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Bartonella spp. in deer keds, *Lipoptena mazamae* (Diptera: Hippoboscidae), from Georgia and South Carolina, USA

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ABSTRACT: Deer keds, *Lipoptena mazamae* (Diptera: Hippoboscidae), were collected from white-tailed deer (*Odocoileus virginianus*) and humans in Georgia and South Carolina, USA (1 October 2001–6 January 2005) and screened for the presence of DNA from *Bartonella* spp. Forty deer keds were screened for *Bartonella* spp. by polymerase chain reaction using primers specific to the riboflavin synthase gene (*rib*C) of *Bartonella*. *Bartonella* species closely related to *Bartonella schoenbuchensis* and to the etiologic agent of cat-scratch disease (*Bartonella henselae*) were detected in 10 keds and one ked, respectively.

Key words: Deer ked dermatitis, ectoparasite, *Odocoileus virginianus*, vector, zoonoses.

Lipoptena mazamae Rondani (Diptera: Hippoboscidae) is a blood-feeding ectoparasite of white-tailed deer (*Odocoileus virginianus*) in North America and brocket deer (*Mazama* spp.) in Central and South America (Samuel and Trainer, 1972). In North America, *Lipoptena mazamae* is host specific to the white-tailed deer, but has been reported as an incidental ectoparasite of domestic cattle, pumas (*Puma concolor*), and humans in the United States (Drummond, 1966; Forrester et al., 1996). *Lipoptena mazamae* has been implicated in the transmission of anaplasmosis of cattle (Drummond, 1966) and *Trypanosoma cervi* in cervids (Strickland et al., 1981); however, the precise role of deer keds in the transmission of either agent has not been investigated.

Bartonella are gram-negative bacteria that infect the erythrocytes of vertebrates and are putatively transmitted by arthropods or by blood to blood contact (Chomel et al., 1996; Chang et al., 2001; Comer et al., 2001). At least nine species of *Bartonella* are associated with bartonellosis in humans (Ciervo and Ciceroni, 2004; Dehio et al., 2004). Eight of these are zoonotic pathogens; however, *Bartonella bacilliformis* (agent of Carrion's disease and Oroya fever) has no recognized nonhuman reservoirs (Karem et al., 2000; O'Rourke et al., 2005). The animal reservoirs, vectors, pathogenicity, and natural history are unknown for most species of *Bartonella*.

The transmission of *Bartonella* spp. to ruminants and *Bartonella schoenbuchensis* to humans and deer by hippoboscidae has been suggested (Dehio et al., 2004; Halos et al., 2004). The role of *Lipoptena cervi* (Linnaeus) in the transmission cycle of *B. schoenbuchensis* is unknown; but this European ked is established in the northeastern United States (Maa, 1969). White-tailed deer in the southeastern United States are infested with *L. mazamae* (Maa, 1969), which might harbor *B. schoenbuchensis* or similar species of *Bartonella*. Host-seeking deer keds are attracted to and will bite humans (Rantanen et al., 1982). In order to determine if *Bartonella* spp. are present in *L. mazamae*, we collected deer keds from Georgia and South Carolina and tested them for known pathogenic *Bartonella* spp. by polymerase chain reaction (PCR) targeting the riboflavin synthase gene (*rib*C; Johnson et al., 2003).

Adult *L. mazamae* were collected from white-tailed deer carcasses killed by motor vehicles in Georgia and South Carolina (Nelder and Reeves, 2005); two additional keds were collected from humans in Georgia (Table 1). The two keds collected from humans still retained their wings; all

TABLE 1. Collection data and *Bartonella* spp. detected in *Lipoptena mazamae* from Georgia and South Carolina, USA (2003–05).

State/county	Collection site	Host	Date collected	No. tested	*Bartonella* spp. identified (No. of PCR-positive keds)
Georgia/Rockdale	Panola Mountain State Park	*Homo sapiens* Linnaeus	25 September 2004	2	None
South Carolina / Anderson	Fants Grove	*Odocoileus virginianus* (Zimmermann)	1 June 2003	9	None
South Carolina / Clarendon	Highway 251 near Sumter County line	*O. virginianus*	6 January 2005	3	*Bartonella* sp. near *B. schoenbuchensis* (1)
South Carolina / Fairfield	Fairfield	*O. virginianus*	5 January 2005	4	*Bartonella* sp. near *B. schoenbuchensis* (1)
South Carolina / Greenville	Greenville	*O. virginianus*	1–2 October 2001	1	*Bartonella* sp. near *B. schoenbuchensis* (1)
South Carolina / Pickens	Clemson, US 76	*O. virginianus*	13 June 2004	9	*Bartonella* sp. near *B. schoenbuchensis* (3)
SC/Richland	Harbison State Forest, Columbia	*O. virginianus*	14 November 2004	9	*Bartonella* sp. near *B. schoenbuchensis* (3)
South Carolina / Williamsburg	Kingston, Highway 251	*O. virginianus*	9 September 2004	3	*Bartonella henselae* (1) *Bartonella* sp. near *B. schoenbuchensis* (1)[a]

[a] Positive results for *Bartonella henselae* and *Bartonella* sp. from the South Carolina/Williamsburg samples were not detected from the same keds.

others were wingless. All specimens were fixed and stored in 95% ethanol and identified using taxonomic keys by Maa (1969).

Forty *L. mazamae* were tested by PCR for *Bartonella* spp. Individual whole flies were frozen in liquid nitrogen and crushed with a sterile Teflon pestle. Teflon pestles were cleaned in 10% sodium hypochlorite for 3 hr, rinsed in distilled water, and autoclaved before each use. Total DNA was extracted from the pulverized keds with an IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, Washington, USA) and resuspended in nuclease-free water. For PCR, the BARTON-1 (5′-TAACCGATATTGGTTGTGTTGAAG-3′) and BARTON-2 (5′-TAAAGCTA-GAAAGTCTGGCAACATAACG-3′) primers were used to amplify a fragment of the riboflavin synthase gene (*rib*C; Johnson et al., 2003). These primers were selected because they were designed to amplify DNA from pathogenic *Bartonella* spp. Each PCR reaction contained 12.5 µl of Taq PCR Master Mix Kit (Qiagen, Valencia, California, USA), 7.5 µl of nuclease-free water, 1.25 µl of each primer, and 2.5 µl of DNA template in water. The PCR products were separated by 2% agarose gel electrophoresis and visualized under ultraviolet light with ethidium bromide. Distilled water was used as a negative control.

The PCR products were purified with a QIAquick PCR Purification Kit (Qiagen). Duplicate sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) using PCR primers, and excess dye was removed with a DyeEx 2.0 column (Qiagen). Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems). Primer sequences were removed and sequences were assembled with Seqmerge (Accelrys, San Diego, California, USA). Assembled sequences

were compared to those in GenBank using the BLAST 2.0 program (NCBI, Bethesda, Maryland, USA). Identification of *Bartonella* spp. was based on a sequence similarity to known taxa. We considered 100% similarity in sequence data to represent the same taxa.

Voucher specimens of *L. mazamae* were deposited in the Clemson University Arthropod Collection and the novel sequence for the *rib*C gene from the *Bartonella* sp. closely related to *B. schoenbuchensis* was deposited in GenBank under accession number DQ125469.

DNA from a novel *Bartonella* sp. closely related to *B. schoenbuchensis* was detected in 10 of 40 deer keds from South Carolina (Table 1). The 533–base pair (bp) sequence from the *rib*C gene of this new *Bartonella* sp. was 96% similar to *Bartonella schoenbuchensis* (GenBank accession number AY116628) from Europe. We detected the same bacterial agent in keds from all geographical regions sampled including the Piedmont and Upper and Lower Coastal Plains. Our data imply that a novel *Bartonella* sp. closely related to *B. schoenbuchensis* is widely distributed in South Carolina and might be transmitted by *L. mazamae*. Hippoboscid ectoparasites of deer have been implicated as vectors of *B. schoenbuchensis* in Europe (Dehio et al., 2004), but hippoboscid ectoparasites of white-tailed deer in North America have not been tested. *Bartonella schoenbuchensis* infects deer and may be the causative agent of deer ked dermatitis in humans (Dehio et al., 2004). In severe cases, deer ked dermatitis causes itchy lesions that can persist for up to a year (Rantanen et al., 1982; Dehio et al., 2004).

Genetic heterogeneity within strains of *Bartonella* spp. closely related to *B. schoenbuchensis* in North America are partially known (e.g., Chang et al., 2000) and cervids in the Nearctic region might harbor a variant strain of *B. schoenbuchensis* or a closely related species. The full extent of genetic variability within *B. schoenbuchensis* or related species cannot be fully determined until these agents are cultured and their taxonomy better defined. Heterogeneity among strains of *B. schoenbuchensis* exists in Europe (Dehio et al., 2001). Culturing of the *Bartonella* spp. from both deer and keds would allow for analyses and comparison between strains or species of *Bartonella*.

The sequence for the 535-bp *rib*C amplicon was 100% identical to the *rib*C sequence for *Bartonella henselae* Houston strain-1 (GenBank# AJ132928). *Bartonella henselae* infects over 20,000 humans in the United States annually (Kaplan et al., 2002). Humans are infected by *B. henselae* when scratched or bitten by a bacteremic domestic cat, but this bacterium is possibly transmitted enzootically by the cat flea, *Ctenocephalides felis* (Bouche) (Chomel et al., 1996). DNA from *B. henselae* has been detected in argasid and ixodid ticks and stable flies (reported as *Stomoxys* spp. but presumably *Stomoxys calcitrans* Linnaeus), but the role of ticks and stable flies in the transmission cycle of this bacterium is unexplored (Sanogo et al., 2003; Chung et al., 2004; Loftis et al., 2005). Deer keds were previously not known to harbor *B. henselae* in North America. Presumably, the deer ked either acquired the bacterium by feeding on an infected deer or via a transovarial route. Cat fleas will rarely infest cervids (Szabo et al., 2000) and transmission of *B. henselae* by infected cat fleas to deer would seem unlikely.

The presence of DNA from *Bartonella* in *L. mazamae* does not demonstrate infection of the ectoparasite or vector competence; the DNA from *Bartonella* could have originated in an undigested blood meal or have been transmitted by transovarial routes. We did not detect *Bartonella* spp. in all flies, including the winged forms, which indicates that *L. mazamae* acquire these agents and that these bacteria are not ubiquitous obligate symbiotes.

Wild and domestic ruminants harbor *Bartonella* spp. (Chang et al., 2000;

Breitschwerdt et al., 2001) and infection rates can be high; more than 90% of mule deer (*Odocoileus hemionus*) in the western United States are infected with unnamed *Bartonella* spp. (Chang et al., 2000). The vectors of these *Bartonella* are unknown but could include ticks, lice, or hippoboscids. The relatively high prevalence of *Bartonella* infections in wild cervids suggests that the vectors of these agents may be widespread, common, or highly efficient vectors. The possibility of pathogen transfer between wildlife and humans or domestic animals exists, and might have been the case when a cattle rancher was infected with *Bartonella vinsonii* (Welch et al., 1999).

Hippoboscids harbor symbiotic species of *Bartonella* (e.g., Candidatus "*Bartonella melophagi*" formerly "*Wolbachia melophagi*") and other bacteria that are not pathogenic or transmitted to vertebrates (Bequaert, 1953; Reeves, 2005; Small, 2005). The PCR primers used in our study were designed to amplify DNA from species of *Bartonella* that infect vertebrates (Johnson et al., 2003) and did not amplify DNA from symbiotic *Bartonella* spp. of hippoboscids. If the symbiotic *Bartonella* spp. of hippoboscids were amplified by our PCR primers, all extracts would produce amplicons. A larger sample size is needed to determine if winged hippoboscids harbor potentially pathogenic *Bartonella* spp.

There are substantial populations of white-tailed deer in the southeastern United States that might serve as reservoirs for a *Bartonella* sp. closely related to *B. schoenbuchensis*. The vector potential of *L. mazamae* and other hematophagous arthropods feeding on white-tailed deer are of potential medical or veterinary concern. Samuel and Trainer (1972) noted that infestation of white-tailed deer by *L. mazamae* increases with deer age and that infestations are more prevalent during warmer months of the year. Future studies should address the transmission of this *Bartonella* sp. closely related to *B. schoenbuchensis* by keds; potential relationships between *Bartonella*-bacteremia and the age of deer; the potential for associated pathology; and further detection and identification of *Bartonella* spp. in white-tailed deer ectoparasites. The assumption that deer are infected with these agents but remain healthy can only be accepted if clinical trials are conducted. For example, *B. henselae* was presumed to be nonpathogenic to cats but recent discoveries by Kordick et al. (1999) have implicated chronic infections by this bacterium as the cause of several previously idiopathic diseases in cats.

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