Seroprevalence of *Borrelia burgdorferi* in Horses Presented for Coggins Testing in Southwest Virginia and Change in Positive Test Results Approximately 1 Year Later

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Background: Lyme disease can affect people, dogs, and horses, but it remains poorly understood, especially in the horse. Determining the seroprevalence of *Borrelia burgdorferi* in horses in different geographic areas will enable better understanding of the epidemiology of the disease, thus improving diagnosis and treatment of affected animals.

Hypothesis: To determine the seroprevalence of *B. burgdorferi* in horses in southwest Virginia.

Animals: Horses presented for routine Coggins testing from January 2013 to January 2014 had additional blood drawn for Lyme Multiplex Assay testing.

Methods: Of 492 samples collected, 250 samples were analyzed using the Lyme Multiplex Assay. Of the 83 horses that had positive test results to at least 1 outer surface protein (Osp), 63 were available for follow-up testing 5–17 months later (June 2014).

Results: Thirty-three percent of horses had positive results for antibodies to at least 1 Osp. Horses with a positive outer surface protein F (OspF) result were older (14.5 ± 0.79) than horses with a negative OspF result (11.6 ± 0.53). Of the horses available for follow-up testing, 63% had the same result as that of the initial test. There was no difference in test result between initial and follow-up testing.

Conclusions: Horses seropositive to *B. burgdorferi* are common in Virginia, and older horses are more likely to have a positive test result for OspF than younger horses. Follow-up testing indicated that the majority of horses that were positive on initial testing did not have a different test result 5–17 months later.

Key words: Lyme; Multiplex; Tick.

Lyme disease, caused by *Borrelia burgdorferi* infection, can affect people, dogs, and horses. However, both *B. burgdorferi* and associated Lyme disease remain poorly understood, especially in the horse.[1] Clinical signs are nonspecific and do not occur in every animal exposed to the organism, making diagnosis difficult. Possible clinical signs in horses include shifting leg lameness, change in attitude, neurologic disease (eg, ataxia and weakness), skin lesions, uveitis, laminitis, lethargy, and hyperesthesia.[1]

Cases in horses were first reported in the New England states and are now commonly diagnosed in that region.[1] Serologic studies from the northeastern United States demonstrated positive antibody titers in 13–45% of horses.[2–5] In other countries, variable results have been reported from a few as 0% of horses in Africa to

Abbreviations:

ANOVA	analysis of variance
CDC	Center for Disease Control and Prevention
MFI	median fluorescent intensity
OspA	outer surface protein A
OspC	outer surface protein C
OspF	outer surface protein F
Osp	outer surface protein
TMS	trimethoprim sulfamethoxazole

as many as 48% of horses in some regions of France.[6–11] Studies also have shown large variation among different geographic locations within the same countries: 7–24% in Italy and 12–48% in France, depending on the specific region within each country.[6–9] With widespread travel of horses in the United States and the presence of vectors for *B. burgdorferi* in many areas of the country, spread of the disease out of the northeastern United States is likely.[12] In fact, a recent study found that 33% of *Ixodes scapularis* ticks in southwest Virginia were infected with *B. burgdorferi*, thus confirming that the vector and organism are present in the area.[13] According to the Center for Disease Control and Prevention (CDC), in 2013 there were 36,307 confirmed or probable cases of Lyme disease in humans in the United States with only 5 states reporting no cases.[14] These results make Lyme disease the most commonly reported vector-borne disease in humans in the United States.[15] Although the majority of cases are found to the northeastern United States, 1,307 suspected or confirmed cases were reported in Virginia in 2013. Virginia, therefore, had the 15th highest incidence and the 10th highest case numbers of Lyme disease in the United States that year.[14] Anecdotally, *B. burgdorferi* infection is

becoming more frequently inquired about, tested for, diagnosed, and treated in horses in Virginia. The purpose of our study was to determine the seroprevalence of *B. burgdorferi* in horses in southwest Virginia.

Procedures

The owners of horses presented to the Virginia-Maryland College of Veterinary Medicine Equine Field Service for routine Coggins testing from January 2013 to January 2014 were asked to allow additional blood to be taken for Lyme Multiplex Assay[a] testing and to complete a short survey. The survey asked for yes/no responses to questions on previous diagnosis or treatment of *B. burgdorferi* infection, previous vaccination against *B. burgdorferi*, and history of lameness, neurologic disease or illness in the last year. Data recorded included age, breed, sex, county of residence, and survey responses. A total of 492 samples were obtained. Once the samples were acquired, blood was refrigerated until serum could be separated (within 3 days of collection). Once separated, serum was stored at −80°C until testing. Samples were stratified by county of residence, and every other sample was selected, such that 50% of horses in each county were selected for testing. An additional 4 samples were selected to reach a total of 250 samples. These samples were shipped overnight on dry ice to the Cornell University Animal Health Diagnostic Center where they were analyzed using the Lyme Multiplex Assay,[a] which has been validated in horses.[16]

Owners of horses that were found to be positive for antibodies to any of the Osps were contacted to request an additional sample for follow-up testing. A total of 63 follow-up test samples were collected in June 2014. The time between initial and subsequent testing ranged from 5 to 17 months with a mean of 13 months, depending on when the initial Coggins test and follow-up samples were collected. These samples were processed and analyzed as above. Test results were interpreted as positive if median fluorescent intensities (MFIs) were outer surface protein A (OspA) > 2,000, outer surface protein C (OspC) > 1,000, and/or OspF > 1,250.[16] All other results were considered negative. This study was approved by the Virginia Tech Institutional Animal Care and Use Committee.

Normal probability plots showed that age followed a normal distribution. Categorical risk factors analyzed statistically included county of residence, breed, sex, history of illness in the last year, and history of lameness in the last year. Associations between each of the categorical risk factors and each of the categorical results (positive versus negative) were tested using proc survey logistic after adjusting for barn as a cluster effect (if the 2-way contingency table had cells with zero counts, a Fisher's exact test was used instead). Mixed model analysis of variance (ANOVA) was employed to test the association between age and OspA, OspC, or OspF result (positive or negative) with barn specified as a random effect. McNemar's chi-square was used to compare the prevalence of positive OspA, OspC, or OspF results between initial sampling and follow-up testing. Significance was set at $P < .05$. All analyses were performed using commercial software.[b]

Results

During the study period, 492 samples were collected from horses presented for Coggins testing. Of these, 250 were submitted for Lyme Multiplex Assay[a] testing. Of the horses with samples submitted for testing, 3 horses had been previously tested for *B. burgdorferi* infection; no horses had been vaccinated against *B. burgdorferi*, 20 horses had history of illness, 57 a history of

lameness, and 2 a history of neurologic disease in the previous year. The 3 horses with a history of being tested for *B. burgdorferi* included 2 horses tested by other veterinary practices with reportedly positive results and 1 horse tested in our practice with a negative result. One of the horses with a reportedly positive result was treated in 2011. The remaining 2 horses were not treated. All 3 horses had negative results in this study. The 2 horses with a history of neurologic disease in the past year included 1 horse that was diagnosed with equine protozoal myeloencephalitis and 1 horse diagnosed with headshaking. Both horses had negative results in our study.

Of the 250 samples submitted, 16 (6.4%) were positive for antibodies to OspA, 20 (8%) were positive for antibodies to OspC, and 63 (25.2%) were positive for antibodies to OspF. Fourteen (5.6%) horses were positive for antibodies to >1 Osp, resulting in an overall 33.2% seroprevalence (Table 1).

Serum from 63 of the 83 seropositive horses was available for follow-up testing (Table 2). Forty (63.4%) of these horses had the same result (positive/negative) on follow-up testing as they did on initial testing. Of the 23 horses that had a different result, 8 became negative to all Osp, 3 became negative to 1 or 2 Osp but remained positive for 1, and 12 became positive to 1 or 2 additional Osp.

Retrospective analysis of the medical records of horses with follow-up test results indicated that 10 horses had received antibiotics for various purposes just before initial testing (<1 month) or during the time between original and follow-up testing. Antibiotics used included ceftiofur crystalline free acid, trimethoprim sulfamethoxazole (TMS), oxytetracycline, and minocycline. Five of the 10 horses treated with antibiotics had a change in test result upon retesting. Of these 5 horses, 4 had fewer positive Osp results with 3 becoming negative for all 3 Osp. The 4 horses with fewer positive Osp results included 2 horses treated with TMS (22 mg/kg PO q12h for 14–21 days), 1 horse treated with ceftiofur (6.6 mg/kg IM on day 0 and 4), and 1 horse that was treated with oxytetracycline (6.6 mg/kg IV q24h for

Table 1. Number and percentage of horses positive for each outer surface protein in the Lyme Multiplex Assay.[a]

Initial Result	No. of Horses	Percentage of Horses
A+	9	3.6
C+	9	3.6
F+	51	20.4
A+ C+	2	0.8
A+ F+	3	1.2
C+ F+	7	2.8
A+ C+ F+	2	0.8
Negative	167	66.8

Test results were interpreted as positive if MFIs were OspA > 2,000, OspC > 1,000, and/or OspF > 1,250.

Table 2. Change in results between initial sampling and retesting (5–17 months later).

Initial Result	No. of Horses	Retest							
		A+	C+	F+	A+ C+	A+ F+	C+ F+	A+ C+ F+	Negative
A+	6	4			1	1			
C+	7		4		1		1		1
F+	37			26		5	1	1	4
A+ C+	2		1		1				
A+ F+	3	1				1			1
C+ F+	6						4	1	1
A+ C+ F+	2			1					1

Test results were interpreted as positive if MFIs were OspA > 2,000, OspC > 1,000, and/or OspF > 1,250.

5 days). The 2 horses treated with TMS included 1 horse initially positive for antibodies to OspF that subsequently was negative and 1 horse that initially was positive for antibodies to OspA and OspF that was OspA positive on retesting. The horse treated with ceftiofur was positive for antibodies to OspC and OspF on initial sampling and negative upon retesting. The horse treated with oxytetracycline initially was positive for antibodies to OspC and was negative upon retesting. The fifth horse with a change in results was treated with TMS had an increased OspA MFI to a positive result and maintained an OspC positive result. The horses that did not have a change in test result had been treated with ceftiofur (4 horses), minocycline (1 horse, also treated with ceftiofur at a different time), and TMS (1 horse).

Categorical risk factor analysis identified no difference ($P \geq .05$) in county of residence, sex, history of illness, or lameness for OspA, OspC or OspF results (positive or negative). Mixed model ANOVA indicated that horses positive for antibodies to OspF were older (14.5 ± 0.79, least squares mean $\pm$ SEM) than horses testing negative for antibodies to OspF (11.6 ± 0.53; $P = .0008$). When comparing initial testing to follow-up testing, results (number of horses positive or negative) were not different on follow-up testing.

Discussion

The results of our study show an overall seroprevalence of 33% to *B. burgdorferi* in horses in southwest Virginia. These results are similar to findings in horses in the northeastern United States.[2–5] Studies of horses in the northeastern United States, however, are older, and seroprevalence may have changed in those areas. Data from the CDC indicate that there has been a gradual increase in the number of cases of Lyme disease in humans diagnosed in the United States over the last decade.[14] Repeated studies in horses in the northeastern United States would help determine possible trends for this disease in horses. It would also be valuable to know the seroprevalence of *B. burgdorferi* in horses in other regions of the United States, as this would help practitioners in those regions make better diagnostic and therapeutic decisions. In fact, a recent study found that only 1% of horses were positive to *B. burgdorferi* in the south-central United States.[17] Widespread seroprevalence studies could be used to determine how the disease is dispersed across the United States and where horses are more likely to be exposed to and infected by *B. burgdorferi*.

Our study result of an overall seroprevalence of 33% to *B. burgdorferi* in horses also is identical to that of a recent study that identified a 33% prevalence of *B. burgdorferi* in ticks in southwest Virginia.[13] Both studies also found prevalence rates to be similar in different geographic areas of the study region. In our study, no significant differences were seen in seroprevalence when comparing county of residence. This finding suggests that results can be extrapolated across a relatively broad region of the state. The similarities in prevalence between ticks and horses are likely due to the fact that horses are routinely housed in areas where tick exposure is common. Therefore, horse seroprevalence data might be useful as a marker for the prevalence of the organism in ticks and vice versa. This information could be used to predict the exposure risk to humans and other animals. Additional research in areas of the country with different prevalences would be needed to determine if horse seroprevalence is well correlated with the prevalence of the organism in ticks.

Antigen testing for *B. burgdorferi* is not well developed and has inherent limitations, so veterinarians must routinely rely on clinical signs and antibody tests for the diagnosis of this disease. This is especially difficult with *B. burgdorferi* infection because the clinical signs can be variable and nonspecific. The Lyme Multiplex Assay[a] was developed in an attempt to more accurately diagnose horses that have been infected, as well as quantitate the duration of infection based on OspA, OspC, and OspF MFI.[18] The assay quantitates the antibody concentration in serum to 3 Osp.[18,19] OspA was thought to be expressed on the surface of *B. burgdorferi* only while the organism is inside of the tick, and not while in the mammalian host, but it is now understood that antibodies to OspA can transiently increase during early infection in horses and dogs.[18–20] However, literature in human medicine indicates that antibodies to OspA are associated with more severe and longer duration arthritis, suggesting that this antigen may be more important in clinical disease development than was thought.[21] Therefore, positive OspA MFI can indicate

vaccination, support a diagnosis of early infection, or indicate chronic, potentially severe disease. Antibodies to OspC are thought to increase during early infection, decrease after 7–11 weeks, and become undetectable after 4–5 months.[18,19] Thus, positive OspC MFI supports a diagnosis of early infection. OspF antibodies are detectable 5–8 weeks after infection and tend to remain high.[18,19] Positive OspF MFI supports a diagnosis of chronic infection.

Results of our study show a 6% seroprevalence to OspA, but none of the horses were reported to have been vaccinated previously for *B. burgdorferi*. These positive MFI results may have been caused by the increase in antibodies to OspA that can been present in early infection.[18–20] However, 9 of the 13 horses positive for antibodies to OspA and available for follow-up testing remained positive for antibodies to OspA. This result is more consistent with the human medical literature that indicates long-term antibodies to OspA can exist in clinical disease and are associated with more severe and longer duration arthritis.[21] Because the horses in our study were not evaluated for clinical signs other than those reported by owners, we cannot comment on whether these horses showed evidence of disease. The tendency to maintain a positive MFI also was seen for OspC with 13 of the 17 horses that were positive for antibodies to OspC remaining positive upon follow-up testing. These data suggest that whereas antibodies to OspA and OspC may increase in acute infection, MFI may persist for several months. Alternatively, horses may be re-exposed to the OspA and OspC antigens by re-exposure to *B. burgdorferi*-infected ticks.

Eight percent of the horses were positive for antibodies to OspC and 25% were positive for antibodies to OspF, but there was no association among OspA, OspC, and OspF results (positive or negative) and a history of illness or lameness in the previous year. Only 2 of the horses in our study had a history of neurologic disease in the previous year, and consequently, this factor was not assessed statistically. Although thorough neurologic and lameness examinations were not performed as a part of our study, many of these horses were working regularly and apparently normal according to the owners, which suggests that positive MFIs to OspA, OspC, and OspF are common without evident disease. Complete lameness and neurologic examination of horses with positive and negative OspA, OspC, or OspF MFI would be helpful in determining the prevalence of subclinical infection.

Table 2 summarizes the changes in results for all horses that were retested. Most of these horses (63%) had the same result (positive or negative) for antibodies to all 3 Osp for both testing times. Statistical analysis of these data indicated no change in the number of horses positive or negative for antibodies to any of the Osp. Consistent with our results, a recent study that evaluated ELISA titers before and after antibiotic treatment in naturally infected horse showed a small decline in ELISA titers posttreatment and an increased

likelihood of increased ELISA titers in untreated controls.[22] In our study, horses with positive OspF results were older than those with negative results. Some possible reasons for persistent positive results may include chronic infection, re-exposure, or persistence of high concentrations of antibodies. Although most horses maintained similar results upon follow-up testing, some horses did have lower MFI at follow-up. Possible reasons for a decrease in MFI causing a change to a negative result could include clearance of the organism or effective treatment.

Retrospective analysis of medical records of the horses available for follow-up testing indicated limited antibiotic use in these animals. Only 10 of the 63 horses received an antibiotic of any type according to the medical record. Five of these horses had no change in results (positive or negative) for OspA, OspC, or OspF. These horses were treated with TMS, ceftiofur, and minocycline. Of the 5 with a change in result, 4 decreased in 1 or 2 Osp MFI to negative results with 3 horses becoming negative for antibodies to all 3 Osp. One horse developed an additional positive Osp result. The horses that had a decrease in Osp MFI were treated with TMS, ceftiofur, and oxytetracycline, whereas the horse with an increase MFI was treated with TMS. Retrospective analysis of the medical records may underrepresent the number of horses treated with antibiotics because owners may have used other veterinarians or used antibiotics previously prescribed for another horse without consulting a veterinarian.

Limitations of this study include selection bias, recall bias in survey data, and retest sample collection bias and timing. The use of horses presented for routine Coggins testing was meant to identify a representative sample of the horses in our practice area. There may have been some bias in the horses selected because only horses attending state events, traveling to public facilities, or traveling across state lines are required to have a Coggins test in Virginia.[23] Therefore, there is a bias toward horses in work. However, many clients in this practice have a Coggins test performed routinely each year, regardless of whether or not they intend to use the horse in any of the above capacities. Recall bias on the part of the owners may have affected survey data, and thorough lameness and neurologic examinations may have provided more clinically relevant findings. However, this would have required substantial commitments on the part of the clients and veterinary staff. It also would have been ideal to sample all horses 1 year after collection of the initial sample to determine how many horses with negative MFI became positive in 12 months and to normalize the time between tests in those horses that were positive. Follow-up testing, however, was not included in the initial study design, which was only designed to determine the prevalence of seropositive horses in the area. Additional studies could be designed to evaluate the change in results over time in horses in endemic areas and to evaluate seroprevalence in other areas of the United States.

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