Isolation of *Leptospira interrogans* serovar *grippotyphosa* from the skin of a dog

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- Antibody to leptospires may not be detectable in dogs with active infections.
- Leptospires are commonly isolated from kidneys, but one should also consider other tissues as possible sources of these spirochetes.
- The *Leptospira interrogans* serovar *grippotyphosa* infects dogs living in southeastern and northeastern United States.

A 14-year-old male Golden Retriever that could not walk in its last year of life was euthanized. Necropsy was performed and attempts were made to detect current or prior exposure to *Borrelia burgdorferi*. One female tick (*Ixodes scapularis*) was attached to the cervical skin. There was external evidence of diarrhea, but no ingesta in the gastrointestinal tract. The liver contained large hepatomas. The kidneys and testicles were small.

Specimens of brain, lung, liver, kidney, thyroid gland, spleen, pancreas, testes, heart, salivary gland, muscle, stomach, intestine, and skin were fixed in buffered 10% formalin, embedded in a purified paraffin and plastic polymer medium, sectioned at 4 to 5 μm, and stained with H&E for histologic examination. Kidney specimens had extensive tubular degeneration, fibrosis, and lesions consistent with chronic interstitial nephritis. There were small, scattered interstitial lymphocytic infiltrates, with a few areas of calcification in the medullary tubules. Fibrosis had replaced numerous tubules and casts were in some of the remaining tubules. Generalized passive congestion was evident in the liver. Lesions of vegetative endocarditis were evident on both atrioventricular valves, and an area of calcification was in the ventricular myocardium. Three noninvasive melanomas were in the intermandibular area. Testes had extensive tubular degeneration and an interstitial (Leydig) cell tumor.

Two members of the family that owned the dog had been previously determined to have *B burgdorferi* borreliosis. Serum from the dog was tested for antibody to *B burgdorferi* by ELISA and the titer recorded at 1:1,024.

In an attempt to isolate borreliae, urine, CSF, and specimens of spleen, urinary bladder, salivary gland, and brain were aseptically removed along with the distal portion of one ear. A total of 0.1 ml of urine and CSF was inoculated into Barbour-Stoenner-Kelly (BSK) medium containing agarose and the antibiotic rifampicin. Tissue specimens of approximately 0.5 cm³ of spleen, salivary gland, bladder, and brain were each placed in a sterile laboratory bag containing 7 ml of BSK medium. Organs were pulverized, and 0.7-ml aliquots were inoculated into tubes of BSK medium with agarose and rifampicin. The section of ear to be tested was cleansed with surgical scrub solution and rinsed with water before an ear-punch specimen was obtained and placed in BSK medium with agarose and rifampicin. Inoculated culture tubes were incubated for 6 weeks at 34°C and examined for spirochetes by dark-field microscopy at approximately 3-week intervals. Borreliae were not isolated, but leptospires were evident in the culture tube inoculated with the skin specimen 19 days after inoculation. Leptospires were not obtained from urine, CSF, or from specimens of brain, spleen, salivary gland, and urinary bladder.

The leptospires grew in sub-passages placed in BSK medium, but cells never became abundant until transfer to 10 ml of Tween 80-bovine albumin medium. Twenty-four days after transfer to liquid Tween 80-bovine albumin medium, 0.5 ml of medium containing 1 × 10⁸ cells, as determined by use of a Petroff-Hauser counting chamber, was injected intraperitoneally into a Syrian hamster. Three days after inoculation, 0.1 ml of blood was obtained from the hamster's heart and inoculated...
into 10 ml of semisolid Tween 80-bovine albumin medium and incubated at 30 °C for 7 days to attempt reisolation. Three days after inoculation, 1.5 × 10^8 cells were isolated. The hamster died from leptospirosis on day 8 after inoculation.

The isolate was characterized at the National Veterinary Services Laboratories by first testing the antigen against antisera to 13 *Leptospira interrogans* serovars representative of serogroups known to exist in the United States (australis, autumnalis, ballum, bataviae, canicola, grippotyphosa, hardjo, copenhageni, pomona, pyrogenes, tarassovi, woffii, and bratislava) by a standard microagglutination procedure. Specific identification of the isolate was then made by using a modification of a previously described restriction endonuclease analysis procedure. In the test, genomic DNA was digested with the restriction enzyme Hha I and electrophoresed on a 20 × 25-cm agarose gel at 60 V for 17 hours. The highest titer was against *L. interrogans* serovar grippotyphosa, and the restriction pattern of the isolate was indistinguishable from those of the reference strains (Andaman and RM52) of *L. interrogans* serovar grippotyphosa.

Serum was tested for antibody against 12 *L. interrogans* serovars australis, autumnalis, ballum, bataviae, bratislava, canicola, grippotyphosa, hardjo, copenhageni, pomona, pyrogenes, and tarassovi by a standard microagglutination test. The dog’s serum diluted at 1:100 did not react with the 12 *Leptospira* serovars tested.

Specimens of kidney and liver were sectioned as described and stained by the uranium nitrate/silver nitrate method of Steiner and Steiner. Leptospirosis were not observed in the tissues.

Leptospires are most frequently isolated from kidney specimens of animals, although they also have been isolated from liver, brain, lung, placenta, uterus, vagina, and various fluids, such as urine, blood, milk, and aqueous humor. Heretofore, they have not been isolated from skin of dogs, but biopsy specimens of skin are frequently used to isolate *B burgdorferi* from rodents and human beings. **T**teen 80-bovine albumin medium is superior to BSK medium for isolation and growth of leptospirosis and should be used in future attempts to isolate these spirochetes from skin.

The *L. interrogans* serovar isolated from the dog of this report, *grippotyphosa*, has previously been recovered from domestic animals including dogs, cattle, and horses and from wildlife. Raccoons and other wild animals are likely the primary reservoirs.

*Leptospira interrogans* serovars canicola and icterohaemorrhagiae are most frequently associated with infection in dogs. *Leptospira interrogans* serovar grippotyphosa has previously been isolated from dogs in the Philippines and was associated with renal dysfunction in a Foxhound in Georgia from which it was isolated.

Absence of antibody to *Leptospira* is paradoxical; however, isolation of leptospires from animals without detectable antibody has been reported for human beings and cattle. Identification of antibody to *B burgdorferi* indicated the prior exposure of this dog to the spirochete. Vectors are not considered important in transmission of leptospirosis, although *L. interrogans* serovars *grippotyphosa* and *canicola* have been isolated from ticks, and it has been suggested that flies may act as mechanical vectors.