In: **49th Annual Convention of the American Association of Equine Practitioners, 2003, New Orleans, Louisiana**, (Ed.)
Publisher: American Association of Equine Practitioners, Lexington KY
Internet Publisher: Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Equine Lyme Disease: A Review of Experimental Disease Production, Treatment Efficacy, and Vaccine Protection (21-Nov-2003)

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Abstract
Borrelia burgdorferi infection in ponies causes consistent and predictable microscopic disease. Clinical disease appears to be less common. Tetracycline administered intravenously can be an effective treatment. Vaccination prior to infection is able to prevent infection.

1. Introduction

Lyme disease (LD) is the most important arthropod-borne bacterial infection in the United States. Affecting horses, people, dogs, and cats, LD is caused by the spirochete *Borrelia burgdorferi* [1-6]. *B. burgdorferi* is maintained in a 2-yr enzootic cycle that involves *Ixodes* sp. ticks and mammals. Infection generally occurs from larval, nymph, or adult tick feeding. In horses, it is not known if larval and nymph bites play an important role in Lyme infection. Ticks are usually attached for at least 24 h for *B. burgdorferi* transmission [7].
Nearly 50% of adult horses in some areas of the northeastern United States are infected or have been infected [8]. Seroprevalence in horses in other parts of the United States has not been reported but would be expected to fluctuate in a manner similar to human infection. Seroprevalence in horses might be expected to be higher than humans because of the increased risk of ticks attaching to horses for greater than 24 h.
A broad spectrum of clinical signs has been attributed to *Borrelia* infection in horses, but cause and effect have been difficult to document. Clinical signs most commonly attributed to LD include low-grade fever, stiffness and lameness in more than one limb, muscle tenderness, hyperesthesia, swollen joints (rarely), and behavioral changes [9,10]. In one report, *Borrelia* infection (confirmed by serology or spirochetemia) was more common in horses with lameness and/or behavioral changes than in horses in the same region without those clinical signs [9].
The laboratory diagnosis of recent and/or active *Borrelia* infection has been based on high (generally > 300 kinetics-based enzyme-linked immunosorbent assay [KELA] units) enzyme-linked immunosorbent assay (ELISA) titer and/or positive Western blot. Positive Western blot determination is based on appearance of bands in several molecular mass regions.
Because of the high seroprevalence in horses in the Northeastern United States and numerous phone calls asking about Lyme disease, we decided to (1) experimentally infect ponies to determine serologic responses and pathology, (2) determine if an outer-surface protein A (OspA) vaccine would be protective, and (3) evaluate three commonly used antibiotics for their potential to eliminate the infection. The purpose of this report is to review those findings.

2. Experimental Infection - Serologic Response and Pathology

Using specific pathogen-free ponies (1 - 5 yr of age) and adult (*Ixodes scapularis*) ticks infected with *B. burgdorferi*, the ticks were placed on the ponies for 7 days. Eight of eight ponies became infected with *B. burgdorferi.* Four of four control ponies that had laboratory raised *I. scapularis*, without *B. burgdorferi* infection attached in a similar manner for 7 days, remained uninfected [11].
All ponies exposed to *B. burgdorferi*-infected ticks developed detectable antibody at 5 - 6 wk. At 3 - 4 mo after tick infection, the KELA units were 200 - 300 and remained that way until euthanasia 9 mo after tick exposure. Western blots became positive at 10 - 12 wk. *B. burgdorferi* was isolated from the skin biopsies taken near the sites of tick attachment throughout the duration of the study. At autopsy, there were lymphohistiocytic nodules in the dermis of all infected ponies, most severe in the areas closest to where the ticks had been attached. Prescapular lymph nodes were enlarged and hyperplastic in all

infected ponies. Perivascular and perineural lymphocytic reactions were observed in some ponies, especially in the skin, fascia, and perisynovial membranes. One pony had a lymphocytic infiltration of the facial nerve. *B. burgdorferi* organisms, alive and/or polymerase chain reaction (PCR) positive, were most commonly found in the skin, prescapular lymph nodes, skeletal muscle, fascia, and synovial membranes. Less commonly, but not infrequently, organisms were found in the heart, pericardium, kidney, and bladder, and organisms were only rarely found in the meninges.

3. Vaccine Study

A recombinant outer-surface protein A (rOspA) subunit vaccine (100 g) was administered to *B. burgdorferi* naîve ponies on three occasions (1, 20, and 82 days). Thirty days after the last vaccination, ponies were challenged with infected ticks, following the procedure used in the previous study. Ticks were removed after 7 days, and the ponies were monitored in a *B. burgdorferi*-free environment for 4 mo [12].

Western blot analysis showed OspA antibody (32 kDa) 3 wk after the first vaccine, and the band became more dense after the second vaccination. Additional bands characteristic of *B. burgdorferi* infection (P83, P65, P60, P41, P39) did not occur during the study period. All vaccinated ponies developed KELA titers of 500 - 590. The titers gradually decreased after the last vaccine was given. All vaccinated ponies had *B. burgdorferi* growth inhibition assay titers of 1:1280 at the time of tick challenge. *B. burgdorferi* was not isolated from any organ, including multiple skin biopsies of vaccinated ponies. All control ponies became infected as they did in the previous study. Control ponies all had the expected positive KELA and immunoblot response after infection and no growth inhibition titer before challenge. The organism was isolated from multiple sites in all control ponies.

4. Treatment Study

In a third and separate study, naîve ponies were infected with *B. burgdorferi,* and after infection, ponies were treated with either tetracycline [a] (6.6 mg/kg IV, q 12 h), doxycycline [b] (10 mg/kg PO, q 12 h), or ceftiofur [c]. (2.2 mg/kg IM, q 12 h). All treatments were for 3 wk. Ponies were euthanized 4 mo after treatment was discontinued. Serology was performed every 3 wk throughout the study period. All ponies treated with tetracycline had a progressive decline in ELISA units at the time of euthanasia. Tetracycline-treated ponies were negative on both culture and PCR. Doxycycline and ceftiofur produced inconsistent results in serologic response and culture findings.

5. Discussion

LD is a common infection in horses in parts of the United States. The infection may be persistent. The gamut of clinical signs is unknown, but from these experimental studies, we know that pathology does occur. These studies support a possible preferred migration of the organism through connective, perineural, and perivascular tissue in the skin, fascia, muscle, and synovial membranes. The pathology in these areas could certainly cause hyperesthesia and lameness, two of the most commonly reported (by owners and veterinarians) clinical signs. It has been our limited experience that clinical signs are most commonly reported in performance horses that are used for eventing. Although not the only possible explanation, one explanation for this observation would be that hyperesthesia and mild lameness/stiffness are more likely to be noticed in this type of horse. We have observed muscle wasting and markedly swollen joints in one horse diagnosed with LD, but these signs have not been common in horses diagnosed with LD. Fever and edema, both responsive to tetracycline therapy, may be found in horses just before Lyme seroconversion but these signs are most likely a result of *Anaplasma phagocytophilia* infection. *A. phagocytophilia* is found concurrently with *B. burgdorferi* in many *Ixodes* ticks, resulting in dual infection in the horse [13].

Our treatment studies suggest that tetracycline given IV is superior to orally administered doxycycline or IM ceftiofur. The superiority of tetracycline over doxycycline might be related to expected higher tissue concentrations with tetracycline, because doxycycline has been shown to have low bioavailability when given orally to horses [14]. Aqueous humor samples collected from our experimental ponies, as part of a separate study, did not have doxycycline concentrations above measurable levels (0.3 g/ml). Some of the ponies treated with doxycycline or ceftiofur had a significant decline in antibody level during treatment, but antibody level increased after treatment was discontinued. This would suggest that the drugs may inhibit proliferation of *B. burgdorferi* but may not eradicate the organism. To be reasonably confident that the organism is no longer present, KELA ELISA titers should drop to very low levels. Although tetracycline was highly efficacious in this study, its efficacy may not be the same if horses have been infected for longer than 5 mo before beginning therapy.

Our vaccine study suggests that OspA vaccination would be effective in preventing *B. burgdorferi* infection in horses. Based on our study, it seems that the antibody inhibited *B. burgdorferi* within the tick, most likely through feeding on the neutralizing antibody rich plasma, because there was no serologic conversion in the vaccinated ponies. *B. burgdorferi* inhabits the gut lumen of the tick, and the spirochete is exposed to host blood antibody and complement when the tick feeds [15]. The vaccine and units used were almost identical to the commercially marketed canine rOspA vaccine. We do not know how frequently the vaccine would need to be given to maintain protection. Safety studies would also need to be performed.

6. Summary

Although many questions regarding LD in the horse remain unanswered, these studies should provide helpful information on expected pathology, clinical signs, diagnostic testing, treatment options, and treatment duration in addition to the possibility of successful vaccination.

Footnotes

[a] Liquamycin LA-200, Pfizer Animal Health, Exton, PA 19341.
[b] Doxycycline Hyclate Tablets 100 mg, IVAX Pharmaceuticals, Miami, FL 33137.
[c] Naxcel, Pharmacia and Upjohn Company, Kalamazoo, MI 49001.

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