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

JOHN D. SCOTT[1], JOHN F. ANDERSON[2], AND LANCE A. DURDEN[3]
[1]RESEARCH DIVISION, DISEASE ASSOCIATION OF ONTARIO, FERGUS, ONTARIO, CANADA
[2]CONNECTICUT AGRICULTURAL EXPERIMENTAL STATION, NEW HAVEN, CONNECTICUT
[3]DEPARTMENT OF BIOLOGY, GEORGIA SOUTHERN UNIVERSITY, STATESBORO, GEORGIA

Abstract: During a pan–Canadian tick–host study, the authors detected the spirochetal bacterium *Borrelia burgdorferi* sensu lato, which causes Lyme disease, in ticks 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, *Accipter cooperii*, on Vancouver Island, British Columbia. Using PCR [polymerase chain reaction] amplification of the linear plasmid *osp*A 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, authors propose that Cooper's hawks are reservoir–competent hosts of *B. burgdorferi*. Authors' tick–host discovery provides the first report of bird–feeding ticks on a Cooper's hawk, and exhibits the premier record of *B. burgdorferi*–positive ticks on a raptor. Not only are passerine (perching) and gallinaceous (chicken–like) birds included in the wide dispersal of Lyme disease vector ticks, raptors also now are 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 four–tick enzootic cycle of *B. burgdorferi* along the West Coast of 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, hardbodied *Ixodes* species ticks (Ixodida: Ixodidae). Most commonly, ticks are reported on passerines (Order: Passeriformes), which also are 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 (Burgdorfer, Barbar, et al 1982). This tick–borne spirochetosis can have a multitude of clinical symptoms, including cardiac, cutaneous, endocrine, gastrointestinal, genitourinary, musculoskeletal, neurologic, cognitive, and neuropsychiatric (Maloney 2009; Savely 2010; Bransfield, Wulfman, et al 2008). If left untreated or inadequately treated, diverse forms (Miklossy, Kasas, et al 2008; Sapi, MacDonald, et al 2008) of *B.burgdorferi* can sequester and persist in immunologically deprived and deep–seated sites (Straubinger 2000; Barthold, Hodzic, et al 2010; MacDonald 2013; Sapi, Bastian, et al 2012; Embers, Barthold, et al 2012; MacDonald 2006; Liegner, Duray, et al 1997; Stricker and Johnson 2013); namely, ligaments and tendons (Häupl, Hahn, et al 1993; Müller 2012), muscle (Frey, Jaulhac, et al 1998), brain (MacDonald 2007; Miklossy 2011; Oksi, Kalimo, et al 1996), bone (Fein and Tilton 1997; Oksi, Mertsola, et al 1994), eyes (Preac–Mursic, Pfister, et al 1993), glial and neuronal cells (Ramesh, Borda, Dufour, et al 2008; Ramesh, Santana–Gould, et al 2013), fibroblasts/scar tissue (Klempner, Noring, et al 1993). There are at least 100 different *B. burgdorferi* genotypes worldwide (Franke, Hildebrandt and Dorn 2013; Casjens, Fraser–Ligget, et al 2011; Crowder, Matthews, et al 2010; Mathers, Smith, et al 2011), and patients often are negative using the 2–tier Lyme disease serology test despite having Lyme disease (Kaiser 2000; Sperling, Middelveen, et al 2012; Clark, Leydet, et al 2013).

This tick–borne microorganism cycles in nature between certain tick species and a wide range of vertebrate hosts, and has been reported from five continents, including subantarctic islands and Australia (Mayne 2011; Masyne 2012). 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 (Scott, Fernando, et al 2001; Morshed, Scott, et al 2005; Scott, Lee, et al 2010; Scott, Anderson, et al 2012). In far–western Canada, Gregson (1956) 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 (Ogden, Lindsay, et al 2008). 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 (BC) (Peterson 2010). The sheep tick, *Ixodes ricinus,* and the tiaga tick, *Ixodes persulcatus,* have been reported on several species of the hawks in Eurasia (Anderson and Magnarelli 1993).

Ticks can transmit more kinds of pathogens than any other group of ectoparasites worldwide affecting people, livestock, wildlife, and domestic animals (Nicholson, Sonenshine, et al 2009). 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* (Eisen and Lane 2002) 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* (Dolan, Lacombe, et al 2000; Oliver, Lin, et al 2003). Moreover, several bird–tick–*Borrelia* studies underpin the fact that ground–frequenting passerines transport Lyme disease vector ticks northward during long–distance flight (Scott, Fernando, et al 2001; Morshed, Scott, et al 2005; Scott, Lee, et al 2010; Scott, Anderson, et al 2012; Ander and Magnarelli 1984; Reed, Meese, et al 2003; Hamer, Goldberg, et al 2012). Not only do migratory songbirds carry ticks northward during spring migration, these avian hosts also transport them southward during fall migration (Morshed, Scott, et al 2005; Durden, Oliver, et al 2001). 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* (Scott, Anderson, et al 2012. Using culturing and PCR–testing, early studies in the southern region of Vancouver Island, BC, detected *B. burgdorferi* in *Ixodes angustus* and *I. pacificus* and established its presence in this area (Banerjee, Banerjee, et al 1994). The aim of authors' tick–host–*Borrelia* study was to explore any new environmental associations that could contribute to an increase in Lyme disease in an area.

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 four 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 percent ethyl alcohol. Using a bubble–pack envelope, ticks were mailed promptly to the lab (JDS) for identification. An Olympus® (Olympus America, Center Valley, PA) 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 (Durden and Keirans 1996; Keirans and Cliffore 1978; Kleinjan and Lane 2008). 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 (polymerase chain reaction) 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 (Persing, Telford, Spielman, et al 1990; Persing, Telford, Rys, et al 1990). Briefly, ticks were ground with a large paper clip in a 0.6–mL microcentrifuge tube containing 25 µL to 35 µL 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 *osp*A gene target: ospA2, 5′–GTTTTGTAATTTCAACTGCTGACC–3′; ospA4, 5′–CTGCAGCTTGGAATTCAGGCACTTC–3′. PCR amplification was performed using a PerkinElmer® (Waltham, MA) thermal cycler set to conduct denaturation at 94°C for 45 seconds, annealing at 45°C for 45 seconds, and elongation at 72°C for 1 minute, 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 (Persing, Telford, Spielman, et al 1990; Persing, Telford, Rys, et al 1990). Amplification products were transferred to a nylon membrane by Southern blot. The membrane was then hybridized overnight with 32P using the probe ospA3, 5′–GCC ATTTGAGTCGTATTGTTGTACTG–3′. The membrane then was washed, and Kodak® X–OMAT® AR film (Eastman Kodak Co., Rodchester, NY) was placed over the membrane for four hours. 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, BC, Canada. This tick collection is the first report of ticks on a Cooper's hawk, and 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, authors 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, authors 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 (similar to abdomen) of the carcass provides a source of energy–laden nutrients, and creates a proclivity to attract birds and rodents foraging for food Stafford and Kitron 2002).

Now, the question becomes, how did the four *I. auritulus* larvae acquire *B. burgdorferi* infection? Connecticut researchers Anderson, Johnson, Magnarelli, et al (1986) 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 (Morshed, Scott, et al 2005; Scott, Lee, et al 2010; Scott, Anderson, et al 2012), authors extrapolate that spirochetes of this zoonosis were transmitted during engorgement on the Cooper's hawk. Authors' 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 authors' 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 this ectoparasite study, they are significantly different.

For comparison, researchers (Rollend, Fish, et al 2013) presented evidence–based data to indicate that *Borrelia miyamotoi*, which is also pathogenic to humans (Platonov, Karan, et al 2011), 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 authors' 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* (Burgess 1989). Moreover, Schwarzoza et al (2006) 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 PCR amplification results, authors 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 four–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 this study not only includes *I. auritulus* on a Cooper's hawk, it implicates raptors as reservoir hosts in the four–tick enzootic cycle of *B. burgdorferi* in this bioregion and expands the number of bird species in Lyme disease dissemination.

ACKNOWLEDGEMENTS

The authors thank Troy Blondin, Christina Carrieres, and Louise Dykslag, wildlife rehabilitators, for collection of ticks and technical field assistance. We are most grateful for the expert technical assistance of Bonnie Hamid and Elizabeth Alves. Authors are indebted to Donna McCoy for computer graphics and Catherine Scott for assistance in manuscript preparation. Technical support was funded in part by the Canadian Lyme Disease Foundation and the Lyme Disease Association of Ontario.

LITERATURE CITED

Anderson, J. F., and L. A. Magnarelli. 1984. Avian and Mammalian Hosts for Spirochete–infected Ticks and Insects in a Lyme Disease Focus in Connecticut. *Yale Journal of Biology and Medicine.* 57: 627–641.

Anderson, J. F., and L. A. Magnarelli. 1993. Epizootiology of Lyme Disease–causing Borreliae. *Clinics in Dermatology*. 11: 339–351.

Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W. Hyde. 1986. Involvement of Birds in the Epidemiology of the Lyme Disease Agent *Borrelia burgdorferi*. *Infection and Immunity*. 51(2): 394–396.

Banerjee, S. N., M. Banerjee, J. A. Smith, and K. Fernando. 1994. Lyme Disease in British Columbia—An Update. *Proceedings* of the VII Annual Lyme Disease Foundation International Conference.

Barthold, S. W., E. Hodzic, D. M. Imai, S. Feng, X. Yang, et al. 2010. Ineffectiveness of Tigecycline Against Persistent *Borrelia burgdorferi*. *Antimicrobial Agents and Chemotherapy.* 54: 643–651.

Bransfield, R. C., J. S. Wulfman, W. T. Harvey, and A. I. Usman. 2008. The Association Between Tick–borne Infections, Lyme Borreliosis and Autism Spectrum Disorders. *Medical Hypotheses*. 70: 967–974.

Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, et al. 1982. Lyme Disease a Tick–borne Spirochetosis? *Science*. 216: 1317–1319.

Burgess, E. C. 1989. Experimental Inoculation of Mallard Ducks (*Anas platyrhynchos platyrhynchos*) with *Borrelia burgdorferi*. *Journal of Wildlife Diseases*. 25: 99–102.

Casjens, S. R., C. M. Fraser–Liggett, E. F. Mongodin, W. G. Qiu, J. J. Dunn, et al. 2011. Whole Genome Sequence of an Unusual *Borrelia burgdorferi* Sensu Lato Isolate. *Journal of Bacteriology*. 193: 1489–1490.

Clark, K. L., B. Leydet, and S. Hartman. 2013. Lyme Borreliosis in Human Patients in Florida and Georgia, USA. *International Journal of Medical Sciences*. 10: 915–931.

Crowder, C. D., H. E. Matthews, S. Schutzer, M. A. Rounds, B. J. Luft, et al. 2010. Genotypic Variation and Mixtures of Lyme *Borrelia* in *Ixodes* Ticks from North America and Europe. *PLoS One*. 5(5): e10650.

Dolan, M. C., E. H. Lacombe, and J. Piesman. 2000. Vector Competence of *Ixodes affinis* (Acari: Ixodidae) for *Borrelia burgdorferi*. *Journal of Medical Entomology.* 37: 766–768.

Durden, L. A., and J. E. Keirans. 1996. Nymphs of the Genus *Ixodes* (Acari: Ixodidae) of the United States: Taxonomy, Identification Key, Distribution, Hosts, and Medical/veterinary Importance. *Thomas Say Publications in Entomology*. Entomological Society of America: Lanham, MD.

Durden, L. A., J. H. Oliver, Jr, and A. A. Kinsey. 2001. Ticks (Acari: Ixodidae) and Spirochetes (*Spirochaetaceae: Spirochaetales*) Recovered from Birds on a Georgia Barrier Island. *Journal of Medical Entomology*. 38: 231–236.

Eisen, L., and R. S. Lane. 2002. Vectors of *Borrelia burgdorferi* Sensu Lato: Lyme Borreliosis: Biology, Epidemiology and Control. CABI Publishing: New York, NY.

Embers, M. E., S. W. Barthold, J. T. Borda, L. Bowers, L. Doyle, et al. 2012. Persistence of *Borrelia burgdorferi* in Rhesus Macaques Following Antibiotic Treatment of Disseminated Infection. *PLoS One*. 7(1): e29914. Doi: 10.1371/journal.pone.0029914.

Fein, L., and R. C. Tilton. 1997. Bone Marrow as a Source of *Borrelia burgdorferi* DNA. *Journal of Spirochetal and Tick–borne Diseases*. 4: 58–60.

Franke, J., A. Hildebrandt, and W. Dorn. 2013. Exploring Gaps in Our Knowledge on Lyme Borreliosis Spirochaetes—Updates on Complex Heterogeneity, Ecology, and Pathogenicity. *Ticks and Tick–borne Diseases*. 4: 11–25.

Frey, M., B. Jaulhac, Y. Piemont, L. Marcellin, P. M. Boohs, et al. 1998. Detection of *Borrelia burgdorferi* DNA in Muscle of Patients with Chronic Myalgia Related to Lyme Disease. *American Journal of Medicine*. 104: 591–594.

Gregson, J. D. 1956. The Ixodoidea of Canada: Publication 930. Science Service, Entomology Divison, Department of Agriculture, Canada.

Hamer, S. A., T. L. Goldberg, U. D. Kitron, J. D. Brawn, T. K. Anderson, et al. 2012. Wild Birds and Urban Ecology of Ticks and Tick–borne Pathogens, Chicago, Illinois, USA, 2005–2010. *Emerging Infectious Diseases*. 18: 1589–1595.

Häupl, T., G. Hahn, M. Rittig, A. Krause, C. Schoerner, et al. 1993. Persistence of *Borrelia burgdorferi* in Ligamentous Tissue from a Patient with Chronic Lyme Borreliosis. *Arthritis & Rheumatism*. 36: 1621–1626.

Kaiser, R. 2000. False–negative Serology in Patients with Neuroborreliosis and the Value of Employing of Different Borrelial Strains in Serological Assays. *Journal of Medical Microbiology*. 49: 911–915.

Keirans, J. E., and C. M. Clifford. 1978. The Genus *Ixodes* in the United States: A Scanning Electron Microscope Study and Key to the Adults. *Journal of Medical Entomololgy,* Supplement. 2: 1–149.

Keirans, J. E., and R. S. Lane. 2008. Larval Keys to the Genera of Ixodidae (Acari) and Species of *Ixodes* (Latreille) Ticks Established in California. *Pan–Pacific Entomology*. 84: 121–142.

Klempner, M. S., R. Noring, and R. A. Rogers. 1993. Invasion of Human Skin Fibroblasts by the Lyme Disease Spirochete, *Borrelia burgdorferi*. *Journal of Infectious Diseases*. 167(5): 1074–1081.

Liegner, K.B., P. Duray, M. Agricola, C. Rosenkilde, L. A. Yannuzzi, et al. 1997. Lyme Disease and the Clinical Spectrum of Antibiotic Responsive Chronic Meningoencephalomyelitides. *Journal of Spirochetal and Tick–borne Diseases*. 4: 61–73.

MacDonald, A. B. 2006. Plaques of Alzheimer's Disease Originate from Cysts of *Borrelia burgdorferi*, the Lyme Disease Spirochete. *Medical Hypotheses*. 67: 592–600.

MacDonald, A. B. 2007. Alzheimer's Neuroborreliosis with Trans–synaptic Spread of Infection and Neurofibrillary Tangles Derived from Intraneuronal Spirochetes. *Medical Hypotheses*. 68: 822–825.

MacDonald, A. B. 2013. *Borrelia burgdorferi* Tissue Morphologies and Imaging Methodologies. *European Journal of Clinical Microbiology and Infectious Diseases*. 32: 1077–1082.

Maloney, E. L. 2009. The Need for Clincial Judgement in the Diagnosis and Treatment of Lyme Disease. *Journal of American Physicians and Surgeons*. 14:82–89.

Mathers, A., R. P. Smith, B. Cahill, C. Lubelczyk, S. P. Elias, et al. 2011. Strain Diversity of *Borrelia burgdorferi* in Ticks Dispersed in North America by Migratory Birds. *Journal of Medical Microbiology*. 36: 24–29.

Mayne, P. J. 2011. Emerging Incidence of Lyme Borreliosis, Babesiosis, Bartonellosis, and Granulocytic Ehrlichiosis in Australia. *International Journal of General Medicine*. 4: 845–852.

Mayne, P. J. 2012. Investigation of *Borrelia burgdorferi* Genotypes in Australia Obtained from Erythema Migrans Tissue. *Journal of Clinical, Cosmetic and Investigational Dermatology*. 5: 69–78.

Miklossy, J. 2011. Alzheimer's Disease—A Neurospirochetosis. Analysis of the Evidence Following Koch's and Hill's Criteria. *Journal of Neuroinflammation*. 8: 90.

Miklossy, J., S. Kasas, A. D. Zurn, S. McCall, S. Yu, et al. 2008. Persisting Atypical and Cystic Forms of *Borrelia burgdorferi* and Local Inflammation in Lyme Neuroborreliosis. *Journal of Neuroinflammation*. 5: 40.

Morshed, M. G., J. D. Scott, K. Fernando, L. Beati, D. F. Mazerolle, et al. 2005. Migratory Songbirds Disperse Ticks across Canada, and First Isolation of the Lyme Disease Spirochete, *Borrelia burgdorferi*, from the Avian Tick, *Ixodes Auritulus*. *Journal of Parasitology.* 91: 780–790.

Müller, K. E. 2012. Damage of Collagen and Elastic Fibres by *Borrelia burgdorferi*–Known and New Clinical and Histopathological Aspects. *The Open Neurology Journal*. 6: 179–186.

Nicholson, W. L., D. E. Sonenshine, R. S. Lane, and G. Uilenberg. 2009. Ticks (Ixodoidea): *Medical and Veterinary Entomology*, 2nd edition. Elsevier Inc: Amsterdam, Netherlands.

Ogden, N. H., L. R. Lindsay, K. Hanincová, I. K. Barker, M. Bigras–Poulin, et al. 2008. Role of Migratory Birds in Introduction and Range Expansion of *Ixodes Scapularis* Ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. *Applied and Environmental Microbiology*. 74: 1780–1790.

Oksi, J., H. Kalimo, R. J. Marttila, M. Marjamäki, P. Sonninen, et al. 1996. Inflammatory Brain Changes in Lyme Borreliosis: A Report on Three Patients and Review of Literature. *Brain*. 119: 2143–2154.

Oksi, J., J. Mertsola, M. Reunanen, M. Marjamäki, and M. K. Viljanen. 1994. Subacute Multiple–site Osteomyelitis Caused by *Borrelia burgdorferi*. *Clinical Infectious Diseases*. 19: 891–896.

Oliver, J. H., Jr, T. Lin, L. Gao, K. L. Clark, C. W. Banks, et al. 2003. An Enzootic Transmission Cycle of Lyme Borreliosis Spirochetes in the Southeastern United States. *Proceedings of the National Academy of Sciences, USA*. 100: 11642–11645.

Persing, D. H., TS. R. Telford, III, A. Spielman, S. W. Barthold. 1990. Detection of *Borrelia burgdorferi* Infection in *Ixodes dammini* Ticks with the Polymerase Chain Reaction. *Journal of Clinical Microbiology*. 28(3): 566–572.

Persing, D. H., S. R.Telford, III, P. N. Rys, D. E. Dodge, T. J. White, et al. 1990. Detection of *Borrelia burgdorferi* DNA in Museum Specimens of *Ixodes dammini* Ticks. *Science*. 249: 1420–1423.

Peterson, T. P. 2010. *Peterson Field Guide to Birds of Western North America*, 4th edition. Houghton Mifflin Harcourt Publishing Company: Boston, MA.

Platonov, A. E., L. S. Karan, N. M. Kolyasnikova, N. A. Makhneva, M. G. Toporkova, et al. 2011. Humans Infected with Relapsing Fever Spirochete *Borrelia miyamotoi*, Russia. *Emerging Infectious Diseases*. 17:1816–1823.

Preac–Mursic, V., H. W. Pfister, H. Spiegel, R. Burk, B. Wilske, et al. 1993. First Isolation of *Borrelia burgdorferi* from an Iris Biopsy. *Journal of Clinical Neuro–ophthalmology*. 13: 155–161.

Ramesh, G., J. T. Borda, J. Dufour, D. Kaushal, R. Ramamoorthy, et al. 2008. Interaction of the Lyme Disease Spirochete *Borrelia burgdorferi* with Brain Parenchyma Elicits Inflammatory Mediators from Glial Cells as well as Glial and Neuronal Apoptosis. *The American Journal of Pathology*. 173: 1415–1427.

Ramesh, G., L. Santana–Gould, F. M. Inglis, J. D. England, and M. T. Philipp. 2013. The Lyme Disease Spirochete *Borrelia burgdorferi* Induces Inflammation and Apoptosis in Cells from Dorsal Root Ganglia. *Journal of Neuroinflammation*. 10: 88.

Reed, K. D., J. K. Meece, J. S. Henkel, and S. K. Shukla. 2003. Birds, Migration and Emerging Zoonoses: West Nile Virus, Lyme Disease, Influenza A, and Enteropathogens. *Clinical Medicine & Research*. 1: 5–12.

Rollend, L., D. Fish, and J. E. Childs. 2013. Transovarial Transmission of *Borrelia* spirochetes by *Ixodes scapularis*: A Summary of the Literature and Recent Observations. *Ticks and Tick–borne Diseases*. 4(1 &2): 46–51.

Sapi, E., A. B. MacDonald, K. Eisendle, H. Mueller, and B. Zelger. 2008. Biofilms of *Borrelia burgdorferi* in Chronic Borreliosis. *American Journal of Clinical Pathology*. 129: 988–990.

Sapi, E., S. L. Bastian, C. M. Mpoy, S. Scott, A. Rattelle, et al. 2012. Characterization of Biofilm Formation by *Borrelia burgdorferi* In Vitro. *PLoS One*. 7: e48277.

Savely, V. 2010. Lyme Disease: A Diagnostic Dilemma. *The Nurse Practioner*. 35: 44–50.

Schwarzová, K., T. Betáková, J. Neméth, and A. Mizáková A. 2006. Detection of *Borrelia burgdorferi* Sensu Lato and *Chlamydophila psittaci* in Throat and Cloacal Swabs from Birds Migrating through Slovakia. *Folia Microbiol* (Praha). 51: 653–658.

Scott, J. D, J. F. Anderson, and L. A. Durden. 2012. Widespread Dispersal of *Borrelia burgdorferi* Infected Ticks Collected from Songbirds across Canada. *Journal of Parasitology*. 98: 49–59.

Scott, J. D., K. Fernando, S. N. Banerjee, L. A. Durden, S. K. Byrne, et al. 2001. Birds Disperse Ixodid (Acari: Ixodidae) and *Borrelia burgdorferi*–infected Ticks in Canada. *Journal of Medical Entomology*. 38: 493–500.

Scott, J. D., M. K. Lee, K. Fernando, L. A. Durden, D. R. Jorgensen, et al. 2010. Detection of Lyme Disease Spirochete, *Borrelia burgdorferi* Sensu Lato, Including Three Novel Genotypes in Ticks (Acari: Ixodidae) Collected from Songbirds (Passeriformes) Across Canada. *Journal of Vector Ecology*. 35: 124–139.

Sperling, J., M. Middelveen, D. Klein, and F. Sperling. 2012. Evolving Perspectives on Lyme Borreliosis in Canada. *The Open Neurology Journal*. 6: 94–103.

Stafford, K. C., and U. Kitron. 2002. Environmental Management for Lyme Borreliosis Control: Lyme Borreliosis: *Biology, Epidemiology and Control*. CAB International: Oxon, Wallingford, UK.

Straubinger, R. K. 2000. PCR–based Quantification of *Borrelia burgdorferi* Organisms in Canine Tissues over a 500–Day Postinfection Period. *Journal of Clinical Microbiology*. 38(6): 2191–2199.

Stricker, R. B., and L. Johnson. 2013. *Borrelia burgdorferi* Aggrecanase Activity: More Evidence for Persistent Infection in Lyme Disease. *Frontiers in Cellular and Infection Microbiology*. 3: 40.