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Prevalence of *Borrelia burgdorferi*, *Bartonella* spp., *Babesia microti*, and *Anaplasma phagocytophila* in *Ixodes scapularis* Ticks Collected in Northern New Jersey

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PCR analysis of *Ixodes scapularis* ticks collected in New Jersey identified infections with *Borrelia burgdorferi* (33.6%), *Babesia microti* (8.4%), *Anaplasma phagocytophila* (1.9%), and *Bartonella* spp. (34.5%). The *I. scapularis* tick is a potential pathogen vector that can cause coinfection and contribute to the variety of clinical responses noted in some tick-borne disease patients.

Lyme disease (LD) has been characterized as a multisystem disease caused by the spirochete *Borrelia burgdorferi* (3). The Centers for Disease Control and Prevention reported 17,730 domestic cases of LD in 2000, making it the most common vector-borne disease in the United States (5). In most patients, antibiotic treatment with doxycycline or amoxicillin has been proven to be a highly effective mode of treatment for acute and late stages of LD (28). However, a subset of patients exhibits persistent symptoms regardless of antibiotic therapeutic intervention.

The primary vertebrate reservoir for *B. burgdorferi* in the northeastern United States has been identified as the white-footed mouse, *Peromyscus leucopus*. Both larval and nymphal stages of *Ixodes scapularis* ticks mainly feed on *P. leucopus* and can infect human hosts with *B. burgdorferi* (1). Other pathogens, including *Babesia microti* and *Anaplasma phagocytophila*, have also been identified in *I. scapularis* ticks, and cotransmission with *B. burgdorferi* has been documented (17, 18, 19).

The present study examined the prevalence of four pathogens in *I. scapularis* that could potentially be transmitted to humans. Coinfection with two or more of these organisms may complicate LD prognosis. For example, simultaneous LD and babesiosis correlated with a more severe clinical progression than either condition alone (15). *I. scapularis* ticks were collected by the tick sweep method (4) from February through July 2001, primarily in Union County, N.J., and were identified by standard taxonomic keys (9, 12, 14). Individual ticks were placed in a microcentrifuge tube containing 470 μl of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA), 25 μl of 10% sodium dodecyl sulfate, and 12 μl of DNase-free proteinase K (10 mg/ml). A handheld motorized pestle (Kontes, Vineland, N.J.) was used to homogenize each tick lysate. DNA was extracted with phenol-chloroform and recovered by ethanol precipitation. Primers for amplification of genomic DNA were synthesized by Research Genetics (Huntsville, Ala.) and are listed in Table 1. The conditions for PCR amplification of *B. burgdorferi*, *B. microti*, *Bartonella* spp., and *A. phagocytophila* were as previously described (7, 8, 13, 20). PCR products were analyzed by electrophoresis through a 1% agarose gel containing 0.5-μg/ml ethidium bromide and UV analysis utilizing a Photodocumentation System (Fisher Biotech, Pittsburgh, Pa.). The identity of selected amplicons was confirmed by independent DNA sequencing (SeqWright, Houston, Tex.). Positive controls were included in each PCR experiment and consisted of genomic DNA extracted from pathogens purchased from the American Type Culture Collection (Table 1). Negative controls consisted of the substitution of pyrogen-free water for

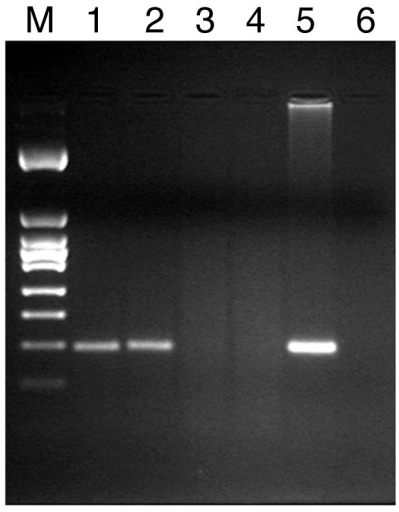

FIG. 1. Molecular detection of *I. scapularis* by amplification of 16S rDNA. Lanes: 1, 2, and 5, DNA extracted from *I. scapularis*; 3 and 4, DNA extracted from *D. variabilis*; 6, negative water control; M, molecular weight ladder developed in house with bands in decreasing order of size of 2,743, 980, 752, 650 (double intensity), 552, 395, 300, 198, and 104 bp.

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TABLE 1. Oligonucleotide primers utilized for species-specific pathogen and *I. scapularis* amplification

Primer	Oligonucleotide primer sequence	Target gene and reference	Product size (bp)
LY1 (F)	5′-GAAATGGCTAAAGTAAGCGGAATTGTAC-3′	*B. burgdorferi ly1* (ATCC 35210) (16)	231
LY2 (R)	5′-CAGAAATTCTGTAAACTAATCCCACC-3′		
MRL-7 (F)	5′-GTTTCAGTAGATTTGCCTGG-3′	*B. burgdorferi ospA* (7)	569
MRL-8 (R)	5′-GCCTGAATTCCAAGCTGCAG-3′		
HGE1F	5′-GGATTATTCTTTATAGCTTGCT-3′	*A. phagocytophila* 16S rDNA (ATCC CRL-10679) (2)	920
HGE3R	5′-TTCCGTTAAGAAGGATCTAATCTC-3′		
P24E	5′-GGAATTCCCTCCTTCAGTTAGGCTGG-3′	*B. henselae* 16S rDNA (ATCC 49882) (20)	279
P12B	5′-CGGGATCCCGAGATGGCTTTTGGAGATTA-3′		
Bab1	5′-CTTAGTATAAGCTTTTATACAGC-3′	*B. microti* (ATCC 30222) (25)	238
Bab4	5′-ATAGGTCAGAAACTTGAATGATACA-3′		
IXO-16S-F	5′-TAAACAATTAAAAGCTTTCTT-3′	*I. scapularis* 16S rDNA (this study)[a]	215
IXO-16S-R	5′-AATCGCTAAAAACGGAACTTA-3′		

[a] Primers derived from data reported previously (3a).

DNA. Precautions against contamination were maintained as previously described (13).

As another species of tick, *Dermacentor variabilis*, is also very common in the geographic region studied, molecular techniques were adopted to verify all ticks as *I. scapularis* (Fig. 1). The primers for amplification were IXO-16S-F and IXO-16S-R (Table 1). The PCR conditions were denaturation at 94°C for 3 min followed by 35 cycles of 94°C for 3 min, 58°C for 1 min, and 72°C for 1 min. Each reaction was concluded with a 10-min final extension step at 72°C in a T3 Thermocycler (Biometra, Göttingen, Germany). Amplification products were resolved through a 2% agarose gel containing 0.5-μg/ml ethidium bromide and visualized on a MultiGenius gel documentation and analysis system (Syngene, Frederick, Md.). The specificity of the primers was verified by amplifying known DNA control extracts from *I. scapularis* and *D. variabilis*. Through this technique, 16 ticks were excluded from the sampling pool due to nonamplification. This control also confirms the validity of the results in those samples in which no pathogens were detected. From the PCR analyses, it was evident that at least one of the four pathogens was present in 45.8% (49 of 107) of the ticks (Table 2) Also, 15 of 107 ticks (14.0%) contained more than one pathogen. The most common combination was *B. burgdorferi* and *Bartonella* spp.

Transmission of *B. burgdorferi* to humans causing LD is just one of several possible outcomes from a tick bite. The environment in the tick is suitable for bacterial diversity, and up to 10 clones of *B. burgdorferi* as well as *B. microti* and *A. phagocytophilum* can be simultaneously isolated from a single *I. scapularis* host (17, 19, 23). In Table 3, the prevalence of the pathogens studied was found to be in agreement with previous reports detailing pathogen detection by PCR methodologies in the northeastern United States, with the exception of the human granulocyltic ehrlichiosis agent, which was at a lower prevalence than all except in northwestern Pennsylvania (11).

This is the first report to assay for *Bartonella* spp. in field-collected *I. scapularis* ticks. Previously, we published a clinical case study presenting molecular and serological diagnostic evidence of patients coinfected with *B. burgdorferi* and *Bartonella henselae* (13). For two patients, ticks found to contain *B. burgdorferi* and *B. henselae* by PCR and identified as *I. scapularis*

TABLE 2. Identification of *I. scapularis* ticks infected with *B. burgdorferi, B. microti, A. phagocytophila*, or *B. henselae*

Pathogen(s)	No. (%) of ticks PCR positive[a]
Single infections	
B. burgdorferi	36 (33.6)
B. microti	9 (8.4)
A. phagocytophila	2 (1.9)
Bartonella spp.	37 (34.5)
None	58 (54.2)
B. burgdorferi and *Bartonella* spp. coinfections (includes those listed above)	
B. burgdorferi and *Bartonella* spp.[b]	9 (8.4)
B. burgdorferi and *B. microti*	2 (1.9)
Bartonella spp. and *B. microti*	1 (0.9)
Bartonella spp. and *A. phagocytophila*	1 (0.9)
B. burgdorferi, Bartonella spp., and *A. phagocytophila*	1 (0.9)
Bartonella spp., *B. microti*, and *A. phagocytophila*	1 (0.9)

[a] Number of ticks positive for each pathogen out of 107 total ticks (confirmed as *I. scapularis* by the procedure depicted in Fig. 1).
[b] Coinfection data overlap with the single-pathogen prevalence percentages.

TABLE 3. Reported infection rates of *B. burgdorferi, A. phagocytophila*; and *B. microti* in the northeastern United States

Location (reference)	% of infection with:		
	B. burgdorferi	*A. phagocytophila*	*B. microti*
Westchester County, N.Y. (26)	52	53	NT[a]
Nantucket Island, Mass. (27)	36	11	9
Hunterdon County, N.J. (11)	43	17	5
Northwest Pennsylvania (11)	61.6	1.9	NT
Southeast Pennsylvania	13	39.8	NT
Union County, N.J. (this study)	31	1.6	8

[a] NT, not tested.

were found in their households, one of which was removed from a household cat. Our report was further reinforced by a more recent publication in which both pathogens were detected by PCR in the cerebrospinal fluid of two patients with symptoms suggestive of neuroborreliosis (22). Although the primers in this study were originally selected for the species-specific amplification of *B. henselae*, this region of the *Bartonella* 16S ribosomal DNA (rDNA) gene is highly conserved among many species within the genus (2, 21). For example, Multalin alignment analysis revealed that only 5 of 279 nucleotides differ between the *B. henselae* and *B. quintana* amplicons, only 2 of which are situated between the oligonucleotide amplification primers (10, 21). Further investigations should seek to amplify more divergent regions of the *Bartonella* genus that can be utilized for species-specific identification. Members of the *Bartonella* genus have also been found in ticks and other insects. For example, *B. henselae* was detected by PCR in 4 of 271 *Ixodes ricinus* ticks removed from humans in Belluno Province, Italy (24), and *Bartonella* species were also detected by PCR in 29 of 151 *Ixodes pacificus* ticks collected in Santa Clara County, Calif. (6).

As treatment for *Bartonella* infections varies from that prescribed for LD patients, physicians should add *Bartonella* infections to the list of possible coinfection agents when evaluating patients in regions of tick endemicity, as single- and multiple-pathogen transmission can complicate clinical presentations. Future studies need to clarify that the *Bartonella* spp. can be passed in culturable form from vector to host and to identify which specific species of *Bartonella* are present.

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REFERENCES

1. **Anderson, J. F.** 1989. Ecology of Lyme disease. Conn. Med. **53:**343–346.
2. **Bergmans, A. M., J. W. Groothedde, J. F. Schellekens, J. D. van Embden, J. M. Ossewaarde, and L. M. Schouls.** 1995. Etiology of cat scratch disease: comparison of polymerase chain reaction detection of *Bartonella* (formerly *Rochalimaea*) and *Afipia felis* DNA with serology and skin tests. J. Infect. Dis. **171:**916–923.
3. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216:** 1317–1319.

3a. **Caporale, D. A., S. M. Rich, A. Spielman, S. R. Telford III, and T. D. Kocher.** 1995. Discriminating between *Ixodes* ticks by means of mitochondrial DNA sequences. Mol. Phylogenet. Evol. **4:**361–365.

4. **Carroll, J. F., and E. T. Schmidtmann.** 1992. Tick sweep: modification of the tick drag-flag method for sampling nymphs of the deer tick (Acari: Ixodidae). J. Med. Entomol. **29:**352–355.
5. **Centers for Disease Control and Prevention.** 2002. Lyme disease—United States, 2000. Morb. Mortal. Wkly. Rep. **51:**29–31.
6. **Chang, C. C., B. B. Chomel, R. W. Kasten, V. Romano, and N. Tietze.** 2001. Molecular evidence of *Bartonella* spp. in questing adult *Ixodes pacificus* ticks in California. J. Clin. Microbiol. **39:**1221–1226.
7. **Chu, F. K.** 1998. Rapid and sensitive PCR-based detection and differentiation of aetiologic agents of human granulocytotropic and monocytotropic ehrlichiosis. Mol. Cell Probes **12:**93–99.
8. **Cogswell, F. B., C. E. Bantar, T. G. Hughes, Y. Gu, and M. T. Philipp.** 1996. Host DNA can interfere with detection of *Borrelia burgdorferi* in skin biopsy specimens by PCR. J. Clin. Microbiol. **34:**980–982.
9. **Cooley, R. A., and G. Kohls.** 1945. The genus *Ixodes* in North America. National Institutes of Health, Washington, D.C.
10. **Corpet, F.** 1988. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. **16:**10881–10890.
11. **Courtney, J. W., R. L. Dryden, J. Montgomery, B. S. Schneider, G. Smith, and R. F. Massung.** 2003. Molecular characterization of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in *Ixodes scapularis* ticks from Pennsylvania. J. Clin. Microbiol. **41:**1569–1573.
12. **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. Entomological Society of America., Lanham, Md.
13. **Eskow, E., R. V. Rao, and E. Mordechai.** 2001. Concurrent infection of the central nervous system by *Borrelia burgdorferi* and *Bartonella henselae*: evidence for a novel tick-borne disease complex. Arch. Neurol. **58:**1357–1363.
14. **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. J. Med. Entomol. Suppl. **2:**1–149.
15. **Krause, P. J., S. R. Telford III, A. Spielman, V. Sikand, R. Ryan, D. Christianson, G. Burke, P. Brassard, R. Pollack, J. Peck, and D. H. Persing.** 1996. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. JAMA **275:**1657–1660.
16. **Liebling, M. R., M. J. Nishio, A. Rodriguez, L. H. Sigal, T. Jin, and J. S. Louie.** 1993. The polymerase chain reaction for the detection of *Borellia burgdorferi* in human body fluids. Arthritis Rheum. **36:**665–675.
17. **Magnarelli, L. A., J. S. Dumler, J. F. Anderson, R. C. Johnson, and E. Fikrig.** 1995. Coexistence of antibodies to tick-borne pathogens of babesiosis, ehrlichiosis, and Lyme borreliosis in human sera. J. Clin. Microbiol. **33:**3054–3057.
18. **McQuiston, J. H., J. E. Childs, M. E. Chamberland, and E. Tabor.** 2000. Transmission of tick-borne agents of disease by blood transfusion: a review of known and potential risks in the United States. Transfusion **40:**274–284.
19. **Mitchell, P. D., K. D. Reed, and J. M. Hofkes.** 1996. Immunoserologic evidence of coinfection with *Borrelia burgdorferi*, *Babesia microti*, and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. J. Clin. Microbiol. **34:**724–727.
20. **Persing, D. H., D. Mathiesen, W. F. Marshall, S. R. Telford, A. Spielman, J. W. Thomford, and P. A. Conrad.** 1992. Detection of *Babesia microti* by polymerase chain reaction. J. Clin. Microbiol. **30:**2097–2103.
21. **Pitulle, C., C. Strehse, J. W. Brown, and E. B. Breitschwerdt.** 2002. Investigation of the phylogenetic relationships within the genus Bartonella based on comparative sequence analysis of the rnpB gene, 16S rDNA and 23S rDNA. Int. J. Syst. Evol. Microbiol. **52:**2075–2080.
22. **Podsiadly, E., T. Chmielewski, and S. Tylewska-Wierzbanowska.** 2003. *Bartonella henselae* and *Borrelia burgdorferi* infections of the central nervous system. Ann. N. Y. Acad. Sci. **990:**404–406.
23. **Qiu, W. G., D. E. Dykhuizen, M. S. Acosta, and B. J. Luft.** 2002. Geographic uniformity of the Lyme disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the northeastern United States. Genetics **160:**833–849.
24. **Sanogo, Y. O., Z. Zeaiter, G. Caruso, F. Merola, S. Shpynov, P. Brouqui, and D. Raoult.** 2003. *Bartonella henselae* in *Ixodes ricinus* ticks (Acari: *Ixodida*) removed from humans, Belluno province, Italy. Emerg. Infect. Dis. **9:**329–332.
25. **Schwartz, I., D. Fish, and T. J. Daniels.** 1997. Prevalence of the rickettsial agent of human granulocytic ehrlichiosis in ticks from a hyperendemic focus of Lyme disease. N. Engl. J. Med. **337:**49–50.
26. **Telford, S. R., III, J. E. Dawson, P. Katavolos, C. K. Warner, C. P. Kolbert, and D. H. Persing.** 1996. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. Proc. Natl. Acad. Sci. USA **93:**6209–6214.
27. **Varde, S., J. Beckley, and I. Schwartz.** 1998. Prevalence of tick-borne pathogens in *Ixodes scapularis* in a rural New Jersey county. Emerg. Infect. Dis. **4:**97–99.
28. **Wormser, G. P., R. B. Nadelman, R. J. Dattwyler, D. T. Dennis, E. D. Shapiro, A. C. Steere, T. J. Rush, D. W. Rahn, P. K. Coyle, D. H. Persing, D. Fish, B. J. Luft, et al.** 2000. Practice guidelines for the treatment of Lyme disease. Clin. Infect. Dis. **31**(Suppl. 1)**:**1–14.