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Equine Viral Arteritis: Signs, Lesions, Pathogenesis and Diagnoses

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The equine arterivirus (EAV), the etiologic agent of equine viral arteritis, is a non-arthropod-born virus classified as a member of the new order *Nidovirales*, including also the bigeneric family *Coronaviridae*, within the family *Arteriviridae* with porcine respiratory and reproductive syndrome virus, simian hemorrhagic fever virus and lactate dehydrogenase elevating virus.

Clinical signs may be absent or include pyrexia, depression, anorexia, leukopenia, limb edema, stiffness of gait, rhynorrhea and epiphora, conjunctivitis, rhinitis. Edema of the periorbital and supraorbital areas, midventral regions, scrotum, prepuce, mammary gland, urticarial rash, and abortion also occur. Less frequently, severe respiratory distress, ataxia, mucosal papular eruptions, submaxillary lymphadenopathy, intermandibular and shoulder edema may be observed. EAV can be associated with epidemic abortion, is occasionally fatal in adults and, more frequently, can be fatal for foals. When neonates are not protected by passive maternal immunity they may present with sudden death or severe respiratory distress followed by death. Affected intact males may become long-term carriers and shed EAV in the semen. Stallions shedding EAV in their semen serve as a reservoir for the virus within the equine population. Additionally, infected stallion and semen have resulted in restrictions for international movement of horses and semen. Clinical pathology findings reported in affected foals include hypoxia, hypercapnia, respiratory acidosis sometimes complicated by metabolic acidosis, neutropenia/neutrophilia, lymphopenia/lymphocytosis, thrombocytopenia and hyperfibrinogenemia. However, abnormal values may be inconsistent are highly variable and are not diagnostic for EVA. Arterial blood gas values in affected foals are diagnostic of severe disease of the respiratory system but these abnormalities may be seen in a variety of neonatal diseases of horses, including bacterial sepsis and other viral diseases such as Equine Herpesvirus 1 infection. Experimentally infected mature horses were consistently leukopenic in one report. The horses in this study presented biphasic leukopenia and neutropenia. Lymphopenia was consistently present post-infection in this group of experimentally infected horses.

EVA is caused by an arterivirus and the vascular system is the principal but not unique viral target. EVA has variable presentations including interstitial pneumonia, panvasculitis with edema, thrombosis and hemorrhage, lymphoid tissue necrosis, renal tubular necrosis, abortion and inflammation of male accessory genital glands. EAVAg can be demonstrated within the cytoplasm of epithelial cells such as alveolar pneumocytes, enterocytes, adrenal cortical cells, trophoblast, thymus stroma, renal tubular cells and male accessory genital glands. It can be also demonstrated within endothelia, in vascular, myometrial and cardiac myocytes, macrophages, dendritic cells of lymphoid organs and chorionic mesenchymal stromal cells. It was possible to determine the pathogenesis of EAV infection in adult horses and foals by following its distribution of viral antigen and lesions in experimental and natural infections. Twenty-four hours post-infection (PI) or later the virus invades the respiratory epithelium and alveolar macrophages. Forty-eight hours PI the virus can be found in the satellite lymph nodes, especially bronchial lymph nodes. Three days PI the virus replicates in bronchopulmonary lymph nodes, endothelium, and circulating monocytes. Systemic distribution of the virus follows with localization within macrophages and dendritic cells of lymphoid tissue. Approximately 6 to 8 days PI the virus localizes within endothelium and medial myocytes of blood vessels and mesothelium. At day 10 PI the most severe damage occurs to blood vessels. After 10 days PI EAVAg is decreased in all the locations except the tunica media of small muscular arteries. The last site to be invaded apparently is the renal tubular epithelium where the virus may persist for additional two weeks. Infective EAV is no longer detectable in most tissues after day 28 post-experimental infection, with the exception of the reproductive tract of some colts and stallions. In the pregnant mare EAVAg can be identified within the uterine small blood vessels (endothelium, myocytes and sometimes pericytes), and within the endometrial glandular and surface epithelium. The salpynx epithelium can be also infected. In the infected fetus EAVAg can be identified within the trophoblastic epithelium and mesenchyme, and shortly after, within pneumocytes, alveolar macrophages, within the thymic epithelium and enterocytes. Often an aborted fetus does not contain detectable EVAAg and evaluation of mare seroconversion, virus isolation and PCR on endometrial tissue should be performed. Indirect immunohistochemistry is a reliable, powerful, rapid and relatively inexpensive diagnostic tool to diagnose EVA infection post mortem and, occasionally *in vivo* using skin biopsies. For indirect immunohistochemical evaluation of tissues, this author uses a murine monoclonal antibodies of the IgG2A isotype (courtesy of Dr. A. Glaser, New York State Diagnostic Laboratory, Cornell University, Ithaca, NY) able to react with a 30 kDa mem-

brane protein of equine arterivirus, diluted 1:5 in PBS containing 4% of horse serum. Results must be interpreted by a board certified veterinary pathologist with knowledge of equine pathology and infectious diseases.

EAV can be isolated using various permissive cell line such as RK-13, Vero, equine lung, others and then identified using monoclonal antibodies. For early detection ideal samples are nasopharyngeal swabs or washings, conjunctival swabs and citrated or EDTA blood samples. Attempts of virus isolation and identification can be performed using urine samples when the virus reached the renal colonization stage. Wide range of tissues from aborted fetuses and foals and sporadic adult demise are utilized for post mortem virus isolation and identification. RT-PCR is a powerful and sensitive diagnostic technique. Genetic variability has to be taken in consideration. If the mutation avoids recognition by the oligonucleotides selected, there would be amplification difficulties and the reaction products would not be readily visible in the acrylamide gel. Multiple PCRs using different couples of primers may be performed to reduce false negatives.

In vivo evaluation of seroconversion is an other important diagnostic tool.

Treatment includes resting, NSAID, antimicrobials, any other symptomatic supportive treatment, intensive care for foals.

Differential diagnoses are numerous and include Equine Herpesviruses 1 & 4, Equine Influenza (orthomyxoviruses A 1 & 2), Equine Rhinoviruses, Equine Infectious Anemia (lentivirus), Hendra disease (morbillivirus), Getah Virus (togavirus), Urticaria, Shock, Purpura Hemorrhagica, African Horse Sickness (orbiviruses), Hoary Alyssum (*Berteroa incana*) toxicity, Hemolytic Uremic Syndrome, Equine adenoviruses in SCID foals (rare). EHV-1 and, sporadically, EHV-4 induce late abortion and stillbirth in horses. In neonates it is difficult to distinguish between EHV and EVA pneumonia macroscopically, whereas at the microscopic level the difference is rather clear. EHV-1 induces necrotizing bronchiolitis and interstitial pneumonia with intranuclear viral inclusions, chromatin margination and fragmentation. Large quantities of intranuclear and intracytoplasmic EHV antigen can be immunocytochemically observed within epithelial cells, macrophages and pulmonary endothelial cells of fetuses. In addition, EHV-1 infected fetuses and neonatal foals may present with multifocal coagulative necrosis of various organs, in particular liver, intestine and lymphoid organs, which are also the ideal specimens for histological and immunocytochemical diagnosis. EHV-1 induces encephalomyelopathy secondary to vasculitis with virus antigen localizing within endothelial cells, myocytes and pericytes. Encephalomyelitis has not been associated with EAV infection. EHV-1 also may rarely induce non-neurologic fatal disease in equine young adults with severe vasculitis histologically difficult to distinguish from EVA infection. Equine adenovirus is a rare cause of death in horses and almost exclusively involves Arabian and Thoroughbred foals affected by combined immunodeficiency (CID), characterized by a severe atrophy of the lymphoid tissues. Foals die because of a severe necrotizing bronchopneumonia. Large intranuclear basophilic Cowdry type B viral inclusions are seen within the bronchial epithelium and are also occasionally located within the exocrine pancreas. The identifiable viral antigen is present in smaller quantities than EHV-1 and EAV and is almost exclusively intranuclear. Influenza orthomyxoviruses infections accompanied by bacteria, cause interstitial and bronchopneumonia. Vasculitis is not a characteristic feature. The orthomyxovirus antigen is abundant within the cytoplasm and nucleus and can be detected in the pulmonary specimens. The acute and subacute forms of lentivirus induced equine infectious anemia (EIA), present with gross and histological lesions which, with the exception of bone marrow hyperplasia and dyserythropoiesis, the absence of vasculitis and the possible occurrence of granulomatous meningoencephalomyelitis, could be mistaken for EVA. Viral RNA can be identified systemically in cells of macrophage lineage. The orbivirus of African Horse Sickness causes acute pulmonary, retro-orbital and muscle edema with ascites, hydrothorax, hydropericardium. Histologically, the edema may be associated with mild perivascular mononuclear infiltrate, which may resemble an arterivirus lesion. The virus is localized within the cytoplasm of endothelium and macrophages. The morbillivirus of Hendra disease may cause a fatal infection and induces hemorrhagic pneumonia with typical endothelial cell syncytia. Vascular lesions resembling EVA can be systemically distributed, particularly in the kidney; the viral antigen is localized within endothelial cells, whereas filamentous paramyxovirus structures may be identified ultrastructurally. Getah virus, *Alphavirus* subgroup *Togaviridae*, can cause fever, rhinorrhea and occasional exanthema, limb edema, lymphopenia or monocytosis and abortion, has also to be included among the differential diagnoses for EVA. Purpura hemorrhagica and septic shock may induce systemic hemorrhages and occasionally vascular changes resembling the severe lesions of the rare fatal EVA cases. The toxic plant Hoary alyssum (*Berteroa incana*) induces fever, hemolysis, limb edema, laminitis, gastroenteritis, abortion and can mimic EVA clinical presentation and gross lesions. Amongst differential diagnoses it is necessary to also include infectious and non-infectious abortion and each of these forms will present more or less characteristic lesions.

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