

Ticks parasitizing gallinaceous birds in Canada and first record of *Borrelia burgdorferi*-infected *Ixodes pacificus* (Acari: Ixodidae) from California Quail

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Abstract

In far-western Canada, gallinaceous birds are hosts of hard ticks (Ixodida: Ixodidae) that can carry zoonotic pathogens. In this study, we collected the avian coastal tick, *Ixodes auritulus* Neumann, the western blacklegged tick, *Ixodes pacificus* Cooley & Kohls, and *Ixodes spinipalpis* Hadwen & Nuttall, from gallinaceous birds on Vancouver Island, British Columbia. Notably, we provide the first records of these three tick species on California Quail, *Callipepla californica* (Shaw), in Canada. We document the first records of *I. auritulus* parasitizing Sooty Grouse, *Dendragapus fuliginosus* (Ridway). Moreover, we furnish the first report of *I. spinipalpis* on a quail. An *I. pacificus* nymph was collected from a California Quail, and it was positive for the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt & Brenner. Using PCR on the nymphal extract and DNA sequencing on the borrelial amplicon, we specifically detected *B. burgdorferi* sensu stricto (s.s.), a genospecies pathogenic to humans and certain domestic animals. Since some ground-dwelling birds are involved in the enzootic maintenance of Lyme disease, veterinarians, wildlife rehabilitators, hunters, and health-care providers should be vigilant that gallinaceous birds may play a role in the enzootic transmission of *B. burgdorferi* s.l. in Canada.

Key words: ticks, *Ixodes auritulus*, *Ixodes pacificus*, *Ixodes spinipalpis*, bird parasitism, Galliformes, Sooty Grouse, California Quail, *Borrelia burgdorferi*, Canada

Introduction

Gallinaceous birds (Order: Galliformes), namely chickens, grouse, pheasants, quail, and turkeys are hosts for certain hard-bodied ticks (Acari: Ixodida: Ixodidae). These blood-sucking ectoparasites can harbor a wide array of zoonotic tick-borne pathogens, including the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmidt, Hyde, Steigerwalt & Brenner (Burgdorfer *et al.* 1982). Globally, the *B. burgdorferi* s.l. complex consists of at least 21 genospecies or genomospecies. In North America, 8 genospecies have been reported, including *B. americana*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* sensu stricto (s.s.), *B. californiensis*, *B. carolinensis*, *B. garinii*, and *B. kurtenbachii* (Baranton *et al.* 1992, Marconi *et al.* 1995, Postic *et al.* 1998, Smith *et al.* 2006, Margos *et al.* 2009, Rudenko *et al.* 2009a, Rudenko *et al.* 2009b, Margos *et al.* 2010). Across North America, *B. burgdorferi* s.s. has been the most commonly reported genospecies that is pathogenic to humans.

The avian coastal tick, *Ixodes auritulus* Neumann, which is found exclusively on birds (Durden & Keirans 1996), parasitizes avifauna in at least 8 orders, including Galliformes (Robbins et al. 2001, González-Acuña et al. 2005). Worldwide, *I. auritulus* is indigenous along many seacoasts, including the Western Hemisphere, Antarctica, Australia, New Zealand, and islands south of Africa (Robbins et al. 2001, González-Acuña et al. 2005). Although *I. auritulus* does not bite humans, it can parasitize many bird species. All motile life stages of *I. auritulus* can harbour *B. burgdorferi* s.l. (Morshed et al. 2005; Scott et al. 2010, 2013, 2015).

The western blacklegged tick, *Ixodes pacificus* Cooley and Kohls, has a geographic range from southwestern Canada to northern Mexico (i.e., Baja California) (Banerjee et al. 1994, Durden & Keirans 1996, Scott et al. 2010). This tick species has been reported in Canada on Passeriformes, but not previously from gallinaceous birds. In California, Castro & Wright (2007) reported *I. pacificus* larvae and nymphs on California Quail, *Callipepla californica* (Shaw) (Galliformes: Odontophoridae). Biogeographically, California Quail inhabit a coastal strip along the Pacific Northwest from southern British Columbia to Mexico, namely Baja California (Peterson 2010). Likewise, the Sooty Grouse, *Dendragapus fuliginosus* (Ridway) (Galliformes: Phasianidae) is a forest-dwelling grouse native to North America's Pacific Coast range, from southeastern Alaska and Yukon south to California.

The geographical range of *Ixodes spinipalpis* Hadwen & Nuttall extends from southwestern Canada to Texas (Durden & Keirans 1996, Gregson 1956, Scott et al. 2012). Hosts include rodents (Dolan et al. 1997, Burkot et al. 2000, Eisen et al. 2003), lagomorphs (Allred et al. 1960) and passerines (Gregson 1956, Scott et al. 2012, 2015).

During the mid-1950s, *I. auritulus* and *I. pacificus* were recorded from gallinaceous birds in Canada (Gregson 1956); however, details on hosts, dates collected, and geographic locations were not provided.

Based on laboratory studies, *I. auritulus* (Scott et al. 2012, 2015), *I. pacificus* (Burgdorfer et al. 1985, Lane et al. 1994, Burgdorfer et al. 1995, Peavey & Lane 1995), and *I. spinipalpis* (Dolan et al. 1997, Eisen et al. 2003) are competent vectors of *B. burgdorferi* s.l. Using spirochete-free, xenodiagnostic larvae, Richter et al. (2000) found that American Robins, *Turdus migratorius* Linnaeus are reservoir-competent hosts for *B. burgdorferi* s.l. Also, *B. burgdorferi* s.l. has been isolated from passerine organs and body fluids; namely, liver (Anderson et al. 1986), skin (Durden et al. 2001), and blood (Anderson & Magnarelli 1984, Schulze et al. 1986, McLean et al. 1993).

Gallinaceous birds are directly involved in the enzootic cycle of *B. burgdorferi* s.l. In England, more than 50% of the nymphs of the castor bean tick, *Ixodes ricinus* (Linnaeus), which were collected from Pheasants, *Phasianus colchicus colchicus* Linnaeus, were infected with three different genospecies of *B. burgdorferi* s.l.; namely, *Borrelia garinii*, *Borrelia valaisiana*, and *B. burgdorferi* s.s. (Kurtenbach et al. 1998a, Kurtenbach et al. 2002). These researchers demonstrated that ground-feeding game birds are reservoir competent hosts for *B. burgdorferi* s.l. (Kurtenbach et al. 1998b). After compiling tick-host association records throughout Eurasia, Anderson & Magnarelli (1993) reported *I. ricinus* and the taiga tick, *Ixodes persulcatus* (Schulze), which both are competent vectors for *B. burgdorferi* s.l., on Common Quail, *Coturnix coturnix* (Linnaeus), another gallinaceous bird.

Not only do migratory songbirds have the capacity to start new tick populations locally, they can also initiate new foci in distant areas (Anderson & Magnarelli 1984, Anderson et al. 1990, Ogden et al. 2008, Scott et al. 2014, Scott 2015, Scott & Durden 2015a, Scott et al. 2015). In the southeastern region of Vancouver Island, California Quail have a localized home range of 7-18 ha, and a prevalence of 2.5 birds/ha (Emlen 1939) and, as avian hosts, perpetuate local tick populations.

In the present study, we hypothesize that gallinaceous birds may be involved in the epidemiological cycle of *B. burgdorferi* s.l. in Canada.

Materials and methods

Study area

Southeastern Vancouver Island belongs to the coastal forest region, and has high rainfall and lush, dense, arboreal habitats. Thick, low-lying vegetation includes: Scotch broom, *Cytisus scoparius* (Linnaeus) Link; Salal, *Gaultheria shallon* Pursh; Ocean spray, *Holodiscus discolor* (Pursh) Maxim.; Himalayan blackberry, *Rubus armeniacus* Focke; Nootka rose, *Rosa nutkana* C. Presl; plus various ferns and grasses.

Tick collection

Ticks were collected from gallinaceous birds by wildlife rehabilitators during clinical assessment of injured avifauna, 2002 to 2015. Ticks were removed with fine-pointed, stainless steel tweezers, and placed in round-bottom, 8.5 mL polypropylene tubes (15.7 X 75 mm) with labels consisting of background information (i.e., host species, location, date collected, collector). In order to receive ticks live, we drilled a 7-mm hole in the polyethylene push caps (15.7 mm diameter) for ventilation. Tulle netting was inserted inside the cap to prevent ticks from escaping. Tubes with field-collected ticks were then placed in a self-sealing, double-zipper, plastic bag with a slightly moistened paper towel. Ticks were sent directly by express mail to the laboratory (JDS) for examination and study. Taxonomic keys were also used for morphological identification (Durden and Keirans 1996, Keirans and Durden 1998, Keirans and Clifford 1978). When the *I. pacificus* nymph molted to a female, and later became fully sclerotized, it was stored in a 2 mL micro tube containing 94% ethyl alcohol.

The first four ticks (3 *I. auritulus*, 1 *I. spinipalpis*) collected in this study were sent by courier to the culturing and PCR amplification laboratory (JFA). These ticks were directly tested for *B. burgdorferi* s.l. using DNA extraction and PCR analysis. The DNA detection methods have been previously described (Persing *et al.* 1990a, 1990b; Scott *et al.* 2013). The fifth and sixth ticks, both *I. pacificus* nymphs (14-5A137, 15-5A55), were put in 94% ethyl alcohol, and sent by courier to a separate laboratory (KLC) for *B. burgdorferi* s.l. testing and molecular analysis.

DNA extraction

DNA was extracted from the ethanol-preserved ticks using a salting out procedure similar to that described previously (Clark *et al.* 2013). Each tick was cut into several pieces with a sterile scalpel blade within a 2-mL micro tube. Then, 500 μ l 1x Tissue and Cell Lysis Buffer (MasterPure, Epicentre, Madison, WI) and 200 μ g of proteinase K were added. After the sample was heated in a water bath at 65°C for 1 hour, the liquid was transferred to a clean tube and then chilled at -20°C for 5 min. Then, 200 μ l of 7.5M ammonium acetate was added, and the tube was vortexed on high speed for 30 sec. The sample was then chilled at -20°C for 5 min again, centrifuged at 16,000 RCF for 5 min at room temperature in a tabletop centrifuge (Eppendorf model 5424) to pellet protein, and the supernatant was transferred to a clean tube. To enhance DNA precipitation, 3 μ l of polyacryl carrier (MRC, Cincinnati, OH) was added, and mixed by vortexing 15 sec, followed by the addition of 700 μ l of 100% isopropanol. The tube was inverted 50 times to gently mix, and then chilled overnight at -20°C. DNA was pelleted by centrifuging at 16,000 RCF for 30 min at room temperature. Supernatant was discarded, and the pellet was washed twice with 1 mL of 75% ethanol, by inverting and rotating the tube gently 5 times to rinse the pellet, and inside of the tube, and then centrifuging for 5 min at 16,000 RCF for each rinse. The residual ethanol was removed with a 200 μ l pipette tip, and the pellet was air dried at room temperature for 10 min. To rehydrate, 100 μ l tris-EDTA buffer (pH 8.0) was added, and the tube was heated at 65°C for 5 min. Thereafter, the sample was stored at 0–4°C.

PCR testing

Tick extracts were initially screened for *B. burgdorferi* sensu lato using two nested (hemi-nested) PCR assays designed to amplify separate portions of the 41-kDa chromosomal flagellin (*flaB*) gene. The assays are hereafter referred to as Bb PCR1 and PCR2. Primary/outer reaction primers for PCR1 were 313F (5'-GCA-GAC-AGA-GGT-TCT-ATA-CAA-ATT-G-3') and 551R (5'-GCT-TCA-TCT-TGG-KTT-GCT-CCA-ACA-T-3'), which amplify a 238-bp fragment; inner reaction primers were 313F and 506R (5'-GCT-TGA-GAY-CCT-GAA-AGT-GAT-GCT-GG), which amplify a 194-bp product. Primers for PCR2 were 481F (5'-CCA-GCA-TCA-CTT-TCA-GGR-TCT-CA-3') and 737R (5'-GCA-TCA-ACT-GTR-GTT-GTA-ACA-TTA-ACA-GG-3'), which amplify a 257-bp product, followed by 532F (5'-GGA-GCA-AMC-CAA-GAT-GAA-GCT-ATT-GC-3') and 737R, amplifying a 206-bp product. Positive results with PCR1 and PCR2 were confirmed with additional *B. burgdorferi* sensu lato specific primers for the 5S-23S rRNA intergenic spacer and *flaB* gene as described previously (Rijkema *et al.* 1995, Clark *et al.* 2014), as well as *Borrelia* species primers for the 16S-23S rRNA intergenic spacer (Bunikis *et al.* 2004).

First round PCR amplifications contained 2.5 µl of tick DNA extract in a total reaction volume of 50 µl. Each inner/nested reaction used 1 µl of outer reaction product as template. First round amplifications utilized a hot start PCR master mix (HotMasterMix, 5 Prime, Gaithersburg, MD) resulting in a final concentration of 1.0 U of *Taq* DNA polymerase, 45 mM KCl, 2.5 mM Mg²⁺, 200 µM of each deoxynucleoside triphosphate, and 0.5 µM of each primer. Second round amplifications used GoTaqGreen® PCR Master Mix (Promega, Madison, WI), which allowed samples to be directly loaded into agarose gels without the addition of a gel loading buffer. All PCRs were carried out in an Applied Biosystems AB2720 thermal cycler (Life Technologies, ThermoFisher Scientific, Waltham, MA). Each primary PCR consisted of initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 30 sec, primer annealing at 5°C below the lowest primer's calculated melting temperature for 30 sec, and extension at 65°C for 1 min, with a final extension at 65°C for 5 min. Nested reactions included initial denaturation at 94°C for 1 min, followed by 35 cycles of amplification with an annealing temperature of 55°C, and extension temperature of 72°C.

PCRs were set up in an area separate from DNA extractions, and within a PCR clean cabinet (CleanSpot Workstation, Coy Laboratory Products, Grass Lake, MI) equipped with a germicidal UV lamp. Other precautions to prevent carryover contamination of amplified DNA included different sets of pipettes dedicated for DNA extraction, PCR setup, and post-amplification activities, the use of aerosol barrier filter pipette tips, and soaking pipettes used for handling DNA samples in 10% bleach solution after setup of each PCR. Each PCR test included negative control samples with nuclease free TE buffer as template. As a further measure to minimize DNA artifact contamination of PCR testing, no positive control samples were used. PCR products were electrophoresed in 2% agarose gels, which were stained with ethidium bromide, and visualized and recorded with a digital gel documentation unit.

DNA sequence analysis

PCR products from PCR1 and PCR2 positive samples were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI). DNA templates were sequenced (McCombie *et al.* 1992) using both the forward and reverse primers used in the nested PCRs. Investigator-derived sequences were compared with those obtained by searching the GenBank database (National Center for Biotechnology Information) using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* 1990), and aligned using Clustal X (Thompson *et al.* 1997).

Nucleotide sequence accession numbers. The DNA nucleotide sequences for the CN137 strain of the *B. burgdorferi* sensu lato *flaB* gene, which was obtained from the *I. pacificus* nymph (14-5A137), using PCR1 and PCR2 primer sets, were deposited in the GenBank database with accession numbers KR611713 and KR611714, respectively.

Results

Six novel host-tick associations of three species of *Ixodes* ticks on gallinaceous birds were recorded from southern Vancouver Island, British Columbia (Fig. 1). Specifically, the ixodid ticks are: *I. auritulus*, *I. pacificus*, and *I. spinipalpis*, and collection details are listed in Table 1. Of special significance, a fully engorged *I. pacificus* nymph was collected from a California Quail on 20 June 2014. This replete nymph molted to a female in 51 d (Fig. 2). Using PCR testing, this unfed female tested positive for *B. burgdorferi* s.l. and, upon DNA sequencing, it was shown to be *B. burgdorferi* s.s., a genospecies pathogenic to people and certain domestic animals. This constitutes the first report of a tick on a quail in Canada and, in particular, the first report in North America of a *B. burgdorferi* s.l.-infected tick on a California Quail. In addition, our study presents the first report of *I. spinipalpis* parasitizing a quail anywhere. Moreover, we document the first record of *I. auritulus* on a quail in Canada, and present the first account of this tick species on a California Quail.

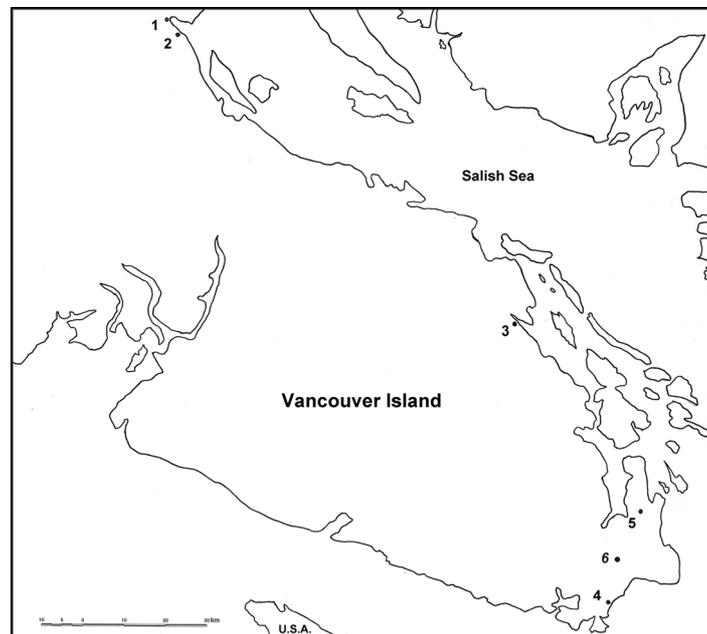


FIGURE 1. Geographic locations in British Columbia, Canada where ticks were collected from gallinaceous birds.

TABLE 1. Host associations and detection of *B. burgdorferi* sensu lato in ticks collected from gallinaceous birds in British Columbia, Canada.

Tick No.	Location	Bird species	Date collected	Tick species	PCR results	Tick-host-pathogen associations
02-5A34A	Courtenay	Sooty Grouse	10-Aug-02	<i>I. auritulus</i> 6F	neg.	HR
02-5A35A	Royston	Sooty Grouse	11-Aug-02	<i>I. auritulus</i> 3N, 1F	neg.	HR, for nymph
12-5A85	Metchosin	California Quail	23-Sep-12	<i>I. auritulus</i> 1F	neg.	HR
13-5A98	Ladysmith	California Quail	25-Jun-13	<i>I. spinipalpis</i> 1N	neg.	HR
14-5A137	Saanich	California Quail	20-Jun-14	<i>I. pacificus</i> 1N	pos.	HR; Bb-pos. <i>I. pac.</i> on California Quail
15-5A55	Highlands	California Quail	2-Jun-15	<i>I. pacificus</i> 1N	neg.	juvenile host (chick)

N, nymph(s); F, female(s); HR, new host record; *I. pac.*, *Ixodes pacificus*



FIGURE 2. *Ixodes pacificus*, unfed female (bar, 1 mm), after molting from a nymph that was collected from a California Quail. Using PCR and DNA sequencing, this tick tested positive for *B. burgdorferi* s.s. Photo credits: Alan Bibby.

Discussion

This study provides new host records for three *Ixodes* species on gallinaceous birds in Canada, and documents the presence of a *B. burgdorferi* s.l.-infected *I. pacificus* on a California Quail. This bird parasitism reveals that these gallinaceous birds may be implicated in the enzootic cycle of Lyme disease spirochetes in North America. Because of their localized home range and ground-feeding habits, grouse and quail are ideal candidates for maintaining tick populations and disseminating *B. burgdorferi* s.l. in the environment.

Migratory birds play a major role in the wide dispersal of Lyme disease vector ticks in Canada (Scott *et al.* 2001, Morshed *et al.* 2005, Ogden *et al.* 2008, Scott 2015; Scott *et al.* 2010, 2012, 2015). During northward spring migration, passerine migrants introduce bird-feeding ticks into Canada from the southern United States, Mexico, Central America, the Caribbean Islands, and South America (Scott *et al.* 2001, Ogden *et al.* 2008, Scott *et al.* 2012, Scott & Durden 2014, Scott & Durden 2015a,b,c,d; Scott 2015). Heavily infested passerines can initiate new Lyme disease foci (Scott *et al.* 2014a). On Vancouver Island, passerines act as hosts of *I. auritulus*, *I. pacificus*, and *I. spinipalpis*, and play a role in the enzootic cycle of *B. burgdorferi* s.l. (Scott *et al.* 2010, 2012). Recently, Scott *et al.* (2015) recorded 31% of *I. auritulus* (larvae, nymphs, and adults) collected from passerines to be infected with *B. burgdorferi* s.l. Because *I. auritulus* feed exclusively on birds, certain wild birds are reservoir-competent hosts.

In addition, gallinaceous birds may play an equally significant role in dispersing bird-feeding ticks locally. Heavily infested grouse and quail could establish new tick populations in their home ranges if suitable avian and mammalian hosts are present. Of note, Banerjee *et al.* (1994) collected *B. burgdorferi* s.l.-infected *I. pacificus* and deer mice, *Peromyscus maniculatus* (Wagner), on southern Vancouver Island; therefore, Lyme disease spirochetes have been circulating in this bioregion for many years. Because of their relatively small home ranges, California Quail could

perpetuate an enzootic cycle of *B. burgdorferi* s.l. in coastal woodlands and dense ecotones in the coastal area of the Pacific Northwest. On southern Vancouver Island, California Quail are hosts for *I. pacificus* immatures and, therefore, have the potential to act as reservoir-competent hosts.

Since we did not draw blood or obtain tissue samples from the California Quail, we are not able to pinpoint the origin of *B. burgdorferi* s.l. detected in the fully engorged *I. pacificus* nymph. However, we were able to discern that the blood meal obtained from the California Quail did not impede the viability of *B. burgdorferi* s.l. during the nymph-female molt. Not only was transstadial transmission verified, California Quail support vector competency of *B. burgdorferi* s.l. Spirochetes may have come directly from the host bird or from the *I. pacificus* larva that fed on a previous infected host. When *I. pacificus* immatures feed on a spirochetemic host, they can then transmit *B. burgdorferi* s.l. to the next life stage (Peavey & Lane 1995). We have not discerned whether California Quail are reservoir hosts of *B. burgdorferi* s.l.; however, the Japanese Quail, *Coturnix japonica* Temminck & Schlegel (Galliformes: Phasianidae), which is found in eastern Asia and Russia, is a reservoir-competent host for *B. burgdorferi* s.l., namely *B. garinii* (Isogai *et al.* 1994). Since the Japanese Quail is a reservoir for *B. burgdorferi* s.l., it is plausible that California Quail are also reservoirs for *B. burgdorferi* s.l.

On southeastern Vancouver Island, thick low-level vegetation provides ideal habitat for ticks and gallinaceous birds. Within this coastal bioregion, gallinaceous birds encounter *I. auritulus*, *I. pacificus*, and *I. spinipalpis* during ground-feeding activities. Not only do gallinaceous birds eat plant leaves and seeds, they also consume invertebrates, including ticks (Hoogstraal & Kaiser 1961, Milne 1950). When a ground-frequenting bird passes over a spot where a gravid female laid eggs, it will be subject to newly-hatched, questing larvae. The spent female, which recently died, has adipose tissue in the posterior end of the idiosoma that acts as an energy source. This female tick can be a food source for vertebrate hosts, including quail and grouse. Notably, transovarial transmission of *B. burgdorferi* s.l.-infected *I. pacificus* was not recorded by Schoeler & Lane (1993); therefore, California Quail would not become spirochetemic after being bitten by unfed *I. pacificus* larvae.

When California Quail forage, it is not unusual to encounter 20 quail, or more, in a single covey (Fig. 3). These ground-dwelling birds inhabit thick undergrowth and low-lying vegetation in southern British Columbia where they become parasitized by host-seeking ticks. In fact, they become maintenance hosts for ticks, and potentially these game birds may act as reservoir hosts for *B. burgdorferi* s.l.



FIGURE 3. A covey of California Quail on Vancouver Island, B.C. after a rare snowstorm. Photo credits: Christina Carrieres.

We provide the first record of *I. spinipalpis* on a gallinaceous bird and, simultaneously, on a California Quail. Based on previous host records, *I. spinipalpis* has been reported on passerines (Gregson 1956, Scott *et al.* 2012, 2015), but not on gallinaceous birds. Medically, *I. spinipalpis* is known to bite humans (Dolan *et al.* 1997, Eisen *et al.* 2006), and is a competent vector of *B. burgdorferi* s.l. (Dolan *et al.* 1997, Eisen *et al.* 2003). Of epidemiological significance, gallinaceous birds are valid candidates to maintain and amplify *B. burgdorferi* s.l. in far-western Canada.

Not only does *I. pacificus* infest passerines and gallinaceous birds, it can also cause tick paralysis in domestic animals (Nelson 1973, Lane 1984). Scott *et al.* (2014b) reported tick paralysis in a Snowshoe Hare, *Lepus americanus* Erxleben caused by attached *I. pacificus* females. Because *I. pacificus* parasitizes a wide range of vertebrate hosts, it can act as a bridge vector for *B. burgdorferi* s.l. to humans and domestic animals (Eisen *et al.* 2003). In coastal southwestern British Columbia, *I. auritulus*, *I. pacificus*, and *I. spinipalpis* are sympatric, and can co-infest an individual bird at the same time. Such tick coinfections would support interconnecting links for multiple genospecies of *B. burgdorferi* s.l. When humans enter shrubby habitats, which are inhabited by gallinaceous birds, they may be bitten by *B. burgdorferi* s.l.-infected ticks. Since our data set is limited, additional ecological/epidemiological studies are needed to ascertain the prevalence of *B. burgdorferi* s.l. in these wild birds.

In conclusion, we provide the first report of a *B. burgdorferi* s.l.-infected *I. pacificus* on a gallinaceous bird in North America and, synchronously, on a California Quail. Not only are California Quail suitable hosts for *I. pacificus*, they facilitate transstadial transmission of *B. burgdorferi* s.l. in Lyme disease endemic areas along North America's Pacific coast. Because transstadial transmission was completed successfully, we demonstrate that California Quail are directly involved in the enzootic cycle of *B. burgdorferi* s.l. Notably, certain gallinaceous birds, world-wide, support the enzootic transmission dynamics of *B. burgdorferi* s.l. Because *B. burgdorferi* s.s. is pathogenic to humans, veterinarians, wildlife rehabilitators, hunters, and medical professionals should be aware that handling gallinaceous birds may pose a public health risk.

Acknowledgments

We thank wildlife rehabilitators who collected ticks from gallinaceous birds. We are indebted to Elizabeth E. Alves for technical support. We are grateful to Alan Bibby and Christina Carrieres for the photographs and John Ward for computer graphics. Funding for this study was supported in part by Lyme Ontario.

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Submitted: 12 Aug. 2015; accepted by Trevor Petney: 11 Nov. 2015; published: 15 Jan. 2016