Abstract
Equine viral arteritis is a highly contagious respiratory and reproductive disease of equids whose etiology was defined in the early 1950s. The virus was named equine arteritis virus (EAV) and the disease equine viral arteritis (EVA) because of the distinctive vascular lesions found on histopathological examination of acutely infected horses. There has been significant recent progress in understanding the molecular biology of EAV and the pathogenesis of its infection in horses. In particular, the use of contemporary recombinant DNA (e.g. reverse genetics) and genomic techniques has generated significant novel information regarding the basic molecular biology of the virus and host susceptibility. Therefore, the objective of this review is to summarize the current understanding of EVA including various risk factors, viral factors and the influence of host factors such as genetics on disease susceptibility and host immune response, as well as advances in diagnostics, potential treatment and prevention strategies.

Keywords: Equine, virus, stallion, infection, EAV, EVA, persistent infection, prevention, treatment

Introduction
Equine arteritis virus infection is highly species-specific and exclusively limited to members of the family Equidae, which includes horses, donkeys, mules and zebras.1,2 Equine arteritis virus has been isolated from donkeys in South Africa3 and Chile (Timoney and Balasuriya, unpublished data, 2014) and the viral nucleic acid has been demonstrated in an aborted alpaca fetus by reverse transcription-polymerase chain reaction (RT-PCR).4 Following the 1984 EVA outbreak in Kentucky, it was discovered that persistently infected stallions serve as the natural reservoir of the virus within the equine population.1,2,5,6

A substantial percentage of all infections are subclinical or asymptomatic, especially those that occur in mares after being bred to persistently infected stallions. However, natural outbreaks of EVA are characterized by one or more of the following outcomes: abortion of pregnant mares; fulminant infection of neonates leading to severe interstitial pneumonia or enteritis; respiratory and systemic illness in adult horses and persistent infection of stallions. Clinical cases of EVA typically have an incubation period of 2 to 14 days (6 to 8 days following venereal exposure) with the most consistent clinical features of EAV being pyrexia and leukopenia. Clinical signs vary considerably among individual horses and among outbreaks and often mimic other respiratory and non-respiratory diseases. Typical cases of the disease present with all or any combination of the following signs: fever up to 41°C (105.8°F) of 2-9 day duration, anorexia, serous nasal discharge, conjunctivitis, urticaria and leukopenia; edema of the lower limbs, scrotum, prepuce, eyelids, ventral body wall and udder; abortion in the mare and fatal interstitial pneumonia or pneumoenteritis in young foals. Some 10 to 70% of stallions acutely infected with EAV subsequently become carriers and constantly shed the virus in semen.1 The EAV carrier state can be short or convalescent (lasts only a few weeks after clinical recovery), intermediate (3 to 7 months) or long-term or chronic (lasts for years and even the entire life). The mechanism of viral persistence in the male reproductive tract is not clear. However, persistence of EAV in stallion is testosterone-dependent.7,8

In all viral infections, there are associated risk factors, environmental, viral and host specific. Risk factors associated with EAV transmission are summarized in Figure 1, with EAV being transmitted among horses in five major ways:3,5

- Respiratory: primary route of transmission in acute cases of infection, common at racetracks, shows and sales.
- Venereal: virus shed in the semen of a carrier stallion (cooled or frozen semen can be infectious).
- Other bodily secretions: urine, feces, etc.
In-utero: virus crosses the placenta from an acutely infected mare to her unborn foal.

Indirect contamination: tack and/or equipment shared among horses. Furthermore, there is a high risk of EVA being transferred indirectly via personnel and fomites. Special care should be taken when handling semen in laboratories prior to insemination or preparation for shipping.

Disease is the result of the interaction between the infectious agent (e.g. virus), the host factors and the environmental factors. The interaction between virus-infected cells and host defense systems determines the disease outcome. These interactions are a complex, multifactorial process, which has not been fully characterized for EVA. Viral factors responsible for the virulence phenotype of some strains have been identified by using reverse genetics and were reviewed previously.9-11 The virulence determinants of EAV have been mapped to genes encoding both nonstructural (nsp1, nsp2, nsp7 and nsp10) and structural proteins12 (GP2, GP4, GP5 and M). However, it appears that the most important virulence determinants of EAV are located in the structural protein genes of the virus. The interaction among the GP2, GP3, GP4, GP5 and M envelope proteins plays a major role in determining the tropism of EAV for CD14+ monocytes, whereas tropism for CD3+ T lymphocytes is determined by the GP2, GP4, GP5 and M envelope proteins but not the GP3 protein.13 Using an in vitro cell culture model of persistent EAV infection, it has been shown that combined amino acid substitutions in E, GP2, GP3, and GP4 proteins or a single amino acid substitution in the GP5 protein can facilitate persistent EAV infection in HeLa cells. However, the viral protein(s) involved in establishment of persistent infection in the stallion's reproductive tract are yet to be definitively identified. In summary, the virulence determinants of EAV appear to be complex and to involve multiple genes encoding both envelope and nonstructural proteins.

All horses appear to be susceptible to EAV infection; however, there are considerable differences in seroprevalence between different breeds of horses. In the United States, some 70-90% of adult Standardbred horses are seropositive for EAV, as compared to only 1-3% of the Thoroughbred population. Similarly, a high percentage of European Warmblood horses are seropositive for EAV. While breed-specific differences might reflect different cultural and management factors, it has recently been demonstrated that an inherent genetically conferred resistance or susceptibility of CD3+ T cells to in vitro infection with the virulent Bucyrus strain of EAV. Specifically, based on the in vitro susceptibility of CD3+ T lymphocytes to EAV infection, horses can be segregated into susceptible and resistant phenotypic groups (Figure 2) as described by Go et al.14 Genome-wide association studies (GWAS) of these horses identified a common, genetically dominant haplotype associated with the in vitro susceptible phenotype in the region of ECA11 (49,572,804-49,643,932).14 Biological pathway analysis identified a variety of cellular genes within this region of ECA11 that encode proteins associated with virus attachment and entry, cytoskeletal organization and NF-κB pathways. These cellular proteins are likely to be important in the pathogenesis of EVA infection in horses.14 Additionally, host cellular factors that are involved in formation of the replication complex and other membrane structures during virus replication may play a role in pathogenesis of EVA.9

Experimental EAV infection of horses with either the in vitro CD3+ T cell susceptibility or resistant phenotype showed a significant difference between the two groups in terms of proinflammatory and immunomodulatory cytokine mRNA expression. Of considerable importance, clinical signs of disease were enhanced in horses possessing the in vitro CD3+ T cell resistant phenotype.14 These studies provide direct evidence for a correlation in individual horses between their genotype and the extent of viral replication, occurrence and severity of clinical signs, and cytokine gene expression after experimental EAV infection. Furthermore, CD3+ T lymphocytes from EAV persistently infected (carrier) stallions were susceptible to in vitro EAV infection,15 whereas stallions that did not become long-term carriers after infection with EAV did not possess the CD3+ T lymphocyte-susceptible phenotype (Figure 2). When considered together, these data suggest that stallions with EAV susceptible CD3+ T lymphocytes are at a higher risk of becoming persistently infected as compared to stallions that lack this phenotype and GWAS analysis confirmed that five of seven proven carrier stallions had the dominant ECA11 haplotype previously associated with the CD3+ T lymphocyte susceptible phenotype.9 Further studies on genes located in ECA11 will enhance our understanding of the pathogenesis of EAV infection.
and variation in susceptibility or resistance to establishment of the carrier state in stallions and will hopefully lead to the identification of a putative EAV cell receptor(s).

**Diagnosis**

The differential diagnosis of EVA includes other acute viral respiratory tract infections common at racetracks, shows, sales such as:

- Equine herpesviruses 1 and 4
- Equine influenza virus
- Equine rhinitis A and B viruses
- Equine adenovirus and Getah virus
- Also may include equine infectious anemia, African horse sickness, purpura hemorrhagica and urticaria and toxicosis due to hoary alyssum (*Berteroa incana*)

Appropriate samples for laboratory diagnosis of EVA include:

- Nasopharyngeal swab or nasal washing/swab
- Conjunctival swab
- Semen (sperm rich fraction of the ejaculate)
- Abortions – fetal fluids, placenta, lung, spleen and lymphoid tissue
- Whole blood (EDTA or citrated)
- Paired serum samples (21 to 28 days apart)

Laboratory diagnosis of EVA is currently based on virus isolation (VI) in cell culture or demonstration of viral nucleic acids by reverse-transcription polymerase chain reaction (standard RT-PCR, RT-nested PCR or real-time RT-PCR [rRT-PCR]) in clinical specimens. Serologic diagnosis of EVA is based on the virus neutralization test (VNT) and demonstration of rising neutralizing antibody titers (4-fold or greater) in paired serum samples. The VI and VNT are the current World Organization for Animal Health (OIE) prescribed standard tests for EVA (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals). Although VI is currently the OIE-approved gold standard for the detection of EAV in semen and is the prescribed test for international trade, it has been demonstrated that at least one published rRT-PCR assay has equal or even higher sensitivity than VI for the detection of EAV nucleic acid in semen samples from carrier stallions.16,17 Very clearly, rRT-PCR has significant advantages over VI in terms of reproducibility between laboratories, ease and speed of completion and cost; thus it is logical that the most accurate rRT-PCR assay would replace VI as the prescribed test for international trade. Similarly, the VNT remains the OIE prescribed regulatory test for serologic detection of EAV infection of the horse, despite the fact that this assay is expensive, labor-intensive and time-consuming to perform. Furthermore, results of the VNT can vary markedly among laboratories when insufficient attention is paid to standardization of both test reagents and procedures; therefore, there is an urgent need to develop a highly specific and sensitive ELISA or microsphere immunoassay (Luminex) for the detection of EAV antibodies in serum samples. Incorporation of the immunogenic nonstructural proteins of EAV in such an assay is likely to improve both its sensitivity and specificity. Recently, a new commercial competitive ELISA (cELISA; VMRD Pullman, WA) has become available in the market.18 Histopathologic examination coupled with immunohistochemical (avidin-biotin complex [ABC] immunoperoxidase) staining is also useful for the detection of viral antigens in formalin-fixed paraffin-embedded samples, as well as in frozen tissue sections.

**Treatment**

- Treatment of EVA consists of supportive care until the end of the acute phase of disease. Adequate rest should be provided, particularly to breeding stallions and to horses in training.
- Use of systemic antibiotics is unrewarding and usually contraindicated, except in pneumonitic foals at risk of secondary bacterial infections.
- Repeated vaccination has no impact on viral clearance in persistently infected stallions.
Antagonists of gonadotropin-releasing hormone (GnRH) and GnRH vaccinations have been used to eliminate persistent infection and viral shedding with various levels of success. However, treatment strategies are still at the experimental level and require further evaluation.

Transient suppression of testosterone production in carrier stallions may be a therapeutic alternative in the elimination of EAV infection. There are preliminary data to support that the use of GnRH vaccines or antagonists can temporarily limit the shedding of the virus in the semen of carrier stallions. Fortier et al reported that daily treatment with a GnRH antagonist for 35-37 days led to a temporary elimination of virus from the semen of 5/5 treated stallions. Of those 5 treated stallions, 3 subsequently resumed viral shedding after treatment after 14, 28 and 42 days, respectively, and two remained negative at 114 days. However, because 2/4 control stallions also ceased viral shedding during the experimental period, the authors were not able to prove that the resolution in the first group was an effect of the treatment. Similarly, Burger et al reported the cessation of EAV shedding by a stallion in which testosterone production was suppressed for 5 months by vaccination against GnRH. It was not possible to exclude the likelihood of viral shedding had not also spontaneously ceased. Nonetheless, antagonists of GnRH and GnRH vaccinations have been used to attempt to eliminate persistent infection and viral shedding in stallions with varied levels of success. However, no conclusive therapies to eliminate the EAV carrier state in stallions have been developed to date.

Outcome and prognosis

- 10 to 70% of exposed stallions become persistently infected with EAV and shed virus in their semen, sometimes for life.
- Persistently infected stallions are asymptomatic and may shed a large amount of virus in their semen.
- The virus can survive semen processing for cooled-shipment and cryopreservation, thus remaining infectious and readily transmitted to naive mares at the time of insemination.
- With the exception of persistently infected stallions, there are no long-term effects after resolution of EAV infection in mares, geldings and foals (<6 months). Immunity is long-lasting and protective.
- Abortion in pregnant mares (3 months to over 10 months of gestation) is not preceded by premonitory signs.
- Congenital infection of late term fetuses leads to weak foals with interstitial pneumonia.
- Older foals acutely infected with EAV may develop a secondary bacterial pneumonia. Prophylactic administration of antibiotics may be indicated in these cases.

Prevention

For all venereal diseases in the horse, biosecurity measures are paramount. Horses and/or semen arriving without appropriate health certification should not be unloaded or used for breeding. Before semen is collected and shipped, appropriate health parameters must be tested and certifying paperwork must accompany the shipments.

- Effective strategies for control and prevention include:
  - Biosecurity: a major factor in determining success of any control program is minimizing or eliminating direct or indirect contact of susceptible horses with the secretions and excretions of EAV-infected animals.
    - Determine serologic and virologic status of all stallions contributing sperm to both cooled-shiped and cryopreserved semen A.I. programs.
    - Establish artificial insemination industry standards.
  - Preventing the establishment of the carrier state in stallions and post-pubertal colts will eliminate the disease.
    - Vaccinate colts after 6 months and prior to 10 months of age.
• The MLV vaccine has been shown to be both safe and effective for use in breeding mares, stallions, geldings, fillies, colts and early gestational pregnant mares (up to 2 months before foaling) and during the immediate postpartum period.

• All sero-negative mares being bred to a persistently infected stallion should be vaccinated 21 days prior to insemination. Those previously vaccinated should be given a booster immunization prior to breeding.

• Stallions for breeding should be tested for neutralizing antibodies to EAV at least 60 days prior to breeding and all seronegative stallions must be vaccinated with the MLV vaccine. The vaccinated stallions should not be used for natural breeding 28 days post vaccination or in the case of stallions solely used for collection of semen for AI, within 14 days post vaccination.

• There is no evidence that a vaccinated stallion will develop the carrier state with the vaccine virus strain.

• Horses that travel for competitions or comingle with outside horses should be vaccinated.

• Late-term pregnant mares should not be vaccinated unless the risk of natural exposure is sufficient to offset the risk of potential complications of a modified-live virus vaccine.

• For EVA and other reportable diseases, more widespread screening of stallion populations and tighter quality control over laboratories providing diagnostics would increase detection of carrier stallions.

Client education
• Critical to prevention and control of EVA is the observance of sound practices which will reduce the risk of viral spread.
  o The reservoir is the carrier stallion, and prevention and detection of persistent infection are critical for control of disease outbreaks.
  o Specific measures aimed at minimizing the spread of virus:
    ▪ Isolate and test new entries
    ▪ Certify/test imported semen
    ▪ Institute an appropriate vaccination protocol
    ▪ Especially vaccinate all colts prior to puberty

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References
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Figure 1: Transmission of EAV and the role of the carrier stallion in maintenance and spread of the virus
Figure 2. Dual-color immunofluorescence flow cytometry analysis of CD3⁺ T cells from carrier and seropositive non-carrier stallions following \textit{in vitro} infection with EAV. (A) Carrier status was confirmed by repeatedly isolating EAV from semen over an extended period of time. (B) Non-carrier stallions were uninfected but seropositive to EAV following natural infection, not vaccination. Lymphocytes from each stallion that were labeled with both anti-CD3 and anti-nsp1 (12A4) MAbs are shown in the upper right quadrant of each dot plot. Note significant double labeling of lymphocytes from the carrier stallion but not the non-carrier stallion for both CD3 and EAV after \textit{in vitro} infection. (Adapted from Go et al., [2012] with permission; Copyright© American Society for Microbiology, [Journal of Virology, 86(22), 2012, 12407-12410 and DOI: 10.1128/JVI.01698-12]).

(Edited note: Figures in this manuscript are available in color in the on-line version of Clinical Theriogenology.)