Chapter 1

BIRDS WIDELY DISPERSE PATHOGEN-INFECTED TICKS

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ABSTRACT

Worldwide, bird migration plays a definitive role in the wide dispersal of bird-transported ticks. As spring unfolds in the Northern Hemisphere, wild migratory birds move northward en route to the boreal forest spanning central and eastern Canada, and become parasitized by bird-feeding ticks when they make landfall at tick endemic areas. Our bird-tick studies reveal engorged Neotropical ticks, which are indigenous to Central America and the northern region of South America, on migratory flycatchers, thrushes, and warblers. With their versatile mobility and vast distribution, migratory birds can act as maintenance hosts for the wide dispersal of pathogenic microbes. Certain passerines, such as the American Robin, *Turdus migratorius* L., can act as reservoir hosts of the Lyme disease bacterium, *B. burgdorferi* sensu lato (s.l.), and help to perpetuate spirochetal infection within a tick population. These hematophagous ectoparasites can transmit infectious microorganisms (i.e., bacteria, piroplasms, viruses) to avian hosts and, subsequently, after bird parasitism, can transmit these pathogenic microbes to vertebrate hosts, including humans. Ticks may simultaneously be co-infected with one or more pathogens that can have a broad diversity of genotypes. When birds become heavily infested with ticks, these avian hosts have the capacity to initiate a new population of ticks. Within an ecosystem, songbirds may be involved in a multi-tick enzootic cycle consisting of several tick species. Within one West Coast bioregion, we show that songbirds amplify *B. burgdorferi* s.l. in a 5-tick enzootic cycle. One heavily infested Fox Sparrow, *Passerella iliaca* (Merrem), in this Pacific Northwest locality was parasitized by avian coastal ticks, *Ixodes auritulus* Neumann, and had a *B. burgdorferi* s.l. infection rate of 81%. Canada-wide we have discovered *B. burgdorferi* s.l. in six different *Ixodes* species of ticks collected from passerine birds. Since wild birds widely disseminate *B. burgdorferi* s.l.-infected ticks, people do not have to frequent an endemic area to contract Lyme disease.

TICKS PARASITIZE WILD BIRDS

Several bird species play a vital role in the wide dispersal of ticks. Worldwide, hard-bodied ticks (Ixodida: Ixodidae) parasitize wild birds, and some of these hematophagous ecoparasites are reservoirs of zoonotic pathogens. Wild birds are normally parasitized by bird-feeding ticks that are questing in low-level vegetation. Passerines, commonly called songbirds, are responsible for wide dispersal of ticks during short- and long-distance flight, especially during spring and fall migration. The main influx of ticks in Canada occurs when migratory passerines make landfall at tick endemic areas throughout the United States of America and, likewise, along the southern fringe of Canada. Subsequently, migratory passerines disperse fully engorged ticks haphazardly across Canada en route to the boreal forest.

The earliest bird-tick studies in Canada were conducted by John D. Gregson [1]. He identified blood-sucking ticks from seven orders of wild birds, namely webbed swimmers (Pelicaniformes); seabirds, shorebirds (Charadriiformes); falcons (Falconiformes); pheasants, chicken-like birds (Galliformes); true owls (Strigiformes); woodpeckers (Piciformes); and perching birds (Passeriformes). Country-wide, he identified nine different tick species detached from wild birds. As well, seabird ticks, *Ixodes uriae* White on King Penguins, *Aptenodytes patagonicus* Miller (Order: Spenisciformes), which breed in the subantartic islands, greatly expands the biogeographical range of ticks on Aves.

In addition, Anderson and Magnarelli [2] reported the European sheep tick, *Ixodes ricinius* (L.), and the taiga tick, *Ixodes persulcatus* (Schulze), in Eurasia on avian hosts, including birds of prey (Accipitriformes), waterfowl (Anseriformes), pigeons, doves (Columbiformes), near passerines (Cuculiformes), cormorants (Suliformes), diving birds, grebes (Podicipedidae), and rails (Gruiformes).

Our bird-parasite studies have focused extensively on ticks and Lyme disease across Canada. The main source of ticks for our tick-host studies has been bird banders, wildlife rehabilitators, and veterinarians. Overall, bird-transported ticks can harbour a myriad of pathogenic microbiota, including the etiological contagion of Lyme disease.

The Lyme disease spirochete, *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt and Brenner is heterogenous [3], and consists of at least 21 genospecies worldwide. Several of these genospecies, including *B. afzelii*, *B. andersonii*, *B. americana*, *B. bissettii*, *B. burgdorferi* sensu stricto (s.s.), *B. lusitaniae*, and *B. valaisiana*, are known to be pathogenic to humans [4]. Not only is *B. burgdorferi* s.l. present in bird-feeding ticks, it is harboured in the blood and tissues of certain wild birds [5, 6]. Globally, Lyme disease has been detected in over 80 countries.

In North America, the blacklegged tick, *Ixodes scapularis* Say, is the primary vector of Lyme disease east of the Rocky Mountains and, likewise, along the West Coast, the western blacklegged tick, *Ixodes pacificus* Cooley and Kohls, transmits *B. burgdorferi* s.l., to avian and mammalian hosts. Bird-feeding ticks can carry a wide array of microbial pathogens, including bacteria, piroplasms, and viruses [7]. In particular, *I. scapularis* is known to carry bacterial, protozoan, and viral pathogens, namely *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), *Babesia* spp. (e.g., *B. microti*, *B. odocoilei*) (babesiosis), *Bartonella* spp. (e.g., *B. henselae* bacteria), *B. burgdorferi* s.l. (Lyme disease), *Borrelia miyamotoi* (relapsing fever group spirochete), deer tick virus (Powassan virus group),

Ehrlichia phagocytophila (granulocytic ehrlichiosis [*E. equi*]), and *Mycoplasma* spp. (e.g., *M. fermentans* [cat scratch disease]). Notably, several different pathogens have been documented in a single tick, and triple coinfections have been detected in *I. scapularis* from vertebrate hosts, including passerines [8-10]. In Eurasia, Russian-based researchers documented multiple pathogens in a single bird-transported *I. ricinus* tick [11]. Overall, many different genospecies and genotypes of *B. burgdorferi* s.l. have been detected in songbird-transported ticks and their avian hosts [12].

MIGRATORY SONGBIRDS TRANSPORT TICKS

Historically, Harry Hoogstraal, pioneer tick researcher, provided the earliest report of ticks on wild birds that were migrating northward through Egypt to Asia and Europe [13]. These bird-carried ticks included *Ixodes frontalis* (Panzer), *Haemaphysalis punctata* Canestrini and Fanzago, *Haemaphysalis sulcata* Canestrini and Fanzago, *Hyalomma aegyptium* L. and *H. marginatum* Koch. The latter 2 tick species are epidemiologically or experimentally associated with Crimean hemorrhagic fever, Q-fever, tularaemia, tick typhus and brucellosis. Of note, these ticks were observed far from their normal geographic range. Researchers subsequently reported migratory birds flying in reverse direction from Europe and Asia to Africa via Sudan and Egypt [14]. Although microbial infection was not reported, they noted that certain tick species have the potential to carry several Eurasian-based human pathogens.

Collectively, during our pan-Canadian tick studies (1996-2014), we have documented 22 species of ixodid ticks belonging to 3 genera (*Amblyomma*, *Haemaphysalis*, *Ixodes*) on wild birds (Table 1). These bird-transported ticks represent four avian orders: seabirds (Charadriiformes); falcons (Falconiformes); chicken-like birds (Galliformes); and perching birds (Passeriformes).

Table 1. Ixodid tick species collected from wild birds across Canada, 1996-2014

Amblyomma	*Haemaphysalis*	*Ixodes*
A. americanum	*H. leporispalustris*	*I. affinis*
A. humerale		*I. baergi*
A. imitator		*I. brunneus*
A. longirostre		*I. dentatus*
A. maculatum		*I. minor*
A. rotundatum		*I. muris*
A. sabanerae		*I. pacificus*
		I. scapularis
		I. spinipalis
		I. uriae
		I. species A[†]
		I. species B[†]
		I. species C[†]

[†] undescribed ticks collected from Neotropical passerines during northward spring migration.

Since we know the indigenous areas of many of the ticks on Neotropical passerines, we extrapolate that these ticks have been carried from their native land in South and Central America during spring migration. Based on the known areas of Neotropical ticks collected from passerine migrants, we posit transcontinental and intercontinental transport of ticks. Epidemiologically, migratory passerines and raptors have the capacity to quickly fly great distances during migration, and play a pivotal role in transporting bird-feeding ticks thousands of kilometres. Inevitably, wild birds can easily disperse ticks over notable distances because they move much faster than terrestrial wingless hosts.

In North America, there are 3 major flyways (Atlantic, Mississippi, Pacific) that transect the continent in a north-south direction. Several North American researchers have documented short- and long-distance movement of ixodid ticks consisting of 4 genera: *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, especially during spring migration [15-18]. In Canada, tick researchers have documented *B. burgdorferi* s.l.-infected *I. scapularis* immatures (larvae, nymphs) on migratory passerines [19-23], and millions of these ticks are widely dispersed coast to coast during spring migration. Recently, Scott and Durden identified a songbird-transport tick, *Ixodes minor* Neumann that was collected from a Common Yellowthroat, *Geothlypis trichas* (L.) in Toronto, Canada during northward spring migration [24]; this extralimital tick is the northernmost documentation of this neotropical tick species in North America, and points out the potential for passerines to carry ticks long distances.

During fall migration, bird-tick researchers studied ticks on songbirds in the upper Midwest [25], and found migrants were carrying ticks southward. Some of the ticks were infected with *B. burgdorferi* s.l. As well, Durden et al. [26] documented *Ixodes* and *Amblyomma* ticks during fall migration infected with *B. burgdorferi* s.l., and determined that songbirds act as southward-shifting disseminators of borreliae.

Certain migratory birds are noted for transcontinental and transoceanic travel and, at the same time, can transport ticks [27]. Many seabirds and shore birds are highly mobile and undertake long, complex flight paths of thousands of kilometres. Some of these birds breed in the subantarctic islands, and make transequatorial migration to the northern parts of the Atlantic and Pacific oceans. Seabirds carry the seabird tick, *Ixodes uriae* White, which has been collected from both the Southern and Northern Hemispheres, infected with *B. burgdorferi* s.l., especially *B. garinii* [28]. The presence of *B. garinii* in *I. uriae* in distant islands indicates transhemispheric exchange of Lyme disease spirochetes by seabirds [28]. Gylfe et al. [29] provides the first *B. burgdorferi* s.l. isolates from seabirds, and documents these birds as reservoir hosts, and *I. uriae* as the vector. Along the eastern seaboard, Smith et al. [30] detected *B. garinii* in *I. uriae*.

The FarAsian-Australian flyway provides an expansive corridor for avian transport of ticks on a north-south axis during bimodal migration within this flyway. Within this extensive flyway, Doube [31] documented the Australian paralysis tick, *Ixodes holocyclus* Neumann on ground-frequenting birds in southeastern Australia; this tick species also bites small mammals, cattle, and humans.

On the West Coast of North America, researchers show a wide range of *Ixodes* species ticks on wild birds. In the upper Midwest, Hamer et al. [32] reported long-distance travel during northern spring migration of Neotropical migrants carrying *Amblyomma nodosum* Neumann, which is indigenous in Brazil. During our bird-tick studies, we have annually collected *Amblyomma longirostre* Koch, which are indigenous to northern South America and

southern Central America, from Neotropical migrants (i.e., warblers and flycatchers) during spring migration. This tick species has been reported in central and eastern Canada during spring migration, which indicates bird parasitism for an estimated 5-10 days during a flight of over 3000 km. Using light-level geolocators on Neotropical passerines, Stutchbury et al. [33] tracked the flight path of several migrants, and revealed one particular Purple Martin, *Progne subis* (L.), which departed the Amazon basin, averaged 577 km/day. As well, Brewer et al. [34] reported a White-throated Sparrow, *Zonotrichia albicollis* (Gmelin) flying 681 km in a single day. Similarly, Hunt and Eliason [35] reported Blackpoll Warblers, *Setophaga striata* (Forster), flying 3000 km for 88 hours (820 km/day), non-stop, over water during fall migration from the eastern seaboard of New England and Maritimes to South America. In essence, wild birds expand the geographic range of ticks, and may concomitantly spread tick-associated pathogens.

ASSOCIATION OF SONGBIRDS IN TICK HABITATS

Ground-foraging songbirds act as important reservoirs of infection within tick populations. In the upper Midwest, 38% of the songbirds infested with *I. scapularis* had *B. burgdorferi* s.l.-infected larvae [36]. During Canadian studies related to bird parasitism, Scott et al. [23] found that *I. scapularis* immatures, which were collected from songbirds, had an infection prevalence of 36% for *B. burgdorferi* s.l.

In order to initiate and maintain a blacklegged tick population, several basic components are needed. Since transovarial transmission of *B. burgdorferi* s.l. does not occur in *I. scapularis* ticks [37], larvae must acquire infection during the initial blood meal from spirochete-laden hosts. Additionally, when a senesent female has laid all her eggs, and dies, her dead remanent body gives off an odoriferous smell that attracts ground-foraging birds and land-inhabiting mammals. Because newly hatched, host-seeking larvae and the dead female are in juxtaposition, the larvae ambush and parasitize any scavenger that frequents this site. This innate survival tactic has allowed ticks to sustain their presence in nature over many millennia. Ixodid tick researchers reveal that songbirds can become heavily infested with larvae, especially where a gravid female deposits her eggs [38]. Not only does the female tick pellet become a lure for hosts, it acts as a compact source of energy-laden nutrients. The spent tick gives off fermenting vapours that increase the luring appeal of the female pellet, which contains carbohydrates, fats, protein, and micronutrients. Interestingly, an energy-rich off-white adipose deposit is present in the posterior section of the idiosoma (posterior body segment) of the female remanent, and it provides a storehouse of nutrition for scavengers. Moreover, several tick researchers have observed that songbirds, quail, and chickens feed on live ticks to obtain energy reserves [14, 39, 40]. In some tick microenvironments, predation of ticks by wild birds is high. One notable example of bird predation of ticks is the Yellow-billed Oxpecker, *Buphagus africanus* Brisson, of sub-Saharan Africa [41].

SONGBIRDS ACT AS RESERVOIR HOSTS FOR
BORRELIA BURGDORFERI

Certain wild birds have the innate physiology to act as reservoir hosts for select pathogenic microorganisms, including *B. burgdorferi* s.l. Based on early bird-tick studies, Anderson and Magnarelli [15] proposed that songbirds have the potential to act as reservoir hosts of *B. burgdorferi* s.l., and transport spirochetes and ticks to new foci. As well, Anderson et al. [16] isolated the first Lyme disease spirochetes from a songbird, namely a Veery, *Catharus fuscescens* Stephens, to elucidate reservoir competency. In the upper Midwest, McLean et al. [42] isolated *B. burgdorferi* s.l. from the blood of a passerine, viz. Song Sparrow, *Melospiza melodia* (Wilson), while along the East Coast, researchers isolated *B. burgdorferi* s.l. from *I. scapularis* larvae collected from several songbirds, including Carolina Wren, *Thryothorus ludovicianus* (Latham); Common Grackle, *Quiscalus quiscula* (L.); Swainson's Thrush, *Catharus ustulatus* (Nuttall); Northern Cardinal; and Veery (16, 26, 43].

In the European Union, Kurtenbach et al. [44] provide the first documentation that gallinaceous birds (Common Pheasants, *Phasians colchicus* L.) act as reservoir hosts *of B. valaisiana*. When *I. ricinus* nymphs were collected from feral pheasants in southern England more than 50% of the engorged nymphs were infected with *B. garinii* or *B. valaisiana*. Both of these *Borrelia* genospecies present a health risk to humans. In central Europe, *I. ricinus* larvae, which were collected from songbirds, were infected with *B. garinii* and *B. valaisiana*, these findings indicate that these songbirds, namely the Blackbird, *Turdus merula* (Brehm), and the Song Thrush, *Turdus philomelos* (L.), are reservoirs of borreliae. Throughout the European Union, certain passerine act as harbingers of *B. burgdorferi* s.l. [45-48]; in some cases, spirochetes were cultured from blood from these avian hosts [48-50].

In far-western North America, Morshed et al. [20] detected *B. burgdorferi* s.l. in a larva of an avian coastal tick, *Ixodes auritulus* Neumann collected from a Fox Sparrow, *Passerella iliaca* (Merrem); this discovery constitutes the first report of *B. burgdorferi* s.l. in an ixodid larva feeding on a bird in western North America. During our recent bird-tick studies, we noted that 21 (81%) of 26 *I. auritulus* immatures (22 larvae, 4 nymphs), which were collected from a Fox Sparrow along coastal British Columbia, were infected with *B. burgdorferi* s.l.; this is the highest spirochetal infection rate that we have ever observed in ticks collected from passerine birds. Enzootically, this bird species has the potential to act as a disseminator of borreliae, especially within established populations of Lyme disease vector ticks. Songbird-transported ticks, which parasitize both mammals and humans, can transmit *B. burgdorferi* s.l. from mammals to humans, especially in a localized enzootic tick cycle. As interconnecting vectors, larvae of certain bird-feeding ticks (i.e*., I. pacificus*, *I. ricinius*, *I. scapularis*) can attach to a *B. burgdorferi* s.l.-infected mice, take a blood meal, drop to the ground, crawl to a cool and moist microhabitat, molt to nymphs in the leaf litter and, subsequently, bite and transmit spirochetes to birds and mammals, including humans. In essence, reservoir-competent songbirds consistently act as an intermediary bridge to channel *B. burgdorferi* s.l. from mammals and humans.

Richter et al. [5] discovered that the American Robin, *Turdus migratorius* L., will retain *B. burgdorferi* s.l. endogenously for up to 6 months. Using xenodiagnostic methods, these researchers put spirochete-free *I. scapularis* larvae on *B. burgdorferi*-infected robins, and allowed them to take a blood meal and feed to repletion. After the larva-nymph molt, unfed

nymphs were put on mice, and the parasitized mice became spirochetemic. Within an enzootic site for *B. burgdorferi* s.l., the American Robin serves as reservoir-competent host, and provides an ongoing source of spirochete-infected ticks. Throughout the breeding, nesting, and fledgling periods, these ground-dwelling birds encounter Lyme disease vector ticks. Notably, American Robins can act as borrelial reservoirs throughout the temperate season, and may become infected again after a period of non-infectivity. The presence of *B. burgdorferi* s.l.-infected *I. scapularis* larvae collected from wild birds further suggests a link between certain avian hosts and reservoir competency.

Coinfestation of birds by multiple tick species can occur. Scott et al. [23] witnessed 3 different species of ticks co-feeding simultaneously on a single passerine during northward spring migration. Explicitly, passerines constitute a core functional group that act as reservoir-competent hosts for Lyme disease spirochetes. Overall, migratory songbirds play a pivotal role in the dissemination of borreliae during short- and long-distance movement.

SONGBIRDS DISSEMINATE ZOONOTIC PATHOGENS

Avian hosts have the physical capacity to widely disseminate zoonotic pathogens. Wild birds may act as reservoirs for several agents, including arboviruses, influenza A virus, piroplasms, enterobacterial pathogens and drug-resistant bacteria, and *B. burgdorferi* s.l. [27]. In nature, birds are the amplifying hosts of West Nile virus. As well, aquatic waterfowl may be asymptomatic carriers of influenza A virus. Pertinent to subarctic latitudes, arboviruses have been found in ticks on migratory birds in Finland [51].

In the European Union, borreliae were initially detected in larval and nymphal *I. ricinus* ticks that parasitized several bird species [46]. DNA probes, which consisted of *fla* and *ospA* genes, revealed the presence of *B. garinii*. The presence of *B. garinii* in *I. ricinus* larvae, which were collected from wild-caught birds (i.e., Eurasian Blackbird, *Turdus merula* L.; European Robin, *Erithacus rubecula* (L.); European Blackcap, *Sylvia atricapilla* (L.); and Great Tit, *Parus major* (L.) indicate that these avian hosts are borrelial reservoirs. As well, Poupon et al. [52] revealed that northward and southward migratory passerines, which were infested with larval and nymphal ticks, were infected *with B. valaisiana* and *B. garinii*. Likewise, Comstedt et al. [53] detected four *Borrelia* genospecies (i.e., *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s., *B. valaisiana*), plus the relapsing fever group spirochete, *B. miyamotoi*, in ticks collected from migratory passerines. In Portugal, Norte et al. [54] surveyed passerines and detected *B. valaisiana*, *B. garinii*, *B. turdi*, and *B. miyamotoi* in ticks collected from wild birds. Pathologically, *B. burgdorferi* s.l.-infected ticks put the public at risk.

In the Baltic region, Lyme disease spirochetes are present in ticks collected from passerines. Movila et al. [55] detected nine different tick-borne pathogens in 3 tick species (i.e., *I. ricinus, I. frontalis*, *H. maginatum*) collected from migratory birds. These pathogens include: *B. garinii*, *B. afzelii*, *B. valaisiana*, *A. phagocytophilum*, Candidatus *Neoehrlichia mikurensis*, *Ricksettsia helvetica*, *Ricksettsia aeshlimanii*, and *Babesia venatorum* and the tick-borne encephalitis virus. As well, Hildebrandt et al. [56] documented several zoonotic pathogens in bird-feeding ticks collected from migratory birds mist-netted on a Baltic Sea island along coastal north-eastern Germany. These tick-borne pathogens included *Babesia divergens*, *Babesia microti*, *A. phagocytophilum*, and members of the spotted fever group

(i.e., *Ricksettsia monacensis*, *R. helvetica*). Alekseev et al. [11] provide the first evidence of human monocytic ehrlichiosis (HME) and human anaplasmosis (formerly human granulocytic ehrlichiosis) microbiota in immature *I. ricinus* ticks collected from passerines. At the same site, *B. afzelii*, *B. garinii*, and *B. burgdorferi* s.s. were detected in *I. ricinus* attached to passerines.

In the Far East, Ishiguro et al. [57] documented the movement of passerine migrants from Mongolia, and onward to China, to Korea, and to Japan, and these avian hosts were transporting tick infected with *B. garinii*. Seabirds play an integral role in the global transmission cycle of *B. burgdorferi* s.l., especially involving *I. uriae*, which migrates long distances between seabird colonies, and transports these ticks between the Southern and Northern Hemispheres [28].

In Sweden, *B. burgdorferi* s.l., which was extracted from seabird-transported *I. uriae* nymphs, matched isolates from *I. ricinus* ticks collected on nearby islands. In South America, wild birds carry several *Amblyomma* spp. ticks, and some of them are infected with ricksettial microorganisms [58].

In North America, Levine et al. [59] reported *B. burgdorferi* s.l. in 3 tick species (i.e., *Ixodes dentatus* Marx, *Haemaphysalis leporispalustris* Packard, and *I. scapularis* (denoted as *I. dammini*) collected from passerine migrants in Virginia.

Additionally, Durden et al. [26] documented eight species of ticks on passerines along coastal southern United States of America, and isolated *B. burgdorferi* s.l. from skin biopsies obtained from these migratory passerines and from songbird-transported ticks. Moreover, the lone star tick, *Amblyomma americanum* (L.), and *I. scapularis*, which are both transported by songbirds, harbour filarial nematodes that can infect the circulatory system of humans [60].

SONGBIRDS START TICK POPULATIONS

Songbirds have the propensity to start ixodid tick populations. During the pioneer phase of Lyme disease epidemiology and ecology, tick researchers noted that heavily parasitized ticks have the potential to initiate new tick populations [15, 17].

In a remote area of southeastern Ontario, Scott et al. [61] provide substantial evidence to show the establishment of *I. scapularis* forming an enzootic tick cycle of *B. burgdorferi* s.l. Although it would be impossible to capture the actual event, and see it develop day by day, recent tick scenarios of previously undiscovered blacklegged tick populations in eastern and central Canada, show substantive evidence for songbirds as tick colony propagators. Since the white-tailed deer, *Odocoileus virginianus* Zimmermann, is not a reservoir-competent host, cervids were discounted as initiators. Biogeographically, blacklegged tick populations on offshore islands underscore the involvement of passerines in starting new tick colonies.

Recently, we collected 17 *I. scapularis* (8 nymphs, 9 larvae) from a Swainson's Thrush, *Catharus ustulatus* (Nuttall) on 7 June 2014 at Toronto, Ontario. If these engorged ticks had not been detached by bird banders, this heavily infested migrant could handily initiate a new Lyme disease endemic area. Songbirds provide a zoonotic mechanism to covertly introduce *B. burgdorferi* s.l.-infected larval and nymphal *I. scapularis* to a tick habitat, especially during spring migration.

While collecting food during the nesting, fledgling, and post-nesting periods, songbirds will naturally scatter spirochete-infected ticks in the locale, which can potentially infect people and domestic animals. Because songbirds widely disperse Lyme disease vector ticks, people do not have to go to an endemic area to contract Lyme disease.

SONGBIRDS TRANSPORT TICKS CARRYING RELAPSING FEVER GROUP SPIROCHETE

The relapsing fever group spirochete, *B. miyamotoi* Fukunaga et al., is pathogenic to humans, and is present throughout the Holarctic region of the world. This spirochete is carried by certain ixodid tick species, including *I. pacificus*, *I. persulcatus*, *I. ricinus*, and *I. scapularis* [62]. *Borrelia miyamotoi* is transmitted transovarially and transstadially, and can coexist with *B. burgdorferi* s.l. in a vector tick. Consequently, an unfed larva can transmit *B. miyamotoi* directly to its host during its first blood meal. In the northeastern United States, Scoles et al., initially reported *B. miyamotoi* in *I. scapularis* [63], and indicated that *B. miyamotoi* is not detected by Lyme disease serology. However, patients with spirochetemia can now use PCR amplification and DNA sequencing to differentiate *B. burgdorferi* s.l. and *B. miyamotoi* sourced from blood [64].

In Canada, Dibernardo et al. [65] reported *B. miyamotoi* in ixodid ticks collected in each province from British Columbia to Nova Scotia and, no doubt, these *B. miyamotoi*-infected ticks were initially dispersed by migratory passerines. Wild birds will undoubtably play an ever-increasing role in the wide dispersal of *B. miyamotoi* Canada-wide and globally.

In Michigan, U.S.A., Hamer et al. [66] provided the initial documentation of *B. miyamotoi* and *B. andersonii* in bird-transported *I. scapularis*; both of these borreliae are pathogenic to humans. In their study, the majority of *B. miyamotoi*-positive ticks were removed from Northern Cardinals. As well, in the same study, six bird species were implicated as reservoirs for *B. andersonii*. Not only are songbirds hosts for *I. scapularis*, they serve as intermediate reservoirs for *B. miyamotoi* and *B. andersonii*. The presence of *B. miyamotoi* and *B. burgdorferi* s.l. in ticks parasitizing passerines underpins the ecological complexity of these zoonotic pathogens within enzootic tick-host associations.

EFFECTS OF TICKS ON SONGBIRDS

Ticks normally attach to the head of the birds, especially around the eyes, mouth, and within auricular feathers (Figure 1). In order to prevent bird predation during preening, ticks instinctively select these non-reachable sites to attach to the skin. Some bird parasitism studies have focused on the health effects of blood-sucking ectoparasites on avian hosts. In particular, Norte et al. [67] evaluated the presence of feeding ticks on songbirds in Western Europe, and found that *B. burgdorferi* s.l.-infected ticks increased the heterophyl/ lymphocyte ratio of Eurasian Blackbirds suggesting increased stress.

Along the Pacific coast, American Robins died from heavy infestations of *I. auritulus* females [20]. In essence, bird parasitism may have a detrimental effect on avian hosts and, ultimately, may result in fatal outcomes of host birds.

Photo credit: Charlotte England.

Figure 1. Swainson's Thrush parasitized by engorged nymphs of the blacklegged tick, *Ixodes scapularis*, below and anterior to the right eye.

COINFECTIONS IN TICKS CARRIED BY BIRDS

Coinfections are apparent in bird-feeding ticks. Migratory birds carry zoonotic pathogens, and contribute greatly to the global spread of emerging infectious diseases [27]. European researchers detected *Borrelia* and *Chlamydophila* in cloacal and throat swabs of migratory passerines, and showed that such infections can be endogenous in birds [68]. Based on the broad diversity of *Borrelia* spp. reported in songbird-transported ticks in North America, there is a great potential to have these infected ticks imported into Canada during spring migration. Interestingly, several genotypes of *B. burgdorferi* s.l. have been reported in widespread regions of Canada [23, 69, 70]. Crowder et al. [71] conducted multilocus genotype analysis of borreliae in field-caught ticks collected from various parts of the United States of America and Europe, and detected 53 distinct genotypes of *B. burgdorferi* s.s. Epidemiologically, some of these genotypes were shared between continents, which suggests transatlantic exchange via ticks on migratory birds. Notably, a significant number of *I. scapularis* and *I. ricinus* ticks had more than one *B. burgdorferi* s.s. genotypes. These findings show a diversity of genotypes in ticks across wide geographic regions, and these diverse genotypes can cause dire clinical consequences. Because of the heterogeneity of borreliae in songbird-carried ticks, Lyme disease patients are apt to fail to seroconvert because borrelial genotypes may not be present in standard Lyme disease serology [72, 73]. Rudenko et al. [74] reported cross-species recombination of *B. burgdorferi* s.l. isolated from an *I. minor* nymph collected from a single Carolina Wren, *Thryothorus ludovianus* (Latham). Based on molecular-level, multi-locus analysis, it was discovered that the *B. burgdorferi* s.s. housekeeping gene, *niff*, was incorporated into another homologous locus of another bird-associated genospecies, *B. americana*. This event supports the hypothesis that diversity and evolution of Lyme disease spirochetes is driven mainly by the host.

Pertinent to other tick-associated pathogens, *A. phagocytophilum* has been reported in American Robins in North America and, likewise, other wild birds in Eurasia (11, 75).

Additionally, passerine-transported ticks may be infected with pathogenic microorganisms, including *Ricksettia*/*Coxiella* and tick-borne encephalitis virus. After a larva-nymph or nymph-female molt, ticks can transmit one or more tick-associated suitable pathogens to suitable vertebrate hosts, including humans.

SONGBIRDS ACT AS CONNECTING LINKS IN MULTI-TICK ENZOOTIC CYCLE

In tick-conducive habitats, wild birds act as important carriers in shifting zoonotic pathogens from wildlife hosts to humans. For instance, Scott et al. [23] documented a 4-tick enzootic cycle of *B. burgdorferi* s.l. on Vancouver Island, British Columbia, Canada that consists of *I. angustus* Neumann, *I. auritulus*, *I. pacificus*, and *I. spinipalpis*. In this coastal-forest habitat, *I. angustus* feeds primarily on small mammals, whereas *Ixodes auritulus* ticks feed exclusively on birds (i.e., passerines, grouse, and raptors). From a bird parasitism standpoint, both *I. pacificus* and *I. spinipalpis* parasitize avian and mammalian hosts. Not only do songbirds play a key role in maintaining and amplifying *B. burgdorferi* s.l. within this Pacific coast habitat, they serve as a spirochetal transit to humans. With the recent inclusion of *Ixodes cookei* Packard, a fifth interconnecting link is added to the enzootic cycle to circulate Lyme spirochetes within this coastal ecosystem. Although there may be one or more contiguous enzootic cycles of *B. burgdorferi* s.l. operating concurrently, in parallel, at this locality, the net effect is basically the same as one encompassing cycle. This 5-tick enzootic cycle of *B. burgdorferi* s.l. emulates a web-like interplay within a tick-bird-mammal community. Ecologically, this interaction of five tick species highlights the complexity of maintaining and perpetuating *B. burgdorferi* s.l. within this particular watershed habitat of British Columbia. Within this multi-tick cycle, Scott et al. [76] provide the first report of *B. burgdorferi s*.l.-infected ticks (*I. auritulus*) parasitizing a raptor (Cooper's Hawk, *Accipiter cooperii* (Bonaparte). This newfound, bird-tick association denotes that raptors are explicitly involved in the spread of Lyme disease. Medically, *I. angustus*, *I. cookei*, *I. pacificus*, and *I. spinipalpis* ticks parasitize humans, and may potentially transmit tick-borne pathogens.

HEALTH IMPLICATIONS

Lyme disease is a multisystem bacterial infection that causes protean manifestations in humans. This zoonotic disease can generate a brutal assault on the body, and promptly converts a healthy person into a febrile individual with cognitive impairment and unending pain. Lyme disease can have significant morbidity, and may be acute, recurrent, or chronic. When Lyme disease advances in a host, *B. burgdorferi* s.l. evades host immunity and generates diverse, pleomorphic forms (i.e., spirochetes, round bodies, granules, blebs) and, combined together, produce gelatinous masses, called biofilms (persister cells). These polysaccarhide-based matrices typify chronicity of infection [77]. Signs and symptoms of Lyme disease may persist after short-term antibiotic treatment, and survive in deep-seated and immune-privileged sites [78-85]. Several tissues in the body are hard to penetrate with antibiotics, including ligaments and tendons [86, 87], muscle [88], brain [89-92], bone [93,

94], eyes [95, 96], glial and neuronal cells [97, 98], and fibroblasts/scar tissue [99]. Persistence of *B. burgdorferi* s.l. has been documented in several different mammalian hosts, including dogs [100, 101], gerbils [102], guinea pigs [103], hamsters [104], nonhuman primates (i.e., rhesus macaques) [105, 106], baboons [107], humans [108-111], laboratory mice [112-116], white-footed mice [117-119], rats [120] and horses [121, 122]. Insofar as *B. burgdorferi* s.l. has an immune-evasion mode during its activity, this stealth pathogen sequesters and survives in suitable hosts. If left untreated or inadequately treated, this zoonotic spirochetosis may ultimately result in fatal outcomes [123, 124].

Lyme disease is typically transmitted to avian and mammalian hosts by certain infected ticks; however, other modes of transmission are present. Passage of *B. burgdorferi* s.l. to humans and domestic animals may occur by: blood transfusion [125-127], congenital transmission [128-133], contact transmission [134], and sexual transmission [135-137]. Furthermore, *B. burgdorferi* s.l. has been detected in breast milk of Lyme disease patients [138]. In essence, Lyme disease can induce chronic, progressive illness that can have a wide diversity of pathological symptoms in vertebrates [139-141]. During treatment, patients can have flu-like Jarisch-Herxheimer reactions. Commercial laboratory testing, which are based on immune response, yields poor results for Lyme disease, and has a sensitivity of only 46% in patients who have been infected for more than 4-6 weeks [142]. Consequently, these tests currently miss more than half of the human Lyme disease cases in North America.

A reservoir-competent songbird, which is heavily infested with *B. burgdorferi* s.l.-infected ticks, can haphazardly disperse fully engorged ticks at stopovers, and consequentially initiate a chain of Lyme disease cases along its flight path. Such pathogen-laden ticks are an ever-growing concern and a public health risk to unsuspecting outdoor adventurers and workers.

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