saturable, but as yet unidentified receptor for LDV on a subpopulation of murine macrophages has been reported. LDV-immune complexes are also infectious and can infect macrophages via Fc receptors. Sialoadhesin (sialic acid-dependent lectin-like receptor 1), a macrophage-restricted, cell surface protein, has been shown to mediate the internalization of PRRSV by alveolar macrophages. Heparin sulphate on the cell surface and sialic acid on the virion are also thought to play a role in entry. PRRSV has been reported to enter cells via a low pH-dependent endocytic pathway. Soon after infection, both EAV and LDV particles have been observed in small vesicles that appear to be clathrin-coated. The existence of an additional level of host restriction at the endosomal membrane fusion or uncoating steps was suggested by the observation that nonsusceptible cells expressing recombinant sialoadhesin could internalize virus but rarely became productively infected.

The arterivirus replication cycle occurs in the cytoplasm of infected cells. After the incoming genomic RNA is uncoated, it is translated to produce polyproteins pp1a (1727–2502 amino acids) and pp1ab (3175–3959 amino acids) and then becomes the template for minus-strand synthesis (**Figure 3**). Either a full-length minus-strand RNA, that then serves as a template for genomic RNA synthesis, or subgenomic minus strands, that then serve as the templates for subgenomic mRNA synthesis, can be produced. The subgenomic RNAs are thought to be produced by a mechanism of discontinuous minus-strand RNA synthesis that utilizes conserved primary (TRSs) and higher-order RNA structures as signals for producing a subgenomic minus-strand template for each subgenomic mRNA. Subgenomic mRNAs are 3′-coterminal and contain a common 5′ leader sequence that is identical to the 5′ terminus of the genomic RNA. *cis*-Acting regulatory

signals required for arterivirus replication have been mapped to the ~300 nt at each end of the genome. Host proteins also appear to be involved in the regulation of arterivirus RNA synthesis.

The co-localization of N with replicase complexes (**Figure 1(e)**) suggests that genome encapsidation may be coordinated with genome synthesis. New virions form via budding of preformed capsids into the lumens of smooth endoplasmic reticulum and/or Golgi complex membranes (**Figure 1(c)**). Arterivirus envelope proteins localize to intracellular membranes and recent data suggest that the formation of the GP<sub>5</sub>–M heterodimer is required for budding (**Figure 1(e)**). Mature virions in the lumens of these vesicles are then transported to the exterior of the cell and released.

The formation of cytoplasmic double-membrane vesicles (**Figure 1(d)**), which have been implicated in viral RNA synthesis, is characteristic of arterivirus-infected cells. Infection of primary macrophages by EAV, SHFV, PRRSV, and probably also LDV is cytotoxic. Laboratory strains of EAV, SHFV, and PRRSV cause obvious cytopathology in the continuous cell cultures that they infect, such as MA-104 cells. Infected cells become rounded by 24–36 h after infection and release from the tissue culture flask. Apoptosis has been reported in PRRSV-infected primary porcine alveolar macrophages and MA-104 cell cultures as well as in testicular germ cells in pigs. However, other studies with PRRSV showed that necrosis, not apoptosis, was the main cause of death of infected cells.

## Genetics

Evidence for virulence variants of all arteriviruses has been obtained. One strain of LDV isolated from C58 tumor-bearing mice and designated LDV-C was shown to efficiently induce neurologic disease in a few susceptible inbred mouse strains, such as AKR and C58, both of which are homozygous for the *Fv-1<sup>r</sup>* allele. Subsequent studies showed that neuropathogenic and non-neuropathogenic isolates coexist in most LDV pools. The number of glycosylation sites in the ectodomain of GP<sub>5</sub> varies in different LDV strains and it has been postulated that antibodies bind less efficiently to virions with extensive glycosylation in this region. A neurovirulent strain of PRRSV has also been reported. Virulent and avirulent mutants of EAV have been identified on the basis of the severity of the diseases they cause. Attenuated vaccine strains of EAV and PRRSV and a number of temperature-sensitive mutants of EAV have been selected. SHFV isolates that produce acute asymptomatic infections and ones that cause persistent, asymptomatic infections in patas monkeys have been reported. EAV and PRRSV infectious cDNA clones have been constructed and provide a means for analyzing the virulence determinants via reverse genetics.

## Evolution

Evidence of RNA recombination has been obtained by genome sequencing for both LDV and PRRSV after co-infections with different strains of the same virus type and it is thought that RNA recombination is the mechanism responsible for the observed gene duplication in the SHFV genome. Sequence comparisons of various field isolates of either PRRSV or EAV indicate that the sequences of the M and N proteins are more conserved than those of the virion glycoproteins. The extent of the divergence of the sequences of European and North American PRRSV isolates indicates that these two virus populations represent subspecies and also suggests that the ability to cause porcine disease arose independently in geographically separated virus populations. Phylogenetic analysis of the arteriviruses indicated that PRRSV is most closely related to LDV and that SHFV is more closely related to both of these viruses than to EAV. Although the host specificity of LDV has experimentally been shown to be restricted to mouse species, it has been postulated that PRRSV arose when wild boars became infected with LDV after they ate infected wild mice and that wild boars then introduced a divergent 'LDV' virus into domestic pigs independently in North America and Europe.

The nidoviruses represent a distinct evolutionary lineage among positive-strand RNA viruses. Although the organization of the conserved replicase motifs in the arterivirus genome is very similar to that of the other nidoviruses (coronaviruses, toroviruses, and roniviruses), the structural protein genes of these viruses are apparently unrelated (Figure 1). This level of divergence may be related to a high frequency of RNA recombination that appears to be a characteristic of nidovirus replicases and may be a byproduct of the mechanism of discontinuous RNA synthesis utilized by these viruses for subgenomic RNA production. The ancestral nidovirus has been postulated to have had an icosahedral capsid. If via a recombination event with another type of virus, the progenitor of the coronavirus/torovirus lineage acquired an N protein that could form a helical nucleocapsid, then packaging restrictions on genome size would have been lost, allowing genome size expansion via further recombination events and divergence from the arterivirus branch.

## Serologic Relationships and Variability

Attempts to demonstrate antigenic cross-reactivity between EAV, LDV, PRRSV, and SHFV have not been successful with one exception. Antibodies produced to a single linear LDV neutralization site located in GP<sub>5</sub> neutralized both LDV and PRRSV. Monoclonal antibodies elicited by one strain of LDV did not bind to the proteins of most other LDV isolates in Western blots, suggesting that serologic

variants of LDV exist. Variation in PRRSV N epitopes has been observed between North American and European virus isolates and a high degree of heterogeneity has been observed between strains of PRRSV within the ectodomain of GP<sub>4</sub>, which contains a secondary neutralization epitope.

## Transmission

There is no evidence for transmission of any of the arteriviruses via insect vectors. Horizontal transmission of both EAV and PRRSV occur via the respiratory route as well as via the venereal route by virus in the semen of persistently infected 'carrier' males. Vertical transmission of PRRSV *in utero* has been reported.

Nothing is currently known about the incidence of transmission of LDV in wild mouse populations. In the laboratory, unless the cage mates are fighting males, LDV is rarely transmitted between mice housed in the same cage, even though infected mice excrete virus in their feces, urine, milk, and saliva. However, transmission of LDV from mother to the fetus via the placenta and to pups via breast milk/saliva has been documented within the first week after infection of the mother. Since LDV in mice and SHFV in patas monkeys is produced throughout the lifetime of persistently infected animals, the transfer of fluids or tissues from an infected animal to an uninfected one results in the inadvertent transfer of infection. Historically the most frequent mode of transmission of LDV among laboratory mice and SHFV from patas monkeys to macaques has been through experimental procedures such as the use of the same needle for sequential inoculation of several animals. Currently, the most frequent sources of LDV contamination are pools of other infectious agents or tumor cell lines that have been repeatedly passaged in mice, especially those first isolated in the 1950s. Such materials should be checked for the presence of LDV. Infectious agent stocks can be 'cured' of LDV by passage in a continuous cell line or a different animal species. Tumor cell stocks can be 'cured' by *in vitro* culture for several passages. It has been suggested but not proven that SHFV can be transmitted between macaques via the respiratory route.

## Tissue Tropism

The primary target cells for all four arteriviruses are macrophages. Measurement of the amount of virus in various tissues during natural EAV infections indicated that lung macrophages and endothelial cells were the first host cells to be infected. Bronchial lymph nodes subsequently became infected and then the virus spread throughout the body via the circulatory system. In fatally infected horses, lesions are found in subcutaneous tissues, lymph nodes, and viscera. The progression of PRRSV



infection in pigs is likely to be similar to that observed with EAV. However, although PRRSV is thought to be naturally transmitted by aerosols, experimental transmission by this route has been difficult to achieve. PRRSV has been reported to replicate in testicular germ cells which could result in excretion of virus into the semen. LDV replicates in an uncharacterized subpopulation of murine macrophages. Virus target cells are located in tissues as well as in the blood. Cells containing LDV-specific antigen have been identified in sections of liver and spleen by indirect immunofluorescence. In spleen, the virus-infected cells are nucleated and located in the red pulp. In liver, only Kupffer cells contained LDV-specific antigen. In C58 and AKR mice infected with a neurotropic strain of LDV, virus replication was demonstrated in ventral motor neurons by *in situ* hybridization.

### Pathogenicity and Clinical Features of Infection

Serological evidence indicates that even though EAV is widespread in the horse population, it rarely causes clinical disease. Both EAV and PRRSV can cause either persistent asymptomatic infections or induce various disease symptoms such as respiratory disease, fever, necrosis of small muscular arteries, and abortion. The severity of disease caused by EAV and PRRSV depends on the strain of virus as well as the condition and age of the animal. The most common symptoms of natural EAV infections in horses are anorexia, depression, fever, conjunctivitis, edema of the limbs and genitals, rhinitis, enteritis, colitis, and necrosis of small arteries. If clinical symptoms occur, they are most severe in young animals and pregnant mares. Infections in pregnant mares are often inapparent, but result in a high percentage (50%) of abortions. Young animals occasionally develop a fatal bronchopneumonia after infection, but natural infections are not usually life-threatening. In contrast, about 40% of pregnant mares and foals experimentally inoculated with EAV die as a result of the infection. Horses infected with virulent EAV isolates develop a high fever, lymphopenia, and severe disease symptoms. Symptoms observed in PRRSV-infected pigs include fever, anorexia, labored breathing, and lymphadenopathy. Lesions are observed in the lungs and infected pregnant sows produce weak or stillborn piglets.

Mice infected with LDV usually display no overt symptoms of disease. A distinguishing feature of LDV infections is the chronically elevated levels of seven serum enzymes, LDH (eight- to tenfold), isocitrate dehydrogenase (five- to eightfold), malate dehydrogenase (two- to threefold), phosphoglucose isomerase (two- to threefold), glutathione reductase (two- to threefold), aspartate transaminase (two- to threefold), and glutamate-oxaloacetate transaminase (two- to threefold). A decrease in the humoral and

cellular immune response to non-LDV antigens is observed during the first 2 weeks following LDV infection. Thereafter, the immune response to other antigens is normal. In immunosuppressed C58 and AKR mice, neurovirulent isolates of LDV can induce a sometimes fatal poliomyelitis. In these mice, immunosuppression is required to delay antibody production until after virus has reached the central nervous system (CNS) and infected susceptible ventral motor neurons. LDV-infected neurons become the targets of an inflammatory response. In mice 6 months of age or older, paralysis of one or both hindlimbs and sometimes a forelimb is observed. In younger C58 mice, poliomyelitis is usually subclinical.

Isolates of SHFV that induce persistent, asymptomatic infections and ones that cause acute, asymptomatic infections of patas monkeys have been reported. All SHFV isolates cause fatal hemorrhagic fever in macaque monkeys. Infected macaques develop fever and mild edema followed by anorexia, dehydration, adipsia, proteinuria, cynosis, skin petechia, bloody diarrhea, nose bleeds, and occasional hemorrhages in the skin. The pathological lesions consist of capillary-venous hemorrhages in the intestine, lung, nasal mucosa, dermis, spleen, perirenal and lumbar subperitoneum, adrenal glands, liver, and periocular connective tissues. These signs and symptoms are not unique to SHFV-infected animals, since they are also observed after infection of macaques with other types of hemorrhagic fever viruses such as Ebola virus. Although the SHFV-induced lesions are widespread in infected animals, the level of tissue damage is not severe. Even so, mortality in macaques infected with SHFV approaches 100% and occurs within 1 or 2 weeks after infection.

### Pathology and Histopathology

In horses experimentally or fatally infected with EAV, the most common gross lesions are edema, congestion, and hemorrhage of subcutaneous tissues, lymph nodes, and viscera. Microscopic investigation of tissues from chronically infected horses, which had mildly swollen lymph nodes and slightly increased volumes of pleural and peritoneal fluids, revealed extensive lesions consisting of generalized endothelial damage to blood vessels of all sizes as well as severe glomerulonephritis. Both types of lesions are thought to be caused by the deposition of viral immune complexes. Extensive capillary necrosis leads to a progressive increase in vascular permeability and volume, hemoconcentration, and hypotension. During the terminal stages of the disease, lesions are also found in the adrenal cortex, and degenerative changes are observed in the bone marrow and liver. Focal myometritis is observed in infected pregnant mares and is thought to be the cause of deficiencies in the fetal and placental blood supply. The resulting anoxia is probably the cause of abortion.

Although most LDV infections are inapparent in mice, some histopathogenic changes are observed in infected animals. As described above, the serum levels of seven enzymes are chronically elevated in LDV-infected mice. Normally, an increase in serum levels of tissue enzymes is the result of tissue damage, but in LDV-infected animals little tissue damage is observed. Although there are five naturally occurring LDH isozymes in mouse plasma, only the level of isozyme LDVV is elevated in LDV-infected mice. Studies have indicated that the increase in enzyme levels is primarily the result of a decreased rate of enzyme clearance. A subpopulation of Kupffer cells involved in receptor-mediated endocytosis of LDH is severely diminished in mice by 24 h after LDV infection. It has been postulated that LDV replication in these cells causes their death and results in increased LDH serum levels. Splenomegaly, characterized by a greater than 30% increase in spleen weight, occurs in about 40% of the mice infected with LDV. The increase in spleen weight is observed by 24 h after infection and persists for up to a month. A marked necrosis of lymphocytes in thymic-dependent areas occurs during the first 4 days after LDV infection together with a transient decrease in the number of circulating T lymphocytes between 24 and 72 h after infection. A transient decrease in peritoneal macrophages is also observed between the first and tenth day of infection. Despite the lifelong presence of circulating viral immune complexes and the demonstration of some LDV antibody deposits in the kidneys of LDV-infected mice as early as 7 days after infection, these animals do not develop kidney disease. It has been suggested that nephritis does not develop in these chronically infected mice because of the inability of the majority of the LDV-antibody complexes to bind C1q. Low levels of C1q-binding activity can only be detected between days 10 and 18 after LDV infection. LDV infection can alter the outcome of concomitant autoimmune disease, probably through modulation of the host-immune responses. LDV infection can also trigger the spontaneous production of different types of autoantibodies, possibly as a result of polyclonal B-lymphocyte activation.

The CNS lesions in neurovirulent LDV-infected C58 and AKR mice are located in the gray matter of the spinal cord and, occasionally, in the brainstem and consist of focal areas of inflammatory mononuclear cell infiltrates in the ventral horn. Virus-specific protein and nucleic acid have been detected in ventral motor neurons, and maturing virions in these neurons have been observed by electron microscopy.

## **Immune Response**

Antibodies in sera obtained from animals infected with EAV or PRRSV recognize virion proteins N, M, GP<sub>5</sub>, and

GP<sub>2</sub>. Neutralizing antibodies are primarily directed to GP<sub>5</sub> and the neutralization epitopes of EAV, LDV, and PRRSV have been mapped to the ectodomain of this protein. For EAV, there are four major GP<sub>5</sub> conformational neutralization sites that are interactive. Also, for EAV, interaction between GP<sub>5</sub> and M is required for neutralization. For LDV and PRRSV, the major neutralization site is located in the N-terminus of GP<sub>5</sub>. A secondary neutralization site for PRRSV has been mapped to the ectodomain of GP<sub>4</sub>. The neutralization epitopes of SHFV have not yet been studied.

Anti-EAV antibodies can be detected in horses 1–2 weeks after infection with virulent or avirulent strains of the virus. Complement-fixing, antiviral antibodies peak 2–3 weeks after the initiation of infection and then decline. Neutralizing antibody peaks between 2 and 4 months after infection. An increase in neutralizing antibodies usually leads to virus clearance. Often after 8 months, anti-EAV antibody can no longer be detected by complement-fixation or neutralization assays. However, in some animals the virus persists and viral immune complexes continue to circulate.

The first month of infection of pigs with PRRSV is characterized by high viremia and disease symptoms. A vigorous antiviral antibody response can be detected by ELISA beginning 7–9 days after infection but these antibodies have little neutralizing activity. Beginning at about a month after infection, neutralizing antibody can be detected and peaks between 1 and 2 months after infection. Viremia is reduced to very low levels but virus continues to be produced from infected cells in tissues for at least 5 months. Usually, the infection eventually is completely cleared, but in some cases it continues to persist.

In LDV-infected mice, which always become persistently infected, antiviral GP<sub>5</sub> and N antibody that is primarily of the IgG2a subclass is produced as early as 6–10 days after infection. The production of this antibody is dependent on functional T helper cells. Plasma from LDV-infected mice has a much higher nonspecific binding activity than plasma from uninfected mice; virus-specific binding measured by enzyme-linked immunosorbent assay (ELISA) usually cannot be detected until the plasma has been diluted at least 1:400. Some virus neutralization by this early antibody has been demonstrated but is incomplete due to the presence of virus quasi-species that are resistant to neutralization. Anti-LDV antibody that is not complexed to virus can be detected by 15 days after infection, indicating that antibody is present in excess of virus in chronically infected mice. Although the presence of anti-LDV antibodies does not prevent infection of macrophages, it does effectively neutralize neurovirulent LDV strains and so protects motor neurons from becoming infected. LDV-infected mice display a polyclonal humoral response and anti-LDV antibody apparently accounts for only a small portion of this polyclonal response. The mechanism by which LDV

infection activates B cells polyclonally is currently not known, but mice immunized with inactivated virus do not develop a polyclonal response. Autoantibodies to a variety of cellular components (autoimmune antibodies) have been detected in mice chronically infected with LDV.

SHFV isolates that produce acute infections in patas monkeys induce high levels of neutralizing antibody, whereas SHFV isolates that induce persistent infections induce low titers of non-neutralizing antibody. Antibodies to virus that causes acute infection do not cross-neutralize virus that causes persistent infection. In macaques, death from an SHFV infection occurs before an effective adaptive immune response can be elicited.

LDV-infected animals develop cytotoxic T cells that can specifically recognize and lyse virus-infected macrophages. However, this cytotoxic response is not able to clear the infection. Whether anti-LDV cytotoxic T cells persist indefinitely in chronically infected mice or eventually disappear due to clonal exhaustion is disputed. A cytotoxic T-cell response as indicated by IFN $\gamma$ -producing T cells can be detected in PRRSV-infected pigs starting about a month (the same time that neutralizing antibody appears) after infection and lasts at least a year.

Although the production of neutralizing antibody and cytotoxic T cells is delayed in EAV- and PRRSV-infected animals, these responses are usually effective in clearing the infection. However, in some EAV and PRRSV infections and in all LDV infections, persistent infections develop even though good levels of neutralizing antiviral antibodies and helper and cytotoxic T-cell responses are elicited. The mechanisms by which these viruses evade clearance by the adaptive immune system include extensive glycosylation of the major glycoprotein GP<sub>5</sub> that masks the major neutralization epitope of some strains of the virus, and enhanced infection of macrophages by infectious viral immune complexes via cell surface Fc receptors. Neutralization escape virus variants have been reported to arise during persistent LDV infections and may also arise during persistent infections with other arteriviruses. An immunodominant decoy epitope located just upstream of the neutralization epitope in PRRSV GP<sub>5</sub> induces a strong non-neutralizing antibody response and may be responsible for the weak/absent induction of neutralizing antibodies during the first month of infection.

## Prevention and Control

Avirulent and virulent strains of EAV and PRRSV have been isolated. A number of live attenuated vaccines and killed vaccines are commercially available for both EAV and PRRSV. The live vaccines are more efficacious in providing protection and induce a longer-lasting immunity than the killed vaccines. Although these vaccines induce immunity against disease, immunized animals are

not completely protected from reinfection. Animals immunized with live vaccines can spread virus and can become persistently infected. Outbreaks of disease due to reversion of live PRRSV vaccines have been reported. To allow discrimination between natural and vaccine infections, markers have been engineered into one EAV live vaccine. Recent vaccine development efforts have focused on the utilization of recombinant virus vectors, such as Venezuelan equine encephalitis virus and pseudorabies, or DNA vectors to express GP<sub>5</sub> or both GP<sub>5</sub> and M.

The current lack of rapid diagnostic assays for the detection of LDV and SHFV in persistently infected animals means that it is still a time-consuming task to identify animals with inapparent infections. Care should be taken not to inadvertently transfer arteriviruses from a persistently infected animal to other susceptible animals. Cells and infectious agent pools obtained from animals that might be persistently infected with an arterivirus should be checked for viral contamination before they are injected into a susceptible animal.

## Future Perspectives

Arteriviruses have so far been isolated from mice (LDV), horses (EAV), pigs (LV), and monkeys (SHFV). It seems likely that other host species, including humans, harbor additional members of this virus family. However, such viruses will be difficult to find if the natural hosts develop asymptomatic infections. Little is yet known about the functional roles of the arterivirus proteins in the virus life cycle. Recent studies suggest that the arterivirus nucleocapsid and envelope proteins have unique properties. The intense current interest in dissecting the structure and function of the SARS-coronavirus replicase may provide new insights for similar analyses of the arterivirus replicase. The availability of reverse genetic systems for several of the arteriviruses will not only aid the further molecular characterization of these viruses but will also facilitate the study of viral pathogenesis and antiviral immunity as well as the development of improved vaccines.

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## Ascoviruses

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### Glossary

**Apoptosis** Genetically programmed cell death.

**Apoptotic bodies** Cell vesicles resulting from apoptosis.

**Caspase** Protease that activates a major portion of programmed cell death.

**Endoparasitic wasps** Species of insect parasites belonging to the order Hymenoptera, which lay their eggs in insects where the wasp larvae develop.

**Per os infection** Infection by feeding.

**Programmed cell death** Genetically programmed cascade proteases and nucleases that cleave DNA and proteins within a cell leading to its death.

**Reniform** Shaped like a kidney.

**Transovarial transmission** Transmission of virus inside the egg.

**Virion-containing vesicles** Vesicles containing virions formed by ascoviruses by rescue of apoptotic bodies induced by ascovirus infection.

### Introduction

The family *Ascoviridae* is one of the newest families of viruses, established in 2000 to accommodate several species of a newly recognized type of DNA virus that attacks larvae of insects of the order Lepidoptera. Viruses of

this family produce large, enveloped virions, measuring 130 nm in diameter by 300–400 nm in length, and when viewed by electron microscopy have a reticulated appearance. They are typically bacilliform or reniform in shape, and contain a circular double-stranded DNA genome that, depending on the species, ranges from ~120 to 185 kbp. Whereas the virions of ascoviruses are structurally complex like those of other large DNA viruses that attack insects, such as those of iridoviruses (family *Iridoviridae*) and entomopoxviruses (family *Poxviridae*), they differ from these in two significant aspects. First, ascoviruses are transmitted from diseased to healthy lepidopteran larvae or pupae by female endoparasitic wasps when these lay eggs in their hosts. Second, ascoviruses have a unique cell biology and cytopathology in which shortly after infecting a cell, they induce apoptosis and then rescue the developing apoptotic bodies and convert these into virion-containing vesicles. This aspect of viral reproduction apparently evolved to disseminate virions to the larval blood where they could contaminate the ovipositors of female wasps so that the virus could be transmitted to new hosts. Ascoviruses appear to occur worldwide, wherever there are endoparasitic wasps and larvae of species belonging to the lepidopteran family Noctuidae. However, as these viruses have been discovered relatively recently and their signs of disease are not commonly known in the scientific community, relatively few ascovirus species have been described.