Use of dapsone in the treatment of *Pneumocystis carinii* pneumonia in a foal

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> Opportunistic infections of *Pneumocystis carinii* can cause pneumonia in immunocompromised horses.
> Dapsone may be useful as an adjunctive treatment to traditional administration of trimethoprim-sulfamethoxazole for *P carinii* pneumonia in horses or as a sole treatment for horses that cannot tolerate trimethoprim-sulfamethoxazole.
> Treatment with intracamerical injections of human recombinant tissue plasminogen activator may be useful in horses with fibrinous uveitis associated with endotoxemia and septicemia.

A 6-month-old male Quarter Horse weighing approximately 180 kg (396 lb) was evaluated because of signs of chronic respiratory tract disease of 6 weeks’ duration. The foal had been purchased from a sale at 2 months of age and was transported approximately 800 miles to the owner’s farm. The owner reported that the colt had been unthrifty and did not gain weight as expected. The colt was treated for 1 week with procaine penicillin injections (unknown dose) in the left cervical musculature. At presentation to the Kansas State University Veterinary Medical Teaching Hospital (VMTH), the foal had signs of depression, a rough dry hair coat, and purulent unilateral nasal discharge (right side). An 8 × 6 cm warm, painful swelling was evident on the left mid-cervical area. In addition, an audible respiratory noise on expiration was detected. Vital signs included a rectal temperature of 38.9°C (102.0°F), heart rate of 78 beats/min, and respiratory rate of 24 breaths/min. No abnormalities were noted on a general ocular examination during the initial physical examination; a fundic examination was not performed. Auscultation of the thorax revealed bilateral increased bronchovesicular sounds with occasional wheezes. Auscultation of the trachea revealed rattles consistent with excessive tracheal mucus. Thoracic radiographs indicated moderate interstitial changes of the ventral lung fields consistent with bacterial or aspiration pneumonia. No abnormalities of the pleural surface of the lungs were identified during thoracic ultrasonography. Results of hematologic analyses indicated leukocytosis (36,100 WBCs/µL; reference range, 6,000 to 14,000 WBCs/µL) characterized by mature neutrophilia (31,000 cells/µL; reference range, 2,500 to 7,500 cells/µL) and hyperfibrinogenemia (600 mg of fibrino-

gen/dL of plasma; reference range, 100 to 400 mg/dL). Lymphocyte concentration (4,700 cells/µL) was within reference limits (1,500 to 7,700 cells/µL). Results of serum biochemical analyses indicated low albumin concentration (2.4 g/dL; reference range, 3.0 to 3.7 g/dL). No abnormalities were detected in the results of venous blood gas or electrolyte analyses. A transtracheal wash was performed, and the samples obtained were submitted for cytologic evaluation and bacteriologic culture. Cytologic findings were indicative of neutrophilic inflammation; neutrophils composed 83% of the cell population in the transtracheal wash samples and would normally be < 5%. A few neutrophils contained irregularly shaped, paired cocci that could not be identified as bacteria with absolute certainty. Initial treatment of the foal included administration of azithromycin (10 mg/kg [4.5 mg/lb], PO, q 24 h) and flunixin meglumine (1.1 mg/kg [0.5 mg/lb], PO, q 12 h); a hot pack was applied to the left side of the neck (15-minute application, q 12 h), as was a mixture of dimethyl sulfoxide and nitrofurazone ointment (30:50 mix; 15 mL, q 12 h). The foal’s attitude improved during the following 24 hours, and vital signs were within reference limits. However, despite 3 days of antimicrobial treatment, the foal continued to have episodes of dyspnea (most notably when recumbent) as a result of unresolved respiratory tract disease. Bronchoalveolar lavage was performed; the samples obtained were submitted for cytologic evaluation to rule out *Pneumocystis carinii* infection. In contrast to findings of the cytologic evaluation of the transtracheal wash samples, a mixed inflammatory cell response was identified with macrophages predominating (composing 73% of the cell population) in the samples obtained via bronchoalveolar lavage. Organisms detected within the macrophages were determined to be *P carinii*.

Because *P carinii* infection has been historically associated with immune suppression, blood samples were obtained from the right jugular vein and submitted for immunoglobulin quantification and lymphocyte subpopulation phenotyping. Results of immunoglobulin quantification in those blood samples indicated pronounced antibody response including IgA concentration of 280 mg/100 mL (reference limits, 38 ± 14 mg/100 mL), IgG concentration of 1,700 mg/100 mL (reference limits, 380 ± 188 mg/100 mL), IgM concentration of 280 mg/100 mL (reference limits, 61 ± 22 mg/100 mL), and IgG(T) concentration of 1,200 mg/100 mL (reference limits, 211 ± 148 mg/100 mL). Immunophenotyping of lymphocytes via flow cytometry revealed reduced lymphocyte subsets (CD5+ cells, 42% [reference limits, 92 ± 2.5%]; CD4+ cells, 37% [reference limits, 66.2 ± 12.3%]; CD8+ cells, 9% [ref-
ference limits, 16.6 ± 2.8%); IgM+B cells, 4% [reference limits, 12.6 ± 3.2%]; and major histocompatibility complex [MHC] II [DR antibody clones analogous to the Th14B human locus] cells, 66% [reference limits, 70 ± 8.3%]). Bacteriologic culture of the transtracheal wash samples recovered Actinobacillus equuli that was sensitive to trimethoprim sulfamethoxazole. On the basis of this finding, administration of azithromycin was discontinued and treatment with trimethoprim sulfamethoxazole (15 mg/kg [6.8 mg/lb], PO, q 12 h) was initiated.

Three days after the initial evaluation, results of hematologic analyses indicated no abnormalities in leukocyte variables, although hyperfibrinogenemia persisted (700 mg/dL). Treatment for the swelling of the left side of the neck was discontinued after 5 days subsequent to resolution of cellulitis.

The colt was discharged from the VMTH on day 6 of hospitalization with instructions to the owner to administer trimethoprim sulfamethoxazole orally for 4 weeks; a CBC was to be performed weekly by the referring veterinarian to monitor disease resolution. At the completion of antimicrobial administration, the colt was to be returned to the VMTH for a follow-up examination including further bronchoalveolar lavage.

Eight days after discharge, the colt was returned to the VMTH for evaluation because of profuse watery diarrhea, anorexia, signs of depression, and lethargy of 2 days' duration. Vital signs included a rectal temperature of 38.4°C (101.1°F), heart rate of 88 beats/min, and respiratory rate of 30 breaths/min. The colt was clinically assessed to be 8% dehydrated and had signs of mild abdominal discomfort; it passed fluid feces. Thoracic auscultation revealed harsh lung sounds that were similar to those detected at the initial evaluation. Results of hematologic analyses indicated leukopenia (4,900 WBCs/µL) characterized by a neutropenia (1,700 cells/µL) with numerous band cells (740 cells/µL; reference range, 0 to 150 cells/µL) and hyperfibrinogenemia (700 mg/dL). Lymphocyte concentration (2,300 cells/µL) was within reference limits. Results of serum biochemical analyses indicated high creatinine concentration (2.1 mg/dL; reference range, 0.9 to 1.7 mg/dL) and hyperalbuninemia (2.6 g/dL).

Fecal samples were submitted to a diagnostic laboratory for bacteriologic culture and fecal ELISA for Clostridium difficile toxins A and B. A presumptive diagnosis of antimicrobial-associated colitis was made on the basis of clinical signs of diarrhea and endotoxemia after serial administration of a variety of antimicrobial agents that included trimethoprim sulfamethoxazole. A trial treatment included lactated Ringer's solution (2 L, IV, q 6 h), penicillin G potassium (22,000 U/kg [10,000 U/lb], IV, q 6 h), gentamicin sulfate (6.6 mg/kg [3.0 mg/lb], IV, q 24 h), flunixin meglumine (0.5 mg/kg [0.23 mg/lb], IV, q 12 h), and polymyxin B (6,000 U/kg [2,727 U/lb], IV, q 8 h). Several hours after initiation of treatment, the foal showed marked improvement in attitude. Further analysis of lymphocyte variables revealed improvement, but CD3+ and CD4+ cell values (CD3+, 53%; CD4+, 40%) remained below reference range, whereas MHC II cell values had increased to within reference limits (MHC II, 76%); the CD8+ and IgM+B cell values were high (CD8+, 37%; IgM+B, 24%).

Propionibacterium acnes suspension (2 mL, IV, q 2 d) was administered to effect nonspecific immunomodulation.

The next day, ophthalmic examination revealed mild accumulations of fibrin strands intermixed with small blood clots in the ventral aspect of the anterior chambers bilaterally. Pupillary light response, intraocular pressures, and results of corneal staining were within normal limits. Treatment of both eyes with neomycin-polymyxin B-dexamethasone ophthalmic suspension (1/4-inch strip, q 6 h) and atropine sulfate 1% ophthalmic ointment (1/4-inch strip, q 24 h) was initiated. Although watery diarrhea continued, the colt was otherwise bright, alert, and responsive and continued to show increased interest in feed. Result of the fecal ELISA for C difficile toxin was negative, which ruled out infection with this organism as a component of the colitis.

Twenty-four hours later, ophthalmic examination revealed marked bilateral uveitis; there was substantial iridal swelling, miosis, and aqueous flare. Additionally, the fibrin accumulations in each anterior chamber had markedly increased so that the iris and lens were partially obscured during slit-lamp biomicroscopic evaluation. Treatment with neomycin-polymyxin B-dexamethasone ophthalmic ointment was discontinued; instead, 1 drop each of prednisolone acetate 1% ophthalmic solution and tobramycin ophthalmic solution (3 mg/mL) was administered every 4 hours in both eyes. Atropine ointment application was continued at the previously mentioned dose and interval.

Results of hematologic analyses indicated resolution of leukopenia, but fibrinogen concentration remained high. Albumin concentration had decreased to 1.9 g/dL, which was attributed to continued intestinal loss secondary to inflammation of the colon. A transfusion with 2 L of normal equine plasma was performed.

Four days after admission to the VMTH, the colt's irides and lenses were completely obscured by dark green fibrinous material that filled the anterior chambers of both eyes. Blindness was inferred from the absence of the menace response bilaterally and the colt's inability to navigate appropriately around the stall. Intracameral injection (0.25 mL) of human recombinant tissue plasminogen activator (r-TPA; 25 µg/0.1 mL of solution) in the anterior chamber of each eye elicited complete clearing of the fibrin clots with restoration of normal vision observed 12 hours after administration. In addition, the foal began to pass normal feces, and bacteriologic culture of feces yielded growth of Salmonella spp resistant to trimethoprim sulfamethoxazole (result of the fecal toxin assay was known to be negative for C difficile).

During the next 3 days, the colt continued to have normal feces and its attitude improved. Vital signs remained within normal limits. All treatments were discontinued except for the ophthalmic pharmaceutical agents. On the eighth day of the second hospitalization period, thoracic radiography revealed a persistent interstitial pattern in the ventral lung fields similar to that detected previously; this finding was consistent with detection of abnormal lung sounds bilaterally on tho-
led to a change in nomenclature whereby multiple species and functional distinctness. This has been identified. Pneumocystis \textit{true plants}. Recently, results of DNA sequencing of \textit{P carinii} producing both C28 and C29 24-alkysterols, as do some \textit{P carinii} it lacks the major fungal sterol ergosterol. Additionally, an immediate cessation of treatment and prevention of \textit{Pneumocystis} pneumonia. Dapsone acts by blocking folic acid synthesis in \textit{Pneumocystis} spp via inhibition of dihydropterote synthase activity. It is recommended as sole treatment or in combination with trimethoprim for humans that cannot tolerate trimethoprim-sulfamethoxazole (the primary treatment for \textit{Pneumocystis} pneumonia). Dapsone is considered only slightly less effective than trimethoprim-sulfamethoxazole. Additionally, when administered orally once daily at 1 to 2 mg/kg (0.45 to 0.91 mg/lb) or twice weekly at 4 mg/kg (1.8 mg/lb), dapsone is an effective prophylaxis for \textit{Pneumocystis} pneumonia in adults and children infected with human immunodeficiency virus.8

Dapsone is labeled for use in humans for treatment of dermatitis herpetiformis and all forms of leprosy. Nonlabeled uses include prophylaxis of malaria and treatment of relapsing polychondritis, inflammatory bowel disorders, Leishmaniasis, \textit{Pneumocystis} pneumonia, rheumatoid arthritis, lupus erythematosus, and brown recluse spider bites. Adverse effects associated with administration of dapsone in humans include peripheral neuropathies, photosensitivity, nausea, vomiting, anorexia, blood dyscrasias, hemolytic anemia, albuminuria, and blurred vision.9

Tissue plasminogen activator is a serine protease, which functions to convert plasminogen to plasmin thereby promoting fibrin breakdown. Tissue plasminogen activator requires fibrin binding for plasminogen cleavage, creating clot-specific activity and minimizing the systemic fibrinolysis associated with other proteases such as urokinase or streptokinase.10 Human r-TPA is available commercially. Intracameral injection of r-TPA has been used therapeutically in the management of various ocular abnormalities and postoperative complications in both humans and animals without observation of adverse effects.11,15 However, intravitreal administrations of r-TPA at doses ≥ 50 μg have been associated with dose-dependent retinal toxicity in cats.13 To our knowledge, there are no reports of the use of dapsone to treat \textit{P carinii} pneumonia in horses. In the

racic auscultation. Dapsone \textsuperscript{1} (3 mg/kg [1.36 mg/lb], PO, q 24 h) was prescribed for continued treatment of the \textit{Pneumocystis} pneumonia. Although dapsone is not labeled for use in horses, we chose to use it (with informed consent from the owner) on the basis of reports of its clinical use in humans and extrapolated a dose from human data. The colt was discharged after 10 days with instructions to the owner for its continued isolation from other horses to prevent spread of \textit{Salmonella} infection. It was recommended to continue administration of dapsone for 1 month until a follow-up examination was performed; at that time, fecal samples would be obtained for bacteriologic culture to detect \textit{Salmonella} spp.

Twenty-eight days later, the colt was presented to the VMTH for reevaluation. It was bright, alert, and responsive and had gained 40 kg (88 lb). Vital signs and results of an ophthalmic examination and hematologic analyses were within reference limits. Cytologic evaluation of samples obtained via bronchoalveolar lavage revealed continued pulmonary inflammation characterized by numerous macrophages, although no \textit{P carinii} organisms were identified. Bacteriologic culture of feces again yielded growth of \textit{Salmonella} spp. Because of persistent pulmonary inflammation and potential ongoing \textit{Pneumocystis} infection, the colt was discharged with instructions to the owner to continue administration of dapsone for 28 days; continuation of the isolation protocol was also recommended until such time as results of bacteriologic culture of feces were negative for \textit{Salmonella} organisms.

Two months later, the referring veterinarian reported that bacteriologic culture of feces yielded no growth of salmonellae and the colt was doing well. We hypothesized that the \textit{P carinii} pneumonia observed in this foal was a result of stress-associated immune suppression because no direct evidence was found to suggest abnormal immune system function.

From data obtained from DNA studies, \textit{P carinii} is presently classified as a saprophytic fungus. However, debate continues regarding its classification with a recent report \textsuperscript{2} that identified \textit{P carinii} as a plant because it lacks the major fungal sterol ergosterol. Additionally, \textit{P carinii} catalyzes 1 or 2 methyl transfer reactions, producing both C28 and C29 24-alkysterols, as do some true plants. Recently, results of DNA sequencing of \textit{Pneumocystis} organisms from different hosts indicated multiple species and functional distinctness. This has led to a change in nomenclature whereby \textit{Pneumocystis jiroveci} infection now characterizes \textit{Pneumocystis} pneumonia recognized in human patients.

In immunocompromised humans and animals, infection with \textit{Pneumocystis} spp most commonly results in development of interstitial pneumonia. Opportunistic infection has been reported in Arabian foals suffering from severe combined immunodeficiency, as well as in a CD4+ and CD8+ deficient foal and an adult Paso Fino horse with selective IgM deficiency.\textsuperscript{14} Infection with \textit{P carinii} has also been reported\textsuperscript{14} in immunocompromised dogs and a mink. Interestingly, the foal of this report had decreased numbers of circulating CD4+ and CD8+ cells at the time of diagnosis of \textit{P carinii} pneumonia. This immunosuppression was detected via analysis of a blood sample obtained from the peripheral vasculature of the foal, and treatment with an immunostimulant (\textit{Propionibacterium acnes} suspension) was initiated.

In humans, most \textit{Pneumocystis} infections are reported in association with acquired immune deficiency syndrome and in organ transplant patients who are receiving immunosuppressive treatment. In 1 study\textsuperscript{4} of immunocompromised humans, \textit{Pneumocystis} organisms were detected cytotically in bronchoalveolar lavage specimens in 57.7% of individuals with and 20.0% of individuals without human immunodeficiency virus infections. \textit{Pneumocystis} pneumonia is considered the most common opportunistic illness in humans with human immunodeficiency virus infections.\textsuperscript{5}

With advances in treatment of human immunodeficiency virus infections in humans and prophylaxis against opportunistic infections, rates of development of \textit{Pneumocystis} pneumonia among humans with immunodeficiencies of varying causes have dramatically declined over the past 2 decades. Dapsone is a sulfonyl antimicrobial and one of the more common drugs used in treatment and prevention of \textit{Pneumocystis} pneumonia. Dapsone acts by blocking folic acid synthesis in \textit{Pneumocystis} spp via inhibition of dihydropterote synthase activity. It is recommended as sole treatment or in combination with trimethoprim for humans that cannot tolerate trimethoprim-sulfamethoxazole (the primary treatment for \textit{Pneumocystis} pneumonia). Dapsone is considered only slightly less effective than trimethoprim-sulfamethoxazole. Additionally, when administered orally once daily at 1 to 2 mg/kg (0.45 to 0.91 mg/lb) or twice weekly at 4 mg/kg (1.8 mg/lb), dapsone is an effective prophylaxis for \textit{Pneumocystis} pneumonia in adults and children infected with human immunodeficiency virus.\textsuperscript{8}
foal of this report, dapsone was an effective treatment that was associated with no apparent adverse effects. Additionally, intracameral administration of r-TPA was found to be a critical component in treatment of fibrin accumulation associated with endotoxemia and septicemia in the foal. For *P. carinii* pneumonia in horses, dapsone may be of use as an adjunctive treatment to administration of trimethoprim-sulfamethoxazole or as a single agent treatment for horses that cannot tolerate trimethoprim-sulfamethoxazole.

References