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High-Prevalence *Borrelia miyamotoi* Infection Among Wild Turkeys (*Meleagris gallopavo*) in Tennessee

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ABSTRACT During spring and fall 2009, 60 wild turkeys (*Meleagris gallopavo*) harvested by Tennessee hunters were surveyed for *Borrelia* spp. by sampling their blood, tissue, and attached ticks. In both seasons, 70% of turkeys were infested with juvenile *Amblyomma americanum*; one spring turkey hosted an adult female *Ixodes brunneus.* Polymerase chain reaction assays followed by DNA sequencing indicated that 58% of the turkeys were positive for the spirochete *Borrelia miyamotoi*, with tissue testing positive more frequently than blood ($P = 0.015$). Sequencing of the 16S–23S rRNA intergenic spacer indicated ≥99% similarity to previously published sequences of the North American strain of this spirochete. Positive turkeys were present in both seasons and from all seven middle Tennessee counties sampled. No ticks from the turkeys tested positive for any *Borrelia* spp. This is the first report of *B. miyamotoi* in birds; the transmission pathways and epidemiological significance of this high-prevalence spirochetal infection remain uncertain.

KEY WORDS *Borrelia miyamotoi*, wild turkey, lone star tick, *Amblyomma americanum*

Two medically important diseases are caused by *Borrelia* species bacteria, as follows: Lyme disease (LD) and relapsing fever (RF) (Barbour and Hayes 1986). The vectors of LD *Borrelia* spp. are hard-bodied ixodid ticks within the *Ixodes ricinus-persulcatus* species complex, which in the United States include *Ixodes scapularis* and *Ixodes pacificus.* RF *Borrelia* spp. are transmitted by soft-bodied argasid ticks and the human body louse (*Pediculus humanus*). Recently, two *Borrelia* species of uncertain disease significance have been found associated with hard-bodied ticks; these are *Borrelia lonestari* in *Amblyomma americanum* (Armstrong et al. 1996, Barbour et al. 1996) and *Borrelia miyamotoi* in several *Ixodes* species (Fukunaga et al. 1995, Scoles et al. 2001, Fraenkel et al. 2002). Both species are genetically more similar to RF *Borrelia* than to LD *Borrelia* (Bunikis et al. 2004a).

Borrelia spp. diversity in wildlife hosts is of interest given concern over ongoing spread of LD in the United States (e.g., Bunikis and Barbour 2005, Hamer et al. 2010). Recently, Jordan et al. (2009) reported significant prevalences of *Borrelia burgdorferi* and *B. lonestari* in wild turkey (*Meleagris gallopavo*) and waterfowl in middle Tennessee, a state considered nonendemic for LD by the Centers for Disease Control (Bacon et al. 2008). This finding was unexpected, as previous studies have suggested that turkeys are poor hosts for *I. scapularis* (Ostfeld and Lewis 1999) and *B. burgdorferi* (Lane et al. 2006). Moreover, the survey by Jordan et al. (2009) coincided with a Tennessee-wide survey that found no *B. burgdorferi* in >1,000 *I. scapularis* collected from vegetation and hunter-harvested deer (Rosen 2009). To help clarify these conflicting findings on a potential role for wild turkeys in LD ecology, we set out to further investigate the *Borrelia* spp. status of this species in Tennessee.

Materials and Methods

During spring (4–25 April) and fall (25 September–19 December) of 2009, we collected blood, tissue, and ticks from 60 wild turkeys harvested by hunters in seven middle and eastern Tennessee counties that had hunter check stations staffed by Tennessee Wildlife Resource Agency (TWRA) biologists. Blood was collected by brachial venipuncture, and skin and muscle tissue samples were taken from the underside of a wing. Care was taken to prevent cross-contamination of samples. Turkeys were sexed and aged (juvenile/adult) by the TWRA biologists, and all visible ticks were removed and stored immediately in 70% ethanol (one vial per bird). Ticks were later identified to species using a dichotomous key (Sonenshine 1979).

Total DNA was extracted from all blood and tissue samples using a DNeasy blood and tissue kit (Qiagen, Valencia, CA), according to manufacturer's instructions, and from a subsample of nymphal ticks, as described by Beati and Keirans (2001). DNA was eluted twice with 50 μl of deionized sterile water and stored

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Table 1. Numbers of hunter-harvested turkeys sampled, and numbers and percentage of tick-infested and *Borrelia*-positive turkeys, by Tennessee county in 2009

County	Turkeys sampled	*A. americanum* infested (*n*; %)	Life stage	*B. miyamotoi* positive (*n*; %)
Spring sampling				
Anderson	20	17 (81%)	N	12 (60%)
Campbell	4	4 (100%)	N	3 (75%)
Cumberland	3	1 (33%)	N	3 (100%)
Montgomery	10	4[a] (40%)	N	8 (80%)
Morgan	2	0 (0%)	—	2 (100%)
Roane	7	7 (100%)	N	2 (29%)
Fall sampling				
Union	13	8 (67%)	L	4 (31%)
Campbell	1	1 (100%)	L	1 (100%)
Total	60	42 (70%)	—	35 (58%)

L, larval; N, nymphal.

[a] Includes one turkey parasitized with a single adult female *I. brunneus.*

at −20°C. One elution was sent to Michigan State University; a second was analyzed at the University of Tennessee. DNA extracts were screened using a *Borrelia* genus-specific nested polymerase chain reaction (PCR) targeting the 16S–23S rRNA intergenic spacer (IGS) locus, as described by Bunikis et al. (2004b); in all assays, water served as the negative control, and DNA from *I. scapularis* ticks infected with strain B31 *B. burgdorferi* (from Centers for Disease Control and Prevention, Ft. Collins, CO) served as the positive control. The IGS products were purified (Qiagen PCR purification kit; Qiagen) and sequenced using the inner primers on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). All sequences were compared with National Center for Biotechnology Information nucleotide collection sequences using the Blast nucleotide query interface (Altschul et al. 1990).

A second subsample of nymphal ticks was sent to Ibis Biosciences for pathogen screening using a highly sensitive PCR-electrospray ionization and mass spectrometry (ESI-MS) method (Ecker et al. 2008, Eshoo et al. 2010). Total nucleic acids were extracted, as described in Crowder et al. (2010). PCR was run using flagellin primers to target all *Borrelia* spp. (Crowder et al. 2010), with *gltA* primers for the *Coxiella* endosymbiont of *A. americanum* used as an internal positive control. Base pair composition data from ESI-MS of the PCR products were used to identify bacteria present.

Results

A total of 46 turkeys from six Tennessee counties was sampled in spring; 32 (70%) were infested with nymphal *A. americanum,* and one (2.2%) carried a single adult female *Ixodes brunneus.* An additional 14 turkeys from two counties were sampled in fall; of these, 10 (71%) were infested with larval *A. americanum* (Table 1).

Of the 60 turkeys, 35 (58%) were PCR positive for the *Borrelia* spp. IGS target. All positive samples, in both laboratories, had PCR bands ≈500 bp in length that were distinct from the >800-bp bands produced by the *B. burgdorferi*-positive controls. PCR amplicons from 30 of the positive samples were sequenced; 28 were identical to published sequences for North American *B. miyamotoi* (e.g., accession numbers AY363706.1 and AY531879.1). The other two samples had a single base pair polymorphism relative to the published sequences. *B. miyamotoi* was detected in samples from all seven counties, more frequently from tissue (30 of 59 samples, 51%) than from blood (11 of 42, 25%; Fisher exact test, $P = 0.015$). No statistically significant difference in prevalence was evident between juvenile and adult turkeys (53 versus 58%), nor between females and males (40 versus 60%) (Fisher exact tests; both $P > 0.50$).

In spring, PCR-positive and -negative turkeys had similar levels of infestation of *A. americanum* nymphs (birds in both groups carried a median of three nymphs, range 0–48). In fall, PCR-positive birds tended to carry more *A. americanum* larvae (median 50, range 0–436) than did the PCR-negative birds (median 10; range 0–124), but again this difference was not statistically significant (Wilcoxon rank sum test; $P = 0.12$). Neither nymphal *A. americanum* from the positive spring turkeys ($n = 42$; 2 per bird if available) nor the single *I. brunneus* tested positive for *Borrelia* spp. by PCR. High-sensitivity PCR/ESI-MS conducted on an additional 33 *A. americanum* nymphs at Ibis Biosciences similarly produced 100% negative results for *Borrelia* spp.

Discussion

Our findings represent the highest prevalence of *B. miyamotoi* yet reported in a wildlife population, and the first published report of this spirochete in birds. (*B. miyamotoi* has recently been detected at low levels in passerine bird-derived ticks in the Midwestern United States [S.A.H., unpublished data].) This spirochete was first described from *Ixodes persulcatus* in Japan, and is genetically distinct from both the LD-group spirochetes (*B. burgdorferi* sensu lato and sensu stricto) and the RF-group spirochetes such as *Borrelia anserina* and *Borrelia hermisii* (Fukunaga et al. 1995).

Whereas *B. burgdorferi* is a generalist (Tsao 2009), less is known about the host range of *B. miyamotoi.* At a Connecticut field site, where both *B. miyamotoi* and *B. burgdorferi* were present in white-footed mice (*Peromyscus leucopus*), *B. burgdorferi* bacteremia was associated with nymphal tick activity, whereas *B. miyamotoi* bacteremia was associated with larval activity (Barbour et al. 2009). Because *B. miyamotoi* uses a tick vector that feeds on a variety of vertebrate species, Barbour et al. (2009) speculated that there could be other eastern North American mammal reservoirs for *B. miyamotoi* besides *P. leucopus,* such as voles, rabbits, or ungulates, with the latter suggestion prompted by reports of *B. miyamotoi* in cattle (Richter and Matuschka 2006) and deer (Wodecka 2007).

Olsen (2007) reviewed *Borrelia* infections of wild birds, identifying only certain species within the *B. burgdorferi* sensu lato group as being significant. *B.*

anserina, a RF-group agent of avian spirochetosis, has not been reported in free-living wild birds (Olsen 2007). The only published report of a RF-group *Borrelia* in a wild bird is from an owl found dead of acute septicemia involving spirochetes closely related to *B. hermsii* (Fischer et al. 2009). Pande et al. (1961) reported a novel *Borrelia* sp. from a Himalayan jay *Garrulus lanceolatus*, noting that based on morphology the spirochete was "not *B. anserina*," but resembled a spirochete isolated from chickens in the United States (Harris 1930, Steinhaus and Hughes 1947). To our knowledge, these jay and chicken spirochetes were never further identified, and their relationship to *B. miyamotoi* and other *Borrelia* spp. has not been determined. Steinhaus and Hughes (1947) discovered the unknown *Borrelia* sp. by chance in chicken eggs that were to be used as a culture medium for *Borrelia.* Their finding raises the possibility of vertical transmission of these spirochetes among birds, which is a possible explanation for the high prevalence detected in the study in the apparent absence of an infected tick vector.

In a similar survey of wild turkeys in a LD-endemic region of California (Lane et al. 2006), only 1% of turkey blood samples tested PCR positive for *B. burgdorferi*, despite 34% of the turkeys being infested with nymphal *I. pacificus* in an area where nymphal infection prevalence was 15%. LD spirochetes are known to have differential survival because of blood complement of different hosts (Kurtenbach et al. 1998, 2002a, 2002b; Ullmann et al. 2003), and in vitro complement-protein assays undertaken during the Lane et al. (2006) study indicated that domestic turkey serum is moderately bacteriolytic for *B. burgdorferi.* Nevertheless, Jordan et al. (2009) reported significant prevalences of *B. burgdorferi* (14%) and *B. lonestari* (8%), but no *B. miyamotoi*, in wild turkeys from middle Tennessee.

The different results of Jordan et al. (2009) and our own study reflect, in part, different methods employed to detect *Borrelia* spp. Jordan et al. (2009) targeted the *Borrelia* flagellin gene (*fla*) and did not sequence the PCR products, instead using probe hybridization and size differentiation to identify species. *B. miyamotoi fla* might not have amplified in their assay because the forward and reverse outer primers used (Haynes et al. 2005) each contain a 1-bp polymorphism from *B. miyamotoi.* In addition, only probes for *B. lonestari* and *B. burgdorferi* were included in their study. In the IGS PCR assays we report in this work, products for *B. lonestari* (412 bp), *B. miyamotoi* (474 bp), and *B. burgdorferi* (812 bp) can be differentiated by size in gel electrophoresis (Bunikis et al. 2004a), and we confirmed our species identifications by sequence analysis. IGS primers have been used successfully to assay coinfections of *B. miyamotoi* and *B. burgdorferi* (Bunikis and Barbour 2005, Ullmann et al. 2005), although coinfections may be underestimated because the minority member in the mixture is sometimes obscured by interference or other PCR artifacts.

We investigated a confirmatory PCR on a second target using single sets of PCR primers in a nested assay. Several sets of primers were screened by blasting and aligning with the few sequences available for North American *B. miyamotoi* (Asian and European strains appear to be divergent). Nested PCR was performed using the the *glpQ* primers outlined in Bacon et al. (2004) and the specific primers for *B. miyamotoi* from Ullmann et al. (2005). We slightly modified a set of outer primers from Schwan et al. (2005) designed for relapsing fever *Borrelia flaB*, and paired it with modified primers from Fukanaga et al. (1996) for the American sequence of *flaB.* We had limited success with these primers, so future work on a confirmatory assay will be necessary.

In the eastern United States, the known vectors for *B. miyamotoi* and *B. burgdorfori* are *Ixodes* spp. ticks (Scoles et al. 2001, Bunikis and Barbour 2005, Hamer et al. 2010). The only *Ixodes* spp. tick that we collected from turkeys was a single adult *I. brunneus*, which was not infected with *Borrelia* spp. (previous attempts to isolate and identify *Borrelia* spp. from *I. brunneus* have similarly been unsuccessful; Kinsey et al. 2000, Goddard et al. 2003). Our study was limited to the spring and fall hunting seasons, and so we cannot rule out the possibility that *Ixodes* spp. ticks infest Tennessee turkeys at other times of the year. Nevertheless, previous studies indicate that *I. scapularis* has not been found on wild turkeys (Davidson and Wentworth 1992), and results of attempted experimental infestations of juvenile *I. scapularis* further indicate that wild turkeys are unlikely to host this tick (Ostfeld and Lewis 1999).

In contrast, the most abundant tick in our study area, *A. americanum*, is very commonly found infesting wild turkeys in the eastern United States (e.g., Kellogg et al. 1969, Kollars et al. 2000, Mock et al. 2001). This tick species does not transmit *B. burgdorferi* (Piesman and Sinsky 1988, Ryder et al. 1992) and has no known association with *B. miyamotoi*, despite numerous surveys having been undertaken for *Borrelia* spp. in *A. americanum* [see Paddock and Yabsley (2007) for a review]. *A. americanum* is a known host for *B. lonestari;* however, Jordan et al. (2009) found no *Borrelia* spp. DNA in 178 adult and 161 nymphal *A. americanum* collected from vegetation at their Tennessee study sites, and we found none in the 75 nymphs we tested from turkeys. As our sample size was small, this failure to detect is not inconsistent with previous estimates of ≈1% *B. lonestari* prevalence among *A. americanum* in Tennessee {Stegall-Faulk et al. 2003) and northern Georgia (Varela et al. 2004).

The extent to which ticks may be involved in the observed *B. miyamotoi* infection of turkeys remains uncertain. Unlike *B. burgdorferi*, *B. miyamotoi* can be transmitted transovarially from infected female ticks to their offspring, which could maintain these spirochetes in wildlife host populations even if infected tick-host interactions are infreqent (Barbour et al. 2009). Vertical transmission in hosts is also a possibility. The transmission pathways involved in *B. miyamotoi* infection in Tennessee wild turkey require further study. Meanwhile, these findings emphasize that accurate LD risk assessment in the eastern United States

requires a more detailed understanding of *Borrelia* species diversity in the region.

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References Cited

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Armstrong, P. M., S. M. Rich, R. D. Smith, D. L. Hartl, A. Spielman, and S. R. Telford. 1996. A new *Borrelia* infecting lone star ticks. Lancet 347: 67–68.

Bacon, R. M., M. A. Pilgard, B. J. Johnson, S. J. Raffel, and T. G. Schwan. 2004. Glycerophosphodiester phosphodiesterase gene (glpQ) of *Borrelia lonestari* identified as a target for differentiating *Borrelia* species associated with hard ticks (Acari: Ixodidae). J. Clin. Microbiol. 42: 2326–2328.

Bacon, R. M., K. J. Kugeler, and P. S. Mead. 2008. Surveillance for Lyme disease: United States, 1992–2006. MMWR Surveill. Summ. 57: 1–9.

Barbour, A. G., and S. F. Hayes. 1986. Biology of *Borrelia* species. Microbiol. Rev. 50: 381–400.

Barbour, A. G., G. O. Maupin, G. J. Teltow, C. J. Carter, and J. Piesman. 1996. Identification of an uncultivable *Borrelia* species in the hard tick *Amblyomma americanum:* possible agent of a Lyme disease-like illness. J. Infect. Dis. 173: 403–409.

Barbour, A. G., J. Bunikis, B. Travinsky, A. G. Hoen, M. A. Diuk-Wasser, D. Fish, and J. I. Tsao. 2009. Niche partitioning of *Borrelia burgdorferi* and *Borrelia miyamotoi* in the same tick vector and mammalian reservoir species. Am. J. Trop. Med. Hyg. 81: 1120–1131.

Beati, L., and J. E. Keirans. 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J. Parasitol. 87: 32–48.

Bunikis, J., and A. G. Barbour. 2005. Third *Borrelia* species in white-footed mice. Emerg. Infect. Dis. 11: 1150–1151.

Bunikis, J., J. Tsao, U. Garpmo, J. Berglund, D. Fish, and A. G. Barbour. 2004a. Typing of *Borrelia* relapsing fever group strains. Emerg. Infect. Dis. 10: 1661–1664.

Bunikis, J., U. Garpmo, J. Tsao, J. Berglund, D. Fish, and A. G. Barbour. 2004b. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiology 150: 1741–1755.

Crowder, C. D., M. A. Rounds, C. A. Phillipson, J. M. Picuri, H. E. Matthews, J. Halverson, S. E. Schutzer, D. J. Ecker, and M. W. Eshoo. 2010. Extraction of total nucleic acids from ticks for the detection of bacterial and viral pathogens. J. Med. Entomol. 47: 89–94.

Davidson, W. R., and E. J. Wentworth. 1992. Population influences: diseases and parasites, pp. 101–118. *In* J. G. Dickson (ed.), The Wild Turkey: Biology and Management. Stackpole Books, Mechanicsburg, PA.

Ecker, D. J., R. Sampath, C. Massire, L. B. Blyn, T. A. Hall, M. W. Eshoo, and S. A. Hofstadler. 2008. Ibis T5000: a universal biosensor approach for microbiology. Nat. Rev. Microbiol. 6: 553–558.

Eshoo, M. W., C. D. Crowder, H. Li, H. E. Matthews, S. Meng, S. E. Sefers, R. Sampath, C. W. Stratton, L. B. Blyn, D. J. Ecker, and Y. W. Tang. 2010. Detection and identification of *Ehrlichia* species in blood by use of PCR and electrospray ionization mass spectrometry. J. Clin. Microbiol. 48: 472–478.

Fischer, R. J., T. L. Johnson, S. J. Raffel, and T. G. Schwan. 2009. Identical strains of *Borrelia hermsii* in mammal and bird. Emerg. Infect. Dis. 15: 2064–2066.

Fraenkel, C. J., U. Garpmo, and J. Berglund. 2002. Determination of novel *Borrelia* genospecies in Swedish *Ixodes ricinus* ticks. J. Clin. Microbiol. 40: 3308–3312.

Fukunaga, M., Y. Takahashi, Y. Tsuruta, O. Matsushita, D. Ralph, M. McClelland, and M. Nakao. 1995. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus,* the vector for Lyme disease in Japan. Int. J. Syst. Bacteriol. 45: 804–810.

Goddard, J., J. W. Sumner, W. L. Nicholson, C. D. Paddock, J. Shen, and J. Piesman. 2003. Survey of ticks collected in Mississippi for *Rickettsia, Ehrlichia,* and *Borrelia* species. J. Vector Ecol. 28: 184–189.

Hamer, S. A., J. I. Tsao, E. D. Walker, and G. J. Hickling. 2010. Invasion of the Lyme disease vector *Ixodes scapularis:* implications for *Borrelia burgdorferi* endemicity. Ecohealth (in press).

Harris, M. B. 1930. A study of spirochetes in chickens with special reference to those of the intestinal tract. Am. J. Hyg. 12: 537–570.

Haynes, J. M., L. P. Prescott, R. L. Seipelt, and S. M. Wright. 2005. Detection *of Borrelia burgdorferi* sequences in a biopsy from a Tennessee patient. J. Tennessee Acad. Sci. 80: 57–59.

Jordan, B. E., K. R. Onks, S. W. Hamilton, S. E. Hayslette, and S. M. Wright. 2009. Detection of *Borrelia burgdorferi* and *Borrelia lonestari* in birds in Tennessee. J. Med. Entomol. 46: 131–138.

Kellogg, F. E., A. K. Prestwood, R. R. Gerrish, and G. L. Doster. 1969. Wild turkey ectoparasites collected in the southeastern United States. J. Med. Entomol. 6: 329–330.

Kinsey, A. A., L. A. Durden, and J. H. Oliver, Jr. 2000. Tick infestations of birds in coastal Georgia and Alabama. J. Parasitol. 86: 251–254.

Kollars, T. M., Jr., J. H. Oliver, Jr., L. A. Durden, and P. G. Kollars. 2000. Host association and seasonal activity of *Amblyomma americanum* (Acari: Ixodidae) in Missouri. J. Parasitol. 86: 1156–1159.

Kurtenbach, K., H. S. Sewell, N. H. Ogden, S. E. Randolph, and P. A. Nuttall. 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. 66: 1248–1251.

Kurtenbach, K., S. De Michelis, S. Etti, S. M. Schafer, H. S. Sewell, V. Brade, and P. Kraiczy. 2002a. Host association of *Borrelia burgdorferi* sensu lato: the key role of host complement. Trends Microbiol. 10: 74–79.

Kurtenbach, K., S. M. Schafer, H. S. Sewell, M. Peacey, A. Hoodless, P. A. Nuttall, and S. E. Randolph. 2002b. Differential survival of Lyme borreliosis spirochetes in ticks that feed on birds. Infect. Immun. 70: 5893–5895.

Lane, R. S., T. F. Kucera, R. H. Barrett, J. Mun, C. Wu, and V. S. Smith. 2006. Wild turkey (*Meleagris gallopavo*) as a host of ixodid ticks, lice, and Lyme disease spirochetes

(*Borrelia burgdorferi* sensu lato) in California state parks. J. Wildl. Dis. 42: 759–771.

Mock, D. E., R. D. Applegate, and L. B. Fox. 2001. Preliminary survey of ticks (Acari: Ixodidae) parasitizing wild turkeys (Aves: Phasianidae) in eastern Kansas. J. Med. Entomol. 38: 118–121.

Olsen, B. 2007. Borrelia, pp. 341–351. *In* N. J. Thomas, D. B. Hunter, and C. T. Atkinson (eds.), Infectious Diseases of Wild Birds. Wiley-Blackwell, Ames, IA.

Ostfeld, R. S., and D. N. Lewis. 1999. Experimental studies of interactions between wild turkeys and black-legged ticks. J. Vector Ecol. 24: 182–186.

Paddock, C. D., and M. J. Yabsley. 2007. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. Curr. Top. Microbiol. Immunol. 315: 289–324.

Pande, P. G., R. R. Shukla, and P. C. Sekariah. 1961. A record of *Borrelia* from the Black-Throated Himalayan Jay, *Garrulus lanceolatus* Vigors, 1832: a posthumous record. J. Parasitol. 47: 692.

Piesman, J., and R. J. Sinsky. 1988. Ability to *Ixodes scapularis, Dermacentor variabilis,* and *Amblyomma americanum* (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). J. Med. Entomol. 25: 336–339.

Richter, D., and F. R. Matuschka. 2006. Modulatory effect of cattle on risk for Lyme disease. Emerg. Infect. Dis. 12: 1919–1923.

Rosen, M. E. 2009. Investigating the maintenance of the Lyme disease pathogen, *Borrelia burgdorferi,* and its vector, *Ixodes scapularis,* in Tennessee. M.S. thesis, Institute of Agriculture, University of Tennessee, Knoxville.

Ryder, J. W., R. R. Pinger, and T. Glancy. 1992. Inability of *Ixodes cookei* and *Amblyomma americanum* nymphs (Acari: Ixodidae) to transmit *Borrelia burgdorferi.* J. Med. Entomol. 29: 525–530.

Schwan, T. G., S. J. Raffel, M. E. Schrumpf, P. F. Policastro, J. A. Rawlings, R. S. Lane, E. B. Breitschwerdt, and S. F. Porcella. 2005. Phylogenetic analysis of the spirochetes *Borrelia parkeri* and *Borrelia turicatae* and the potential for tick-borne relapsing fever in Florida. J. Clin. Microbiol. 43: 3851–3859.

Scoles, G. A., M. Papero, L. Beati, and D. Fish. 2001. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 1: 21–34.

Sonenshine, D. E. 1979. Ticks of Virginia (Acari: Metastigmata). Research Division, Virginia Polytechnic Institute, Blacksburg, VA.

Stegall-Faulk, T., D. C. Clark, and S. M. Wright. 2003. Detection of *Borrelia lonestari* in *Amblyomma americanum* (Acari: Ixodidae) from Tennessee. J. Med. Entomol. 40: 100–102.

Steinhaus, E. A., and E. M. Hughes. 1947. Isolation of an unidentified spirochete from hen's eggs after inoculation with liver tissue from hens. Public Health Rep. 62: 309–311.

Tsao, J. I. 2009. Reviewing molecular adaptations of Lyme borreliosis spirochetes in the context of reproductive fitness in natural transmission cycles. Vet. Res. 40: 36.

Ullmann, A. J., R. S. Lane, K. Kurtenbach, M. Miller, M. E. Schriefer, N. Zeldner, and J. Piesman. 2003. Bacteriolytic activity of selected vertebrate sera for *Borrelia burgdorferi* sensu stricto and *Borrelia bissettii.* J. Parasitol. 89: 1256–1257.

Ullmann, A. J., E. S. Gabitzsch, T. L. Schulze, N. S. Zeidner, and J. Piesman. 2005. Three multiplex assays for detection of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* sensu lato in field-collected *Ixodes* nymphs in North America. J. Med. Entomol. 42: 1057–1062.

Varela, A. S., V. A. Moore, and S. E. Little. 2004. Disease agents in *Amblyomma americanum* from northeastern Georgia. J. Med. Entomol. 41: 753–759.

Wodecka, B. 2007. [Significance of red deer (*Cervus elaphus*) in the ecology of *Borrelia burgdorferi* sensu lato]. Wiad. Parazytol. 53: 231–237.

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