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Equine Protozoal Myeloencephalitis Associated with Neosporosis in 3 Horses

Carrie J. Finno, Monica Aleman, and Nicola Pusterla

A 24-year-old Appaloosa gelding was evaluated for a 2-month history of pelvic limb incoordination. The gelding had been administered phenylbutazone (2 mg/kg PO q12h) for 1 week before admission, and no improvement was apparent. Two months before presentation, the gelding had been vaccinated against Eastern and Western equine encephalitis (EEE, WEE), tetanus, influenza, West Nile virus (WNV), and rabies. Deworming was adequate, with oral dewormers rotated between ivermectin and pyrantel pamoate every 2 months. On admission, the gelding was tachycardic (52 beats per minute), normothermic (37°C, 99.0°F), and eupneic (16 breaths per minute). Muscling appeared symmetric; however, the gelding placed more weight on the right pelvic limb than on the left and the tail was held to the right side. A complete neurologic evaluation revealed asymmetric hypermetria, characterized by a grade 2 of 5 ataxia on the left thoracic and left pelvic limbs and a grade 3 of 5 ataxia on the right thoracic and right pelvic limbs. There were no cranial nerve deficits, and both tail tone and were normal. The gelding was observed to urinate and defecate normally.

A CBC revealed a leukocytosis (14,000/μL; reference range, 5,000–11,600/μL) characterized by a mature neutrophilia (12,200/μL; reference range, 2,600–6,800/μL). Serum biochemical abnormalities included hyperglycemia (124 mg/dL; reference range, 50–107 mg/dL). Findings of cervical radiographs revealed mild osteoarthrosis of the articular facets at C4-5 and C5-6. Lumbosacral cerebrospinal collection was performed, and clear cerebrospinal fluid (CSF) fluid was obtained and submitted for cytology, serology (immunoglobulin M [IgM] capture ELISA) for WNV, and indirect fluorescent antibody testing (IFAT) for equine protozoal myeloencephalitis (EPM) (*Sarcocystis neurona and Neospora hughesi*). The CSF total nucleated cell count (TNCC) was normal (1/μL; reference range, <6/μL) with the cell population consisting primarily of small mononuclear cells (70%), large mononuclear cells (17%), and neutrophils (13%). The CSF protein concentration was normal (47 mg/dL; reference range, 20–70 mg/dL). Red blood cell (RBC) concentration within the CSF was 8/μL. The IgM capture ELISA was negative for WNV. *S neurona* IFAT titers were negative in serum (<40) and CSF (<5), whereas the serum and CSF tested positive for *N hughesi*, with titers of 2,560 and 5, respectively. A diagnosis of EPM associated with *N hughesi* was made, and the gelding was started on a course of ponazuril (5 mg/kg PO q24h for 30 days) and phenylbutazone (2 mg/kg PO sid for 14 days).

A repeat examination was performed after 30 days of treatment, and clinical improvement was noted, with the gelding graded as 1 of 5 ataxic on the thoracic limbs and grade 2 of 5 ataxic on the pelvic limbs. The right pelvic limb was slightly worse than the left pelvic limb, but otherwise the ataxia appeared relatively symmetric. Treatment with ponazuril was continued for another 30 days, and a repeat examination was performed after that time. The thoracic limb ataxia was completely resolved; however, the gelding remained at grade 2 of 5 ataxic on the pelvic limbs. At that time, a repeat EPM IFAT titer in serum remained negative for *S neurona* (<40) and positive for *N hughesi* (10,240).

A 16-year old Quarter Horse gelding was evaluated for a 1-week history of gait abnormalities of the pelvic limbs. Progressive weight loss had been noted by the owner within the 6 months before presentation, and the gelding was anorexic on the day of admission. Seven months before presentation, the gelding was vaccinated for EEE, WEE, influenza, and WNV. Deworming with ivermectin occurred 1 month before evaluation. On admission, the gelding was normothermic (37°C, 99.8°F) with a normal heart rate (40 beats per minute) and respiratory rate (16 breaths per minute). Marked symmetric muscle atrophy was noted over the epaxial, gluteal, semimembranosus, and semitendinosus muscles. The left pelvic limb was swollen distal to the tarsus with associated superficial skin abrasions. Mentation was normal, and there were no cranial nerve deficits. A spastic pelvic limb gait was noted where the horse would bring the pelvic limbs up to the ventral abdomen and occasionally appear to hop with the hind end. The right pelvic limb appeared worse than the left. The spasticity would increase when the gelding negotiated steps or changes in footing. Conscious proprioceptive deficits were noted in the pelvic limbs. Tail tone and anal tone were normal. The gelding was graded as 1 of 5 ataxic on the thoracic limbs and 2 of 5 ataxic on the pelvic limbs. Urination and defecation were normal.

A CBC revealed no notable abnormalities. Serum biochemical abnormalities included an increase in serum creatine kinase level (600 U/L; reference range, 119–287 U/L) and aspartate transaminase level (636 U/L; reference range, 168–494 U/L). Findings of cervical radiographs revealed mild degenerative changes of the articular facets at C4-5 and C5-6. A lumbosacral spinal tap was performed, and clear CSF was obtained. Cytology revealed a normal TNCC (1/μL), with 74% small mononuclear cells, 23% large mononuclear cells,

From the Veterinary Medical Teaching Hospital (Finno) and the Department of Medicine and Epidemiology (Aleman, Pusterla), School of Veterinary Medicine, University of California, Davis, CA.

Reprint requests: Carrie J. Finno, DVM, University of California, Davis, One Shields Avenue, Davis, CA 95616; e-mail: cjfinno@vmth.ucdavis.edu.

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and 3% neutrophils. Total protein concentration within the CSF was within normal limits (52 mg/dL), and RBC concentration was 6/μL. Muscle biopsies were taken of the semimembranosus, semitendinosus, gluteal, triceps, and epaxial muscles, and severe polysaccharide storage myopathy (PSSM) was diagnosed on the basis of the presence of amylase-resistant abnormal polysaccharide on a periodic acid-Schiff stain. Serum selenium and whole blood vitamin E concentrations were within the normal range. Serum and CSF were submitted for IFAT testing for EPM. Serum and CSF were negative for *S neurona* but were positive for *N hughesi* (serum titer, 1,280; CSF titer, 10). Diagnoses of EPM associated with *N hughesi* and PSSM were made, and the gelding was treated with a 45-day course of ponazuril (5 mg/kg PO q24h) and a 28-day course of vitamin E (10 IU/kg PO q24h). Diet recommendations included feeding 16–20 lb (1.5–2% body weight) of good-quality grass hay along with 1 cup of corn oil daily. Recommendations also included pasture turnout. An evaluation by the referring veterinarian after 4 weeks of treatment with ponazuril revealed complete resolution of the ataxia and a normal pelvic limb gait. Repeat serology for *N hughesi* demonstrated a decreasing serum titer at 6-weeks postdischarge (320); however, the titer returned to the previous value at 5-months postdischarge (1,280).

A 4-month old Percheron filly was evaluated for an acute onset of ataxia. She was found to be recumbent on the morning of admission and, after rising with encouragement, appeared ataxic. The filly was weaned 2 weeks before admission and, directly after weaning, was diagnosed by the referring veterinarian with respiratory disease, which was characterized by coughing, pyrexia (39°C, 102°F), and bilateral mucopurulent nasal discharge. Treatment consisted of a 14-day course of trimethoprim sulfamethoxazole (TMS; 7,680 mg [8 tablets] PO q12h). Two other weanlings (3–4 months of age) with mucopurulent nasal discharge and a cough were housed in the same paddock as the filly and were treated with a similar course of TMS. None of the weanlings had been vaccinated for strangles. The dam of the filly had been vaccinated against EEE, WEE, WNV, influenza, and tetanus and dewormed with ivermectin 1 month before foaling. The filly had not received any vaccinations since birth but had been dewormed with fenbendazole (500-lb dose PO) 1 month before admission.

The filly was dull and depressed on examination. Rectal temperature was 102.2°F, heart rate was 64 beats per minute, and respiratory rate was 44 breaths per minute. Bilateral mucopurulent nasal discharge was noted. Auscultation of the trachea revealed a tracheal rattle. In the caudodorsal thorax, wheezes were noted on rebreathing examination on both the right and left sides. The left submandibular lymph node was enlarged. A complete neurologic evaluation was performed. Mentation was quiet but alert. Stance at rest appeared normal, and assessment of cranial nerves revealed no cranial nerve deficits. The filly demonstrated normal lateral neck flexion to both sides, and the cervicofacial reflex was intact bilaterally. Tail tone and anal tone were normal, and muscle mass appeared symmetric. Symmetric grade 3 of 5 ataxia, characterized by a hypermetric gait, stumbling, interference, circumduction and pivoting while circling, and abnormal postural placement responses were noted on the thoracic and pelvic limbs. The filly was able to urinate and defecate normally.

A CBC, serum biochemical profile, thoracic radiographs, transtracheal wash fluid collection for cytology and culture, nasal swab for molecular detection of respiratory pathogens (*Streptococcus equi* subspecies *equi*, equine herpesvirus 1 and 4, equine influenza virus), cervical radiography, and serology for WNV and EPM were performed. The CBC revealed a hyperfibrinogenemia (600 mg/dL; reference range, 100–400 mg/dL), leukocytosis (32,000/μL) due to a neutrophilia (28,200/μL), a regenerative left shift (bands, 962/μL), and monocytosis (1,900/μL; reference range, 0–500/μL). Serum biochemistry abnormalities included a hyperproteinemia (7.8 g/dL; reference range, 5.8–7.7 g/dL) caused by a hyperglobulinemia (5.4 g/dL; reference range, 1.6–5.0 g/dL). Findings of thoracic radiographs revealed focal patchy alveolar infiltrates and diffuse interstitial infiltrates, and a mass was noted in the caudoventral lung field consistent with an abscess. Cytology on the transtracheal wash revealed septic purulent inflammation, culture grew small numbers of *S equi* subsp. *Equi*, and the nasal swab was positive on polymerase chain reaction for *S equi* subsp. *equi*. A diagnosis of bronchopneumonia with pulmonary abscessation associated with *S equi* subsp. *equi* was made.

Findings of cervical spine radiographs revealed no abnormalities. A standing lumbosacral cerebrospinal tap was unsuccessful. While awaiting results of serology, the filly was discharged from the hospital on a course of doxycycline (10 mg/kg PO bid) for 30 days. Serology for WNV was negative. Serology for EPM was negative for *S neurona* (<40) but positive for *N hughesi* (1,280) on IFAT. Collection of atlanto-occipital CSF under injectable anesthesia to confirm EPM associated with *N hughesi* was recommended. Seven days after discharge, the bronchopneumonia appeared to be clinically resolving, and collection of atlanto-occipital CSF was performed in the field. Cytology of the CSF revealed a normal TNCC (1/μL) with 69% small mononuclear cells, 20% large mononuclear cells, 10% neutrophils, and 1% eosinophils. Total protein concentration within the CSF was normal (62 mg/dL), and RBC concentration was <1/μL. The EPM IFAT was positive for *N hughesi* (20). A diagnosis of EPM due to *N hughesi* was assigned. The filly was started on a course of ponazuril (5 mg/kg PO q24h) for 30 days. Serum from the dam was tested for EPM via IFAT and was negative for *S neurona* and positive for *N hughesi* at a titer of 640. A neurologic examination of the filly was performed 30 days after cessation of treatment with ponazuril, and although the filly remained ataxic (grade 1 of 5), the ataxia had improved. Clinical signs of respiratory disease had completely resolved at that time. The owners elected not to pursue further treatment of the EPM, and a further follow-up neurologic examination 6 months

after presentation revealed complete resolution of neurologic signs.

Neosporosis, associated with *N hughesi*, is an uncommon cause of equine protozoal myeloencephalitis.[1] There have been 7 reports of clinical neosporosis in horses, and all cases were diagnosed on the basis of isolation of *Neospora* tachyzoites on postmortem examination.[2–9] It is not possible to differentiate between *Neospora caninum* and *N hughesi* serologically; however, in the most recent reports of clinical equine neosporosis, the isolates were identified as *N hughesi* on the basis of genetic differences in the internal transcribed spacer, surface antigen 1 and surface reactive sequence 2 genes.[10,11] Of the 3 reported cases of equine encephalomyelitis associated with neosporosis,[3,5,12] the *Neospora* isolate was identified as *N hughesi* with these molecular methods.[4,10,11] Treatment of suspected cases of *N hughesi* has not been previously described.

Previous cases report a wide range of clinical signs including hind limb ataxia,[3,5] hind limb weakness progressing to paraplegia and recumbency,[6] and dysuria.[5] On the basis of the horses presented in this study, clinical signs of encephalomyelitis associated with neosporosis included gait abnormalities and ataxia, which are similar to those neurologic abnormalities described for cases of EPM associated with *S neurona*.[13] The wide range of clinical signs that have been reported in cases of EPM associated with *S neurona* reflect the multifocal or diffuse distribution of the lesions that may occur in the gray and white matter of the brain, brainstem, or spinal cord.[14] Obscure lameness or asymmetric ataxia is a common finding in cases of EPM associated with *S neurona*,[13] and it is interesting to note that asymmetric ataxia was noted in 2 out of the 3 horses reported in this study.

The prevalence of antibodies to *Neospora* spp. in horses in North America has been estimated at 23% with a direct agglutination test.[15] When the IFAT was used, 12% of horses from Alabama had antibodies to *Neospora* tachyzoites.[12] The antemortem diagnosis of encephalomyelitis associated with neosporosis is challenging, and until recently, an antemortem test for the disease was not available. An IFAT test for *N hughesi* antibodies has been validated by testing serum and CSF collected before and after experimental infection with *N hughesi*, with a cut-off titer in serum of 640 and a cut-off in CSF of 5.[1] There is no cross-reactivity of the IFAT for *N hughesi* with antibodies against *S neurona*; however, there might be cross-reaction between antibody titers to *N caninum* with the *N hughesi* IFAT, though it would occur at lower dilutions than would be seen with *N caninum* IFAT.[1]

When evaluating the CSF from horses in the present study, we found that the total nucleated cell count and protein concentrations were within the reference ranges, a situation that often occurs in cases of EPM associated with *S neurona*.[14] In 1 reported case of EPM associated with neosporosis, the protein concentration and TNCC in the CSF were high (88 mg/dL and 20 TNCC/microliter, respectively).[5] The cytologic interpretation of the CSF in horses 1 and 2 in this report was classified as a mild mononuclear pleocytosis, and the percentage of neutrophils in the CSF was increased in all horses. A similar increase in the number of large mononuclear cells in the CSF has been reported.[5] When determining antibody concentration against *N hughesi* in the CSF, the IFAT has been shown to be reliable even when blood contamination caused the RBC concentration in the CSF to be up to 10,000 RBCs/microliter at serologic titers of $\leq$320.[16] Although serum serologic titers exceeded 320 in these 3 horses and the effect of blood contamination has not been demonstrated at these higher titers, blood contamination was minimal in all horses ($\leq$8/μL).

It is of interest to note that the 3 horses that were diagnosed with EPM associated with neosporosis in this study were diagnosed with a concurrent disease. Without myelography, it is not possible to determine if the neurologic abnormalities noted in horses 1 and 2 were due to EPM or to the mild osteoarthrosis of the cervical facets; however, both horses responded to treatment with ponazuril, even after discontinuation of administration of nonsteroidal anti-inflammatories. Horse 2 was diagnosed with PSSM, and horse 3, with bronchopneumonia associated with *S equi* subsp. *equi*. In cases of EPM associated with *S neurona,* it has been suggested that the development of clinical disease is related to the size of infective dose, immunocompetence, and environmental stress.[17] The current experimental model for causing clinical EPM in horses inoculated with *S neurona* involves transport stress, which results in a more rapid seroconversion and development of neurologic abnormalities than in horses not transport-stressed.[18] There has been a reported case of neosporosis causing encephalomyelitis and polyradiculoneuritis in an older mare with hyperadrenocorticism, and it was suggested that immune suppression associated with the pituitary adenoma was a significant factor in disease development.[6]

A treatment protocol for EPM due to *N hughesi* has not been described. Unlike *S neurona, Neospora* spp. can form tissue cysts, and it has been suggested that because of this tissue cyst stage, the parasite would remain refractory to treatment.[5] The horses in this report were treated empirically with ponazuril, an antiprotozoal drug that is approved by the US Food and Drug Administration for treatment of cases of EPM due to *S neurona*.[19] Clinical improvement and, in 1 horse, resolution were observed in all 3 horses after 1 or two 30-day courses of treatment with ponazuril, in addition to anti-inflammatories or vitamin E. It is interesting to note that serum antibody concentrations against *N hughesi* were not well correlated with the progression of clinical disease.

In the 3rd horse, without the positive results of the CSF IFAT, it would have been difficult to determine if the serum antibodies for *N hughesi* were due to actual infection, exposure, or passively transferred from the filly's dam. Although maternal antibody lifespan against *N hughesi* has not been described, it has been demonstrated that maternal antibodies to *S neurona* can persist for several months.[20] Antibodies against *S neurona* have been demonstrated in the CSF of clinically normal neonatal foals with Western blot at 2–3 months

of age, and it has been suggested that these are colostral antibodies that have diffused across a permeable blood-brain barrier.[21] By 2–3 months of age, maternal antibodies against most pathogens are declining in foals,[22,23] though maternal antibodies against influenza have been reported for as long as 6 months of age.[24] In the horse reported in this study, the dam's serum antibody titer was one 2-fold dilution lower than that of the 4-month-old filly. Because of the age of the filly, the higher serum antibody titer when compared with her dam, the presence of antibodies in the CSF against *N hughesi*, and the filly's response to treatment with ponazuril, infection with *N hughesi* was likely.

In conclusion, the course of clinical disease on EPM associated with *N hughesi* is similar to that seen with EPM associated with *S neurona.* Suspected cases of EPM should be tested for antibodies against *S neurona* and *N hughesi*. A diagnosis of EPM associated with *N hughesi* can be made on the basis of the presence of gait abnormalities or ataxia, elimination of other causes of neurologic disease, and a positive (>5) CSF IFAT to *N hughesi* with minimal blood contamination. Response to treatment with ponazuril may be effective in resolving clinical disease or reducing the grade of ataxia in affected patients. Serum antibody titers are not a reliable indicator of disease progression, and clinicians should instead rely upon the progression of clinical signs when formulating a treatment plan.

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