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INTERSTITIAL LUNG DISEASES

Diffuse granulomatous or nodular interstitial lung diseases in the horse are relatively rare. They are thought to result from aerogenous or hematogenous injury to the alveoli caused by infectious organisms or particulates which fail to be completely eliminated by the immune cells. The ensuing inflammatory reaction involves alveolar and interstitial macrophages, lymphocytes, neutrophils and Giant cells with the subsequent formation of interstitial fibrosis and granulomas or nodules. Some previously recognized etiological agents that cause “nodular” interstitial lung disease include fungal (Coccidioides immitis; Cryptococcus neoformans; Histoplasma capsulatum; Blastomyces dermatitidis) and bacterial (Mycobacterium spp) organisms; inhaled silicates or mineral oil; and repeated subcutaneous injections of BCG. However, often no obvious etiological agent can be identified in horses with nodular interstitial lung disease.

Recently, a new type of interstitial lung disease called EMPF has been recognized and detailed descriptions of the pathological features and clinical signs have been published.

In the original report by Williams et al. (2007), the gross pathological findings in the lungs of 24 cases (11 males and 13 females, predominantly Thoroughbreds; ages ranging from 4 to 28 years with a mean age of 14.5 years) consisted of fibrotic pulmonary nodules. The investigators described 2 distinct manifestations of EMPF:

- The first and most common form consisted of numerous coalescing firm pale tan-white nodules that ranged in size from <1 to 5 cm in diameter. Horses typically exhibited little normal remaining lung tissue.
- In the second manifestation of EMPF, multiple discrete nodules separated by grossly unaffected lung were present. These larger fibrotic masses, up to 10 cm in size, were pale-tan to white and were characteristically larger than those in the coalescent form of the disease. There were also more extensive areas of grossly normal lung tissue between the nodules.

Histopathological examinations of the nodules, which were largely confined to the alveolar parenchyma, were similar regardless of the gross pathology. Nodules consisted of marked interstitial expansion by well-organized mature collagen and infiltration by lymphocytes, smaller numbers of macrophages, neutrophils and occasional eosinophils. The alveolar lumen contained moderate numbers of neutrophils and macrophages and occasionally a large macrophage with an oval eosinophilic intranuclear viral inclusion body could be seen. When samples were screened for herpesviruses using PCR techniques, the investigators found EHV-5 DNA in the macrophages of all samples from the affected horses as well as EHV-2 DNA in approximately 1/3 of the samples from the diseased horses. Samples from control horses, which had a variety of pulmonary disorders including pulmonary fibrosis and recurrent airway obstruction, were also examined and screened.
for herpesviruses. In the control samples exam-
ined, the gamma herpesvirus EHV-5 was not detected. This suggested to the investiga-
tors that EHV-5 was associated with the EMPF although the exact mechanism by which EHV-5 induced fibrosis was unknown. Williams et al. (2008) did note that gamma herpesviruses encode viral homologs of cy-
tokines and chemokine receptors which, if expressed, might alter or bias immune respons-
es. Indeed, both EHV-2 and EHV-5 possess a gene homologous to that encoding IL-10, an immunosuppressive cytokine. IL-10 down-
regulates Class II major histocompatibility complex expression and causes suppression of host immune response. How this relates to the pro-fibrotic phenotype of EMPF remains to be estab-
lished.12

CLINICAL SIGNS OF EMPF

The clinical signs of at least 6 horses with EMPF have also been reported in the litera-
ture. In general the horses tend to be middle-
aged or older although a few cases involved 2-
and 4-year old horses. EMPF is not gender specific: In the cases reported thus far, the Thoroughbred breed appears to be overrepre-
sented.8,10,11 Typical cases have a 1 month or longer history of weight loss, inappetence, inter-
termittent fever, tachypnea or respiratory dis-
tress. Adventitious lung sounds characterized by crackles and wheezes are detected in the majority of cases. Some affected horses also exhibit a cough and nasal discharge. Although clinically the horses may resemble a severe case of recurrent airway obstruction, the history of fever and the alteration in blood work make this diagnosis less likely. In many of the EMPF cases reported thus far, changes in the leukogram and serum biochemistries are notable and include the presence of anemia; lymphopenia; neutrophilic leukocytosis, hyper-
globulinemia and hyperfibrinogenemia. In a few cases, a mild hypoxemia (PaO₂ <75 mmHg) was reported. However the arterial oxygen tensions reported, although indicative of lung disease and gas exchange failure, were not severe enough to be causing the tachypnea.

DIAGNOSIS

In evaluating these cases clinically, one initial test that can be used to help rule out RAO (or SPAOPD) is to determine if reversible bronchospasm is contributing to the disease process. In horses with RAO or SPAOPD, bronchodilator administration significantly improves the horse’s breathing effort and pattern within 20-30 minutes administration. Horses with EMPF show little improvement with bronchodilator therapy. The next most common diagnostic procedures that are performed include cytological and microbiologi-
al analysis of transtracheal aspirates and/or bronchoalveolar lavage (BAL) samples. This approach is critical in ruling out fungal, bacterial and silicate-induced granulomatous interstitial lung disease. Most samples from EMPF cases are characterized by a predomin-
ance of non-degenerate neutrophils with lesser numbers of macrophages and lympho-
cytes, a finding that may confuse the diagno-
sis with recurrent airway obstruction or in-
flammatory airway disease. No bacterial or fungal organisms are identified either intra- or extracellularly although some reports have identified macrophages with eosinophilic intranuclear inclusion bodies suggesting a viral etiology. In the 6 cases reported in the litera-
ture, BAL samples were polymerase chain re-
a ction (PCR) positive for EHV-5.10,11 Imaging studies of the thoracic cavity are also help-
ful in establishing a diagnosis of EMPF. Radiographic findings include the presence of a severe, diffuse, nodular interstitial pattern that is either uniformly distributed or evident primarily in the mid-ventral to cranio-ventral lung lobes. Multiple radiographic views may be necessary to adequately demonstrate the presence of nodules. Such findings rule-out recurrent airway disease but not the other causes of nodular interstitial lung disease. Ultrasonographic examination reveals bilateral, diffuse roughening of the pleural surface and the existence of multiple superficial discrete nodules of varying size. Ante-mortem confirmation of EMPF is de-
pendent upon histopathological evaluation of lung sections obtained by percutaneous, ultra-
sound-guided biopsy procedure. This is especially challenging in the tachypneic horse as sedation often does not reduce the horse’s breathing frequency and increases the risk of lung laceration and pulmonary hemorrhage. Sampling of affected nodular areas superficially located in the dorsal lung field is preferred. The technique for obtaining a lung biopsy is straightforward. The intercostal space is clipped, aseptically prepared, and desensitized by infiltrating 10 ml of local anesthetic in the skin and deeper tissues. As the intercostal arteries and veins follow the caudal border of the rib, the biopsy procedure is performed closer to the cranial aspect of the rib. The contact surface of the ultrasound transducer is covered in acoustic gel and enclosed within a sterile glove so that the biopsy site remains sterile during the procedure. The biopsy instrument is inserted through a small incision in the skin and advanced a few centimeters through intercostals muscles. Once the biopsy needle is imaged via ultrasound, the instrument is rapidly inserted into the lung during inspiration and quickly removed. At our clinic we prefer to use a spring-loaded biopsy instrument for rapid retrieval of the samples although airway hemorrhage and epistaxis are still a risk. Several lung biopsies are obtained unless pulmonary hemorrhage or other complications develop. Samples are submitted for histopathological evaluation of formalin-fixed tissues stained with hematoxylin and eosin; Masson trichrome and Wright-Giemsa; microbial culture and PCR assay for EHV-5. The horse is evaluated for at least 30 minutes following the procedure to make certain that no secondary complications (hemothorax) have occurred. A 5-day course of oral trimethoprim sulfa (30 mg/kg PO q 12) is recommended unless another antimicrobial (doxycycline, see below) is selected. Should pulmonary hemorrhage occur (detected either by the presence of epistaxis, hemothymia or hemothorax on ultrasound evaluation), a solution of aminocaproic acid (10-40 mg/kg dissolved in 1 L 0.9% NaCl) is administered over a 20-30 minute period. This can be repeated at 6 hour intervals if hemorrhage continues.

IS EHV-5 THE CAUSATIVE AGENT OF EMPF?

It is tempting to attribute this syndrome to EHV-5 infection but proof of causality and thus fulfillment of Koch’s postulates remains to be demonstrated. Although EHV-5 DNA was detected in 6 clinical cases and in 24 of the necropsied principal cases, EHV-5 can also be isolated from nasal swabs, from peripheral blood mononuclear cells (PBMCs) and from various tissues in healthy horses and foals. In a recent survey of 55 clinically healthy Lipizanner horses stratified by age (25 horses ages 4-17 with a median age of 7 years; 30 horses aged 1-3 years with a median age of 2 years), the overall prevalence of EHV-5 detected in the nasopharyngeal swabs (NPS) and PBMCs using a nested PCR was 60% and 53%, respectively. The investigators noted that the prevalence was significantly greater in the younger horses (73% in NPS; 80% in PBMCs) compared to the older horses (40% in NPS; 20% PBMCs) and ascribed the differences to latency of the virus in the older horse population. Fortier et al. (2009) analyzed 708 tracheal wash and BAL samples from healthy horses (n=62) and horses with inflammatory pulmonary disease (n=646) for evidence of EHV-5 DNA. In the tracheal washes, 19% of the controls and 43% of the diseased horses were PCR positive for EHV-5; in the BAL samples, 40% of the controls and 34% of the diseased horses were PCR positive for EHV-5. However, the prevalence of EHV-5 DNA was not significantly different between the two groups of horses in either set of respiratory secretions.

TREATMENT OF EMPF

Based upon limited experience, the diagnosis of EMPF is associated with a fair to poor...
of the few cases that have been reported in the literature, less than 40% have responded to therapy as evidenced by an improvement in demeanor, weight gain and radiographic changes. In the report described by Wong et al. (2008) two of five treated horses survived and resumed regular training schedules 6 and 10 months after initial presentation. Interestingly, those horses on presentation were mildly tachypneic with breathing frequencies of 22-24 breaths per minute. The authors in that report recommended treating horses with a diagnosis of EMPF for at least 6 weeks to provide adequate time for the anti-inflammatory and antiviral therapy to work. The suggested treatment regimen included the administration of dexamethasone (0.08 – 0.1 mg/kg IV q 24-48 hours); doxycycline (5-10 mg/kg PO q 12-24 hours); and acyclovir (20 mg/kg PO q 6-8 hours).

Corticosteroids are advocated to suppress the pulmonary inflammation by inhibiting the synthesis of inflammatory cytokines and mediators that promote ongoing fibrosis and cellular infiltration. However, immunosuppressants steroids may cause recrudescence of viral infections and increase the horse’s susceptibility to other infectious diseases. One should strive to decrease the dosage of dexamethasone by one third to one half after 7-10 days of dosing or sooner if complications develop. We typically administer a dose of 0.1 mg/kg IV q 36 h for 5 treatments before decreasing the dosage. Prednisolone has also been utilized (after a course of dexamethasone) starting at doses of 1-2 mg/kg PO q 24 hours.

Doxycycline is administered as a potential anti-inflammatory because in murine models of pulmonary disease, this drug interacts with bound zinc or calcium ions required for metalloproteinase activities. These enzymes are prevalent in pulmonary inflammatory conditions and the doxycycline reduces their activities.

The use of acyclovir is speculative for several reasons:

- The pathogenic role of EHV-5 remains to be proven.
- The susceptibility of equine gamma herpesviruses to acyclovir is unknown—although the Epstein-Barr virus, a human gamma herpesvirus, is reportedly susceptible to acyclovir.18
- This drug (as opposed to valacyclovir) is poorly absorbed in the horse—the ensuing serum concentrations are well below the concentrations required to at least inhibit EHV-1 replication in vitro.19,20

Valacyclovir is the prodrug of acyclovir—it is acyclovir linked to L-valine by an ester—that exhibits 60% bioavailability when administered at a dose of 30 mg/kg q 8h. However, as previously mentioned, its effectiveness against EHV-5 in vitro has not been demonstrated. Garre et al (2009) recently examined the efficacy of orally administered valacyclovir (40 mg/kg PO q 8h) in ponies experimentally infected with EHV-1. They reported that although sufficiently high acyclovir concentrations were attained in the plasma and nasal mucus, there was no effect of drug administration on clinical signs, viral shedding or viremia.22

Additional treatment considerations include administration of intravenous fluids if the horse is dehydrated and moving the horse to a cool environment during the hot, humid summer months to reduce the heat burden presented to the respiratory tract.

REFERENCES