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Isolation of *Bartonella schoenbuchensis* from *Lipoptena cervi*, a Blood-Sucking Arthropod Causing Deer Ked Dermatitis

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***Bartonella schoenbuchensis*, which commonly causes bacteremia in ruminants, was isolated from the deer ked *Lipoptena cervi* and was shown to localize to the midgut of this blood-sucking arthropod, causing deer ked dermatitis in humans. The role of *B. schoenbuchensis* in the etiology of deer ked dermatitis should be further investigated.**

The deer ked (*Lipoptena cervi*) is a common hematophagous louse fly of red deer, roe deer, elk, and sika deer in Europe, Siberia, and northern China and of white-tailed deer, elk, horses, and cattle in North America. The incidental infestation of humans with deer keds is well documented (1, 11, 12). In humans, these ectoparasites engorge on blood in 15 to 25 min. The bite is barely noticeable and initially leaves little trace. Within 3 days, the site develops into a hard, reddened welt. The accompanying itch is intense and typically lasts 14 to 20 days; occasionally, a pruritic papule may persist even for 1 year (6, 14, 15). Histological analysis of deer ked dermatitis lesions revealed C3 deposits in dermal vessels. Skin tests with deer ked whole-body extracts were positive in all patients tested, showing both immediate and delayed reactions. Moreover, 57% of the patients tested had elevated serum immunoglobulin E (IgE) levels. These findings suggest that complement, cell-mediated immune mechanisms, and IgE are involved in the pathogenesis of deer ked dermatitis (14).

While the etiological agent of this disease is unknown, all available data are consistent with the transmission of an infectious agent, either a bacterium or a parasite, through the bite of the deer ked. Deer keds have not been known to transmit any infectious agents to humans. Recent evidence has shown that several ruminant hosts of deer keds are frequently bacteremic for *Bartonella* spp. Bartonellae are gram-negative bacteria that have been isolated from the blood of a wide range of mammals, including humans, rodents, lagomorphs, carnivores, and ruminants (4). These hemotropic bacteria are increasingly being recognized as important human pathogens (10, 13). Until the 1990s, only *Bartonella quintana* (causing trench fever) and *B. bacilliformis* (causing Carrion's disease) were known to cause disease in humans. Since then, six additional *Bartonella* spp. have been associated with an increasing range of clinical manifestations, reflecting the expansion of the genus *Bartonella* to the currently described 20 species (7, 10, 13).

Bartonellae are transmitted by various arthropods, such as lice, fleas, and flies. Indeed, all six recently identified *Bartonella* spp. pathogenic for humans are anthropozoonotic agents, which are thought to be transmitted to humans by blood-sucking arthropods. Little is known about the risk of zoonosis from *Bartonella* spp. infecting ruminant hosts of deer keds. Recently, *B. schoenbuchensis* bacteremia was demonstrated in ~80% of the roe deer analyzed in Germany (9). Given the close association of the deer ked with its ruminant hosts, including regular blood meals, and the incidental infestation of humans with this arthropod, the deer ked could serve as a vector for the transmission of *B. schoenbuchensis* within ruminants and to humans. The purpose of this study was to analyze whether deer keds collected from wild ruminants are colonized with *B. schoenbuchensis*; such colonization should represent a prerequisite for transmitting this pathogen to ruminant or human hosts.

From December 2001 to November 2002, 49 deer keds were collected from the fur of seven roe deer and eight red deer shot at three different locations in Germany (Table 1). Following surface sterilization by immersion in 70% ethanol for 5 min, 30 deer keds were individually homogenized in phosphate-buffered saline and cultured on Columbia agar containing 5% defibinated sheep blood. Following incubation at 37°C in a humidified atmosphere containing 5% CO_2, 8 deer keds (27%) did not give rise to bacterial growth, while large numbers of bacterial colonies (>1,000 per deer ked) appeared on the plates for 22 deer keds (73%) after 4 to 6 days and continued to grow for several days. Except for a few instances of low-titer contamination with fast-growing bacteria (which may have resulted from incomplete surface sterilization), the uniform slow growth and colony phenotype suggested the isolation of a single bacterial species at a high titer. The growth characteristics and colony phenotype were consistent with *B. schoenbuchensis*, which was previously found at a similar prevalence in the blood of roe deer (80%) (3, 9). Deer keds were culture positive for seven out of eight roe deer (88%) and five out of seven red deer (71%). Interestingly, when several deer keds isolated from the same deer were analyzed, they were found to be either sterile or culture positive at a high titer (Table 1), suggesting that bacteremia of a deer results in infection of the infesting deer ked population.

One bacterial isolate from each of the deer infested with culture-positive deer keds (except for sample 23) was subjected

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TABLE 1. Origins of *L. cervi* samples and *Bartonella* sp. isolates

L. cervi sample	Host (deer)	Origin of host in 2002	No. of flies collected	No. of culture-positive flies/ no. tested	*Bartonella* sp. isolate	GenBank accession no.
1	Roe	Southern Black Forest	2	2/2	Dlf01	AJ564632
9	Roe	Southern Black Forest	8	0/4		
10	Roe	Southern Black Forest	10	2/2	Dlf10	AJ278183
11	Roe	Southern Black Forest	1	2/2	Dlf11	AJ278183
12	Roe	Southern Black Forest	5	2/2	Dlf12	AJ278183
13	Roe	Harz	1	1/1	Dlf13	AJ278183
14	Roe	Harz	1	1/1	Dlf14	AJ278183
15	Red	Harz	5	2/2	Dlf15	AJ564633
16	Red	Harz	2	0/2		
17	Red	Harz	2	2/2	Dlf17	AJ564634
18	Red	Harz	2	0/2		
21	Red	Schoenbuch Nature Park	3	3/3	Dlf21	AJ564634
22	Red	Schoenbuch Nature Park	5	3/3	Dlf22	AJ564635
23	Red	Schoenbuch Nature Park	1	1/1	Dlf23	
24	Roe	Schoenbuch Nature Park	1	1/1	Dlf24	AJ278183

to DNA sequence-based typing to the species level. For this purpose, a partial fragment of the *gltA* gene was amplified by PCR with genus-specific primers and subjected to DNA sequence analysis as described previously (9). The partial *gltA* sequences of strains Dlf10, Dlf11, Dlf12, Dlf13, Dlf14, Dlf21, and Dlf24 were identical to the *gltA* sequence of *B. schoenbuchensis* type strain R1 (9), while those of strains Dlf01, Dlf15, Dlf17, and Dfl21 were found to carry several base pair substitutions (Table 1 and Fig. 1).

Using CLUSTAL_X (16) and njplot software, a neighbor-joining tree was generated from the complete alignment of 374-bp *gltA* fragments from all deer ked isolates described here as well as from closely related *Bartonella* isolates and *Bartonella* spp. deposited in GenBank (Fig. 1). All deer ked isolates clustered within one clade together with *B. schoenbuchensis* strains $R1^T$, R3, and R4 from roe deer (9) and deer isolate deer-6. The other clade belonging to the node includes *B. capreoli* isolates from roe deer; this species is most closely related to *B. schoenbuchensis* (3, 9). The next most related clades include either deer isolates (deer-4 and deer-5) or *B. bovis*, isolated from a bovine, together with isolates from elk (elk-1 and elk-2); all of these are ruminants known to be infested by deer keds. Together, these data suggest that the 11 deer ked isolates analyzed all belong to the species *B. schoenbuchensis*, which is closely related to other *Bartonella* spp. and to isolates obtained from ruminant hosts of deer keds.

Immunohistochemical analysis allowed us to localize *B. schoenbuchensis* within deer keds. Cryosectioning of intact insects fixed in phosphate-buffered saline containing 3% formaldehyde and 0.5% glutaraldeyde for 30 min, followed by indirect immunofluorescence labeling with rat anti-*B. schoenbuchensis* serum and Cy3-labeled goat anti-rat IgG antibodies and fluorescence microscopy analysis, revealed single bacteria as well as large bacterial aggregates in the lumen of the midgut (Fig. 2). For ultrastructural analysis, the fixed midgut was post-fixed with 1% OsO4 for 1 h, dehydrated, stained with 70% ethanol and 2% uranyl acetate for 1 h, and embedded in Epon. Ultrathin sections were stained with 6% uranyl acetate for 1 h and lead acetate for 2 min. Transmission electron microscopy confirmed the presence of bacterial aggregates in the midgut (Fig. 3A) and revealed typical characteristics of the gram-negative cell wall (Fig. 3B).

Taken together, our findings of high-titer cultivation of *B. schonbuchensis* from surface-sterilized deer keds and the large

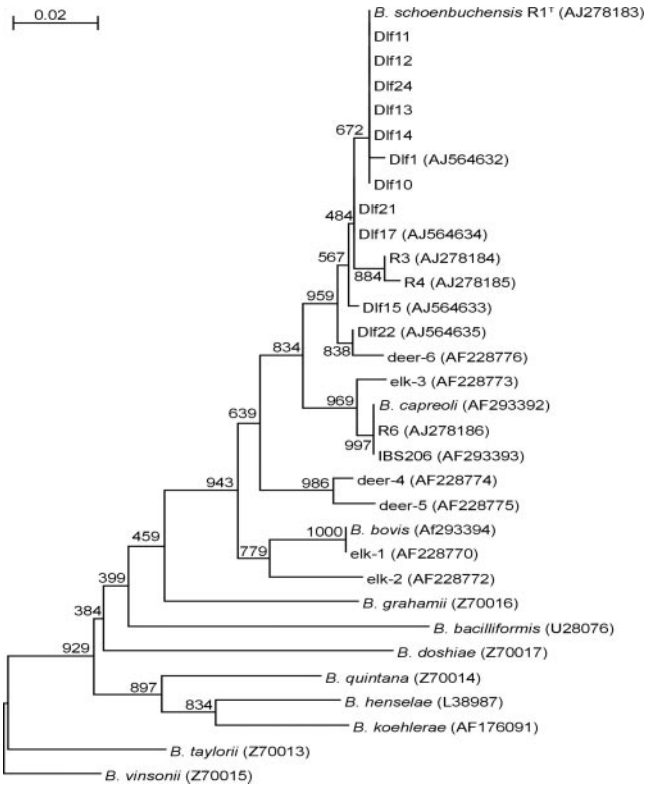

FIG. 1. Neighbor-joining tree of partial *gltA* sequences. The tree is based on the complete aligment of 374-bp fragments (GenBank accession numbers are indicated in the tree) corresponding to bp 698 to 973 of the partial *gltA* sequence of *B. schoenbuchensis* (GenBank accession no. AJ278183). Bootstrap values resulting from 1,000 bootstrap trials are indicated for each major branch. The *B. vinsonii* sequence (GenBank accession no. Z70015) that was most divergent from the *B. schoenbuchensis* sequence was used as an outgroup.

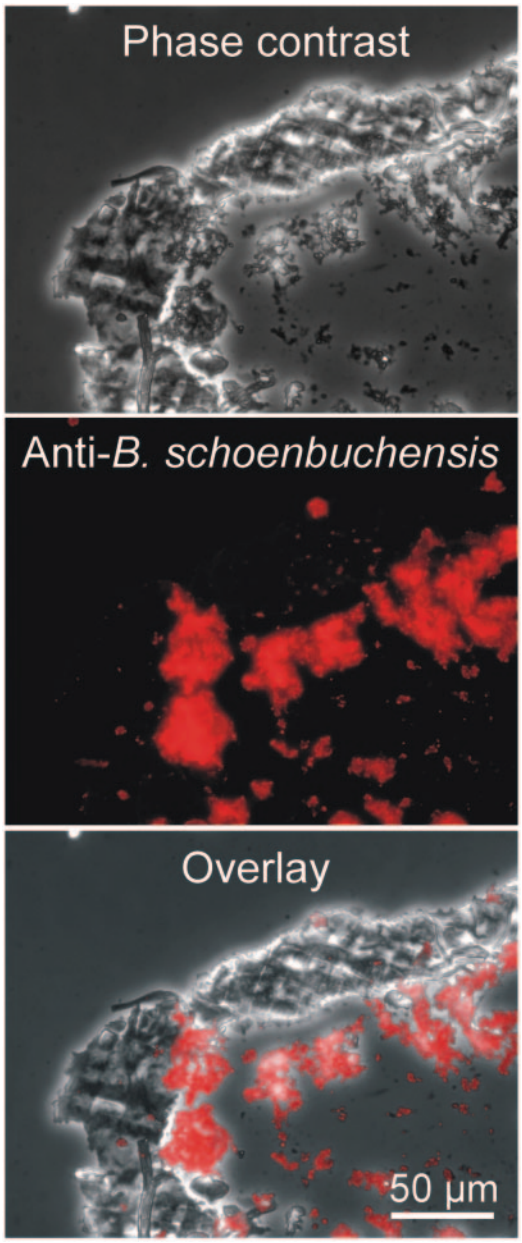

FIG. 2. Immunolocalization of *B. schoenbuchensis* in the midgut lumen of *L. cervi*. Cryosections of *L. cervi* (sample 12) were stained with rat anti-*B. schoenbuchensis* serum and then with Cy3-labeled anti-rat IgG antibodies. Digital images of the specimens were recorded with phase-contrast bright-field optics (top) and with fluorescence excitation and detection with a tetramethyl rhodamine isothiocyanate fluorescence filter set (middle). (Bottom) Overlay of phase-contrast and fluorescence images.

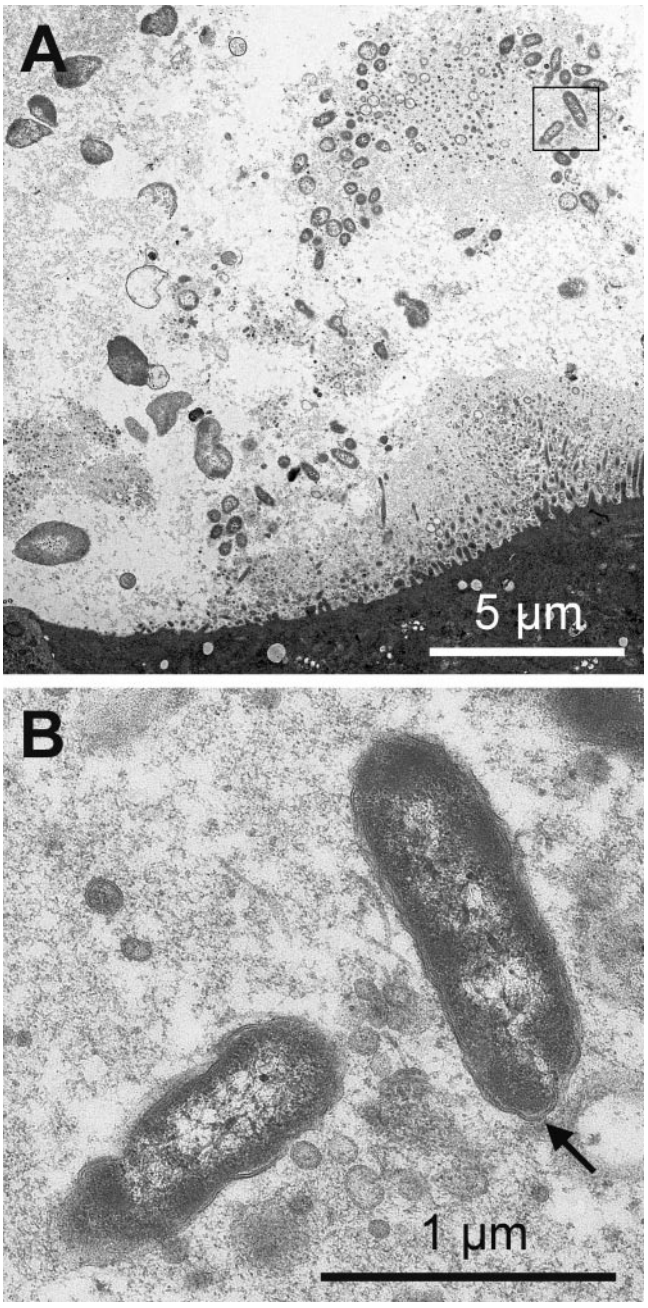

FIG. 3. Ultrastructural analysis of *B. schoenbuchensis* in the midgut lumen of *L. cervi*. Transmission electron micrographs of thin sections from the midgut of *L. cervi* (sample 12) were recorded at magnifications of ×2,000 (A) and ×16,000 (B). The image shown in panel B represents an enlargement of the inset in panel A. The electron-dense tissue in the lower part of panel A represents the midgut epithelium. The arrow in panel B indicates a typical aspect of the gram-negative cell wall.

bacterial aggregates observed in the midgut indicate that these arthropods represent a natural reservoir supporting the replication of this pathogen. With respect to lifestyle, the deer ked, which infests ruminants for most of its life and takes regular blood meals, appears to represent an ideal vector for efficient transmission of *B. schoenbuchensis* within ruminant populations. Given that deer keds incidentally take blood meals from humans, the risk for occasional transmission of *B. schoenbuchensis* to humans is apparent, e.g., to hunters, forestry workers, and cross-country runners. This conclusion raises the important question as to whether *B. schoenbuchensis* may be involved in the formation of deer ked dermatitis. Interestingly, among the known *Bartonella* spp., *B. schoenbuchensis* is most closely related to *B. bacilliformis* (9), an important human

pathogen, which is the only other *Bartonella* sp. for which the vector is known to be a fly, the sandfly *Lutzomyia verrucarum* (5). *B. schoenbuchensis* strains display considerable heterogeneity, e.g., in their *gltA* sequences (9), a finding supported by the identification of five additional variants in this study (Table 1 and Fig. 1). Some variant strains may thus bear a larger anthropozoonotic risk than others (9).

Interestingly, the clinical scenario of deer ked dermatitis resembles a primary manifestation of cat scratch disease, caused by *B. henselae*. This globally distributed anthropozoonotic pathogen is transmitted from cats to humans by the bite of an infected cat flea or, alternatively, by direct contact with cats (i.e., cat scratch or bite). After a 3- to 10-day incubation period, an erythematous papule or pustule develops at the site of inoculation and regresses after 2 to 8 weeks. This primary lesion of cat scratch disease is reminiscent of the clinical scenario of deer ked dermatitis (2), while other manifestations of cat scratch disease, i.e., lymphadenopathy, are not observed in deer ked dermatitis. A positive delayed-type hypersensitivity skin test, like that characteristically observed for *B. henselae* antigens in cat scratch disease (2), was also reported for all cases of deer ked dermatitis when whole deer ked extracts were used for the skin test (14). Also, C3 deposits in dermal vessels like those described for deer ked dermatitis (14) are consistent with infection by vasculotropic bartonellae (8). Taken together, certain clinical and histological characteristics of deer ked dermatitis are reminiscent of human infection by bartonellae, indicating that these pathogens should be considered possible etiological agents of deer ked dermatitis.

In summary, our study has provided evidence that deer keds collected from roe deer and red deer in Germany are commonly infected by *B. schoenbuchensis*. Furthermore, we have shown that *B. schoenbuchensis* colonizes the midgut of these arthropods and that this pathogen can be cultured at high titers from surface-sterilized arthropods. Our data suggest an important risk for the transmission of *B. schoenbuchensis* or related bartonellae to humans by the bite of an infected deer ked and suggest that a potential role of bartonellae in the etiology of deer ked dermatitis should be investigated further.

Nucleotide sequence accession numbers. GenBank accession numbers for the sequences determined here and sequences used for comparisons are indicated in Table 1 and Fig. 1.

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