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Identification of Different *Bartonella* Species in the Cattle Tail Louse (*Haematopinus quadripertusus*) and in Cattle Blood

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***Bartonella* spp. are worldwide-distributed facultative intracellular bacteria that exhibit an immense genomic diversity across mammal and arthropod hosts. The occurrence of cattle-associated *Bartonella* species was investigated in the cattle tail louse *Haematopinus quadripertusus* and in dairy cattle blood from Israel. Lice were collected from cattle from two dairy farms during summer 2011, and both lice and cow blood samples were collected from additional seven farms during the successive winter. The lice were identified morphologically and molecularly using 18S rRNA sequencing. Thereafter, they were screened for *Bartonella* DNA by conventional and real-time PCR assays using four partial genetic loci (*gltA*, *rpoB*, *ssrA*, and internal transcribed spacer [ITS]). A potentially novel *Bartonella* variant, closely related to other ruminant bartonellae, was identified in 11 of 13 louse pools collected in summer. In the cattle blood, the prevalence of *Bartonella* infection was 38%, identified as *B. bovis* and *B. henselae* (24 and 12%, respectively). A third genotype, closely related to *Bartonella melophagi* and *Bartonella chomelii* (based on the *ssrA* gene) and to *B. bovis* (based on the ITS sequence) was identified in a single cow. The relatively high prevalence of these *Bartonella* species in cattle and the occurrence of phylogenetically diverse *Bartonella* variants in both cattle and their lice suggest the potential role of this animal system in the generation of *Bartonella* species diversity.**

The *Bartonella* genus represents a fascinating example of diverse bacteria, formed by more than 30 species and subspecies, that can be found in a wide range of mammalian and arthropod hosts (e.g., fleas, lice, sandflies, and ticks) and exhibits a great genetic variation (1). In mammalian hosts, *Bartonella* species produce long-persistent infections, characterized by an apparent subclinical and cyclic bacteremia, allowing the bacteria to establish and be transmitted from one animal to another through bloodsucking arthropods (i.e., vectors) (2). Thereby, the maintenance and circulation of bartonellae in nature within arthropod-mammal systems is reflected by their diversity within a particular niche (e.g., feline, human, rodent, and ruminant bartonellae) (3). However, the incidental occurrence of *Bartonella* infection in nonadapted hosts may lead to the manifestation of disease; such is the case of cat-scratch disease in humans caused by the feline-associated *Bartonella henselae* (4, 5). Thus, the elucidation of the *Bartonella* cycle in nature, including the identification of the arthropod vectors and the *Bartonella* species distributed in a particular host and geographical area, is of great relevance in trimming the risk of their zoonotic potential, especially on those close and common human-animal interactions.

Bartonella species were previously detected in cattle, and three species have been identified: *Bartonella bovis*, *Bartonella schoenbuchensis*, and *Bartonella chomelii* (6–8). Interestingly, phylogenetic analyses of the ruminant-associated *Bartonella* species have clustered them as a separated lineage from the other *Bartonella* species (9, 10). *Bartonella bovis* is the most common reported species in cattle, while the other two have only been occasionally reported in these animals. Moreover, *B. bovis* has been associated with cases of bovine endocarditis (11, 12), and its zoonotic potential is still unknown. The prevalence of *Bartonella* in cattle varied across the studies and geographical areas, ranging from apparent uninfected-cattle regions such as Kenya and Japan to up to 90% infection rates in regions such as Marneuli District, Georgia (13).

Cattle are commonly infested by many ectoparasites, resulting in a constant menace for animal health either directly through blood consumption and physical burden, or indirectly through the transmission of pathogens (14, 15). Among these ectoparasites, lice (Phthiraptera) are important pests in cattle worldwide. Five louse species have been identified in cattle, including four sucking louse species and one biting louse species. They are characterized by a site affinity, each localized on a particular body region of the cow (15). Accordingly, the cattle tail sucking louse, *Haematopinus quadripertusus*, is commonly found infesting the distal area of the tail and is prevalent in tropical and subtropical areas such as Israel (15). Sucking lice are known vectors of human pathogens such as *Borrelia recurrentis*, *Rickettsia prowazekii*, and *Bartonella quintana* (3, 16, 17). Moreover, recent studies have identified *Bartonella* DNA in several species of lice collected from wild rodents (18, 19), suggesting that these arthropods have a role in the active maintenance of *Bartonella* in other mammals. *Bartonella* DNA has been also detected in cattle-associated hematophagous arthropods, such as *Rhipicephalus* (*Boophilus*) *microplus* ticks collected from Taiwanese cattle (20), biting flies such as *Haematobia* species, and *Stomoxys* species from Californian cattle (21) and *Hippobosca equina* from Europe (22). Nevertheless, the vector involved in *Bartonella* transmission to cattle has not been elucidated to date. Thus, along with these blood sucking parasites, sucking lice in cattle could represent a possible candidate for the active maintenance and transmission of cattle-associated *Bartonella* species.

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The accelerated origination of *Bartonella* species from a common ancestor in different ecological niches ("adaptive radiation") is especially evidenced in *Bartonella* species from ruminants (10). This, together with the unclarified ecological *Bartonella* cycle in ruminants, makes this system an interesting model to study the bacterium-vector-host interaction in nature. Thus, in the present study the occurrence of *Bartonella* species in cattle and their cattle tail lice was investigated. The potential role of cattle and the cattle tail lice in maintenance and transmission of bartonellae in ruminants is discussed.

MATERIALS AND METHODS

Collection of lice from cattle. Lice were collected in two periods from dairy cattle farms. The first collection period (summer) took place on September 2011 in two dairy cattle farms from Mevo Horon (31°50′57.04″N, 35°2′9.34″E) and Galon (31°37′58.07″N, 34°50′51.72″E). Lice were manually collected from the tail of each cow. Totals of 510 and 600 specimens were collected from 17 and 20 cows (~30 lice per cow) from the Galon and Mevo Horon farms, respectively. The specimens were placed in microtubes containing 1 ml of 70% ethanol, transported to the laboratory, and kept at room temperature. They were then classified to the genus and species level by morphological characteristics (23) and by molecular characterization of the 18S rRNA gene (described below). The second sample collection occurred on December 2011 (winter) in nine dairy cattle farms: Mevo Horon, Galon, Givat Hashlosha (32°5′53.88″N, 34°55′15.59″E), Ahisamakh (31°56′5.63″N, 34°54′26.27″), Matzliah (31°54′28.08″N, 34°52′26.4″E), Nehalim (32°3′30.6″N, 34°54′49.31″E), Yarhiv (32°9′8.27″N, 34°58′4.07″E), Nahshonim (32°3′36.35″N, 34°56′51.71″E), and Gat (31°37′37.91″N, 34°47′38.76″E) (Fig. 1). During the second collection, lice were collected from 50 cows. Approximately 10 to 20 lice were collected from each cow and transported in 1 ml of 70% ethanol.

Collection of cattle blood. During winter collection, cow blood samples were collected from the above-mentioned 50 cows (from which lice were also collected). Accordingly, blood samples were drawn in EDTA tubes, chilled in cool-boxes, and transported to the laboratory, where they were kept at −80°C until further analyzed.

DNA extraction from lice and cattle blood. DNA was extracted from louse pools (2 to 20 lice per pool according to the farms and/or season of collection) and from individual lice (from winter collection), as follows. First, the lice were washed once in 1 ml of ethanol 70% for 10 min and twice in 1 ml of sterile phosphate-buffered saline for 10 min. Thereafter, they were transferred to a new DNA-free vial and homogenized with a sterile pestle until a clear solution was obtained. Finally, the DNA was extracted using a DNA extraction kit (Illustra Tissue & Cells GenomicPrep Mini Spin kit; GE Healthcare, Buckinghamshire, United Kingdom) according to the manufacturer's instructions.

DNA was extracted from 50 μl of EDTA-blood of each cow using a DNA extraction kit (BiOstic bacteremia DNA isolation kit; MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. The DNA was obtained in 50 μl of elution buffer. For quality assurance, a *Bartonella*-free blood sample was used as an extraction control.

Molecular identification of the cattle tail louse. The 18S rRNA gene (~2,970 bp) was amplified by conventional PCR from louse pools and single louse, using primers EUKA (AACCTGGTTGATCCTGCCAGT) and EUKB (GATCCTTCTGCAGGTTCACCTAC) (24). In order to clarify the targeted sequence of the 18S rRNA gene, additional internal primers were required. Accordingly, the primer pairs HF18S (CGACGAAAC TTACCGTCGGA)/HR18S (ATTAAGCCGCAAGCTCCACT) and 454F (AAGCTCGAAAGGAATCCGCA)/1620R (TGTTGAGATCGCGTCGG AAA), which targeted 1,188- and 1,200-bp fragments, respectively, were designed. All PCRs were performed under the following thermal cycling conditions: an initial step of 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 90 s, with a final step of 72°C for 10 min. Amplified fragments were sequenced in an MJ Research PTC-225 Peltier thermal cycler using an ABI Prism BigDye Terminator cycle sequencing kit with AmpliTaq DNA polymerase (FS enzyme; Applied Biosystems, Carlsbad, CA) according to the protocols supplied by the manufacturer. Further analyses of sequences were done with MEGA alignment software version 5.05 (The Biodesign Institute, Tempe, AZ), and the final sequence was obtained by the assembly of all of the sequenced 18S rRNA gene fragments.

Molecular screening of *Bartonella* DNA from lice and cattle blood DNA. The molecular screening for *Bartonella* DNA on lice was assessed by conventional PCR and high-resolution melt (HRM) real-time PCR assays. Conventional PCR assays were performed (i) targeting 379- and 800-bp fragments of the citrate synthase (*gltA*) gene using the primers Bhcs.781p and Bhcs.1137n (25) and the primers CS443f and CS1210r (26, 27), respectively, (ii) targeting a 795-bp fragment of the RNA polymerase (*rpoB*) gene using the primers 1400F and 2300R (28), and (iii) targeting a 602-bp fragment of the 16S-23S internal transcribed spacer (ITS) using the primers 321s and 983as (29). The PCRs were carried out in a 25-μl final volume using PCR-Ready high-specificity ready mix (Syntezza Bioscience, Ltd., Jerusalem, Israel) containing 1 μl of a 10 μM solution of each primer, 21 μl of double-distilled water (DDW), and 2 μl of each extracted DNA sample. Thermal conditions were performed according to the authors' recommendations. In order to increase the sensitivity of the *Bartonella* screening, HRM real-time PCR assays were performed for the amplification of partial fragments for the ITS (190 bp) and the transfer-mRNA (*ssrA*) gene (301 bp) using primer sets and protocols as previously described (29–31). The real-time PCRs were carried out in a 20-μl final volume containing 1 μl of 0.5 μM solution of each primer, 0.6 μl of 1.5 μM solution of Syto9 (Invitrogen, CA), 3.4 μl of DDW, 10 μl of MAXIMA Hot-Start PCR master mix 2X (Thermo Scientific, Surrey, United Kingdom), and 4 μl of each genomic DNA. All HRM real-time reactions were carried out in the Rotor Gene 6000 cycler (Corbett Research, Sydney, Australia). Molecular screening of cattle blood for *Bartonella* species DNA was assessed by using HRM real-time PCR assays targeting the ITS and *ssrA* fragments, as described above.

All *Bartonella*-positive real-time and conventional PCR products were purified with a PCR purification kit (Exo-SAP; New England BioLabs, Inc., Ipswich, MA) and subsequently sequenced by using BigDye Terminator cycle sequencing chemistry from an Applied Biosystems ABI 3700 DNA analyzer and the ABI's data collection and sequence analysis software (ABI, Carlsbad, CA). Further analysis was done with MEGA alignment software (version 5.05; The Biodesign Institute).

Nucleotide sequence accession numbers. Newly determined sequence data were deposited in GenBank under accession numbers KJ522491, KJ522487, KJ522489, KJ522488, and KJ522490.

RESULTS

Identification of cattle tail lice. Louse specimens were identified as *Haematopinus quadripertusus* by morphological characteristics. The characterization of the 18S rRNA gene sequence resulted in a partial fragment of 2,835 bp. In all 18S fragments sequenced, a common region of ambiguous nucleotides was noticed (from the nucleotide positions 992 to 1387); thus, this area was manually annotated and clarified from 13 louse 18S rRNA gene amplicon chromatograms. The consensus 18S rRNA gene sequence was deposited in GenBank under accession number KJ522491. The obtained 18S rRNA sequence was 91.2% similar to the *Haematopinus* sp. strain NKU-011 18S rRNA gene (JQ309927.1) collected from cattle from Sichuan Province, China (32; Qiang Xie, personal communication), the longest available *Haematopinus* sp. 18S rRNA partial gene available in the GenBank database.

***Bartonella* DNA detection in *Haematopinus quadripertusus*.** Eleven of 13 tested louse pools (median of 10 lice) collected from two farms during summer (8 from Galon and 5 from Mevo Horon) (Fig. 1) were determined to be positive for *Bartonella*

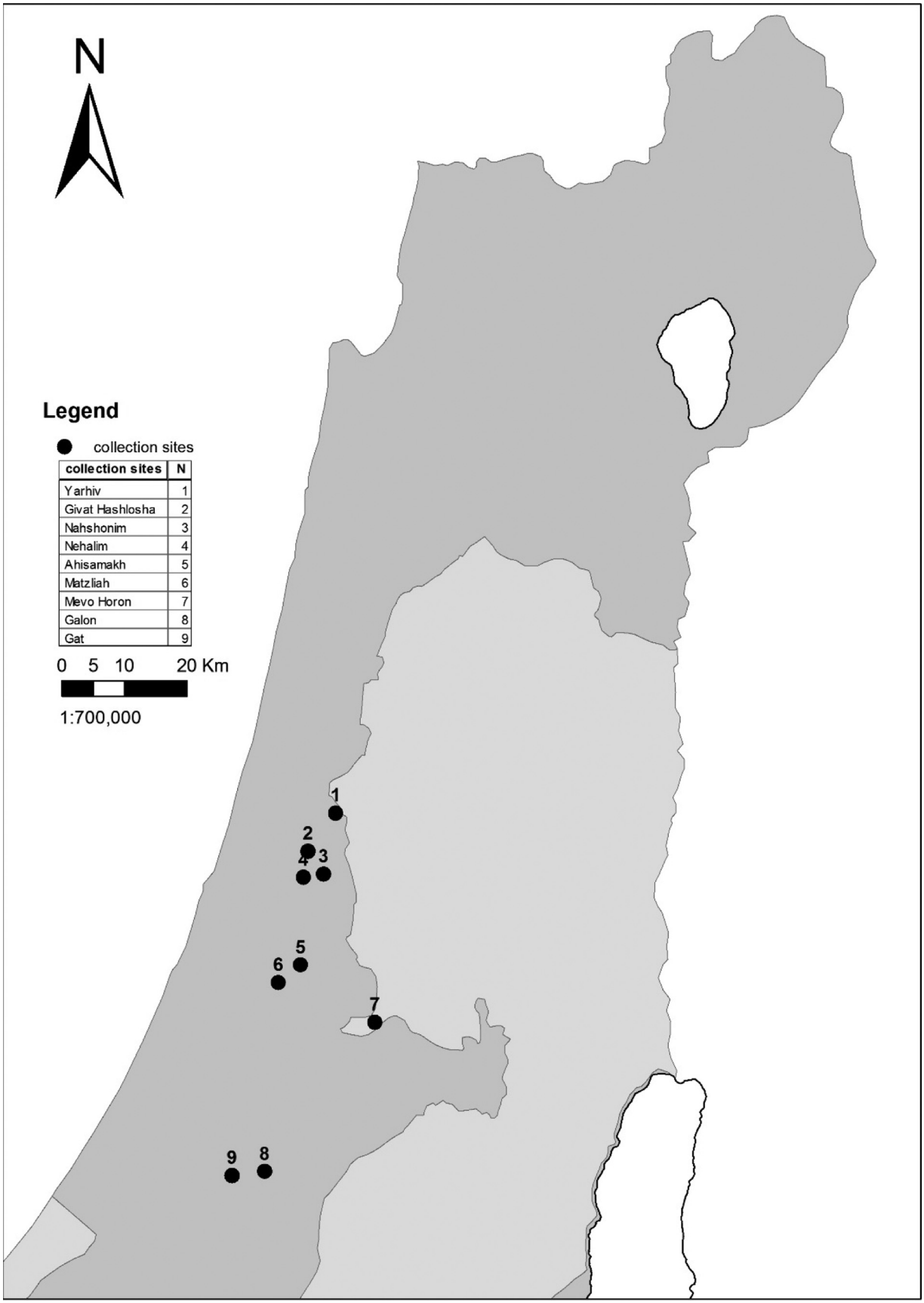

FIG 1 Map of Israel indicating the locations of the cattle dairy farms where lice and cattle blood samples were collected. The map was constructed using ArcMap 10.0 software (Esri, Redlands, CA).

TABLE 1 Pairwise distance analysis between uncultured *Bartonella* sp. clone Hq (from *Haematopinus quadripertusus*) versus ruminant bartonellae and *Bartonella grahamii* (outgroup) sequences from the GenBank database

Matched *Bartonella* sp.	% similarity between sequences			
	gltA (354 bp)	*rpoB* (852 bp)	ITS (424 bp)	*ssrA* (250 bp)
B. chomelii	95.1	95.6	93.3	98.8
B. schoenbuchensis	94.8	95.4	93.0	98.4
B. bovis	94.8	94.1	95.2	96.7
B. capreoli	95.1	95.2	92.6	98.8
B. melophagi	95.8	95.1	91.1	98.8
B. grahamii	88.7	82.3	41.9	92.4

DNA through the PCR screening methods. The sequences obtained by the different genetic target loci (*gltA*, *rpoB*, ITS, and *ssrA*) showed 100% identity between the louse pool samples amplified from both farms. Those *Bartonella* sequences were deposited in GenBank under the following accession numbers: *gltA* (KJ522487, 748 bp), *rpoB* (KJ522489, 852 bp), ITS (KJ522488, 424 bp), and *ssrA* (KJ522490, 253 bp). Phylogenetic analyses of the *Bartonella* species detected from *H. quadripertusus* lice demonstrate that this species is closely related to other ruminant bartonellae (Table 1 and Fig. 2). During the winter collection period, none of the louse pools (from 39 cows) or the single louse (from 29 cows) tested was determined to be positive for *Bartonella* DNA by any conventional or real-time PCR assay.

***Bartonella* DNA detection in cattle blood.** *Bartonella* DNA was detected in 38% (19/50) of all cattle blood samples by real-time PCR assays. Both *ssrA* and ITS real-time PCR assays were found positive in 10 cow blood samples, and yet in 9 additional cases only one of the target regions was successfully amplified (Table 2). The *Bartonella* DNAs identified from the cow blood samples were closely related to *B. bovis* or *B. henselae* DNA sequences. In addition, one cow presented an *ssrA* genotype closely related to *B. melophagi* and *B. chomelii* DNA sequences (both with 98% similarities), and the ITS sequence detected was 100% similar to the *B. bovis* ITS sequence. The *ssrA* sequence was deposited in GenBank under the accession number KJ540110. Accordingly, *B. bovis* showed an infection rate of 24% (12/50), and *B. henselae* showed an infection rate of 12% (6/50). Seven dairy farms presented at least one cow infected with *Bartonella* DNA. The only locations where no *Bartonella* infections were detected in the cattle samples were Gat and Mevo Horon. Furthermore, most positive cases were identified in Givat Hashlosha (7/19), followed by the Ahisamakh dairy farm (6/19), which are located ~20 km apart.

TABLE 2 Molecular detection of *Bartonella* DNA from cattle blood samples collected from dairy farms in Israel (winter collection)

Cow	Farm	Real-time PCR		*Bartonella* sp. (% identity)[a]
		ITS	*ssrA*	
1	Ahisamakh	+	0	*B. henselae* (100)*
2	Ahisamakh	0	+	*B. bovis* (100)†
3	Ahisamakh	+	+	*B. bovis* (100)‡
4	Ahisamakh	+	+	*B. bovis* (99)*
5	Ahisamakh	+	+	*B. bovis* (100)‡
6	Ahisamakh	+	+	*B. henselae* (100)*
7	Galon	0	+	*B. bovis* (100)†
8	Givat Hashlosha	+	+	*B. bovis* (100)†
9	Givat Hashlosha	+	0	*B. henselae* (100)*
10	Givat Hashlosha	0	+	*B. bovis* (100)†
11	Givat Hashlosha	+	0	*B. henselae* (100)*
12	Givat Hashlosha	+	+	*B. melophagi-B. chomelii/B. bovis*[b]
13	Givat Hashlosha	+	0	*B. henselae* (100)*
14	Givat Hashlosha	+	+	*B. bovis* (100)†
15	Matzliah	0	+	*B. bovis* (100)†
16	Nachshonim	+	+	*B. bovis* (100)†
17	Nehalim	+	+	*B. bovis* (100)‡
18	Yarhiv	+	0	*B. henselae* (100)*
19	Yarhiv	+	+	*B. bovis* (100)†

[a] *, identity based on the ITS sequence; †, identity based on the *ssrA* sequence; ‡, identity based on both the ITS and the *ssrA* sequences.
[b] The *Bartonella* genotype was 98% similar to those of *B. melophagi* and *B. chomelii* based on the *ssrA* fragment, and the *Bartonella* genotype was 100% similar to that of *B. bovis* based on the ITS fragment.

DISCUSSION

This study demonstrated the occurrence of *Bartonella* DNA sequences closely related to ruminant *Bartonella* species in the cattle

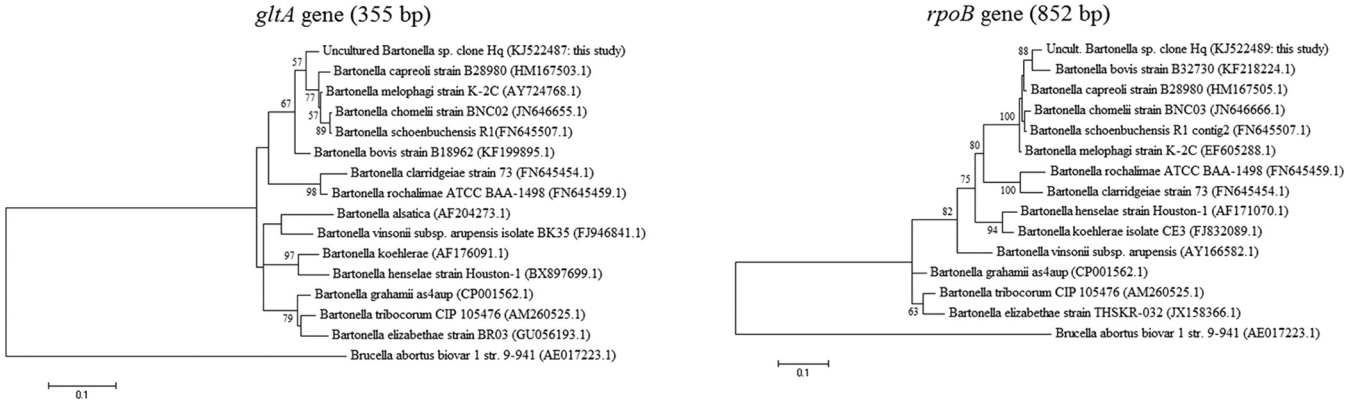

FIG 2 Maximum-likelihood phylogenetic trees based on partial *rpoB* (825-bp) and *gltA* (355-bp) genes. Bootstrap values higher than 50% are indicated. Phylogenetic trees were obtained from the uncultured *Bartonella* sp. clone Hq sequences detected from *Haematopinus quadripertusus* lice and common *Bartonella* species sequences, including ruminant-associated *Bartonella* species (sequences were obtained from the GenBank database, and accession numbers are indicated in parentheses). The *Brucella abortus* biovar 1, strain 9-941, sequence was used as an outgroup.

tail louse *H. quadripertusus*. In addition, cattle and feline associated *Bartonella* DNA were detected in the blood of dairy cattle from seven Israeli dairy farms.

Previous studies that screened *Bartonella* DNA on *Haematopinus* species lice collected from pigs and cattle reported negative results (33, 34). Therefore, to the best of our knowledge, this is the first report of *Bartonella* detection in this louse genus. The current molecular characterization of the *Bartonella* DNA found in the *H. quadripertusus* lice exhibits certain features suggesting that it may represent a single bacterial clone or variant. First, the positive lice were collected from two geographically distant cattle farms (Mevo Horon and Galon, ~40 km apart). Second, the *Bartonella* genetic targets showed 100% identity between the louse pool samples amplified from both farms. Interestingly, the sequence analyses of these amplicons revealed a close but distinguishable phylogenetic relationship between this *Bartonella* with the other known ruminant-associated *Bartonella* species, suggesting a new *Bartonella* species according to the La Scola taxonomic classification (35). Despite this evidence, we are aware that, in order to confirm the single bacterial origin of these DNA products, bacterium isolations were required (attempts to isolate the *Bartonella* from lice and cattle blood were assessed without success [data not shown]). Moreover, *Bartonella* DNA was only detected in specimens from the summer collection and not from those collected during winter. This phenomenon can be related to the higher relative abundance and activity of *H. quadripertusus* during the summer season (15), suggesting a potential seasonal effect on *Bartonella* species acquisition by these lice.

Bartonella infection in cattle has been extensively reported worldwide (6, 13, 31, 36, 37). *Bartonella bovis* has been the most common species identified in these animals. Accordingly, in the present study, *Bartonella* DNA was detected in a relatively high percentage of the animals (38% of the cattle tested), with *B. bovis* DNA being the most common *Bartonella* sequence identified (24%). Interestingly, the global distribution of *B. bovis* has shown a great inconsistency in the infection rates across and within geographical areas. For instance, Bai et al. (13) investigated the *Bartonella* infection in cattle from Thailand, Kenya, Japan, Georgia, and Guatemala and reported overall prevalence that varied from 0% (Japan and Kenya) to 57% (Georgia). Similarly, *B. bovis* infection rates from cattle from the United States varied across the regions studied, being as high as 82.4% in North Carolina (38) and California (81 to 96%) (6) and less pronounced in Georgia (47%) (31). In Europe, the prevalence of *B. bovis* in cattle has shown similar variability, with reports from France (59%) (39), Italy (24.2%) (40), and Poland (6.8%) (37). It should be noted that *B. schoenbuchensis* and *B. chomelii*, the other two *Bartonella* species detected in cattle, were initially isolated from cows in France (7, 8). In the present study, DNA sequences of two additional *Bartonella* species were identified. First, DNA of *B. henselae*, a feline-associated and zoonotic *Bartonella* species, was detected in six cows in our study. This finding represents the second report of this *Bartonella* species in cattle worldwide since its first detection in beef cattle from North Carolina (38). The occurrence of *B. henselae* in cattle may reflect the frequent spread of this species in Israel. Recently, the prevalence of this species was determined in cats from this country and shown to be evenly distributed in stray and domestic cats (30). Thus, despite their acknowledged feline association, *B. henselae* seems to have a permissive cycle in nature since it has been detected in several ecological niches (hosts and vectors) (18, 41). Finally, a third *Bartonella* genotype was detected in one cow blood sample, which was closely related to *B. chomelii* and *B. melophagi*, two ruminant-associated *Bartonella* species, according to the *ssrA* sequence, and to *B. bovis* according to the ITS sequence. The uncertainty in species identification could be explained by coinfection with two *Bartonella* variants (*B. bovis* and a variant related to *B. chomelii* or *B. melophagi*) or by infection with a variant that contained recombinant sequences. Nevertheless, both possible scenarios illustrate the challenge of *Bartonella* identification using direct detection of housekeeping genes and the variation of these genes that complicate the taxonomic classification (as in the case of the *ssrA* sequence). These phenomena have been extensively observed in wild rodent bartonellae (1, 42, 43).

Lice are considered one of the most abundant ectoparasites infesting dairy cattle in Israel. Since all known *Bartonella* species are associated with a vector-borne life cycle (1), lice were proposed as potential vehicles for cattle-associated *Bartonella* in this country. It should be noted that all cows included in the study were found to be highly infested with these arthropods, especially in summer. However, the incrimination of these lice as active *Bartonella* vectors was challenged since the screening of *Bartonella* DNA showed no detection of *Bartonella* during winter, although the cattle blood samples were positive during this season. Moreover, the *Bartonella* variants identified in either the louse pools or cattle blood were different. A potential explanation for the latter finding may suggest that these species/variants coexist in cattle, but a selective pressure may occur in the lice toward the acquisition of the detected *Bartonella* variant and only the dominant ones could be detected in the cattle hosts. Coinfection with multiple *Bartonella* variants in a single carrier and an apparent selective distribution of those variants have been revealed in other ecological niches, such as wild rodents and their fleas (44). Furthermore, the *Bartonella* variant found in lice could represent a unique bacterium from this niche, without strict association with the cattle hosts. Lice are known to have symbiotic association with bacteria, which become essential to their growth and reproduction (15, 45). For instance, *B. melophagi* has been suggested as an endosymbiont of *Melophagus ovinus* sheep keds (22). Thus, a similar evolutionary process between *H. quadripertusus* and the *Bartonella* detected during the summer might be taking place. On the other hand, the role of other bloodsucking arthropods (e.g., biting fleas or ticks) in this cattle system may be more relevant ecologically, especially for the *B. bovis* and *B. henselae* found in the cattle. Overall, our results suggest that *H. quadripertusus* lice probably do not play an important role in the transmission of these cattle-associated *Bartonella* species.

In conclusion, our findings demonstrated that *Bartonella* species are prevalent in dairy cattle from Israel and that *H. quadripertusus* lice can host a novel *Bartonella* genotype/species, which was not found in the tested cattle. The infection rates of *B. bovis* in cattle blood were higher than for other related species and much higher than those reported from other geographical areas worldwide. In addition, the wide spread of *B. bovis* and the presence of feline-zoonotic *B. henselae* in cattle must be highlighted since these organisms represent a veterinary and zoonotic health hazard. The identification of potentially novel *Bartonella* variants in both cattle and their lice suggest that this system plays a role in the generation of *Bartonella* diversity. This system adds evidence for a complex maintenance of *Bartonella* in nature, as was observed in other mammals such as wild rodents. Finally, further studies

should target other blood-sucking arthropods in dairy farms in order to elucidate the potential role of other vectors in the life cycle of these cattle-associated *Bartonella* species and to determine the ecological factors affecting bartonella occurrence in each of the players in this important agriculture system.

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