Viable *Borrelia Burgdorferi* in the Urine of Two Clinically Normal Horses
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What is This?
patient with concurrent rabies infection. Diagnosticians should be aware of the possibilities of these concurrent viral infections in raccoons with neurologic disorders.

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References


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Lyme disease is a multisystemic bacterial disease caused by the spirochete *Borrelia burgdorferi.* Lyme disease has been reported in humans and in many domestic animal species, including dogs, cattle, and horses. Reported clinical signs of Lyme disease in horses include lameness, uveitis, encephalitis, and foal mortality. Although the spirochete has been demonstrated in deerflies, horseflies, and mosquitoes, transmission of *B. burgdorferi* usually occurs via the bite of an infected *Ixodes* tick. Urine shedding of the spirochete has been documented in cattle and wild white-footed mice (*Peromyscus leucopus*). Urine shedding from wild mice may play a role in maintaining *B. burgdorferi* in natural populations in the absence of tick vectors. The importance of urine shedding of the organism as a mode of nonvector transmission among horses has not been examined. In this report, we describe the presence of viable *B. burgdorferi* in the urine of 2 clinically normal mares housed in a Lyme disease-endemic region of Connecticut.

Approximately 100 ml of midstream urine was collected into sterile containers from 5 randomly chosen horses housed at the University of Connecticut. Rectal temperature, pulse, respiration rate, and complete blood counts were within normal limits for all 5 horses at the time of urine collection. Each horse was inspected while walking and was negative for signs of lameness. Urine was transported to the laboratory in a cooler. Under a laminar flow hood, 0.5 ml of each horse’s urine sample was placed in 7 ml of sterile Barbour-Stoenner-Kelly II medium (BSK II) containing ciprofloxacin (40 μg/ml) and rifampicin (20 μg/ml) and incubated at 32°C. The remaining urine sample was centrifuged at 11,000 × g for 20 minutes, and the sediment was resuspended in 3 ml of the supernatant; aliquots were frozen at −20°C for future antigen testing.
Following a 30-day incubation period, the urine cultures were examined under dark-field microscopy for the presence of spiral organisms. Cultures containing spiral organisms were passaged into new tubes of BSK II medium with antibiotics, and 1 ml of the culture was frozen in 30% glycerol at −70 °C for seed culture. The remaining culture was frozen at −20 °C for future antigen testing to positively identify the spiral organisms as *B. burgdorferi*.

Urine sediments from all 5 original urine samples and urine cultures that were clearly positive for spiral organisms by dark-field examination were used for polymerase chain reaction (PCR) analysis. Chromosomal DNA was extracted using standard phenol–chloroform extraction\(^\text{19}\) and was precipitated with absolute ethanol. Amplification of DNA was performed utilizing a primer pair as previously described\(^\text{17}\), which amplifies a 309-base pair DNA product within the outer surface protein A gene specific to *B. burgdorferi*, and a PCR reagent kit\(^\text{b}\) in a thermocycler\(^\text{c}\) (35 cycles). PCR products were separated on a 0.8% agarose gel, stained with ethidium bromide, and photographed. The DNA was then transferred onto a blotting membrane.\(^\text{d}\) For Southern blot analysis,\(^\text{21}\) *B. burgdorferi* genomic DNA PCR product was used as a DNA probe; the probe was labeled with \(^{32}\)P using a DNA labeling kit\(^\text{e}\) according to the manufacturer’s instructions. The membranes were hybridized overnight at 65 °C, washed, air dried, and then exposed to imaging film\(^\text{f}\) for 8–24 hours.

**Figure 1.** A. Positive control *Borrelia burgdorferi* culture stained with polyclonal fluorescein-labeled goat anti-*Borrelia* antibody. Fluorescing spirochete (arrow). B. Fluorescing spirochete (arrow) in a second passage urine culture (horse A) started from a frozen primary culture.
Of the 5 urine samples cultured, 2 (horses A and B) were positive for spiral organisms by dark-field examination after a 1-month incubation period. Fluorescent antibody staining of a second passage urine culture from horse A using polyclonal goat anti-*Borrelia* species fluorescein isothiocyanate-labeled antibody revealed fluorescing spirochetes (Fig. 1). The fluorescent antibody used is produced by immunizing goats with whole cells of *B. burgdorferi* and is highly specific for *B. burgdorferi* and *B. hermsii*. Spiral organisms verified as *B. burgdorferi* by PCR were present in passages 1–3 from horse A and passages 1–4 from horse B, as demonstrated by the presence of the 309-base pair PCR product in an autoradiograph of the Southern blot (Fig. 2). The anticipated 309-base pair product was also present in cultures started from frozen primary urine cultures of both horse A and horse B (Fig. 2). Direct PCR analysis of the original urine sediments from each horse was negative.

This is the first report of the presence of viable *B. burgdorferi* in the urine of clinically normal horses in an endemic region. The number of organisms shed in the urine of these horses appeared to be low; enrichment culture was necessary before *B. burgdorferi* could be detected by PCR. The ability of the spirochetes to be passaged and to multiply to a level of detection confirms the viability of *B. burgdorferi* in equine urine. Urine samples cultured from 5 healthy horses in a nonendemic region of Vermont were culture negative (data not shown).

Direct urine/mucosal contact is one known mechanism for transmission of *Leptospira*; nontick transmission of *B. burgdorferi* may occur by a similar route. Nonvector contact transmission of the spirochete has been demonstrated in experimentally infected dogs and mice. The urine/mucosal transmission of *B. burgdorferi* in horses could have clinical importance in the spreading of the disease between horses during the breeding process, where urine splashing is a common event. Potential zoonotic spread of the disease in this manner is a possibility deserving further investigation. Regardless of clinical presentation, urine from horses in a Lyme disease-endemic region should be considered potentially infectious and should be handled with care.

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Sources and manufacturers

- a. Sigma Chemical Co., St Louis, MO.
- b. GeneAmp, Perkin-Elmer Cetus, Norwalk, CT.
- c. Model 480, Perkin-Elmer Cetus, Norwalk, CT.
- d. Zeta-Probe, Bio-Rad, Hercules, CA.
- e. DECAprime II, Ambion, Austin, TX.
- f. Kodak X-OMAT, Sigma Chemical Co., St Louis, MO.
- g. Kirkgaard & Perry Laboratories, Gaithersburg, MD.
- h. GIBCO Laboratories, Gaithersburg, MD.

References

2. Bosler EM, Schultze TL.: 1986, The prevalence and significance
of *Borrelia burgdorferi* in the urine of feral reservoir hosts. Zentralbl Bakteriol Mikrobiol Hyg 263:40–44.

**Concurrent trichinosis and oral squamous cell carcinoma in a cat**

Peter G. Moisan, Michael D. Lorenz, Paul C. Stromberg, Heather A. Simmons

Trichinosis is a cosmopolitan disease of humans and warm-blooded animals caused by *Trichinella spiralis* and other *Trichinella* species. There is little host specificity, and the agent has been reported in a number of species of wild and domestic animals. The most common neoplastic condition of the feline oral cavity is squamous cell carcinoma. Concurrent trichinosis and oral squamous cell carcinoma in a cat

The animal presented to the veterinary clinician (HAS) was a 10-year-old neutered male domestic shorthair cat. The animal had been missing from its home in New York state for approximately 1 month. At the time of initial examination (September 30, 1995), a small mass was found in the subcutaneous tissue at the rostroventral surface of the left mandible. Two 5-mm ulcers were present in the skin overlying and inferior to the mass. The lower left canine tooth was missing, and there was an accumulation of necrotic tissue within the vacant dental alveolus. The necrotic material extended to the mass adjacent to the mandible. The patient had ptyalism, and mucopurulent discharge from the eyes and nose was noted. A wedge-shaped section excised from the proliferative tissue inside the mouth at the junction of the gingiva and lip was submitted for histologic examination. The animal was treated with 100 mg of oral lincomycin twice daily for 2 weeks.

Histopathologic examination revealed mild to moderate chronic–active inflammation associated with helminth fragments. Moderate fibrosis, with surgical artifact, and accumulations of inflammatory cellular debris within the section prevented accurate taxonomic identification of the organisms. Neoplastic change was not identified in the initial tis-