

Retrospective Evaluation of Horses Diagnosed with Neuroborreliosis on Postmortem Examination: 16 Cases (2004–2015)

L.K. Johnstone, J.B. Engiles, H. Aceto, V. Buechner-Maxwell, T. Divers, R. Gardner, R. Levine, N. Scherrer, D. Tewari, J. Tomlinson, and A.L. Johnson

Background: Equine neuroborreliosis (NB), Lyme disease, is difficult to diagnose and has limited description in the literature.

Objective: Provide a detailed description of clinical signs, diagnostic, and pathologic findings of horses with NB.

Animals: Sixteen horses with histologically confirmed NB.

Methods: Retrospective review of medical records at the University of Pennsylvania and via an ACVIM listserv query with inclusion criteria requiring possible exposure to *Borrelia burgdorferi* and histologic findings consistent with previous reports of NB without evidence of other disease.

Results: Sixteen horses were identified, 12 of which had additional evidence of NB. Clinical signs were variable including muscle atrophy or weight loss (12), cranial nerve deficits (11), ataxia (10), changes in behavior (9), dysphagia (7), fasciculations (6), neck stiffness (6), episodic respiratory distress (5), uveitis (5), fever (2), joint effusion (2), and cardiac arrhythmias (1). Serologic analysis was positive for *B. burgdorferi* infection in 6/13 cases tested. CSF abnormalities were present in 8/13 cases tested, including xanthochromia (4/13), increased total protein (5/13; median: 91 mg/dL, range: 25–219 mg/dL), and a neutrophilic (6/13) or lymphocytic (2/13) pleocytosis (median: 25 nucleated cells/ μ L, range: 0–922 nucleated cells/ μ L). PCR on CSF for *B. burgdorferi* was negative in the 7 cases that were tested.

Conclusion and Clinical Importance: Diagnosis of equine NB is challenging due to variable clinical presentation and lack of sensitive and specific diagnostic tests. Negative serology and normal CSF analysis do not exclude the diagnosis of NB.

Key words: Ataxia; Borrelia; Equine; Lyme disease; Meningitis.

Lyme neuroborreliosis (NB) is caused by infection of the nervous system by bacteria belonging to the *Borrelia burgdorferi* sensu lato spirochete complex, which are transmitted by *Ixodes* ticks. Within the spirochete complex are 5 genospecies that can cause human disease.¹ In humans in North America, the only well-established cause of Lyme borreliosis is *B. burgdorferi*, whereas in Europe and Asia, *B. garinii* and *B. afzelii* are more common.² Each species, and the subtypes within each species, variably express neurotropism. *B. garinii* shows the highest neurotropism in humans.²

Serological analysis of horses in endemic regions of North America indicates that exposure of horses to

Abbreviations:

CSF	cerebrospinal fluid
EPM	Equine Protozoal Myelopathy
FFPE	formalin-fixed paraffin-embedded
IQR	interquartile range
MFI	median fluorescent intensity
NB	neuroborreliosis
Osp	outer surface protein
PCR	polymerase chain reaction

B. burgdorferi is common.³ However, the incidence of NB is unknown and appears to be low. Description of equine NB is limited to 7 cases.^{4–9} This, in part, reflects the low incidence of the disease but is also likely due to difficulties in confirming the diagnosis. In the absence of a gold standard diagnostic test, the American Academy of Neurology published guidelines for establishing an antemortem definitive diagnosis of NB in people. These included possible *Ixodes* tick exposure, the presence of neurologic disease and supportive clinicopathologic data.¹⁰ The European Federation of Neurological Societies has similar guidelines, but these are more heavily weighted by laboratory evidence, requiring both cerebrospinal fluid (CSF) pleocytosis and intrathecal *B. burgdorferi* antibody production in order to be definitive.¹¹ The development of similar guidelines in equids has been hindered by several factors including that the value of serology is limited by the high rates of seropositive healthy horses³ and the frequency of false-negative results.⁶ Furthermore, there are different methods of serologic analysis,^{5,7–9} and only one published report includes antibody analysis of the CSF,⁸ providing only a small volume of data. Only 2 case reports included an analysis of CSF, and these were discrepant—one showing a neutrophilic⁷ and the other a

From the University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square, PA (Johnstone, Engiles, Aceto, Scherrer, Johnson); Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA (Buechner-Maxwell); Cornell University College of Veterinary Medicine, Ithaca, NY (Divers, Tomlinson); B.W. Furlong & Associates, Oldwick, NJ (Gardner); Henderson Veterinary Associates, Elizabethtown, PA (Levine); and the Pennsylvania Veterinary Laboratory, Pennsylvania Department of Agriculture, Harrisburg, PA (Tewari).

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Corresponding author: L.K. Johnstone, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, 382 West Street Road, Kennett Square, PA, 19348. e-mail: laurakjohnstone@gmail.com.

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lymphocytic pleocytosis.⁸ There is currently no experimental model in which diagnostic tests can be evaluated as infection of 27 ponies with *B. burgdorferi* failed to reproduce neurologic signs.^{12–14} In summary, current literature on equine NB provides insufficient data to guide the interpretation of antemortem diagnostics.

The histologic features and distribution of lesions reported in equine NB are highly unique and include multifocal, asymmetrical pleocellular leptomeningitis and encephalomyelitis with perivascularitis and sclerosing vasculitis, and cranial and peripheral ganglionitis, radiculoneuritis and neuritis.^{5–7} Lesions, and when identified, argyrophilic spirochetes, predominate in the leptomeninges and dura mater with fewer lesions affecting the parenchyma of the brain or spinal cord.^{5–7} Similar histologic lesions of the nervous system were also reported in 3 of the 27 ponies that were experimentally infected with *B. burgdorferi* including mild lymphocytic neuritis and perineuritis of nerves and spinal nerve roots and mild lymphocytic perivascular cuffing of the meninges and thalamus.^{12,13} These pathologic findings correspond to those in human NB.¹⁵ Methods of spirochete detection, including argyrophilic (eg, Warthin–Starry or Steiner) stains, polymerase chain reaction (PCR), and immunohistochemistry, frequently lack agreement.^{5,6,9} Therefore, the authors propose that histology might represent the most definitive test for NB in horses.

The objective of this case series was to review the clinical, pathologic, and diagnostic features of horses with histologic evidence of NB.

Materials and Methods

Cases were identified via retrospective review of medical records at the University of Pennsylvania from January 2004 to March 2015. Cases were also sought via an ACVIM listserv query. Inclusion criteria required horses to have lived or traveled in an area endemic for *B. burgdorferi* and have histologic lesions as outlined in previous reports of equine NB and the exclusion of other etiologies based on a combination of clinical, laboratory, and histologic data. In detail, histologic criteria included multifocal asymmetrical, pleocellular to lymphohistiocytic leptomeningitis and encephalomyelitis with perivascularitis, sclerosing vasculitis, and/or cranial and peripheral ganglionitis, radiculoneuritis, and neuritis. Data collected included signalment, geographical history, nature and duration of clinical signs, hematologic and serum biochemical values, CSF analysis, *B. burgdorferi* antibody concentration in CSF and serum, treatment administered and response, gross and histopathologic lesions, and spirochete detection by PCR and Warthin–Starry stain.

Western blot and kinetic ELISA (KELA) were performed using whole *B. burgdorferi* cell lysate for antibody detection.¹² Detection of antibodies to *B. burgdorferi* outer surface protein (Osp) A, Osp C, and Osp F was performed using the validated fluorescent bead-based multiplex assay as described previously,^{16,17} including the use of positive and negative controls. To assess the potential value of serum to CSF ratios, paired serum and CSF multiplex median fluorescent intensities (MFI) were compared with the expected ratio of antibody assuming a normal blood–brain barrier.¹⁸ The multiplex assay of serum was run at a dilution of 1 : 400, whereas CSF was run undiluted. Therefore, to estimate the serum to CSF ratio, the serum results were multiplied by 400 and then divided

by the CSF result. As the optimal cutoff value has not been established, ratios of <130 : 1, which correlate to CSF MFI approximately equal to or greater than 4x serum MFI, were considered to indicate possible intrathecal production of antibodies. However, this calculation and cutoffs have not been validated. PCR was performed on CSF and aqueous humor as described previously.⁸ In brief, DNA was extracted using the DNeasy Blood and Tissue extraction kit,^a amplified using the G1 primer pair targeting OspA¹⁹ and then tested using a real-time 5'-nuclease assay targeting the flagellin gene.²⁰ Positive and negative controls were applied. PCR was performed on shavings from formalin-fixed paraffin-embedded (FFPE) tissue sections as described previously.²¹ Briefly, 10- μ m FFPE sections were deparaffinized twice in 1 mL xylene at room temperature for 30 minutes each, rinsed with 500 mL (100%) ethanol, pellets were dried and resuspended in 100 mL buffer (Proteinase K 200 mg/mL, 50 mM Tris pH 8.5, and 0.5% Tween-20 and 1 mM EDTA), and incubated overnight at 37°C followed with 95°C for 10 minutes and then 1 μ L of supernatant was used for real-time PCR with primers amplifying the flagellin gene.²⁰ Positive and negative controls were used with *B. burgdorferi* DNA (received from CDC) as the positive control. Detection of leptospira DNA from tissue sections was also investigated as described elsewhere.²²

Data were evaluated descriptively using ratios. Continuous variables were examined for normality using the Shapiro–Wilk test. As most parameters were not normally distributed, data were presented as medians, interquartile ranges (IQR), and total ranges.

Results

Sixteen horses fulfilled the inclusion criteria, 12 of which had additional evidence of NB including indication of intrathecal antibody production (4/10 tested) and detection of spirochetes in CNS tissue by *B. burgdorferi* PCR (5/10 tested) or Warthin–Starry stain (10/13 tested). Cases were included from the University of Pennsylvania (12), Cornell University (3), and the Virginia–Maryland College of Veterinary Medicine (1). The horses were housed in Maryland (5), Pennsylvania (4), New Jersey (4), New York (2), and Virginia (1). Of the 16 cases, 12 were geldings, while the remainder was mares. The median age at the time of death was 12 years with a range of 8–23 years (IQR 10–15). Breeds included 6 Thoroughbreds, 2 Paints, 2 Ponies, 2 Quarter Horses, and 1 each of Haflinger, Arabian, and Morgan. In one case, the breed was unknown. No horse had a history of vaccination against *Borrelia*.

Clinical Findings

Clinical signs were variable including muscle atrophy or weight loss (12), cranial nerve deficits (11), ataxia (10), changes in behavior (9), dysphagia (7), fasciculations (6), neck stiffness (6), episodic respiratory distress (5), uveitis (5), fever (2), joint effusion (2), and cardiac arrhythmias (1). Duration of disease before death, as reported by the owner, ranged from 2 to 730 days with a median of 120 days (IQR 33–180 days). Ataxia was characterized by general proprioceptive deficits and was frequently reported in conjunction with limb paresis. One horse displayed signs of generalized lower motor neuron weakness. Facial nerve deficits were present in 4 horses, presenting either as paresis or muscle

fasciculations. Dysphagia was clinically evident in 7 horses, while tongue paresis and fasciculations were evident in 5 horses. One horse with dysphagia was diagnosed with megaesophagus via contrast radiography. Five horses had an episode of respiratory distress, which in 4 horses was associated with laryngeal dysfunction evident during upper airway endoscopy. One of these horses had dysphonia. Two horses with laryngeal dysfunction died suddenly, possibly from laryngeal obstruction. Uveitis predated the occurrence of neurologic signs in all 5 horses with ocular disease. Three horses had bilateral uveitis, 1 requiring bilateral enucleation.

Eight horses received antibiotic treatment, including doxycycline, minocycline, oxytetracycline, or ceftiofur. In these cases, clinical signs either continued to progress or, despite an initial improvement, plateaued or showed recurrence and subsequent progression.

Antemortem Diagnostic Findings

Hematologic and serum biochemical abnormalities were minimal: a mild lymphopenia was reported in 2 horses, while 3 horses had increase in plasma fibrinogen concentration. CSF abnormalities were present in 8/13 cases tested, including xanthochromia (4), increased total protein (5; median: 91 mg/dL, range: 25–219 mg/dL, IQR: 65.5–185.5 mg/dL), and a lymphocytic (2) or neutrophilic (6) pleocytosis (median: 25 nucleated cells/ μ L, range: 0–922 nucleated cells/ μ L, IQR: 2–315 nucleated cells/ μ L).

Serologic analysis was positive for *B. burgdorferi* infection in 6/13 cases tested (4 by multiplex assay with 1 positive for Osp A and Osp F and 3 for Osp F only; 2 by KELA followed by Western blot). CSF multiplex analysis was positive in 5 horses: 2 horses were positive for Osp A, Osp C, and Osp F, while 3 were positive for Osp F only. Comparison of the serum to CSF MFI ratios of the multiplex analysis indicated possible intrathecal antibody production in 4/10 horses tested (1, 3, and 4 horses for Osp A, C, and F, respectively). Of these 4 cases, only 1 had a serum MFI within the positive range.

Borrelia burgdorferi PCR of CSF was performed in 7 horses. Results were negative in all cases. PCR of aqueous humor was positive in 1 horse with uveitis.

Pathologic Findings

Gross lesions of the nervous system were limited to the meninges and included opacification and yellow discoloration, as well as hyperemia with injected vessels and plaques of edema. In accordance with inclusion criteria, all horses had multifocal, pleocellular leptomeningitis, and perivasculitis with segmental vasculitis characterized by infiltration of the tunica media and adventitia by inflammatory cells with medial hypertrophy and interspersed by hyaline to finely fibrillar eosinophilic material interpreted as hyaline degeneration and sclerosis, respectively (Fig 1). Perivascular inflammation frequently expanded Virchow-Robin spaces but

also occasionally extended into the parenchyma (Fig 2). Although lymphohistiocytic infiltrates predominated, neutrophilic, plasmacytic, and eosinophilic inflammation was also present in 10, 9, and 1 cases, respectively. Inflammatory cells infiltrated the nerves, parenchyma, nerve roots, and ganglia in 10, 9, 8, and 4 cases, respectively. In 11 cases, inflammatory foci were accompanied by reactive astrogliosis and Wallerian degeneration (Fig 3), characterized by dilated axons (spheroids), dilated myelin sheaths with Myelomacrophages (digestion chambers), and neuronal chromatolysis. The locality of inflammatory lesions varied among horses that displayed dysphagia. Cranial neuritis involving nerves IX, X, and XII was noted in 3 of the 7 horses that displayed dysphagia, while another had meningoencephalitis and radiculoneuritis of the brainstem. A lymphocytic perivasculitis and perineuritis and myofiber degeneration of the tongue was present in 1 horse, while 2 horses

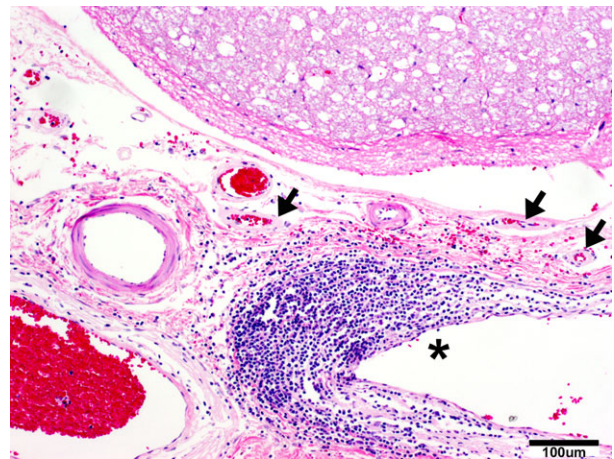


Fig 1. Leptomeningeal venule (asterisk) surrounded by dense cuffs of small lymphocytes that infiltrate the vascular wall. Walls of arterioles are often expanded by pale eosinophilic material (arrows). Hematoxylin–eosin stain at 10 \times magnification.

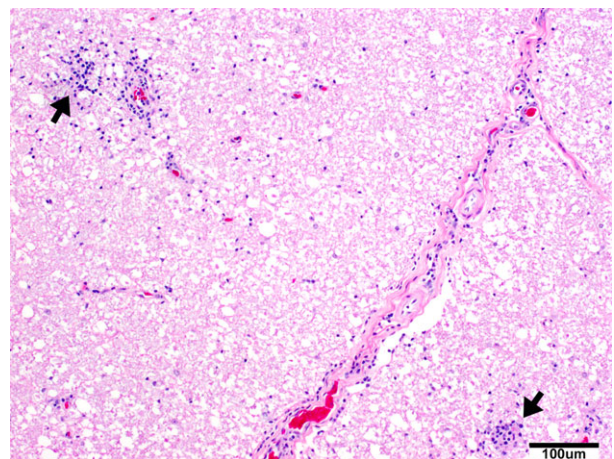


Fig 2. Lymphocytes infiltrate Virchow–Robin spaces forming perivascular cuffs and occasionally infiltrating the parenchyma (arrows). Hematoxylin–eosin stain at 10 \times magnification.

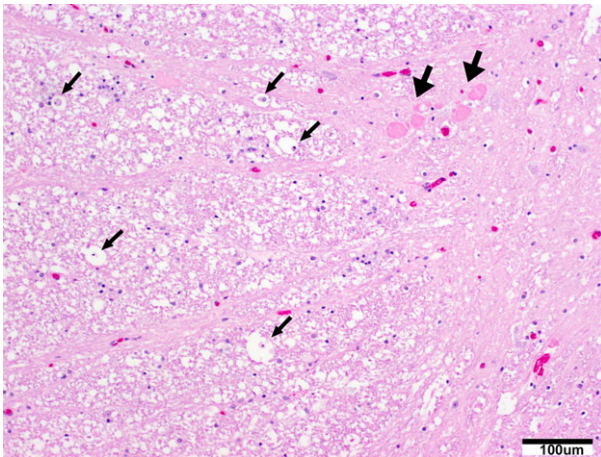


Fig 3. Reactive gliosis and Wallerian degeneration characterized by vacuolar degeneration with multifocal spheroids (large arrows), dilated myelin sheaths containing myelomacrophages (small arrows), and rare neuronal chromatolysis (not shown) was observed in 11 of the 16 cases. Hematoxylin–eosin stain at 10× magnification.

had lymphocytic perivascular and perineural inflammation within the guttural pouch. The aforementioned horse diagnosed with megaesophagus had histologic evidence of lymphocytic neuritis and myositis of the skeletal esophageal musculature. Of the 5 horses with clinical laryngeal dysfunction, atrophy of the cricoarytenoideus dorsalis muscle was noted in 2 horses (bilateral in 1 horse and unilateral, involving the left, in another). Two additional horses, both Thoroughbreds, had evidence of atrophy of the left cricoarytenoideus dorsalis muscle on postmortem evaluation.

Inflammation was also observed in organs outside the nervous system. One horse that was presented for atrial fibrillation 1 month prior to death had lymphohistiocytic epicarditis and interstitial myocarditis, while 2 others had histologic lesions consistent with myocarditis but no cardiac signs. Musculoskeletal involvement was rare. One horse that was presented for neck pain and ataxia had inflammation involving the synovium, cartilage, and periosteum of the C5–C6 cervical synovial articulation with vasculitis and myositis of the epaxial musculature. Myositis was also observed in the intercostal and temporalis muscles in 1 horse and in the esophagus of the aforementioned horse with megaesophagus. Ocular lesions included uveitis with severe vitreal inflammation, conjunctivitis, keratitis, retinitis, and optic neuritis. Other lesions included mild interstitial nephritis (4), hepatitis (3), and interstitial pneumonia (1).

Tests used for detection of spirochetes within tissue sections included Warthin–Starry staining and *B. burgdorferi* PCR performed on shavings from FFPE tissue sections. Warthin–Starry stain detected 7–15 μm -long loosely spiraled to curved argyrophilic organisms in nervous tissue sections from 10/13 horses and in the vitreous humor from 1 horse. These spirochete-like organisms were concentrated in the leptomeninges and

glia limitans of the brain (Fig 4A) and vitreous of the eye (Fig 4B). *B. burgdorferi* PCR of nervous tissue obtained positive results in 5/10 horses tested. Of the 5 cases that had a negative PCR, 4 were positive with Warthin–Starry stain and 1 showed an indication of intrathecal antibody production. The presence of *Leptospira* spp DNA was not detected within lesion-rich nervous tissue of all cases tested (12/12).

Discussion

Results from this retrospective case series exemplify the variability in clinical presentation and the possible inaccuracy of currently available diagnostic tests. These are similar challenges faced in human medicine when diagnosing NB.

In humans, NB typically causes a triad of meningitis, cranial or peripheral neuritis, and radiculitis, collectively termed Bannwarth's syndrome.²³ This syndrome manifests clinically as headaches, myalgia, neck pain, fever, ataxia, nausea, photophobia, and cranial or peripheral

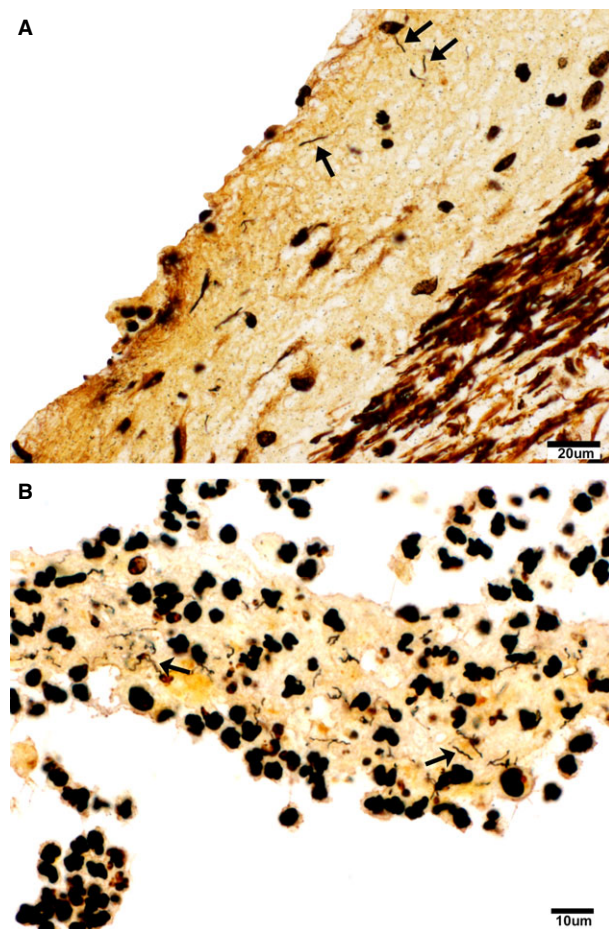


Fig 4. (A) Argyrophilic spirochete-like organisms are identified within the glia limitans of the cerebral cortex (arrows). Warthin–Starry stain at 40× magnification. (B) Large numbers of spirochetes (arrow) were concentrated within the vitreous from a horse with uveitis and vitritis in addition to ataxia. Warthin–Starry stain at 60× magnification.

nerve deficits. Although some of these signs are difficult to determine in horses, others were evident and warrant further discussion. Cranial or peripheral neuropathies were common. Facial nerve palsy, as observed in 4/16 of the horses in this study and in 3/7 previously reported cases of equine NB,^{6,7} occurs in 8% of human NB cases.²⁴ Dysphagia was frequently observed and has been described in the human literature in association with *Borrelia* brainstem encephalitis.²⁵ Histologic findings from this report indicate that the cause of dysphagia might be multifocal including cranial neuritis, guttural diverticulitis, and tongue and esophageal myositis. Recurrent laryngeal nerve paralysis has also been reported as a rare complication of human NB that can result in dysphonia and respiratory failure requiring tracheostomy.^{26–28} Fever and neck stiffness (noted in 2 and 6 horses, respectively) were present in 15% of the 118 patients with acute NB.²⁹

The variation in presenting complaints reflects the multisystemic nature of Lyme borreliosis. Uveitis was the most frequent extraneural manifestation of *Borrelia* infection. Five cases of equine uveitis associated with *Borrelia* have been reported.^{5,6,9,30} Three of these also showed neurologic signs or had histologic evidence of neural inflammation.^{5,6,9} Ocular Lyme borreliosis is rare in humans¹ and can present with variable ocular manifestations.³¹ In a case series of human ocular Lyme borreliosis, 10/20 patients had uveitis and 8/20 had concurrent neurologic signs.³¹ Although serum biochemistry did not reveal any renal or hepatic dysfunction, mild interstitial nephritis and hepatitis was observed histologically in 4 and 3 horses, respectively. There are multiple reports of dogs with *B. burgdorferi* antibodies developing lymphoplasmacytic interstitial nephritis.³² However, despite detection of *B. burgdorferi* in tissue by immunohistochemistry and urine by culture,³³ a causal relationship has not been proven.³² Lyme arthropathy was only evident in 1 horse, involving the cervical synovial articulations, but has been reproduced in an experimental model in 6- to 12-week-old puppies. The puppies showed oligoarthritis in the limb closest to the tick bites, 2–5 months after exposure, which self-resolved in 4 days without treatment.³² Carditis, as observed histologically in 3 horses, is reported to occur in 1% of human Lyme borreliosis cases and usually manifests as partial heart block³⁴; atrial fibrillation has also been reported.³⁵

The histologic lesions observed in horses from this study are unique in equine neuropathology and distinguish these cases from other known causes of neurologic disease in horses in northeastern United States. Infiltrative disease such as lymphoma is less likely given the rarity of primary meningeal lymphoma without discrete masses or multiorgan involvement, the patchy distribution of lesions in these cases having mixed inflammatory infiltrates without cell atypia, the vascular changes in these cases, and the presence of spirochetes identified within some cases.^{36,37} Equine Protozoal myelitis (EPM) causes necrotizing, granulomatous, and eosinophilic lesions predominantly within the spinal cord, thus given the histologic features present in these

cases, EPM is also not likely.³⁸ The distribution and histologic features present in these horses that include vascular sclerosis (indicating chronicity) and pleocellular inflammatory infiltrates are also not compatible with previously described equine viral encephalitis.³⁹ The histologic lesions described in this study are consistent with those reported previously in horses and humans with NB. The majority of human NB histologic lesions involve lymphocytic plasmacytic perivascular meningo-radicitis.⁴⁰ It has been proposed that neuroimmune modulators and apoptotic regulators might contribute to the lesions associated with *Borrelia*⁴¹ as opposed to direct damage caused by the spirochete. The white matter degeneration and progressive demyelination is thought to be secondary to changes in perfusion associated with the vasculitis.⁴²

This study highlighted some potential differences in the immune response to *B. burgdorferi* by horses compared with other species. First, not all horses had an abnormal CSF analysis. In an analysis of 118 human patients with acute NB, CSF analysis showed lymphocytic pleocytosis in all patients.²⁹ Second, of the horses that had CSF pleocytosis, the majority of cases showed a neutrophilic response, which contrasts with the lymphocytic pleocytosis observed in humans.^{29,40} The reasons for these discrepancies are unclear. Production of a chemokine, CXCL13, has been found to precede intrathecal antibody production in humans and is hypothesized to be responsible for the influx of lymphocytes.⁴⁰ The effects of *B. burgdorferi* on chemokine and cytokine levels have not been evaluated in horses but might be different to that of humans. Finally, Osp A antibodies were produced at positive levels, in serum or CSF, in 2 horses yet neither horse had been vaccinated against *B. burgdorferi*. *B. burgdorferi* has a complex antigenic composition, which varies depending on host and stage of infection. Osp A is expressed in the midgut of infected ticks and becomes down-regulated during transmission. Due to its high immunogenicity, it is used in canine vaccines and a positive antibody level in dogs is usually interpreted as a vaccine response. However, in people, antibodies to Osp A have been documented in later manifestations of Lyme disease, and it has been shown that the expression of Osp A by *B. burgdorferi* is upregulated in an inflammatory milieu.⁴³ Therefore, an antibody response to Osp A in an unvaccinated horse might represent late-stage disease and should not be considered a paradox.

Clinicopathologic data from this case series exemplified the challenge in diagnosing NB, as negative results were common among serologic tests and methods of spirochete detection. This problem is not unique to horses. In human studies, a significant proportion of patients from which *B. burgdorferi* was isolated did not have detectable immunoglobulin.^{44,45} It was hypothesized that this might be due to variation in *B. burgdorferi* antigen expression or low immunogenicity of the antigen. Testing prior to seroconversion might be an additional explanation for the negative serologic results. Ponies were experimentally infected with *B. burgdorferi* seroconverted 10–12 weeks after infection when tested

using the 2-tiered approach of a KELA followed by Western Blot.¹² Similar tests on experimentally infected horses have not been performed for the multiplex assay. PCR of serum, CSF, and tissue samples,² as well as culture and histology,²⁴ have low sensitivity to detect *B. burgdorferi* in humans. This appeared true in the horses from this study as well. *Borrelia* DNA is reported to be detected in only 1 in 6 human brain tissue samples submitted for PCR analysis.⁴² One explanation for the low sensitivity is that small numbers of spirochetes can cause substantial inflammation within the nervous system as seen in the experimental infection model using nonhuman primates.⁴⁶ Disease severity is believed to be related to the host inflammatory response, rather than direct damage by the spirochete.¹ In addition, antibodies to *Borrelia* OspA epitopes have been shown to cross-react with neural tissue⁴⁷ and have been associated with autoimmune disease in chronic Lyme disease in humans.⁴⁸ Because no gold standard is available, another factor that must be considered is the possibility that not all cases included in this study were caused by *B. burgdorferi*. It is prudent to consider other spirochete infections, such as leptospirosis and treponemiasis, or bartonellosis a pleomorphic argyrophilic bacillus. However, in the absence of published reports of equine neurospirochetosis other than NB, the former etiologic agents were considered unlikely.

Although neuroleptospirosis occurs in 10–15% of humans infected with leptospira,⁴⁹ reports in veterinary species are exceedingly rare and to the authors knowledge are limited to include a 2-year-old steer⁵⁰ and an 8-week-old puppy.⁵¹ In addition, none of the laboratory animals used as models for leptospirosis develop neurologic manifestations.⁵² Cases in this report are more consistent with human neuroborreliosis than human neuroleptospirosis, both clinically and histologically. Neuroleptospirosis typically coincides with hepatorenal dysfunction or acute sepsis.⁴⁹ Histopathologic lesions reported for neuroleptospirosis include parenchymal microglial nodules, perivascular ring hemorrhages and demyelination, and mild lymphoplasmacytic or granulomatous infiltrate of the meninges.^{49,53} Perivascular lymphocytic cuffing is rarely featured.⁴⁹ In addition, no evidence of *Leptospira* DNA was found by PCR analysis in the 12 cases tested.

Despite the inaccuracies of serologic testing and spirochete detection, the assessment of intrathecal antibody production is an effective means of diagnosis in people. Moreover, a positive antibody index is considered necessary for the definitive diagnosis according to the European Federation of Neurological Societies.¹¹ In humans, the specificity and sensitivity of the *B. burgdorferi* antibody index is 93%⁵⁴ and around 85%,^{29,54} respectively. In light of this, an attempt to assess intrathecal production was performed in this study despite known limitations including the potential lack of linearity of MFI results at low or high levels, the lack of validated cutoff values, and the assumption (likely erroneous) of a normal blood to CSF barrier. In contrast to the reliability of intrathecal antibody production in

humans, calculation of Osp A, Osp C, or Osp F serum to CSF MFI ratios indicated possible intrathecal antibody production in only 4/10 cases using our presumptive cutoff values. In addition to the aforementioned limitations regarding this calculation, the negative serum to CSF ratios might be due to testing prior to sufficient intrathecal antibody production or immunomodulatory effects of early antibiotic treatment.²⁹ Alternatively, the apparent lack of intrathecal antibody production compared to that observed in humans might represent differences in the immune response, as alluded to previously. In accordance with this hypothesis were the differences observed in CSF analysis between horses and humans; humans almost always show a lymphocytic pleocytosis predominated by B cells and plasma cells,²⁹ while only 2 of the 13 horses tested in this series showed lymphocytic pleocytosis. Interestingly, only 1 of the 4 cases with a serum to CSF ratio potentially indicative of intrathecal antibody production was positive on serology. This might be an example of a local immune response or could represent the slower rate of clearance of *B. burgdorferi* antibodies from the CSF compared to serum, as found in humans.²⁹

One of the primary limitations to studying NB in horses is the current lack of a gold standard diagnostic test. This study used histologic lesions as the gold standard. Twelve of the 16 horses had additional evidence of NB including indication of intrathecal antibody production (4/10), and detection of spirochetes in CNS tissue by *B. burgdorferi* PCR (5/10) or Warthin–Starry stain (10/13) and was, therefore, highly suspicious, but not definitive, cases of NB.

Equine NB is difficult to diagnose due to the variability in clinical presentation and the lack of reliable diagnostic tests. However, when presented with a horse displaying ataxia, cranial nerve deficits, and weight loss, with historic or current evidence of uveitis, collapse, or dysphagia, one should consider NB—regardless of CSF analysis or serological results. In postmortem evaluations where a lymphocytic perivascular leptomeningitis is observed, other organs including the eye and heart should be evaluated for similar inflammatory lesions and Warthin–Starry staining, and PCR analysis should be performed. In cases in which *B. burgdorferi* is not identified, testing for other spirochetes is warranted.

Footnote

^a Qiagen, Valencia, CA

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare off-label use of antimicrobials.

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