

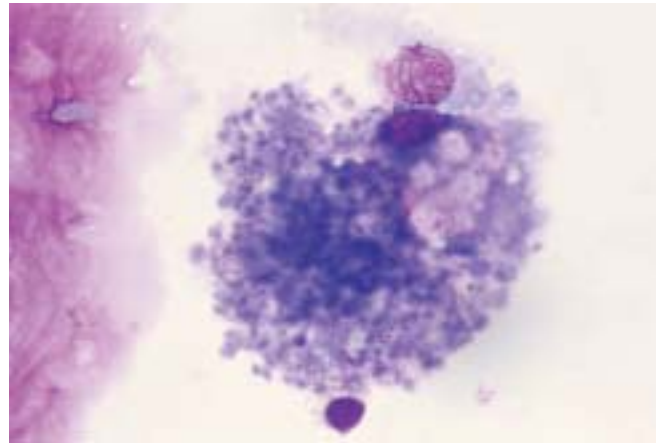
## Pneumonia in a Paso-Fino Mare

Amy L. MacNeill, A. Rick Alleman, Robert P. Franklin, Maureen Long, Steeve Giguère, Elizabeth Uhl, Alric López-Martinez, Melinda Wilkerson

A 5-year-old Paso-Fino mare foaled 2 months prior to presentation at the University of Florida Veterinary Medical Teaching Hospital (VMTH). There was no history of previous health problems, although weight loss was detected in the mare during pregnancy and continued postpartum. The owners presented the mare to the referring veterinarian after observing an episode of coughing. The referring veterinarian administered intravenous fluids, ceftiofur sodium, and flunixin meglumine. However, the horse developed severe respiratory distress during treatment. The mare was referred to the VMTH later that day.

Upon presentation, the mare was extremely underweight with a body score of 2/9 and a dull coat. The horse was in severe respiratory distress but without stridor or nasal discharge. Increased bronchovesicular sounds without crackles or wheezes were auscultated on both sides of the thorax. A 2/6 systolic heart murmur was detected, with the point of maximal intensity over the left 4th intercostal space. The mare's rectal temperature was 102.2°F. Radiographs of the thorax showed areas of a patchy, dense, coalescing alveolar infiltrate with distinct air bronchograms. Small foci of dense interstitial changes were noted in both caudoventral lung fields. There was mild ballooning of the distal trachea and mainstem bronchi. Thoracic ultrasound revealed right heart enlargement.

CBC values were within normal limits except for slight increases in band neutrophil (790 cells/ $\mu$ L, reference interval 0-100 cells/ $\mu$ L) and monocyte (1100 cells/ $\mu$ L, reference interval 0-900 cells/ $\mu$ L) counts. Other abnormal results included hyperfibrinogenemia (900 mg/dL, reference interval 100-500 mg/dL) and increased serum alkaline phosphatase (306 U/L, reference interval 68-200 U/L) and gamma glutamyl transferase (136 U/L, reference interval 10-40 U/L) activities. All other serum



**Figure 1.** Cytocentrifuged sample of bronchoalveolar lavage fluid from a horse. Wright's-Giemsa,  $\times 250$ .

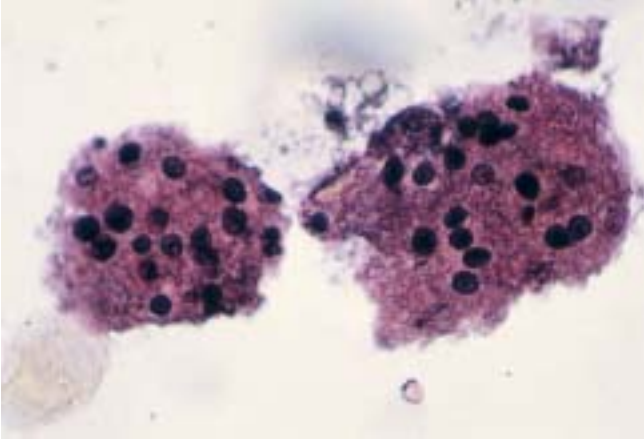
chemistry values were within normal limits. Arterial blood gas analysis indicated decreased PaO<sub>2</sub> (51 mm Hg, reference value 94 mm Hg), decreased PaCO<sub>2</sub> (32.3 mm Hg, reference interval 36-46 mm Hg), low normal pH (7.36, reference interval 7.32-7.44), and decreased bicarbonate concentration (18.3 mmol/L, reference interval 24-30 mmol/L).

Because of the clinical and radiographic evidence of lung disease, a tracheal wash and bronchoalveolar lavage (BAL) were performed. The tracheal wash revealed mild to moderate purulent inflammation with severely increased amounts of mucus. The BAL fluid was colorless and clear and contained 137 nucleated cells/ $\mu$ L. The sample was cytocentrifuged at 70 g for 5 minutes and stained with Wright's-Giemsa for cytologic examination (Figure 1).

*(Continued on next page)*

From the Departments of Physiological Sciences (MacNeill, Alleman), Large Animal Clinical Sciences (Franklin, Long, Giguère), and Pathobiology (Uhl, López-Martinez), University of Florida College of Veterinary Medicine, Gainesville, FL; and the Department of Veterinary Pathobiology, Kansas State University, Manhattan, KS (Wilkerson). Corresponding author: Dr Amy L. MacNeill, University of Florida, Health Science Center, PO Box 100103, Gainesville, FL 32610-0103 (almac@ufl.edu).

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**Figure 2.** Cytocentrifuged sample of bronchoalveolar lavage fluid from a horse, with amorphous foamy material and round fungal organisms. Grocott's methenamine silver stain.  $\times 250$ .

### Cytologic Interpretation

The sample contained large amounts of mucus and low numbers of RBCs in the background. The nucleated cell population comprised 41% small well-differentiated lymphocytes, 39% large reactive mononuclear phagocytes, and 20% mildly degenerate neutrophils. Occasional eosinophils also were noted. The large mononuclear phagocytes often were clustered, and many of them contained variable numbers of round intracytoplasmic fungal organisms (Figure 1). The organisms were encapsulated spores approximately 6.5  $\mu\text{m}$  in diameter and were usually in clusters. Small basophilic bodies arranged in a ring were observed within several spores. Extracellular organisms also were observed and were associated with amorphous foamy material. The cytologic interpretation was fungal sepsis consisting of organisms with morphologic characteristics consistent with *Pneumocystis carinii*.

Two additional cytocentrifuged slides were stained with Grocott's methenamine silver (GMS) stain to confirm the identification of the organisms (Figure 2). The organisms stained positively with GMS stain, and most were round to oval in shape. A few spores were slightly folded and had a bell-shaped appearance characteristic of *P carinii*. The organisms were evaluated further by Dr Christine Orlando, Department of Pathology, Immunology and Laboratory Medicine at the University of Florida College of Medicine (Gainesville, FL) who found them to be morphologically identical to *P carinii* organisms seen in human patients.

### Additional Test Results

Because *P carinii* can be pathogenic in immunosuppressed hosts, serum mineral concentrations (atomic absorption spectroscopy, Florida Diagnostic Laboratory,

Kissimmee, FL), immunoglobulin (Ig) levels (radial immunodiffusion, Cornell University, Ithaca, NY), and immunophenotypes of peripheral blood leukocytes (flow cytometric analysis, Kansas State University, Manhattan, KS) were determined to assess the immunocompetence of the mare. Serum concentrations of copper (1.45 ppm, reference interval 0.65-2.00 ppm), zinc (0.60 ppm, reference interval 0.50-1.50 ppm), and selenium (215 ppb, reference interval 140-250 ppb) were within normal limits. IgA concentration was normal (210 mg/dL, reference interval 67-239 mg/dL), IgG concentration was mildly decreased (800 mg/dL, reference interval 984-1685 mg/dL), and IgM was undetectable (reference interval 90-150 mg/dL). Immunophenotyping revealed normal numbers of CD5<sup>+</sup> T cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, B cells, granulocytes, and monocytes. However, only 28% of cells were positive for major histocompatibility complex class II (MHC class II) expression; normally, 71-86% of cells express MHC class II molecules.

The horse was treated with intranasal oxygen insufflation (10 L/min), trimethoprim sulfamethoxazole (30 mg/kg), flunixin meglumine (1.1 mg/kg), and decreasing doses of dexamethasone. Because of continued respiratory distress, the owners elected to euthanize the horse. Gross and histologic necropsy findings included chronic interstitial pneumonia, pericardial effusion, hypertrophy and dilation of the right ventricle, and hepatic fibrosis. Pulmonary alveolar spaces were filled with aggregates of foamy macrophages and a few multinucleated giant cells. Alveolar septa were markedly distended by large foamy macrophages, lymphocytes, and moderate amounts of fibrous connective tissue. Moderate to marked hyperplasia of type II epithelial cells was present throughout the lung sections. Lung sections were stained with Brown and Bren stain for bacterial organisms and GMS to detect fungal elements, but no organisms were identified.

### Discussion

*Pneumocystis carinii* recently has been reclassified as a fungal organism based on the DNA sequence of its 16S-like RNA subunit.<sup>1</sup> Cytologic examination of BAL fluid is the preferred method used to diagnose human infection with *P carinii*.<sup>2</sup> Diagnostic sensitivity of cytology is reported to be 89.0-94.4%, and complications caused by the BAL procedure are minimal.<sup>2</sup> In comparison, the sensitivity of histopathologic examination of lung biopsies is 73.3%.<sup>2</sup> A culture system has not been developed for diagnosis of *P carinii* infection.<sup>1</sup> Instead, diagnosis of *P carinii* pneumonia is achieved by microscopic identification of characteristic morphologic features of the pathogen.<sup>3</sup>

Histologic characteristics of *P carinii* pneumonia in

human patients include alveolar spaces filled with foamy amphiphilic material and interstitial inflammation with widened septa, fibrin deposition, pneumocyte proliferation, mild hemorrhage, and hyaline membrane formation.<sup>4</sup> Although organisms were not identified after microscopic examination of the lungs, this horse had histologic findings consistent with *P carinii* pneumonia in humans and foals.<sup>4,5</sup> The difference in sensitivity between BAL cytology and lung histopathology may also apply to the diagnosis of *P carinii* pneumonia in horses, and is one possible explanation for the inability to identify organisms in lung sections from this mare. Additionally, treatment with potentiated sulfonamides may have suppressed the infection enough to prevent histologic identification of organisms.

Immunocompromise caused by severe malnutrition, congenital immunodeficiency, or acquired immunodeficiency can increase susceptibility to *P carinii* pneumonia.<sup>1</sup> This mare had foaled 2 months previously, was malnourished, and had laboratory evidence of immunosuppression based on decreased expression of MHC class II molecules, low levels of IgG, and IgM deficiency. Without adequate antigen presentation by MHC class II expression on the surface of cells, this horse may not have been able to effectively control the proliferation of *P carinii* within the airways. The IgM deficiency and low IgG levels may have been secondary to decreased levels of MHC class II molecules, leading to reduced B cell stimulation. However, congenital diseases such as common variable immune deficiency (CVID) also should be considered. CVID is characterized by a period of normal immune function followed by recurrent infections resulting from decreased production of immunoglobulins. *Pneumocystis carinii* pneumonia has been reported in both humans and dogs with CVID.<sup>6,7</sup>

In conclusion, *P carinii* organisms are opportunistic fungal pathogens that can cause fatal pneumonia in immunosuppressed hosts. Animals diagnosed with *P carinii* pneumonia should be evaluated for immunosuppression. Immunocompromise plays a major role in the pathogenesis of *P carinii* pneumonia, but the exact immunologic deficiency of affected animals is variable. Cytologic examination of BAL fluid is the most sensitive method for diagnosis of *P carinii*.

Danish Veterinary Laboratory in Copenhagen, Denmark recently developed a fluorescent in situ hybridization method to aid in the diagnosis of *P carinii* pneumonia in foals and pigs.<sup>8</sup> An oligonucleotide probe targeting the 18S ribosomal RNA of *P carinii* was used to detect the organism in histologic sections. Sixteen samples from animals with *P carinii* pneumonia were tested;

all of the samples showed distinct positive reactions using this labeled probe.<sup>8</sup> Unfortunately, the test was not available at the time of this horse's diagnosis. ◇

### Abstract

A 5-year-old Paso-Fino mare was presented for severe respiratory distress. The mare had foaled 2 months prior to presentation. The horse was in poor body condition with a dull hair coat. A mild fever was noted during physical examination and increased bronchovesicular sounds were auscultated. Thoracic radiographs showed an interstitial pattern and an alveolar infiltrate with distinct air bronchograms. Moderate purulent inflammation with increased mucus was observed in tracheal wash fluid, but no infectious agents were identified. A bronchoalveolar lavage (BAL) contained a large amount of mucus and reactive mononuclear phagocytes with variable numbers of intracellular fungal organisms morphologically consistent with *Pneumocystis carinii*. The mare had undetectable levels of immunoglobulin M (IgM) and decreased IgG levels in the serum. Immunophenotyping revealed decreased expression of major histocompatibility complex (MHC) class II molecules. Moderate to marked hyperplasia of type II epithelial cells was present throughout histologic sections of lung, but the fungal organisms were not observed. A culture system has not been developed for diagnosis of *P carinii* infection. Instead, diagnosis of *P carinii* pneumonia is achieved by microscopic identification of characteristic morphologic features of the pathogen. Cytologic examination of BAL fluid is the preferred method used to diagnose human infection with *P carinii*. In humans, the diagnostic sensitivity of cytology is significantly higher than the sensitivity of histopathologic examination of lung biopsies. The difference in sensitivity between BAL cytology and lung histopathology may also apply to the diagnosis of *P carinii* pneumonia in horses. (MacNeill AL, Alleman AR, Franklin RP, Long M, Giguère S, Uhl E, López-Martinez A, Wilkerson M. Pneumonia in a Paso-Fino mare [*Pneumocystis carinii* pneumonia]. *Vet Clin Pathol.* 2003;32:73-76)

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**Key Words:** Bronchoalveolar lavage, horses, immunodeficiency, *Pneumocystis carinii*, pneumonia

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