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Fatal Spirochetosis Due to a Relapsing Fever-like *Borrelia* sp. in a Northern Spotted Owl

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ABSTRACT: Acute septicemic spirochetosis was diagnosed in an adult male northern spotted owl (*Strix occidentalis caurina*) found dead in Kittitas County, Washington, USA. Gross necropsy findings included marked enlargement of the liver and spleen and serofibrinous deposits on the serous membranes lining the body cavities and the pericardial and perihepatic sacs. Microscopic observations included macrophage infiltration in the liver and spleen with mild thrombosis and multifocal necrosis, as well as hemorrhage and acute inflammation in the choroid plexus of the brain. No viruses or pathogenic bacteria were isolated from brain, liver, or spleen, and no parasites were found in blood smears or impression smears of the liver. Chlamydial culture attempts were unsuccessful and no chlamydial antibodies were detected in serum. In silver-stained microscopic sections and by transmission electron microscopy of liver, numerous long, thin, spiral-shaped bacteria were seen in the liver, spleen, cerebral ventricles, and within blood vessels in many organs. The organism was identified as a member of the *Borrelia* genus by sequence analysis of the PCR-amplified 16S rRNA gene. The most closely related species is *B. hermsii,* an agent of relapsing fever in humans in the western United States. This is the first report of a relapsing fever-related *Borrelia* in a wild bird.

Key words: Avian, bird, *Borrelia hermsii,* case report, disease, northern spotted owl, pathology, relapsing fever, spirochete, *Strix occidentalis caurina.*

The northern spotted owl (*Strix occidentalis caurina*) is a nonmigratory, medium-sized owl that inhabits coniferous forests in the mountains of the Pacific Northwest USA and southwestern British Columbia, Canada (Gutiérrez, 1996). Conservation of the northern spotted owl has been a highly contentious issue between environmentalists and the timber industry because of this species' association with late-succession forests. Habitat loss and a declining population prompted the U.S. to list the northern spotted owl as threatened under the Endangered Species Act in 1990.

Public attention for the past 20 yr has resulted in extensive studies into many aspects of the owl's natural history and demographics. Information about the causes of mortality makes important contributions to these studies. The primary causes of death cited for northern spotted owls are starvation, predation by great horned owls (*Bubo virginianus*) and goshawks (*Accipiter gentilis*), and accidental collisions (Gutiérrez, 1996). These causes are similarly reflected in National Wildlife Health Center (NWHC; Madison, Wisconsin, USA) diagnostic data that documented the causes of death for 75 free-flying northern spotted owls since 1986 (53% emaciation from unknown cause, 17% traumatic injuries, 12% undetermined causes, 11% miscellaneous causes, 4% disease, and 3% suspected intoxication; NWHC unpubl. data). Deaths due to infectious disease were rare in these data, but here we describe the case of a northern spotted owl that succumbed to infection by an unusual spirochete.

An adult male northern spotted owl was found dead in Kittitas County (Washington, USA) on 5 August 1994. The bird had been observed behaving normally during radiotelemetry studies 2 wk earlier. The carcass was shipped chilled by overnight commercial carrier to the NWHC for necropsy. On gross examination, the owl had striking enlargement of the liver and spleen. The liver had rounded margins and was brown with a distinct pale reticular pattern visible on the capsular and cut surfaces. The elongate spleen was pale, mot-

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tled, and 3 × 1.2 cm. Clear yellow fibrinous deposits coated serous membranes in the body cavities, clung to the splenic capsule, and were accumulated in the perihepatic sacs. Clear yellow serofibrinous exudate filled the pericardial sac. The bird was in fair body condition and weighed 490 g.

Samples of brain, heart, lung, trachea, kidney, liver, spleen, pancreas, and intestine were fixed in 10% buffered formalin, paraffin embedded, sectioned at 5 μm, and stained with hematoxylin and eosin for light microscopy. Liver and spleen sections were stained with Ziehl-Nielssen acid fast, Taylor's gram, modified Giminez, and Gomori's iron stain, and kidney sections were stained with Gomori's iron and Ralph's stain for hemoglobin (Luna, 1968; Andersen, 1998). By light microscopy, the pale reticular pattern in the liver appeared as bridges of fatty degeneration between portal regions, along with accumulations of periportal macrophages and granulocytes. The predominate microscopic feature of the spleen was hyperplasia of macrophages, reticular cells, and plasma cells along with diminution of the lymphoid follicles. Occasional fibrin thrombi and foci of coagulative necrosis were noted in the liver and spleen. The choroid plexus of the third ventricle in the brain had acute hemorrhage and granulocyte infiltration and the lateral ventricles were mildly distended by proteinaceous fluid (Fig. 1). Mild heterophilic meningitis was concentrated near the cerebellum. The heart had only mild vascular-oriented pericarditis and superficial myocarditis. Mild renal tubular cast formation and bright red granules that did not stain as hemosiderin or hemoglobin in the proximal renal tubular epithelium suggested proteinuria.

Bacterial isolations from brain, liver, and spleen were attempted using routine culture techniques incorporating 5% sheep blood agar and eosin methylene blue agar (Remel, Lenexa, Kansas, USA) and in a *Salmonella*-selective culture method using both Hektoen-Enteric Agar (Remel) and

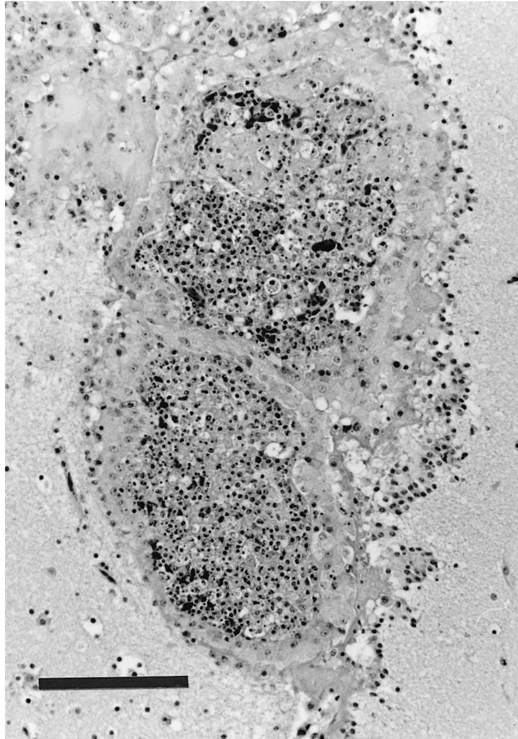

FIGURE 1. Choroid plexus in the brain of a northern spotted owl. The stroma is distended by inflammatory cells and fibrin. HE. Bar = 125 μm.

XLT4 Agar (Difco Laboratories, Detroit, Michigan, USA) after enrichment in Dulcitol-Selenite Broth (NWHC). No bacteria were isolated from brain or liver and an *Enterococcus* sp. isolated from spleen was considered to be a postmortem contaminant. Brain, liver, and spleen were negative for viruses following culture in embryonated chicken eggs and muscovy duck embryo fibroblasts (Docherty and Slota, 1988; Senne, 1998). *Chlamydia psittaci* was not isolated from brain, liver, and spleen at the US Department of Agriculture (USDA) National Veterinary Services Laboratory (Ames, Iowa, USA). Serum collected from clotted blood in the heart was negative for antibody to *C. psittaci* by the latex agglutination test at the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA). No parasites were found on gross examination, in squash preparations of liver, lung, and kid-

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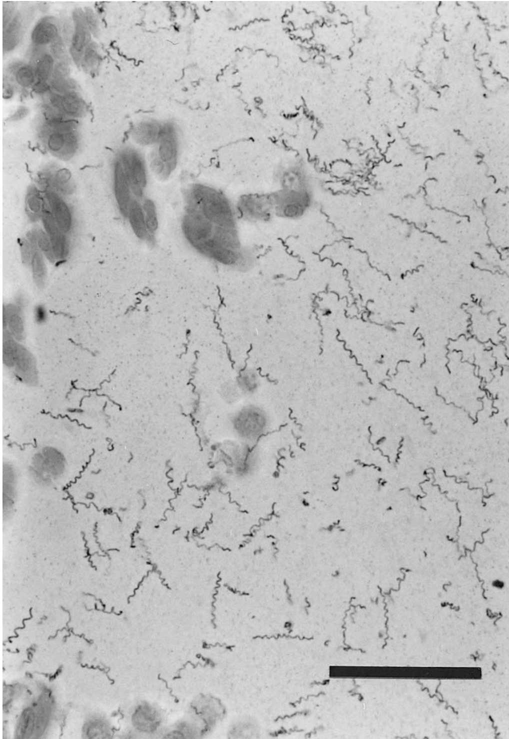

FIGURE 2. Numerous spirochetes in the lumen of an hepatic vein of a northern spotted owl. Steiner stain. Bar = 25 μm.

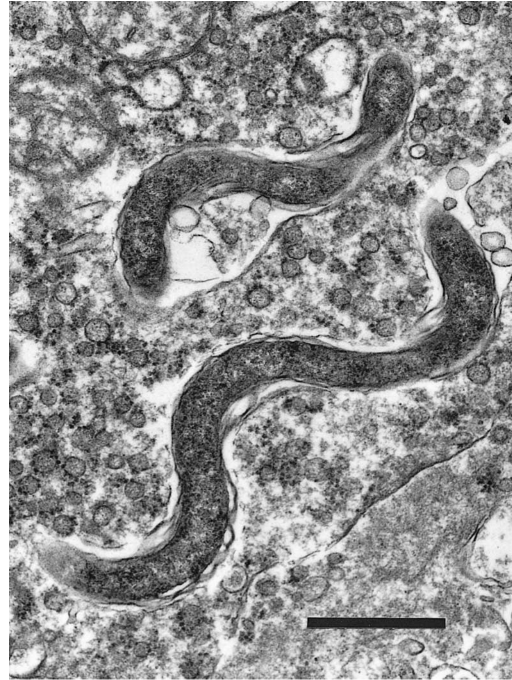

FIGURE 3. Transmission electron micrograph of two spirochetes in the space of Disse in the northern spotted owl's liver. Bar = 1 μm.

ney, or in Giemsa-stained (Garcia and Bruckner, 1993) blood smears. Unidentified unsporulated coccidial oocysts and *Capillaria* sp. detected in scrapings of the gastrointestinal mucosa were considered incidental.

When a modified Steiner's silver stain for spirochetes (Garvey et al., 1985) was applied to tissue sections numerous long, thin, spiral bacteria were visible in blood vessels throughout the body (Fig. 2) and were accumulated in the cerebral lateral and third ventricles. Spirochetes were lying in tangles in the hepatic sinusoids and were scattered throughout the splenic parenchyma. Spirochetes were occasionally present in renal tubules but were infrequent there in comparison with the renal glomerular and interstitial blood vessels. For comparison, liver sections from 13 northern spotted owls that died in Kittitas County (Washington, USA) ($n = 6$) or Oregon (USA) ($n = 7$) were similarly stained, and contained no visible spirochetes. The 13 owls had been previously diagnosed as traumatic deaths, handling accidents, or emaciated.

Giemsa-stained impression smears of liver contained faintly staining spirochetes. By transmission electron microscopy of Karnovsky-fixed liver, post-fixed with 1% osmium tetroxide and stained with 2% uranyl acetate before embedding in Epon Araldite and ultra-thin sectioning, multiple spiral bacteria, with a wavelength of approximately 1.9 μm and diameter of approximately 0.25 μm, were seen in hepatic sinusoids (Fig. 3).

Retrospective attempts to culture the spirochete from tissue samples that had been stored frozen at −70 C were unsuccessful. Samples of brain, liver, spleen, and clotted blood were inoculated into BSK-H supplemented with 10% rabbit serum (Sigma, St. Louis, Missouri, USA) and incubated at 37 C with 5–6% CO_2. A portion

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TABLE 1. Percent identity between DNA sequences of 16S rRNA genes of the owl spirochete and selected *Borrelia* spp.

Species	Species								
	Bh	Bt	Bp	Bcr	Ba	Bco	Bl	Bbi	Bb[a]
Owl spirochete	99.6	99.1	99.0	98.9	98.8	98.4	98.0	96.4	96.3
B. hermsii (Bh)	100	99.3	99.2	99.0	98.5	98.7	98.1	96.6	96.5
B. turicatae (Bt)		100	99.9	99.2	98.8	98.9	98.4	96.7	96.6
B. parkeri (Bp)			100	99.1	98.7	98.8	98.4	96.7	96.7
B. crocidurae (Bcr)				100	98.8	98.9	98.1	96.4	96.5
B. anserina (Ba)					100	98.2	97.5	96.3	96.3
B. coriaceae (Bco)						100	98.3	96.4	96.2
B. lonestari (Bl)							100	96.0	95.8
B. bissettii (Bbi)								100	99.6

[a] *B. burgdorferi* sensu stricto.

of the culture was examined for spirochetes periodically using phase-contrast microscopy. Cultures were subcultured one time at day 4, and both culture sets were considered negative for spirochetes after 4 wk. The culture attempt was repeated by inoculating the same tissue samples into BSK II medium (Barbour, 1984) and incubating at 34 C, but there was no spirochete growth after 3 wk.

The spirochete was identified in frozen liver tissue using polymerase chain reaction (PCR) and sequence analysis of the 16S rRNA gene. Total DNA from the liver specimen was obtained by using DNeasy Tissue Kit (QIAGEN Inc., Valencia, California, USA). Sequences of primers and PCR conditions for amplification of partial 16S rRNA gene of *Borrelia* species have been described previously (Barbour et al., 1996). The PCR product was purified by gel extraction (QIAquick Gel Extraction Kit, QIAGEN, Inc.), ligated into pCR2.1 vector (TA Cloning Kit, Invitrogen, Carlsbad, California), and transformed into *E. coli.* Both strands of the insert of the recombinant plasmid were sequenced. The following *Borrelia* spp. 16S rRNA gene sequences were used for comparison: *B. burgdorferi* sensu stricto (GenBank accession number M88329), *B. lonestari* (U23211), *B. anserina* (U42284), *B. turicatae* (U42299), *B. parkeri* (U42296), *B. coriaceae* (U42286), *B. hermsii* (U42292), *B. crocidurae* (U42302), and *B. bissettii* (AJ224141). Multiple sequences were aligned with aid of the Higgins-Sharp routine using default values of the MacDNASIS Pro suite (version 3.6) of programs from Hitachi Software (San Bruno, California). Partial 16S rRNA sequence of the owl spirochete has been assigned GenBank (*http://www.ncbi.nlm.nih.gov/Genbank)* accession number AF116903.

The PCR using the owl liver DNA preparation as a template and a pair of primers specific for the 16S rRNA gene of *Borrelia* spp. produced an approximately 1300 bp fragment. This fragment was cloned, sequenced, and 1290 bp were compared with the analogous sequences of selected *Borrelia* spp. with exclusion of the primer target sequences from the analysis (Table 1). The 16S rRNA gene sequences of the owl spirochete was nearly identical at 99.6% to a strain of *B. hermsii,* an agent of relapsing fever in western United States and Canada. There was less similarity with sequences of other New World relapsing fever borrelias (*B. turicatae* and *B. parkeri*), an Old World relapsing fever species (*B. crocidurae*), the avian borreliosis agent (*B. anserina*), a species found in *Amblyomma americanum* ticks (*B. lonestari*) (Barbour et al., 1996), and two Lyme disease *Borrelia* complex species (*B. burgdorferi* and *B. bissettii*) of North America.

Avian spirochetosis, or borreliosis,

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caused by *B. anserina,* is uncommon in the U.S., but outbreaks occasionally have been reported in poultry or captive gamebirds (Mathey and Siddle, 1955; Cooper and Bickford, 1993). The Lyme disease agent, *B. burgdorferi,* was isolated in surveys of clinically normal wild birds in the U.S., but has not been associated with morbidity or mortality in birds (Mather et al., 1989; Burgess et al., 1993; McLean et al., 1993; Bishop et al., 1994; Durden et al., 1997). Similarly, *B. garinii,* an agent of Lyme disease in Europe, was detected in wild seabirds and game birds, but is not considered an avian pathogen (Olsen et al., 1995; Kurtenbach et al., 1998). Other borrelial diseases in the U.S. are the tick-borne relapsing fevers of humans, caused by *B. turicatae* in the southwest and *B. hermsii* in the mountainous west (Barbour and Hayes, 1986). *Borrelia hermsii* is endemic in the Cascade mountain range in Washington where this northern spotted owl was found (Dworkin et al., 1998).

The lesions in this owl clearly indicated that the bird died from an acute to subacute, overwhelming infection. The lesions of typical avian spirochetosis due to *B. anserina* are similar to those seen in this owl and include hepatomegaly, splenomegaly, fibrinous polyserositis, thrombosis, necrosis, and vast numbers of circulating organisms (Cooper and Bickford, 1993). However, erythrophagocytosis and hemosiderosis that are common in avian spirochetosis were not evident here. The involvement of the choroid plexus in the northern spotted owl was somewhat unique and probably related to the vascularity of that tissue. No primary involvement of the brain was apparent and meningitis was very mild; however, the choroid plexus may be the site of entry of borrelias into the central nervous system (Cadavid and Barbour, 1998).

Proteinuria is difficult to confirm postmortem but was suggested by the granules and casts in the owl's renal tubules. Proteinuria was reported in 46% of clinical human patients in Dworkin et al.'s (1998) review of tick-borne relapsing fever in the northwestern U.S.; the mechanism is unknown but could result from mechanical injury to glomerular capillaries. The severe consequences of spirochete infection in this case suggest that the owl was an accidental host for this pathogen, rather than serving as a reservoir host in the organism's transmission cycle.

The source of the *Borrelia* sp. and means of exposure for the northern spotted owl are unknown. The principle vectors known to transmit borreliosis in the U.S. are ticks, specifically the soft-bodied *Argas* sp. and *Ornithodorus* sp. for avian spirochetosis and the relapsing fevers, respectively, and *Ixodes* sp. for Lyme disease. Mites and biting insects have also been implicated in mechanically transmitting avian spirochetosis (Barnes, 1997). Ectoparasites reported for the northern spotted owl are limited to lice and hippoboscid flies found on live-captured owls or museum specimens (Young et al., 1993; Hunter et al., 1994). These parasite survey methods are unlikely to detect nest-dwelling argasid ticks. Northern spotted owls are territorial and monogamous, utilizing either tree cavities or platforms, such as old raptor nests (Buchanan et al., 1993; Forsman and Giese, 1997). Nests are reused at a rate of 18–25% (Forsman and Giese, 1997), making a nest-associated transmission cycle conceivable. A more likely alternative, however, may be direct transmission by predation or through contact with secretions or excreta from an infected individual. Experimental transmission of relapsing fever infection to rats and dogs through consumption of brains of infected animals has been documented (Joyeux and Sautet, 1938; Horrenberger, 1955). Small rodents serve as reservoirs for the agents of the relapsing fevers and Lyme disease, and the principle prey of northern spotted owls are dusky-footed and bushy-tailed woodrats (*Neotoma fuscipes* and *N. cinerea,* respectively), northern flying squirrels (*Glaucomys sabrinus*), and red tree voles (*Phenacomys longicau-*

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dus) (Forsman et al., 1984; Gutiérrez, 1996).

In a previous study chickens were successfully infected with *B. duttonii,* an agent of human relapsing fever in Africa, through direct inoculation (Kervan, 1947). In this case we have found the first evidence that fatal disease may be caused by a relapsing fever species of *Borrelia* in birds. Such cases could be prone to underreporting because spirochetes are not readily detected using most routine avian diagnostic techniques. Techniques useful for diagnosis of suspected borreliosis include dark field microscopy of fresh, wet tissue preparations or spirochete-specific histochemical stains of fixed tissue.

The relationship between the owl *Borrelia* and agents of human disease is under further investigation. While any cause of mortality in an endangered or declining species may be of concern, the significance of this finding to the northern spotted owl and other species remains unclear until the source of the agent and means of transmission are better explained.

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