



Research Article

First Detection of Lyme Disease Spirochete *Borrelia burgdorferi* in Ticks Collected from a Raptor in Canada

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Abstract

During a pan-Canadian tick-host study, we detected the spirochetal bacterium, *Borrelia burgdorferi* sensu lato, which causes Lyme disease, in ticks collected from a raptor. Lyme disease is one of a number of zoonotic, tick-borne diseases causing morbidity and mortality worldwide. Larvae of the avian coastal tick, *Ixodes auritulus*, were collected by wildlife rehabilitators from a Cooper's hawk, *Accipiter cooperii*, on Vancouver Island, British Columbia. Using PCR amplification of the linear plasmid *ospA* gene of *B. burgdorferi*, 4 (18%) of 22 larvae were positive. Since these engorged *I. auritulus* larvae had not had a previous blood meal and *B. burgdorferi* is rarely transmitted from infected female ticks to their progeny, we propose that Cooper's Hawks are reservoir-competent hosts of *B. burgdorferi*. Our tick-host discovery provides the first report of bird-feeding ticks on a Cooper's Hawk, and exhibits the premiere record of *B. burgdorferi*-positive ticks on a raptor. Not only are passerine (perching) and gallinaceous (chicken-like) birds involved in the wide dispersal of Lyme disease vector ticks, raptors are now also implicated in the dissemination of *B. burgdorferi*-infected ticks. Although *I. auritulus* does not bite humans, this tick species plays an integral role in the 4-tick enzootic cycle of *B. burgdorferi* along the West Coast of North America. In essence, raptors and *I. auritulus* ticks may help to amplify this infectious agent in nature, and increase the likelihood of people contracting Lyme disease, especially in coastal areas.

Keywords

Falconiformes; Raptors; Cooper's Hawk; *Accipiter cooperii*; Canada; Lyme disease; *Borrelia burgdorferi*; Ticks; *Ixodes auritulus*

Introduction

A diversity of wild birds act as avian hosts of blood-sucking, hard-bodied *Ixodes* species ticks (Ixodida: Ixodidae). Most commonly, ticks are reported on passerines (Order: Passeriformes), which are also known as perching or songbirds, and some of these ticks are infected with *Borrelia burgdorferi* sensu lato (hereafter *B. burgdorferi*), the spirochetal bacterium that causes Lyme disease [1]. This tick-borne spirochetosis can have a multitude of clinical symptoms, including cardiac, cutaneous, endocrine, gastrointestinal, genitourinary, musculoskeletal, neurologic, cognitive, and neuropsychiatric [2-4].

If left untreated or inadequately treated, diverse forms [5,6] of *B. burgdorferi* can sequester and persist in immunologically deprived and deep-seated sites [7-14]; namely, ligaments and tendons [15,16], muscle [17], brain [18-20], bone [21,22], eyes [23], glial and neuronal cells [24,25], fibroblasts/scar tissue [26]. There are at least 100 different *B. burgdorferi* genotypes worldwide [27-30], and patients are often negative using the 2-tier Lyme disease serology test despite having Lyme disease [31-33].

This tick-borne microorganism cycles in nature between certain tick species and a wide range of vertebrate hosts, and has been reported from 5 continents, including subAntarctic islands and Australia [34,35]. In the coastal area of southeastern Australia, the avian coastal tick, *Ixodes auritulus* (Ixodida: Ixodidae), and the paralysis tick, *Ixodes holycyclus*, which are both Lyme disease vector ticks, aid in the spread of Lyme disease. In Canada, several different wild bird species, which are short- and long-distance carriers, widely disperse Lyme disease vector ticks nationwide [36-39]. In far-western Canada, Gregson [40] reported *I. auritulus*, on a Bald Eagle, *Haliaeetus leucocephalus* and a Rocky Mountain wood tick, *Dermacentor andersoni*, on a hawk. Although raptors (Falconiformes: Accipiteridae) were examined recently in southern Ontario for attached ticks, none was noted [41]. Cooper's Hawks, which have ample opportunity to encounter host-seeking ticks, have a continent-wide range and transcontinental distribution across the central temperate region of North America, including Vancouver Island, British Columbia (B.C.) [42]. The sheep tick, *Ixodes ricinus* and the tiaga tick, *Ixodes persulcatus* have been reported on several species of the hawks in Eurasia [43].

Ticks can transmit more kinds of pathogens than any other group of ectoparasites worldwide affecting people, livestock, wildlife and domestic animals [44]. In Canada, at least 6 of 23 known *Ixodes* species collected from vertebrates (avian, mammalian, reptilian) exhibit some degree of vector competence for *B. burgdorferi*. The principal vectors to humans are the western blacklegged tick, *Ixodes pacificus*, in British Columbia and Alberta and, east of the Rockies, the blacklegged tick, *Ixodes scapularis*, parasitizes a wide range of vertebrate hosts. Similarly, *Ixodes dentatus* and *Ixodes spinipalpis* [45] are confirmed as competent vectors of *B. burgdorferi*. Additionally, *Ixodes affinis*, which is occasionally transported from the southeastern USA and Mexico by northward migrating passerines in the spring, is an extralimital tick that has vector competency for *B. burgdorferi* [46,47]. Moreover, several bird-tick-*Borrelia* studies underpin the fact that ground-frequenting passerines transport Lyme disease vector ticks northward during long-distance flight [36-39,48-50]. Not only do migratory songbirds carry ticks northward during spring migration, these avian hosts also transport them southward during fall migration [37,51]. Along the West Coast, *I. auritulus* ticks, which are ectoparasites of passerines and galliforms, play a role in the natural enzootic cycle of *B. burgdorferi* [39]. Using culturing and PCR-testing, early studies in the southern region of Vancouver Island, B.C. detected *B. burgdorferi* in *Ixodes angustus* and *I. pacificus* and established its presence in this area [52]. The aim of our tick-host-*Borrelia* study was to explore any new environmental associations that could contribute to an increase in Lyme disease in an area.

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Materials and Methods

Tick collection

Ticks were detached primarily from the head and neck using fine-pointed tweezers by wildlife rehabilitators. One to three ticks were placed in 2 mL polypropylene micro tubes, and 4 or more ticks were placed in clear 4 dram (12 mL) polystyrene vials with white polyethylene caps vented with tulle netting. These containers were placed in a ziplock bag with a slightly moistened section of paper towel. Dead or badly damaged ticks were put directly in 2 mL micro tubes containing 95% ethyl alcohol. Using a bubble-pack envelope, ticks were mailed promptly to the lab (JDS) for identification. An Olympus stereoscopic microscope SZX16 (objective, 1x; eyepieces, 10x), which provided zoom observation magnification of 7x-115x, was used to view the following tick characteristics: 1) alive or dead, 2) unfed, partially engorged, fully engorged, 3) developmental life stage, and 4) tick species [53-55]. Partially and fully engorged ticks were kept alive and allowed to molt to the next developmental life stage. After background information was noted, ticks were sent by overnight courier to the culturing and PCR amplification research laboratory (JFA).

Spirochete detection

Each unfed and engorged tick was tested for the presence of *B. burgdorferi* using PCR by methods as previously described [56,57]. Briefly, ticks were ground with a large paper clip in a 0.6 mL microcentrifuge tube containing 25 μ L to 35 μ L K Buffer, which consisted of: 18 mL sterile irrigation water, 2 mL 10X Base Buffer, 0.09 mL NP 40 (Sigma, lot #122K00401), and 0.09 mL Tween 20 (Sigma lot #033K0109). A different paper clip was used for each tick. Each tick was boiled at 94°C for 10 minutes. DNA was extracted from engorged ticks using instructions in the QIAamp DNA Mini Kit (250) (QIAGEN, Valencia, CA). Primers were the linear plasmid *ospA* gene target: *ospA2*, 5'-GTTTTGTAATTTCAACTGCTGACC-3'; *ospA4*, 5'-CTGCAGCTTGAATTCAGGCACTTC-3'. PCR amplification was performed using a Perkin-Elmer thermal cycler set to conduct denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, and elongation at 72°C for 1 min for a total of 45 cycles. Appropriate negative and positive controls were used. Amplification products were analyzed by electrophoresis, stained with ethidium bromide, and examined under UV illumination as described previously [56,57]. Amplification products were transferred to a nylon membrane by Southern blot. The membrane was then hybridized overnight with ³²P using the probe *ospA3*, 5'-GCCATTTGAGTCGATTGTTGTAAGT-3'. The membrane was then washed, and Kodak X-OMAT AR film was placed over the membrane for four hr. Infected ticks were detected with the ³²P probe. Attempted culturing of spirochetes from the larval ticks from the Cooper's Hawk was not done because they were all dead upon arrival for tick identification.

Results

Tick collection

A total of 22 engorged *I. auritulus* larvae were collected from the edge of the lower right eyelid of a juvenile male Cooper's hawk, *Accipiter cooperii*, which was examined on 29 October 2012, after it was recovered at Oak Bay, Vancouver Island, B.C., Canada (Figure 1). This tick collection is the first report of ticks on a Cooper's Hawk, and



Photo credit: Christina Carrieres

Figure 1: A Cooper's Hawk parasitized by *Borrelia burgdorferi*-infected larvae of the avian coastal tick, *Ixodes auritulus*.

constitutes a new tick-host record.

Spirochete detection

Four (18%) of 22 *I. auritulus* larvae were infected with *B. burgdorferi*. Based on an extensive literature search, we provide the first report of *B. burgdorferi*-positive ticks on a raptor. Of the 4 positive ticks, 2 of 17 (12%) partially engorged and 2 of 5 (40%) fully engorged larvae were positive for *B. burgdorferi*. The Cooper's Hawk was released on 5 November 2012, and blood was not drawn from this raptorial host; thus, we could not verify spirochetemia in this host bird.

Discussion

This bird parasitism provides the first report of ticks on a Cooper's hawk, and announces new-found evidence of *B. burgdorferi* in ticks collected from a raptor. The results of this study provide credible evidence that raptors act as reservoirs of *B. burgdorferi*, and add to the increased role of wild birds as dispersal agents of this zoonotic pathogen. Cooper's Hawks prey primarily on small- and mid-sized birds, but also supplement their diet with small mammals. As they consume their capture, they frequently make contact with low-lying vegetation where ticks are questing. In this particular case, the Cooper's Hawk was most likely at a site where a gravid *I. auritulus* female laid her eggs in the spring. During the summer, these eggs hatched to larvae, and were ready for active host-seeking in the late summer and fall. Since the attached larvae had a similar amount of engorgement, the Cooper's Hawk must have encountered a cluster of larvae from recently hatched eggs. When the *I. auritulus* female lays her eggs, and dies, the fat pellet in the posterior end of the idiosoma of the carcass provides a source of energy-laden nutrients, and creates a proclivity to attract birds and rodents foraging for food [58].

Now, the question becomes: how did the four *I. auritulus* larvae acquire *B. burgdorferi* infection? Connecticut researchers [59] provided the first isolation of *B. burgdorferi* from a passerine (Veery, *Catharus fuscencens*), and showed that certain wild birds exhibit reservoir competency. Since the *I. auritulus* larvae had not had a previous blood meal, and transovarial transmission (female to eggs) of *B. burgdorferi* is not apparent during prior bird-tick studies [37-39], we extrapolate that spirochetes of this zoonosis were transmitted

during engorgement on the Cooper's Hawk. Our findings that 2 of 17 (12%) of the partially engorged and 2 of 5 (40%) of the fully engorged larvae removed from the hawk were positive for *B. burgdorferi* may be significant in this respect. Although the numbers are small, tick larvae that had imbibed a larger volume of host blood were more likely to be *B. burgdorferi*-positive, which provides circumstantial support for our suggestion that these ticks imbibed spirochetes with their bloodmeal from the hawk. If transovarial transmission of *B. burgdorferi* was the only source, then the infection rate of the partially engorged and the fully engorged larvae would be approximately the same. However, in our ectoparasite study, they are significantly different.

For comparison, researchers [60] presented evidence-based data to indicate that *Borrelia miyamotoi*, which is also pathogenic to humans [61], is transmitted transovarially by *I. scapularis* females; however, *B. burgdorferi* was not transmitted or detected in unfed larvae derived from egg clutches of wild-caught *I. scapularis* females. For our study, the host Cooper's Hawk was most likely spirochetemic, and the host-seeking larvae acquired *B. burgdorferi* during engorgement. Further studies are necessary to confirm whether transovarial transmission occurs with *I. auritulus*.

As birds of prey, raptors are continuously consuming small mammals and wild birds, which presumably are infected with *B. burgdorferi* and, after eating them, may become infected. Not only do Cooper's Hawks have frequent opportunities to encounter *B. burgdorferi*-infected, ectoparasitic ticks, they could feasibly become orally infected. Subsequently, these spirochetemic avian hosts could infect unfed, spirochete-free larvae. For comparison, 22 days post-inoculation, spirochetes were isolated from cloacal material and kidneys from Mallard Ducks, *Anas platyrhynchos platyrhynchos* that had orally been infected with *B. burgdorferi* [62]. Moreover, Schwarzozá et al. [63] similarly detected *B. burgdorferi* in the throat and cloacal cells from birds migrating through Slovakia. These findings show that certain orally-infected birds can develop spirochetemia, and shed *B. burgdorferi* in their droppings. Based on our PCR amplification results, we suggest that Cooper's Hawks are reservoir-competent hosts, and act as dispersal vehicles of *B. burgdorferi* to new environmental foci. Along Canada's Pacific coast, this raptorial host presumably plays a notable role in the 4-tick enzootic cycle of *B. burgdorferi*, which consists of four vector-competent ticks (*I. auritulus*, *I. angustus*, *I. pacificus*, and *I. spinipalpis*). The bird parasitism in our study not only includes *I. auritulus* on a Cooper's Hawk, it implicates raptors as reservoir hosts in the 4-tick enzootic cycle of *B. burgdorferi* in this bioregion, and expands the number of bird species in Lyme disease dissemination.

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