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The Establishment of a Blacklegged Tick Population by Migratory Songbirds in Ontario, Canada

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Abstract

This 2-year study implicates migratory songbirds in the initiation of an inland Lyme disease endemic area in southeastern Ontario. The spirochetal bacterium, *Borrelia burgdorferi* sensu lato Johnson, Schmid, Hyde, Steigerwalt & Brenner, which causes Lyme disease, was detected in blacklegged ticks, *Ixodes scapularis* Say, collected by flagging. Based on PCR amplification, 19 (33.3%) of 57 *I. scapularis* adults (males, females) were infected with *B. burgdorferi*. Since transovarial transmission of *B. burgdorferi* is nil in *I. scapularis* and white-tailed deer. *Odocoileus virginianus* Zimmermann, are not reservoir-competent hosts, we suggest that songbirds are the mode of introduction of *B. burgdorferi*-infected *I. scapularis*. All of the natural abiotic and biotic attributes are present to establish a Lyme disease endemic area. Blacklegged ticks survived the winter successfully at the epicentre. We provide substantial evidence that migratory songbirds initially introduced Lyme disease vector ticks and *B. burgdorferi* spirochetes to this remote woodland habitat and initiated an established population of blacklegged ticks.

Introduction

The blacklegged tick, *Ixodes scapularis* Say (Ixodida: Ixodidae), is the primary vector of the Lyme disease-spirochete, *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt & Brenner, east of the Rocky Mountains [1]. This blood-sucking, ixodid ectoparasite feeds on at least 144 different vertebrates (avian, mammalian, reptilian), including humans, and domestic and wildlife animals [2,3]. Immatures (larvae, nymphs) of *I. scapularis* parasitize at least 76 different bird species, especially passerines (Order: Passeriformes), primarily perching birds (songbirds) [4-11]. This tick species has been collected from migratory songbirds as far west and north as the town of Slave Lake, Alberta [7] and, similarly, a *B. burgdorferi*-positive *I. scapularis* nymph was detached from a passerine migrant in central Saskatchewan (Tweedsmuir, SK) [3].

Lyme disease can have a multitude of clinical symptoms, including cardiac, cutaneous, endocrine, gastrointestinal, genitourinary, musculoskeletal, neurologic, cognitive, and neuropsychiatric [12-14]. If left untreated or inadequately treated, diverse forms [15,16] of *B. burgdorferi* can sequester and persist in immunologically deprived and deep-seated sites [17-24]; namely, ligaments and tendons [25,26], muscle [27], brain [28-30], bone [31,32], eyes [33], glial and neuronal cells [34], and fibroblasts/scar tissue [35]. Patients are often seronegative because standard commercial immunoassays (i.e., ELISA, EIA) for Lyme disease yield poor results with a sensitivity of 44-56% in patients who have been infected for more than 4-6 weeks [36-40]. In addition to Lyme disease spirochetes, the blacklegged tick acts as a zoonotic vector of several human pathogens: *Anaplasma phagocytophilum* (human granulocytic anaplasmosis) [41], *Babesia* spp. (e.g., *B. microti*, human babesiosis) [42], *Bartonella* spp. (e.g., *B. henselae* bacteremia) [43-45], *Borrelia miyamotoi* (relapsing fever group spirochete) [46], deer tick virus (Powassan virus group) [47], *Ehrlichia phagocytophila* (granulocytic ehrlichiosis [*E. equi* group]) [48], and *Mycoplasma* spp. (e.g., *M. fermentans*) [49].

Certain songbirds are reservoir-competent hosts of *B. burgdorferi*. Using xenodiagnosis tests, Richter et al. [50] determined that the American Robin, *Turdus migratorius* L., can harbour *B. burgdorferi* in its body for 6 months and, as a result, engorging larval and nymphal *I. scapularis* can subsequently become infected. In southeastern United States, Durden et al. [8] cultured *B. burgdorferi* from skin biopsies of several passerines. As well, *B. burgdorferi* has been isolated from a Veery, *Catharus fuscescens* (Stephens) [51]; the House Wren, *Troglodytes aedon* (Vieillot) [52]; and American Robin [52]. Because transovaral transmission of *B. burgdorferi* is not present in *I. scapularis* [53], this mode of spirochetal transmission to eggs or larvae, is not apparent. Additionally, *B. burgdorferi* has been isolated from partially- and fully-fed *I. scapularis* larvae parasitizing songbirds, which compliments the fact that these avian hosts are reservoirs of infection, and can potentially initiate new Lyme disease foci [52]. Moreover, in Europe, Schwarzová et al. [54] similarly detected *B. burgdorferi* in the throat and cloacal cells from birds migrating through Slovakia; these findings also show that songbirds are *B. burgdorferi* reservoirs.

Connecticut researchers [52] introduced the concept of passerines starting established populations of *I. scapularis* and *B. burgdorferi* spirochetes in new foci. When a heavily infested passerine releases several replete *B. burgdorferi*-infected, fully engorged *I. scapularis* in a suitable habitat, an enzootic network may be initiated. Of note, migrating songbirds are a flagship in the introduction of spirochete-infected *I. scapularis* larvae and nymphs. Our study shows that songbirds can transport *B. burgdorferi*-infected *I. scapularis* into a

new geographic area, and provide the essential enzootic components to establish a Lyme disease endemic area.

Materials and Methods

Study area

The tick investigation was conducted on independently owned land (44.29N, 76.43W), which is located on the southern fringe of the Canadian Shield, west of Verona, Frontenac County, in eastern Ontario, Canada. This area has rugged, undulating topography with igneous rock outcrops that are interspersed with pockets of well-drained, sandy, moraine-type topsoil, which is bordered by a shallow lake and random beaver ponds. The climate is temperate and, during the winter, the landscape is normally covered by a thick blanket of snow. The predominant mammals are: White-tailed Deer, *Odocoileus virginianus* Zimmermann; Eastern Cottontail, *Sylvilagus floridanus* (J. A. Allen); Beaver, *Castor canadensis* L.; Raccoon, *Procyon lotor* L.; Striped Skunk, *Mephitis mephitis* (Schreber); Eastern Chipmunk, *Tamias striatus* L.; Deer Mouse, *Peromyscus maniculatus* Gloger; and Northern Short-tailed Shrew, *Blarina brevicauda* Say. Deer trails are prominent throughout the area.

The principal arboreal species include: Red Oak, *Quercus rubra* L; Shagbark Hickory, *Carya ovata* (Mill.) K. Koch; Bitternut Hickory, *Carya cordiformis* (Wangenh.) K. Koch; Ironwood, *Ostrya virginiana* (Mill.) K. Koch; Red Maple, *Acer rubrum* L; Sugar Maple, *Acer saccharum* Marsh.; Trembling Aspen, *Populus tremuloides* Michx.; and Red Dogwood, *Cornus sericca* L. The closest dwelling and road is 2 km from this woodland epicentre.

Tick collection

In November 2012, a representation of 34 blacklegged tick adults (10 males, 24 females) were collected by flagging using white flannel cloth draped on a 2.1 m pole. Using fine-pointed, stainless steel tweezers, the ticks were put into vials and placed in a plastic ziplock bag with slightly moistened paper towel, and sent by express mailing for identification. Upon confirmation of identification, they were sent by overnight courier for PCR amplification. In May 2013, a total of 23 blacklegged tick adults (12 males, 11 females) were collected for identification and PCR amplification.

Spirochete detection

Dead ticks were tested for *B. burgdorferi* s.l. using DNA extraction and PCR amplification of *B. burgdorferi* s.l. using gene primers of the outer surface protein A (OspA), whereas live ticks were cultured in BSK medium. The DNA detection protocols have been previously described [55-57].

Results

Our 2-year study revealed that *I. scapularis* overwintered successfully at this remote epicentre, and this tick species was in plentiful numbers the following spring. The *B. burgdorferi* infection prevalence was not only maintained from fall to the following spring, it actually increased. Overall, 19 (33.3%) of 57 blacklegged tick adults (fall 2012, 29.4%; spring 2013, 39.4%) were positive for *B. burgdorferi*. For the spring 2013 collection, 10 live cultures of *B. burgdorferi* were isolated.

Discussion

Our findings confirm a population of blacklegged ticks infected with *B. burgdorferi* in southcentral Frontenac County, Ontario. This new-found focal epicentre is 2 km from any road or dwelling, and the closest known cluster of *B. burgdorferi*-positive *I. scapularis* is approximately 25 km away [58]. A *B. burgdorferi*-infected larva can infect any small mammal population; however, at least 2 or more larvae or nymphs (one molts to a male, the other molts to a female) are the bare minimum number to initiate an established population. Because of their limited travel, mammalian hosts in these environs most likely were not involved in introducing *B. burgdorferi*. For instance, *P. maniculatus* has a home range that averages 590 m^2 [59]. White-tailed deer have been considered, but their home range is only 140 ha (radius, 6.7 km) [60]. In addition, although white-tailed deer are hosts of all 3 developmental life stages of *I. scapularis* [5], and help to amplify this tick, they are not competent reservoirs of *B. burgdorferi* [61]. Consequently, they are unlikely to have introduced spirochetes to this epicentre. Also, if a white-tailed deer happened to bring a fully engorged *I. scapularis* female to this locale, the replete female could not transmit *B. burgdorferi* to larvae through eggs because transovarial transmission is not present in this tick species [62]. Therefore, another mode of introduction for *B. burgdorferi*-infected *I. scapularis* larvae and nymphs is needed. We suggest that migratory songbirds brought *B. burgdorferi*-infected *I. scapularis* immatures to this locale.

Capturing the actual moment when a migratory songbird released ticks into a new habitat, and started an established population, is virtually impossible. However, we can provide the basic parameters for such an event to occur. Any new habitat for *I. scapularis* must provide the basic abiotic (i.e., weather) and biotic (i.e., vegetation) components to sustain the developmental life cycle of the tick. First, this area must have ecological amenities that provide food (i.e., acorns, nuts, seeds) for small mammals (i.e., deer mice, eastern chipmunk, northern short-tailed shrew), medium-size mammals (i.e., striped skunk), and large mammals (i.e., white-tailed deer). Second, this northern locality must have adequate snowfall to provide an insulating blanket of snow during the winter. Third, migratory songbirds must have natural materials to build their nest for their young. This isolated area has all of these natural factors.

This habitat is conducive to sustaining an established population because it has several suitable maintenance hosts for all life stages of *I. scapularis*. Deer mice, which are plentiful in this locality, are suitable hosts for *I. scapularis* immatures, and support *B. burgdorferi* infectivity of this spirochetal zoonosis [63]. The northern short-tailed shrew acts as a common host for larval and nymphal *I. scapularis*, and also acts as a primary reservoir of *B. burgdorferi* [64,65]. Likewise, the eastern chipmunk is a competent reservoir of *B. burgdorferi*, and can hold spirochetes for at least 4 months [66]. As evidenced by the many deer trails in these environs, white-tailed deer are abundant, and these cervids act as maintenance hosts for all life stages of *I. scapularis*.

Schauber et al. [67] indicate that acorn production and mouse abundance in the northeastern United States are a strong predictors of Lyme disease incidence. The white-tailed deer migrate to nut-bearing groves because they are looking for acorns and nuts, which provide energy-boosting nutrients (i.e., carbohydrates, fats, protein,

and micronutrients). As well, acorns act as a common food source for small mammal reservoirs (i.e., deer mice, eastern chipmunks, northern short-tailed shrews), and these small mammals help to bolster and perpetuate the life cycle of blacklegged ticks. Additionally, Jones et al. [68] indicate that an abundant acorn crop in the fall increases the number of mice and eastern chipmunks the following summer. Whenever there is an abundant autumn crop of acorns, white-tailed deer congregate and, as a result, *I. scapularis* larvae escalate the following summer [69]. When reservoir-competent small mammals and white-tailed deer comingle in the area of high acorn production, they increase the likelihood of maintaining both *I. scapularis* and *B. burgdorferi* in the Lyme disease ecosystem.

To augment an enzootic cycle of *B. burgdorferi* infection, the same songbirds inherently return to the same nesting areas the following year. At this time, birds can bring additional *B. burgdorferi*-infected, immature *I. scapularis* and, consequently, amplify ticks and spirochetal infection. Based on the remoteness of this site, predation by house cats, *Felis catus* L., would be unlikely and, thus, this locale is a favourite nesting site for ground-frequenting songbirds. In Canada, cats kill an estimated 100-305 million birds per year, especially in human-dominated landscapes [70]. Therefore, this remotely-located, nut-bearing habitat provides all the enzootic factors to establish a population of *I. scapularis* infected with *B. burgdorferi*.

In our study, we collected *I. scapularis* adults. Since songbirds only become parasitized by larvae and nymphs, there is no possible way that these avian hosts could introduce adults in the spring. This area is normally covered by a thick blanket of snow from December to March when none of the motile stages of *I. scapularis* are questing. These ticks are in the leaf litter and humus layer over the winter, and are cozy under an insulating blanket of snow. Furthermore, *I. scapularis* ticks have antifreeze-like compounds in their bodies, and can withstand sub-zero ambient temperatures of -40°C.

Upon arrival at the breeding grounds and nesting site, a passerine migrant can release several *B. burgdorferi*-infected larvae and nymphs into the leaf litter, which is populated with small mammals, especially rodents. These replete, songbird-transported immatures molt, and develop into infected larval and nymphal ticks in 5-7 weeks. The nymphs typically parasitize small mammals, and transmit *B. burgdorferi* to these hosts. In order to complete the developmental life cycle, the replete nymphs molt to adults. They commonly quest for large mammals and, during the fall or following spring, the female takes a blood meal, and the male and female mate. When fully engorged, the female drops to the leaf litter in the nut-bearing woodlot where there will be an abundance of future hosts for larval and nymphal progeny. In the spring (late April-May), the female normally lays 1000-2000 eggs on a well-drained forest floor. These eggs develop during the warm weather, and hatch in late-July and August and, subsequently, the larvae quest for wild birds and small mammals. Soon after the female lays eggs, she dies. Her energy-rich carcass acts as an attractant for songbirds and small mammals.

The initiation of larval hatch occurs concomitantly with the death of the parent female that just laid eggs. Because the newly hatched larvae and female carcass are in juxtaposition, hosts are automatically drawn to this microhabit. The fermenting carcass gives off odoriferous gases that act as a magnet for small mammals and songbirds. The female carcass provides a nutritious source of carbohydrates, fat, protein, and micronutrients. An off-white fat pellet is present that is clearly visible in the posterior end of the idiosoma of the carcass. Over many millennia, evolution has developed this unique survival mechanism to link ectoparasitic ticks with ground-foraging hosts.

As hosts search for the odorous, female carcass, these designated targets are covertly ambushed, and parasitized by a hundreds of nearby larvae that hatched from the recently laid eggs. If hosts are spirochetemic, they can transmit *B. burgdorferi* spirochetes to the attached, blood-sucking larvae. Tactfully, as an innate survival technique, a replete larval or nymphal *I. scapularis* will not release from its host (songbird) until it senses odorant compounds (CO_2, NH_3, lactic acid, phenols) produced by its next host [71]. Sensing a potential host, the *I. scapularis* immatures release to the forest floor and, subsequently, become the initial building blocks of a blacklegged tick colony. "Any *I. scapularis* larva which is not successful in parasitizing a suitable host in autumn, can overwinter in the leaf litter and top soil of well-drained soils in the temperature zone of central and eastern Canada under a deep blanket of insulating snow and quest for a host the following spring".

Notably, migratory songbirds have the physical capability to transport *I. scapularis* immatures thousands of kilometres during the period of a complete blood meal [3]. Using light-level geolocators, Stutchbury et al. [72] tracked passerine migrants, and revealed they can travel at least 575 km/day en route from southern wintering grounds to northern breeding grounds during northern spring migration. These passerines could easily have introduced the initial seed stock of blacklegged ticks from hyperendemic Lyme disease areas along the East Coast and Hudson River basin, or Thousand Islands area to this northern epicentre.

Many bird-tick studies exhibit multiple ticks on a passerine host. During a previous study [64], 19 nymphs of *I. scapularis* (denoted as *I. dammini*) were collected from an American Robin and, likewise, 21 larvae on a Gray Catbird, *Cumetella carolinensis* (L.), and a Swamp Sparrow, *Melospiza Georgiana* (Latham). Additionally, multiple *B. burgdorferi*-infected *I. scapularis* nymphs were collected from a House Wren at a bird banding site in southern Ontario [73].

The fact that wild birds are reservoir hosts of *B. burgdorferi* sensu lato is clearly recognized around the world [74]. Not only do tick-infested passerine migrants act as a bioresource in introducing *B. burgdorferi*-infected ticks, they have the reservoir capacity to transmit spirochetes during the tick-host blood meal. Additionally, avian hosts can act as genetic mixing bowls for diverse strains of Lyme disease spirochetes, and may precipitate the exchange of *Borrelia* genes, such as cross-species recombinant genotypes [74].

As a process of elimination, we know of no other logical way that this population of *I. scapularis* was started. Conceivably, *B. burgdorferi*-infected songbirds provide the mode to initiate this established population of blacklegged ticks. Collectively, our findings strongly suggest and support the involvement of migratory songbirds in initiating Lyme disease endemic areas. Ultimately, this Lyme disease endemic area is a public health risk because resident songbirds can disseminate *B. burgdorferi*-infected *I. scapularis* within this region during the breeding and nesting season.

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