

Detection of *Anaplasma phagocytophilum* and *Babesia odocoilei* DNA in *Ixodes scapularis* (Acari: Ixodidae) Collected in Indiana

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ABSTRACT The blacklegged tick, *Ixodes scapularis* Say, first reported in Indiana in 1987, has now been detected in more than half of Indiana's counties. The first case of human granulocytic ehrlichiosis (human anaplasmosis) in Indiana was reported in 2002. We now report the detection of *Anaplasma phagocytophilum* and *Babesia odocoilei* (Emerson and Wright 1968) in *I. scapularis* ticks collected in northern Indiana. Using polymerase chain reaction analysis, 41 of 193 adult ticks (21.2%) collected from deer were positive for *A. phagocytophilum*, and 22 (11.4%) were positive for *Babesia* sp. Restriction fragment analysis of 12, and sequencing of another five of the amplified products identified these parasites as *B. odocoilei*. Five ticks (2.6%) were coinfecting. Eight of 68 questing adult ticks (11.8%) were positive for *A. phagocytophilum*; seven (10.3%) were positive for *Babesia* sp. Six of the latter seven positive samples were determined to be *B. odocoilei* by restriction fragment analysis and sequencing of two samples. None of 39 pools of nymphs was positive for *Babesia* sp. Three of 15 ticks (20%) collected from a dog were positive for *A. phagocytophilum* and three ticks (20%) were positive for *Babesia* sp. One was confirmed as *B. odocoilei*. One tick was coinfecting. This is the first report of the presence of these two agents in ticks in Indiana.

KEY WORDS *Ixodes scapularis*, *Anaplasma phagocytophilum*, *Babesia odocoilei*, polymerase chain reaction, blacklegged tick

THE BLACKLEGGED TICK, *Ixodes scapularis* Say, harbors and transmits a variety of human and animal pathogens, including *Borrelia burgdorferi*, the Lyme disease spirochete (Burgdorfer et al. 1982, Johnson et al. 1984); *Anaplasma phagocytophilum*, the newly named but previously recognized agent of human granulocytic ehrlichiosis now referred to as human anaplasmosis (Chen et al. 1994, Dumler et al. 2001); and *Babesia microti*, the agent of human babesiosis in the United States (Spielman et al. 1985, Herwaldt et al. 1995). The geographic distributions and prevalence rates of the diseases attributed to these agents, namely, Lyme disease, human anaplasmosis, and human babesiosis, respectively, coincide with the distribution of *I. scapularis* in the northeastern and north central United States (Piesman et al. 1987, Adelson et al. 2004, Holman et al. 2004). *I. scapularis* also has been found to transmit *Babesia odocoilei*, a tick-borne hemoprotozoan parasite that causes babesiosis in deer and other cervids, and the distribution of *B. odocoilei* seems to coincide with that of *I. scapularis* (Waldrup et al. 1990; Emerson and Wright 1968, 1970; Holman et al. 2000).

Coinfection and cotransmission of more than one pathogen by *I. scapularis* have been reported by several investigators (Piesman et al. 1987, Adelson et al. 2004, Holman et al. 2004). In areas where infection rates of these agents are high, one would expect to find ticks infected with more than one species of microbe.

As *I. scapularis* and the agents they transmit become better established in the north central states, the risk of human infections with one or more of these microbes will increase. For example, *I. scapularis* was unknown in Indiana before 1987, but by 2001 it had been recorded from 56 of Indiana's 92 counties (Pinger and Glancy 1989; Pinger et al. 1991, 1996; R.R.P., unpublished report). In Pulaski County in northern Indiana where ticks for this study were collected, *I. scapularis* has become well established; adult ticks were present on 53% of harvested deer examined during a special hunt carried out in 2003 (R.R.P., unpublished data). We reported for the first time in 1996 the presence of *B. burgdorferi* in 31% of *I. scapularis* adults sampled in Jasper County, in northern Indiana (Pinger et al. 1996). Since that time, we have continued to monitor and test *I. scapularis* for this pathogen. Within the past year, we have observed a prevalence of infection as high as 55% in Pulaski County, a county adjacent to Jasper County (R.R.P., unpublished data).

The first case of human anaplasmosis in Indiana was reported in 2002, and another case was reported in

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2003 (James Howell, personal communication). We report here the detection of *A. phagocytophilum* and *B. odocoilei* in *I. scapularis* collected in Pulaski County. This report is part of a larger study to investigate the co-occurrence of multiple microbes within individual ticks.

Materials and Methods

Sample Collection and DNA Extraction. Adult *I. scapularis* were collected during October–November 2003 at Tippecanoe River State Park (41° 6' 24.8" N, 086° 34' 58.9" W) in Pulaski County, Indiana. Unfed ticks were collected by dragging a 1-m² cloth along deer trails. Also, feeding and fed adult ticks were collected from freshly killed white-tailed deer, *Odocoileus virginianus* (Zimmermann), that were brought to a deer checking station located in the same park. Ticks were removed from a dog that lived on the premises. *I. scapularis* nymphs were collected by dragging at the same site during June 2004. The ticks were taken to the laboratory, surface rinsed with 95% ethanol, and stored at –80°C. Adults were stored individually; nymphs were stored in pools of three. DNA was extracted from adult ticks using a Genomic DNA purification kit (Gentra Systems, Minneapolis, MN). DNA extraction from nymphs was done using a DNeasy tissue kit (QIAGEN, Valencia, CA). Ticks were ground with Teflon pestles in 1.7-ml microcentrifuge tubes in the presence of liquid nitrogen. The DNA extraction protocol for mouse tail was followed as described in each kit. Engorged female ticks of ≈30 mg or greater were quickly frozen with liquid nitrogen, ground to fine powder with a mortar and pestle, and DNA was isolated following the CTAB-DNA extraction method described by Burket et al. 1998.

Polymerase Chain Reaction Analysis for *A. phagocytophilum*. A nested PCR assay specifically targeting a 546-bp fragment of the 16S rRNA gene was performed on adults and nymphs according to Massung et al. (1998). PCR amplifications were performed in an Eppendorf Mastercycler gradient thermal cycler (Eppendorf, Westbury, NY). The cycling conditions were modified to a touchdown program that was the same for both primary and nested cycles. After an initial denaturation at 95°C for 1 min, 35 cycles of the following were performed: denaturation at 94°C for 15 s; annealing for 15 s at 68°C (cycles 1–3), 64°C (cycles 4–6), 60°C (cycles 7–9), and 56°C (cycles 10–35); and extension at 72°C for 20 s. Additionally, a final extension at 72°C for 5 min was performed. Polymerase chain reaction (PCR) products used for subsequent nested PCR or for agarose gel electrophoresis visualization were maintained at 4°C or at –20°C (depending upon whether the procedure was performed immediately or later).

DNA Sequencing. After electrophoresis, PCR products were sliced from the gel and cleaned using the QIAquick gel extraction kit (QIAGEN). For some samples, aliquots of the cleaned products were sequenced for both strands at Indiana University (Indiana Mo-

lecular Biology Institute) or sent to Davis facilities (Davis Sequencing, Davis, CA).

Polymerase Chain Reaction Analysis for *Babesia* sp. A PCR assay using the PIRO-A/B primers and targeting either a 408-bp (*B. odocoilei*) or a 437-bp (*B. microti*) fragment of the 18S rRNA gene from *Babesia* sp. (Armstrong et al. 1998) was performed on adults and nymphs by using a touchdown protocol. After an initial denaturation at 95°C for 1 min, 35 cycles of the following were performed: denaturation at 94°C for 15 s; annealing for 15 s at 68°C (cycles 1–4), 64°C (cycles 5–8), and 60°C (cycles 9–35); and extension at 72°C for 20 s. Additionally, a final extension at 72°C for 5 min was performed. Again, amplicons were maintained at 4°C or at –20°C until they were separated by agarose gel electrophoresis, stained with ethidium bromide, and examined by UV transillumination. Electrophoresed PCR products were sliced from gels and purified using the QIAquick kit as mentioned above. Some aliquots of cleaned products were sequenced or used for further restriction enzyme assays. The results were analyzed with Chromas 2.23 shareware (Cornell University, Ithaca, NY). A Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information) analysis was performed to match the PCR product sequences with known sequences from the GenBank database (Altschul et al. 1990).

Restriction Enzyme Assay. To distinguish between *B. microti* and *B. odocoilei*, 10 µl of cleaned PCR products were subjected to digestion with either *Hinf*I or *Bst*EII (Armstrong et al. 1998). The enzyme *Bst*EII digests the PIRO-A/B amplicon to generate 78- and 330-bp fragments in *B. odocoilei*, whereas the enzyme *Hinf*I cuts the *B. microti* amplicon into 81- and 356-bp fragments. Restriction fragments were separated in 8% polyacrylamide gels (Novex precast gels, Invitrogen, Carlsbad, CA), stained with ethidium bromide, and examined by UV transillumination. Positive control DNA isolated from *B. microti* was obtained by extracting DNA from a pool of 25 infected *I. scapularis* nymphs (kindly supplied by Dr. D. Fish and M. Papero, Yale University, New Haven, CT).

Results

Nested PCR for *A. phagocytophilum*. Fig. 1 shows a typical image of 33 PCR products subjected to agarose gel electrophoresis by using the two primer pairs ge3age10r and ge9f-ge2 in the primary and nested reactions, respectively. Four samples (249, 261, 263, and 278) showed the 546-bp fragment, indicating they were positive for *A. phagocytophilum*. Three to four negative controls were included with each analysis to check for amplification of nonspecific PCR products or for the presence of contamination. Negative controls yielded no amplification products. Touchdown PCR eliminated false positives by increasing the stringency of annealing of the primers to the template at the beginning of the amplification (Sachse 2003).

PCR and Restriction Analysis for *Babesia* sp. Primers PIRO-A and PIRO-B were used to amplify 408- and 437-bp fragments from the 18S rRNA gene of *B.*

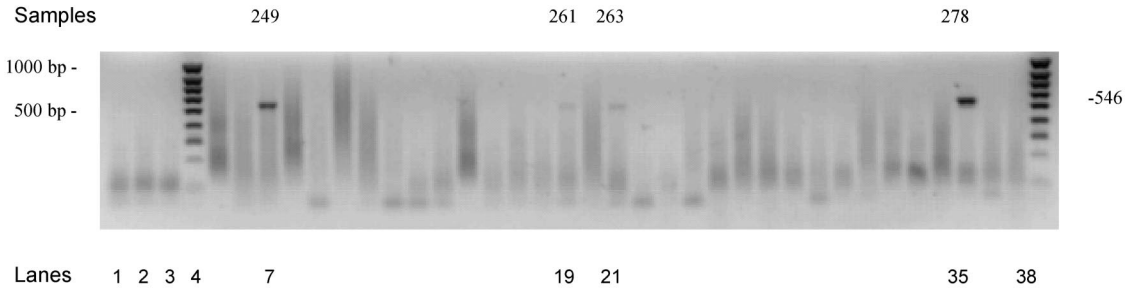


Fig. 1. Agarose gel electrophoresis of nested PCR products for *A. phagocytophilum*. Nested PCR products (10 μ l each) were visualized in a 1.5% agarose gel stained with ethidium bromide. Lanes 1–3 contain negative controls; lanes 4 and 38 contain 3.5 μ l (350 ng) of ladder 4 molecular weight marker (BioLine, Randolph, MA); Lanes 7, 19, 21, and 35 contain positive sample numbers 249, 261, 263, and 278 with an amplified band at 546 bp. All other samples were scored as negative.

odocoilei and *B. microti*, respectively (Armstrong et al. 1998). After PCR, samples of the amplified products were digested with *BstEII* and *HinfI* separately. The two species were readily identified by differences in their restriction digestion patterns as visualized by their mobility in the polyacrylamide gel. *B. odocoilei* DNA was digested by *BstEII*, whereas *HinfI* cut at sites within the amplicon of *B. microti* (Fig. 2). Because PCR samples were digested by *BstEII* but not by *HinfI*, the *Babesia* species observed in the samples was *B. odocoilei*. The larger fragment of 330 bp is clearly depicted in Fig. 2, although the fainter 78-bp fragment also may be observed. The *B. microti* positive control was digested by *HinfI*, as expected, yielding fragments of 356 and 81 bp.

Survey and Sequencing Results. The results of PCR analysis of 276 adult *I. scapularis* ticks and 39 pools of nymphs for *A. phagocytophilum* and *B. odocoilei* are

given in Table 1. For *A. phagocytophilum*, sequences were obtained for 16 isolates (one from a questing tick, three from ticks removed from the dog, and 12 from ticks removed from deer). Thirty-two isolates were PCR positive for *Babesia* sp. Nineteen of these isolates were identified as *B. odocoilei* by restriction fragment analysis. In addition, sequencing of seven of the 32 isolates confirmed that these isolates were *B. odocoilei*. BLAST analyses of the sequenced PCR products (E values = 0) confirmed the presence of either *A. phagocytophilum* (accession no. AY741099) or *B. odocoilei* (accession no. AY661508).

Between 11.8 and 21.2% of ticks were infected by *A. phagocytophilum* and between 10.3 and 20.0% of ticks tested were infected with *B. odocoilei*. For *A. phagocytophilum*, the prevalence of infection for questing ticks was 11.8%; for ticks collected from deer, 21.2%; and from the dog, 20.0%. One of 39 pools of

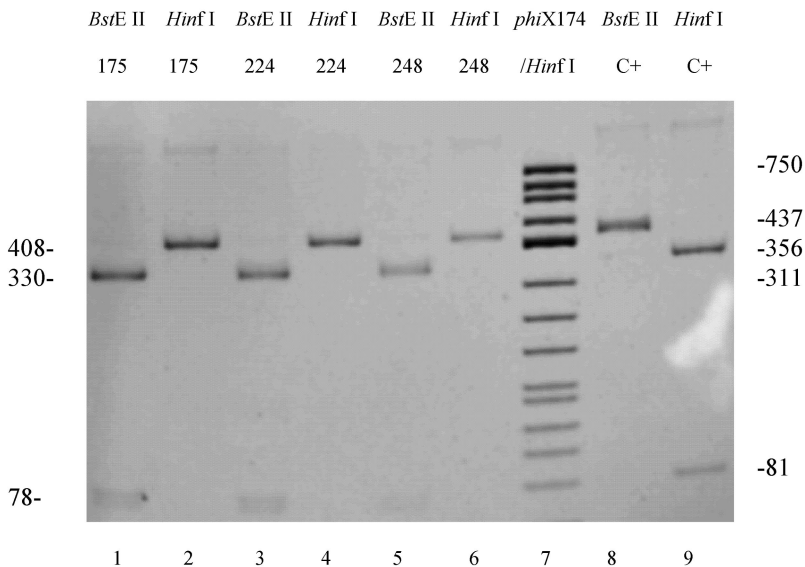


Fig. 2. Polyacrylamide gel electrophoresis after PCR amplification of *Babesia* sp. with PIRO-A/PIRO-B primers and restriction digestion. An 8% polyacrylamide gel is shown. Lanes 1–6 contain 6 μ l of the PCR products from samples 175, 224, and 248 digested with *BstEII* or *HinfI*; lanes 8 and 9 contain 6 μ l the control (C+, nymphs infected with *B. microti*) digested with the same enzymes. Lane 7 contains 1.5 μ l of Φ X174 DNA/*HinfI* marker.

Table 1. Results of PCR analysis of *I. scapularis* ticks collected in northern Indiana for *A. phagocytophilum* and *Babesia* species

Source	No. ticks	<i>A. phagocytophilum</i> No. PCR positive (% positive)	<i>A. phagocytophilum</i> No. sequenced	<i>Babesia</i> sp. No. PCR positive (% positive)	<i>Babesia odocoilei</i>		No. coinfectd (%)
					No. <i>BstE</i> II digested ^a	No. sequenced ^b	
Questing	68	8 (11.8)	1	7 (10.3)	6	2	0
Dog	15	3 (20.0)	3	3 (20.0)	1		1 (6.7)
Deer	193	41 (21.2)	12	22 (11.4)	12	5	5 (2.6)
Nymphs (pools)	39 ^c	1 (0.85) ^d		0			

^a Number of samples confirmed by *BstE*II digestion.

^b Number of samples confirmed by sequencing.

^c Pools (three nymphs per pool).

^d Minimum infection rate.

nymphs was positive for *A. phagocytophilum*, yielding a minimum infection rate of 0.85% (1/117). Similarly, for *B. odocoilei*, the prevalence of infection for questing ticks was 10.3%; for ticks collected from deer, 11.4%; and from the dog, 20.0%. None of the 39 pools of nymphs tested positive for *Babesia* sp. Although no questing *I. scapularis* were observed to be coinfectd, 2.6% of ticks collected from deer and 6.7% of those collected from the dog harbored both microbes (Table 1).

Discussion

The first cases of human anaplasmosis were detected in northern Minnesota and Wisconsin (Chen et al. 1994). Ten years later, in 2003, 362 cases were reported from 24 states (CDC 2005). Thus, the disease is becoming more widespread or, at least, better recognized. The first cases in Indiana occurred in 2002 and 2003 (James Howell, personal communication). Here, we report the presence of *A. phagocytophilum* in *I. scapularis* collected from a site in northern Indiana where this species has become well established. In this study, \approx 21.2% of the ticks collected from deer, and 11.8% of those questing were found to be infected with *A. phagocytophilum* as demonstrated by amplification of specific PCR products and corroboration by subsequent sequencing. This prevalence of infection is considerably lower than both the 50% prevalence of infection reported by Magnarelli et al. (1995) for ticks collected in Connecticut, and the 39.8% prevalence of infection reported by Courtney et al. (2003) for ticks collected in southeastern Pennsylvania. But, it is higher than the 1.9% prevalence of infection reported for ticks from northwestern Pennsylvania (Courtney et al. 2003) and the 0.0 to 9.5% prevalence of infection reported by Holman et al. (2004) for ticks collected in Maine. Thus, our observed prevalence of infection falls within the range of rates reported elsewhere. Although it seems from the data in Table 1 that the prevalence of *A. phagocytophilum* infection in adult ticks collected from deer was higher than that in questing adult ticks, the difference was not statistically significant ($\chi^2 = 3.007$, $df = 1$).

The first isolations of *B. odocoilei* from white-tailed deer blood were made in Texas in 1968 (Emerson and Wright 1968, 1970). The known geographic range of the parasite now includes the north central and north-

eastern states. Armstrong et al. (1998) found piroplasm in 14.4% of the deer ticks collected from intensely tick-infested sites in Maine, Massachusetts, and Wisconsin. Of those examined, 83.3% of the piroplasms contained DNA characteristic of *B. odocoilei*. Our findings, based on restriction enzyme analyses and sequencing of PCR amplicons, indicate that *B. odocoilei* is the most prominent *Babesia* species in *I. scapularis* ticks in northern Indiana. Armstrong et al. (1998) commented on the possibility that the prevalence of *B. odocoilei* in ticks collected from deer might overestimate the true prevalence of infection because these ticks could have acquired *B. odocoilei* DNA in their current bloodmeal. However, our results (10.3% prevalence rate in questing ticks compared with 11.4% in ticks fed on deer) suggest that this is not a significant concern.

I. scapularis larvae feed primarily on mice and or small mammals (Main et al. 1982). To date, there have been no reports of *B. odocoilei* infections in mice or small mammals (Pat Holman, personal communication). To test whether any of the nymphs we collected had become infected as larvae and maintained their infection while molting to nymphs, we tested 117 nymphs in pools (three individuals/pool). All 39 pools tested negative for *B. odocoilei*, supporting the notion that *I. scapularis* are unlikely to become infected as larvae. *I. scapularis* nymphs, in contrast have been found to feed on deer (Walls et al. 1998). Thus, we assumed that the questing adult ticks became infected with *B. odocoilei* while feeding on deer as nymphs.

B. odocoilei has been found in other cervids, including elk and reindeer in Wisconsin (Holman et al. 2003), caribou in a Minnesota zoo (Holman et al. 1994), and penned elk in Indiana (Gallatin et al. 2003). Gallatin et al. (2003) speculated that the Indiana elk had possibly become infected by ticks that fed on the abundant white-tailed deer in the vicinity. However, at the time the article was written, the authors had no data on the occurrence of *B. odocoilei* in either white-tailed deer or blacklegged ticks in Indiana. Our findings of *B. odocoilei*-infected ticks support their supposition.

Phylogenetic studies indicate that *B. odocoilei* is closely related to *B. divergens*, which causes babesiosis in European cattle, and to another recently discovered species of *Babesia* that causes zoonotic babesiosis in Europe (Herwaldt et al. 2003). Whereas *B. odocoilei* is

not known to cause illness in healthy humans, it is a concern for animals and might be pathogenic in immunocompromised humans (Herwaldt et al. 2003). We found no evidence of the presence of *B. microti* in Indiana in this study; however, we will continue to search for this agent in *I. scapularis*.

The site of this study is a state park visited by joggers (with their dogs), campers, horseback riders, and others. A primary public health concern is that infected ticks may be transported, by pets or on camping gear, from this site to other sites in Indiana or to residences. Our discovery of infected ticks on the resident dog justifies this concern. The widespread distribution of both white-tailed deer and blacklegged ticks means that both *A. phagocytophilum* and *B. odocoilei* will remain a medical and veterinary concern in the future.

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References Cited

- Adelson, M. E., R. V. Rao, R. C. Tilton, K. Cabets, E. Eskow, L. Fein, J. L. Occi, and E. Mordechai. 2004. Prevalence of *Borrelia burgdorferi*, *Bartonella* spp., *Babesia microti*, and *Anaplasma phagocytophilum* in *Ixodes scapularis* ticks collected in northern New Jersey. *J. Clin. Microbiol.* 42: 2799–2801.
- 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., P. Katavolos, D. A. Caporale, R. P. Smith, A. Spielman, and S. R. Telford, III. 1998. Diversity of *Babesia* infecting deer ticks (*Ixodes dammini*). *Am. J. Trop. Med. Hyg.* 58: 739–742.
- 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.
- Burket, C. T., C. N. Vann, R. R. Pinger, C. L. Chatot, and F. E. Steiner. 1998. Minimum infection rate of *Amblyomma americanum* (Acari: Ixodidae) by *Ehrlichia chaffeensis* (Rickettsiales: Ehrlichieae) in southern Indiana. *J. Med. Entomol.* 35: 653–659.
- [CDC] Centers for Disease Control and Prevention. 2005. Summary of notifiable diseases—United States, 2003. *MMWR* 52: 20.
- Chen, S. M., J. S. Dumler, J. S. Bakken, and D. W. Walker. 1994. Identification of a granulocytic *Ehrlichia* species as the etiologic agent of human disease. *J. Clin. Microbiol.* 32: 589–595.
- 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.
- Dumler, J. S., A. F. Barbet, C.P.J. Bekker, G. A. Dasch, G. H. Palmer, S. C. Ray, Y. Rikihisa, and F. R. Rurangirwa. 2001. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia*, and *Ehrlichia* with *Neorickettsia*, descriptions of six new species new species combinations and designation of *Ehrlichia equi* and “HGE agent” as subjective synonyms of *Ehrlichia phagocytophila*. *Int. J. Syst. Evol. Microbiol.* 51: 2145–2165.
- Emerson, H. R., and W. T. Wright. 1968. The isolation of a *Babesia* in white-tailed deer. *Bull. Wildl. Dis. Assoc.* 4: 142–143.
- Emerson, H. R., and W. T. Wright. 1970. Correction. *J. Wildl. Dis.* 6: 519.
- Gallatin, L. L., A. R. Irizarry, M. L. Renninger, P. J. Holman, G. G. Wagner, J. E. Sojka, and J. A. Christian. 2003. *Babesia odocoilei* infection in elk. *J. Am. Vet. Med. Assoc.* 223: 1027–1032.
- Herwaldt, B. L., F. E. Springs, P. P. Roberts, M. L. Eberhard, K. Case, and D. H. Persing, and W. A. Agger. 1995. Babesiosis in Wisconsin: a potentially fatal disease. *Am. J. Trop. Med. Hyg.* 53: 146–151.
- Herwaldt, B. L., S. Caccio, F. Gherlinzoni, H. Aspöck, S. B. Slemenda, P. P. Piccaluga, G. Martinelli, R. E. Edelhofer, U. Hollenstein, G. Poletti, et al. 2003. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. *Emerg. Infect. Dis.* 3: 165–170.
- Holman, M. S., D. A. Caporale, J. Goldberg, E. Lacombe, C. Lubelezyk, P. W. Rand, and R. P. Smith. 2004. *Anaplasma phagocytophilum*, *Babesia microti*, and *Borrelia burgdorferi*, in *Ixodes scapularis*, southern coastal Maine. *Emerg. Infect. Dis.* 10: 744–746.
- Holman, P. J., J. Madeley, T. M. Craig, B. A. Allsopp, M. T. Allsopp, K. R. Petrini, S. D. Waghela, and G. G. Wagner. 2000. Antigenic, phenotypic and molecular characterization confirms *Babesia odocoilei* isolated from three cervids. *J. Wildl. Dis.* 36: 581–530.
- Holman, P. J., K. Petrini, J. Rhyon, and G. G. Wagner. 1994. In vitro isolation and cultivation of a *Babesia* from an American woodland caribou (*Rangifer tarandus caribou*). *J. Wildl. Dis.* 30: 195–200.
- Holman, P. J., K. G. Bendele, L. Schoelkopf, R. L. Jones-Witthuhn, and S. O. Jones. 2003. Ribosomal RNA analysis of *Babesia odocoilei* isolates from farmed reindeer (*Rangifer tarandus tarandus*) and elk (*Cervus elaphus Canadensis*) in Wisconsin. *Parasitol. Res.* 91: 378–383.
- Johnson, R. C., G. P. Schmidt, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner. 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. *J. Syst. Biol.* 34: 496–497.
- Magnarelli, L. A., K. C. Stafford III, T. N. Mather, M. T. Yeh, K. D. Horn, and J. S. Dumler. 1995. Hemocytic *Rickettsia*-like organisms in ticks: serologic reactivity with antisera to ehrlichiae and detection of DNA of agent of human granulocytic ehrlichiosis by PCR. *J. Clin. Microbiol.* 33: 2710–2714.
- Main, A. J., A. B. Carey, M. G. Carey, and R. H. Goodwin. 1982. Immature *Ixodes dammini* (Acari: Ixodidae) on small mammals in Connecticut, USA. *J. Med. Entomol.* 19: 655–664.
- Massung, R. F., K. Slater, J. H. Owens, W. L. Nicholson, T. N. Mather, V. B. Solberg, and J. G. Olson. 1998. Nested PCR assay for detection of granulocytic ehrlichiae. *J. Clin. Microbiol.* 36: 1090–1095.
- Piesman, J., T. C. Hicks, R. J. Sinsky, and G. Obiri. 1987. Simultaneous transmission of *Borrelia burgdorferi* and *Babesia microti* by individual nymphal *Ixodes dammini* ticks. *J. Clin. Microbiol.* 25: 2010–2013.

- Pinger, R. R., and T. Glancy. 1989. *Ixodes dammini* (Acari: Ixodidae) in Indiana. *J. Med. Entomol.* 26: 130–131.
- Pinger, R. R., J. Holycross, J. Ryder, and M. Mummert. 1991. Collections of adult *Ixodes dammini* in Indiana, 1987–1990, and the isolation of *Borrelia burgdorferi*. *J. Med. Entomol.* 28: 745–749.
- Pinger, R. R., L. Timmons, and K. Karris. 1996. Spread of *Ixodes scapularis* (Acari: Ixodidae) in Indiana: collection of adults in 1991–1994 and description of a *Borrelia burgdorferi*-infected population. *J. Med. Entomol.* 33: 852–855.
- Sachse, K. 2003. Specificity and performance of diagnostic PCR assays, pp. 1–29. *In* K. Sachse and J. Frey [eds.], PCR detection of microbial pathogens: methods and protocols. *Methods in molecular biology*, vol. 216. Humana, Totowa, NJ.
- Spielman, A.M.L., Wilson, J. F., Levine, and J. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu. Rev. Entomol.* 30: 439–460.
- Waldrup, K. A., A. A. Kocan, R. W. Barker, and G. G. Wagner. 1990. Transmission of *Babesia odocoilei* in white-tailed deer (*Odocoileus virginianus*) by *Ixodes scapularis*. (Acari: Ixodidae). *J. Wildl. Dis.* 26: 390–391.
- Walls, J. J., N. Asanovich, J. S. Bakken, and J. S. Dumler. 1998. Serologic evidence of a natural granulocytic ehrlichiosis in Wisconsin and Maryland. *Clin. Diag. Lab. Immunol.* 5: 762–765.

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