Case Description—A 12-year-old Thoroughbred was examined because of signs of depression, neck stiffness, and poor performance.

Clinical Findings—Physical examination revealed that the horse was dull, appeared depressed, was reluctant to raise its neck and head above a horizontal plane, and had a temperature of 38.5°C (101.3°F). No radiographic or scintigraphic abnormalities of the neck were found; however, high plasma fibrinogen concentration and relative lymphopenia were identified and the horse was seropositive for antibodies against *Borrelia burgdorferi*. Analysis of CSF revealed neutrophilic inflammation, and results of a PCR assay of CSF for *B. burgdorferi* DNA were positive. Immunologic testing revealed severe B-cell lymphopenia and a low serum IgM concentration consistent with common variable immunodeficiency.

Treatment and Outcome—The horse responded well to doxycycline treatment (10 mg/kg [4.5 mg/lb], PO, q 12 h for 60 days) and returned to normal exercise. However, 60 days after treatment was discontinued, the horse again developed a stiff neck and rapidly progressive neurologic deficits, including severe ataxia and vestibular deficits. The horse’s condition deteriorated rapidly despite IV oxytetracycline treatment, and the horse was euthanatized. Postmortem examination revealed leptomenigitis, lymphohistiocytic leptomeningeal vasculitis, cranial neuritis, and peripheral radiculoneuritis with Wallerian degeneration; findings were consistent with a diagnosis of neuroborreliosis.

Clinical Relevance—Nervous system infection with *B. burgdorferi* should be considered in horses with evidence of meningitis and high or equivocal serum anti-*B. burgdorferi* antibody titers. Evaluation of immune function is recommended in adult horses evaluated because of primary bacterial meningitis. (J Am Vet Med Assoc 2010;237:1180–1185)

A 12-year-old Thoroughbred gelding was brought to the George D. Widener Hospital for Large Animals at the University of Pennsylvania for evaluation of neck stiffness and poor performance. During the several weeks prior to admission, the owner had noted that the horse seemed uncomfortable when ridden. This was particularly notable at faster speeds (ie, a canter) and when jumping. In addition, the horse’s behavior seemed to have changed; the horse appeared less active when turned out in a pasture and preferred to isolate itself from its pasture mates.

On initial physical examination, the horse’s temperature was at the upper limit of the reference range (38.5°C [101.3°F]) but heart and respiration rates were within reference limits. The horse appeared quiet and slightly depressed and preferred to maintain its head in a lowered position. Body condition was thin, despite a reportedly good appetite and diet. There was mild effusion of the left shoulder and decreases in the volume and mobility of both front feet. The horse reacted to palpation across the back, particularly in the caudal thoracic and lumbar regions. Muscle fasciculations over the hindquarters were noted when the tail and hindquarters were palpated and when the tail was manipulated, and the horse appeared anxious when the tail was elevated. Lateral neck flexibility (ie, to the left and right) appeared normal, but the horse was reluctant to lift its head above a horizontal plane or to reach toward the ground for food. No other neurologic abnormalities were noted. Additionally, results of an ophthalmic examination were unremarkable.

Lameness examination revealed a grade 2 out of 5 right hind limb lameness that was exacerbated by lunging on a soft surface in a clockwise direction. The horse was noted to hold its head and neck to the outside of the circle when lunged in a counterclockwise direction. Throughout the lameness examination, the horse appeared dull and had signs of depression with decreased responses to external stimuli.

Because of the history and clinical signs, radiography of the cervical portion of the vertebral column, a CBC, and a serum biochemical profile were performed. No radiographic abnormalities of the cervical portion...
of the vertebral column were seen. Total WBC count was within reference limits (total WBC count, 10,240 × 10^3 cells/µL; reference range, 4.3 × 10^3 cells/µL to 14.8 × 10^3 cells/µL), but a relative lymphopenia (1,428 cells/µL; reference range, 1,700 to 3,800 cells/µL) was detected. Serum fibrinogen concentration was slightly elevated (763 mg/dL; reference range, 150 to 375 mg/dL), but results of the serum biochemical analysis were otherwise unremarkable.

A nuclear scintigraphic evaluation was performed to further evaluate the neck stiffness and the lameness. This revealed moderate IRU in the region of the distal tarsal joints in the left and right hind limbs, mild to moderate IRU associated with the spinous processes of thoracic vertebrae 13 to 15, and mild IRU associated with the medial aspect of the right forelimb. Mild to moderate IRU was noted in the proximal aspect of the first phalanx in both forelimbs. No IRU was noted in the cervical portion of the vertebral column or the head.

During the first 12 hours of hospitalization, the horse's rectal temperature remained at the upper end of the reference range (38.4° to 38.6°C [101.2° to 101.4°F]) and the horse remained quiet and had signs of depression. Serum samples were submitted for anti-"Borrelia burgdorferi" antibody titer testing. Testing of the sample collected at the time of admission yielded equivocal results on both ELISA (296 KELA units; positive result, > 380 units; equivocal result, 130 to 380 units; negative result, < 130 units) and western blot analysis. A subsequent sample obtained 3 days after admission yielded an equivocal ELISA result (349 KELA units). However, western blot analysis of this second sample revealed a low to moderate amount (349 KELA units) of antibody, consistent with "B burgdorferi" infection (ie, a positive result).

A sample of CSF was obtained from the lumbosacral space with the horse sedated. Laboratory analysis of the CSF revealed slight xanthochromia with an NCC of 82% neutrophils; 12% small mononuclear cells; 6% large mononuclear cells with foamy, vacuolated cytoplasm; and a few RBCs. Findings were indicative of neutrophilic inflammation. No bacteria were seen during the cytologic examination. Bacterial and fungal cultures of the CSF did not yield any growth. Results of a western blot analysis of the CSF for evidence of "Sarcocystis neurona" infection were negative. Results of a PCR assay (OspA primer) of the CSF for "B burgdorferi" DNA were positive.

Because of the relative rarity of meningitis in mature adult horses, immunologic testing of the horse was undertaken to evaluate lymphocyte phenotype and serum immunoglobulin concentrations. Peripheral blood lymphocyte phenotyping (Table 1) revealed CD4+ lymphopenia and severe B-cell lymphopenia. The CD4+:CD8+ ratio was high. Serum IgG and IgA concentrations were within reference ranges (IgG, 1,200 mg/dL; reference range, 984 to 1,685 mg/dL; IgA, 210 mg/dL; reference range, 67 to 239 mg/dL), but serum IgM concentration was low (23 mg/dL; reference range, 90 to 150 mg/dL).

Treatment with doxycycline (10 mg/kg [4.5 mg/lb], PO, q 12 h for 60 days) and phenylbutazone (2.2 mg/kg [1 mg/lb], PO, q 12 h for 7 days, then 2.2 mg/kg, PO, q 24 h for 7 days) was initiated. Within the first week of treatment, the horse's behavior and attitude were reported to improve with a return to normal head carriage and gradual weight gain. Plasma fibrinogen concentration 14 days after initiation of treatment had decreased to 440 mg/dL. The horse was then returned to its normal turnout routine.

Sixty days after treatment with doxycycline was discontinued, the horse was readmitted to the hospital because of an acute recurrence of neurologic signs. It had been noted to have a slightly stiff neck for 4 to 5 days prior to admission and shied away when approached from the left side, had difficulty turning to the left, and had a reduced appetite for grain. Treatment with phenylbutazone PO and oxytetracycline IV had been initiated by the referring veterinarian, and the horse's condition had appeared relatively stable until the day of admission, when it acutely and rapidly deteriorated. The horse had become ataxic, developed tremors, was unwilling to walk, and leaned to the left side.

On admission, the horse had obvious left-sided vestibular deficits and was leaning heavily on the left

| Table 1—Peripheral blood lymphocyte phenotype analysis of a horse with meningitis and a subsequent diagnosis of neuroborreliosis. |
| Variable | Patient lymphocytes (1,428 cells/µL) |
| Patient lymphocytes (1,428 cells/µL) | Reference range (% positive cells) | Patient lymphocytes (1,428 cells/µL) |
| Negative control | 0.1 | 0.1 (B and T cells) | 0.1 (B and T cells) |
| CD5+ T cells | 96.5 | 6.0 (B and T cells) | 6.0 (B and T cells) |
| CD6+ T cells | 97.8 | 0.1 (B and T cells) | 0.1 (B and T cells) |
| CD8+ T cells | 97.8 | 0.1 (B and T cells) | 0.1 (B and T cells) |
| CD19-like | 1.7 | 2.5 (all T cells) | 2.5 (all T cells) |
| B cells (CD19) | 1.7 | 2.5 (all T cells) | 2.5 (all T cells) |
| B cells (CD21) | 1.0 | 2.5 (all T cells) | 2.5 (all T cells) |
| B cells (IgM) | 0.4 | 2.5 (all T cells) | 2.5 (all T cells) |
| CD3+ T cells | 77.6 | 0.1 (B and T cells) | 0.1 (B and T cells) |
| CD4+ T cells | 92.0 | 0.1 (B and T cells) | 0.1 (B and T cells) |
| CD8+ T cells | 18.2 | 0.1 (B and T cells) | 0.1 (B and T cells) |
| MHC class I | 99.9 | 0.1 (B and T cells) | 0.1 (B and T cells) |
| MHC class II | 99.9 | 0.1 (B and T cells) | 0.1 (B and T cells) |

MHC = Major histocompatibility complex.
side of the trailer for support. There was a consistent lack of left menace response and an inconsistent right menace response. A normal blink response was noted in both eyes, although subjectively, the horse may have been slower to respond to touch on the left side of the face. No nystagmus was present at admission, although intermittent ventrolateral strabismus of the left eye was noted. The horse was reluctant to move and was transferred to a stall with difficulty. At this time, periods of somnolence combined with head pressing were noted and the horse continued to lean heavily on the wall on its left side.

Preliminary clinicopathologic analysis revealed that plasma ammonia concentration (4.0 μmol/L) and PCV (34%) were within reference limits, but total protein concentration (5.3 mg/dL) was slightly low. Repeated CSF analysis was not attempted owing to safety concerns, given the horse's neurologic deficits. Treatment consisted of mannitol (0.4 g/kg [0.18 g/lb], IV) for potential cerebral edema, flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV, once), and IV fluid administration. Additionally, dimethyl sulfoxide (1 g/kg [0.45 g/lb], IV) was administered to reduce CNS edema and for its antioxidant and anti-inflammatory effects. The horse's condition continued to deteriorate, and it eventually became unresponsive, unaware of handlers, compulsive and violent in its movements, severely ataxic, and recumbent. It began to struggle with increasing violence while recumbent and developed nystagmus. Detomidine (0.02 mg/kg [0.009 mg/lb], IV) and phenobarbital (12 mg/kg [5.5 mg/lb], IV) were given and initially reduced the horse's activity level, so that it remained relatively quiet in lateral recumbency. Oxytetracycline (4 mg/kg [1.8 mg/lb], IV) was administered in view of the prior diagnosis of borreliosis, and diphenhydramine (1.35 mg/kg [0.6 mg/lb], IV over 30 minutes) was administered as a vestibular suppressant. Approximately 6 hours after administration of the detomidine and phenobarbital, the violent behavior recurred. Additional doses of detomidine (0.03 mg/kg [0.014 mg/lb], IV) and phenobarbital (6 mg/kg [2.7 mg/lb]) were administered with less effect than previously observed.

Because of the severity of the horse's vestibular dysfunction and the progressively worsening and violent self-traumatizing episodes, euthanasia was recommended. After owner permission was obtained, the horse was euthanatized with an IV overdose of barbiturates.

At necropsy, gross evaluation revealed severe diffuse hyperemia of the meninges with a mild to moderate amount of blood in the calvarium. This was interpreted as secondary to head trauma sustained during the violent episodes. Histologic evaluation of multiple serial transverse sections of the brain revealed chronic active lymphohistiocytic leptomeningitis and vasculitis (Figures 1 and 2) and mild lymphocytic cranial neuritis and peripheral radiculoneuritis. The cerebral inflammation consisted of asymmetric patchy foci concentrated within the prosencephalon and mesencephalon. There was also bilateral vestibulocochlear lymphohistiocytic perineuritis with mild multifocal Wallerian degeneration within sections of the inner ear. Histologic examination of the spinal cord revealed mild multifocal asymmetric chronic lymphohistiocytic leptomeningitis limited to the cervical and thoracic segments (Figure 3). Also present within these segments was focal cervical neuritis with mild multifocal Wallerian degeneration. Microorganisms were not detected on Gram, Gomori methenamine silver, Ziehl-Neelsen (acid-fast), or Warthin-Starry stains. Perls' iron stain revealed numerous large macrophages that contained phagocytized erythrocytes and hemosiderin. Congo red stains were negative for amyloid deposits. Results of routine fluorescent antibody testing of the cerebral cortex and cerebellum, including the medulla, by the Pennsylvania Department of Health for rabies virus were negative. Results of a PCR assay of brain tissue and cell culture virus isolation for West Nile virus were negative. Shavings of formalin-fixed, paraffin-embedded postmortem tissues from inflamed regions of the brain were submitted for *B burgdorferi* PCR testing. Postmortem *B burgdorferi* PCR tests were negative. However, because of the prior diagnosis of borreliosis and the pattern, type, and distribution of the histologic lesions, a presumptive diagnosis of leptomeningitis and peripheral radiculoneuritis secondary to borreliosis was made.
Discussion

The definitive diagnosis of borreliosis in horses remains challenging. It has been reported that 13% to 24% of horses in areas in which the disease is endemic have serologic evidence of exposure to *B. burgdorferi*. Infection rate varies by season, and horses may have no definitive clinical signs. Currently, the CDC recommends a 2-tiered approach to the serologic diagnosis of Lyme disease in people, consisting of use of an ELISA or immunofluorescence assay as a screening test, followed by western blot analysis in patients with positive or equivocal ELISA or immunofluorescence assay results. This approach has also been adopted in the veterinary community. However, whereas the specificity of this diagnostic approach is high, the sensitivity is relatively poor (50% to 75%), which necessitates testing of paired samples to avoid false-negative results, as was the case for the horse described in the present report.

The sensitivity of PCR analysis of tissue samples for *B. burgdorferi* has been found to be higher than that of tissue culture techniques in a study evaluating experimental induction of chronic borreliosis in dogs. Definitive postmortem diagnosis of persistent *B. burgdorferi* infection by use of PCR analysis was unsuccessful in the horse described in the present report. However, an experimental study suggested that the number of *B. burgdorferi* spirochetes in brain tissues is typically low in nonhuman primates infected with *B. burgdorferi*. This is supported by similar findings in a human patient with neuroborreliosis, where *B. burgdorferi* DNA was detected in only 1 of 6 brain tissue samples submitted for PCR analysis. Formalin fixation can also affect the sensitivity of PCR assays. Because only formalin-fixed paraffin-embedded specimens were available for PCR analysis for the patient in the present report, it is unclear whether results were falsely negative.

The ante-mortem diagnosis of neuroborreliosis in human patients is often difficult. Traditionally, demonstration of intrathecal anti-*B. burgdorferi* antibody production provides the strongest evidence of infection. Because of the potential for antibody leakage across the blood-brain barrier with CNS infection, comparison of antibody titers in the CNS and serum (CNS:serum index) is required to accurately estimate intrathecal antibody production. However, this technique was not used in the horse described in the present report. Newer techniques, such as the use of a PCR assay for detection of *B. burgdorferi* DNA, have also become more widely used for diagnosis, particularly because of the low sensitivity of culture techniques for isolation of *B. burgdorferi* from the CSF.

Previous reports have documented arthritis and panuveitis, lameness, and encephalitis in horses with *B. burgdorferi* infection. Localization of *B. burgdorferi* to the nervous system has been described in human patients and reproduced in nonhuman primates. Signs of neuroborreliosis in human patients include encephalomyelitis, lymphocytic meningitis, radiculo-neuropathy, and cranial neuropathies, most commonly facial nerve paralysis. Histologic lesions include meningial hyperemia and focal subdural hemorrhage, multifocal cerebral vasculitis, perivasculitis, leptomeningitis, and focal white matter degeneration. Vasculitis may cause changes in perfusion, which can result in white matter degeneration and progressive demyelination. A recent study involving rhesus macaques with intrathecal exposure to *B. burgdorferi* identified induction of proinflammatory cytokines, chemokines, and proapoptotic genes by glial cells (mainly astrocytes and microglia) and endothelial cells. It has been proposed that these neuroimmune modulators and apoptotic regulators may contribute to the pleocellular leptomeningitis, vasculitis, and perivasculitis associated with borreliosis and may also induce neuronal and oligodendrocyte apoptosis that could result in de-myelination and progressive neurologic deterioration. Localization of spirochetes to the spinal roots (motor and sensory), dura mater, leptomeninges, and dorsal root ganglia has been noted in immunosuppressed nonhuman primates experimentally infected with *B. burgdorferi*. Dose-dependent adherence of *B. burgdorferi* has been noted in primary rat brain cultures and C6 glioma cell lines; particular affinity for the extracellular matrix was noted. *Borrelia burgdorferi* can express an
assortment of adhesins to facilitate dissemination and adhesion after transmission to the host; these include proteins to bind to integrins, fibronectin, decorin, and nondecorin glycosaminoglycans. Decorin binding has been found to confer protection to \textit{B burgdorferi} from host humoral immunity in experimentally infected mice. The ability to adhere to host tissues and evade host humoral immunity may explain chronic \textit{B burgdorferi} infection. However, additional studies are required to confirm whether this can occur in horses. Immunosuppression has been found to be of importance in the experimental induction of clinical borreliosis in adult dogs and the increased spirochete tissue load in studies involving nonhuman primates. In the horse described in the present report, concurrent common variable immunodeficiency was strongly suspected on the basis of the immunologic testing performed. However, additional testing to confirm the diagnosis of common variable immunodeficiency (ie, in vivo vaccine response) had not been performed at the time the horse's condition suddenly deteriorated. It appears highly likely that common variable immunodeficiency played a role in the development of meningitis, radiculoneuritis, and vasculitis secondary to borreliosis in this horse. However, it is uncertain whether neuroborreliosis can develop in immunocompetent horses and this warrants further investigation.

Optimal antimicrobial treatment for horses with high anti-\textit{B burgdorferi} antibody titers and evidence of clinical disease remains unclear. Some experimentally infected ponies treated with doxycycline PO or ceftiofur IM continued to have positive results for tissue culture for \textit{B burgdorferi} after 28 days of treatment. However, treatment with tetracycline IV resulted in negative postmortem tissue culture results. Because of poor penetration of the blood-brain barrier, oxytetracycline was not given initially to the patient described in the present report, and a clinical response was noted following treatment with doxycycline PO despite reports of negligible CSF concentrations following administration of doxycycline PO in clinically normal horses. However, the subsequent recrudescence of clinical signs in this patient was consistent with a report of failure to consistently eliminate \textit{B burgdorferi} from the tissues of experimentally infected ponies following treatment with doxycycline. It is unclear whether therapeutic doxycycline concentrations can be achieved in the CNS of patients with altered blood-brain barrier permeability or whether the clinical improvement reported was associated with the reported anti-inflammatory properties of doxycycline. In vitro testing of \textit{B burgdorferi} isolates with amoxicillin, tilmicosin, and enrofloxacin showed susceptibility to tilmicosin. However, SC tilmicosin injection in horses produces severe inflammatory reactions at the injection site and it is contraindicated for use in horses in the commonly available preparation. Further investigation into the most appropriate antimicrobial treatment for the treatment of borreliosis in horses is still required. Appropriate antimicrobial treatment in human patients with borreliosis remains equally controversial. Several studies have failed to show a benefit of prolonged antimicrobial treatment, particularly in patients with chronic infection. Published guidelines for human patients recommend treatment with amoxicillin or doxycycline (or alternatively cefuroxime) PO in early infections and parenteral administration of ceftriaxone (alternatives include cefotaxime and penicillin G) in patients with acute neurologic or cardiac involvement.

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\end{itemize}

\textbf{References}


From this month’s AJVR

Determination of the prevalence and severity of metacarpophalangeal joint osteoarthritis in Thoroughbred racehorses via quantitative macroscopic evaluation

Richelle H. Neundorf et al

Objective—To determine the prevalence and severity of osteoarthritis in the metacarpophalangeal joints of Thoroughbred racehorses via development and validation of a quantitative macroscopic evaluation system.

Sample Population—Metacarpophalangeal joints from 50 Thoroughbred racehorses.

Procedures—Joints were collected from horses that died or were euthanized within 60 days of racing. Metacarpophalangeal joints were assessed for osteoarthritic degeneration by use of macroscopic and histologic scoring systems, polarized light microscopy, and cartilage biochemical analysis. The global macroscopic score for the entire metacarpophalangeal joint was based on factors that reflected the size and severity of lesions as well as the involvement of weight-bearing surfaces.

Results—One-third of all 2- and 3-year-old horses had partial- or full-thickness cartilage lesions and osteoarthritis. Osteoarthritis severity increased until age 6 in this population. Significant correlations were found between macroscopic grade and age, cause of death, glycosaminoglycan depletion, and loss of superficial cartilage zone polarized light intensity.

Conclusions and Clinical Relevance—The macroscopic system devised for this study had good correlations with quantitative methods. Two- and 3-year-old horses had full-thickness cartilage lesions that may have been career limiting. Year-to-year attrition and a small population of older horses may have led to underestimation of the prevalence of osteoarthritis in older horses. The macroscopic scoring system was reliable when used by nonexpert and expert users. (Am J Vet Res 2010;71:1284–1293)