Applied and Environmental Microbiology, Oct. 2010, p. 6864–6869 Vol. 76, No. 20
0099-2240/10/$12.00 doi:10.1128/AEM.00879-10

Bartonella Genotypes in Fleas (Insecta: Siphonaptera) Collected from Rodents in the Negev Desert, Israel[▽]

Danny Morick,[1] Boris R. Krasnov,[2] Irina S. Khokhlova,[3] Georgy I. Shenbrot,[2] Michael Y. Kosoy,[4] and Shimon Harrus[1]*

Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel[1]; Mitrani Department of Desert Ecology, Institute for Dryland Environmental Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer Campus, 84990 Midreshet Ben-Gurion, Israel[2]; Wyler Department of Dryland Agriculture, French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boqer Campus, 84990 Midreshet Ben-Gurion, Israel[3]; and Bacterial Diseases Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80521[4]

Received 11 April 2010/Accepted 16 August 2010

Fleas collected from rodents in the Negev Desert in southern Israel were molecularly screened for *Bartonella* species. A total of 1,148 fleas, collected from 122 rodents belonging to six species, were pooled in 245 pools based on flea species, sex, and rodent host species. Two *Bartonella* gene fragments, corresponding to RNA polymerase B (*rpoB*) and citrate synthase (*gltA*), were targeted, and 94 and 74 flea pools were found positive by PCR, respectively. The *Bartonella* 16S-23S internal transcribed spacer (ITS) region was also targeted, and 66 flea pools were found to be positive by PCR. Sixteen different *Bartonella gltA* genotypes were detected in 94 positive flea pools collected from 5 different rodent species, indicating that fleas collected from each rodent species can harbor several *Bartonella* genotypes. Based on *gltA* analysis, identified *Bartonella* genotypes were highly similar or identical to strains previously detected in rodent species from different parts of the world. A *gltA* fragment 100% similar to *Bartonella henselae* was detected in one flea pool. Another 2 flea pools contained *gltA* fragments that were closely related to *B. henselae* (98% similarity). The high sequence similarities to the zoonotic pathogen *B. henselae* warrant further investigation.

Bartonellae are small Gram-negative bacilli belonging to the alpha-2 subdivision of the *Proteobacteria.* Different *Bartonella* species were detected in a wide range of vertebrate animals. There are currently 30 known species or subspecies, among which 14 have been associated with human diseases (7). *Bartonella* organisms are parasites of mammalian erythrocytes and endothelial cells and are transmitted by fleas and lice and potentially by other blood-feeding arthropods such as ticks and flies (2). Infection in the natural host commonly causes a chronic bacteremia, which is asymptomatic in most cases.

Rodents are being extensively studied and were found to have a high prevalence of *Bartonella* infection, with a high diversity of *Bartonella* spp. and strains (3). The close contacts between human and rodent populations around the world create excellent conditions for transmission of *Bartonella* spp. from animals to humans (28). The transmission routes of *Bartonella* bacteria by arthropod vectors among rodents and between rodents and other mammalian hosts have public health implications. In order to understand the extent to which rodents serve as source of human infections, investigations of rodent-borne *Bartonella* are essential (28). A few cases of human infections with *Bartonella* bacteria of rodent origin have been reported: *B. elizabethae* was associated with endocarditis, *B. washoensis* was associated with cases of myocarditis and meningitis, *B. vinsonii* subsp. *arupensis* was reported to cause fever and neurologic symptoms, and *B. grahamii* was isolated from the intraocular fluids of a patient with neuroretinitis (5, 11, 12, 25, 29).

An earlier survey carried out in the Tel Aviv region, Israel, demonstrated the occurrence of *Bartonella* strains closely related to *B. elizabethae* and *B. tribocorum* in commensal rats (*Rattus rattus*) (8). Another study has surveyed wild rodents and their fleas for *Bartonella* spp. in 19 geographical locations in Israel from the Upper Galilee in the north to Beer Sheba in the south. *Bartonella* DNA was detected in spleen samples of 19 out of 79 (24%) black rats (*R. rattus*), in 1 of 4 (25%) Cairo spiny mice (*Acomys cahirinus*), and in 15 of 34 (44%) flea pools collected from black rats (*R. rattus*) (21). The objectives of the current study were to screen fleas collected from rodents inhabiting the Negev Desert south to Beer Sheba for *Bartonella* infection and to compare *Bartonella* prevalences between male and female fleas.

MATERIALS AND METHODS

Study site and flea collection. Rodents were captured in the northern and central portions of the Negev Desert in October to December 2007. The climate of the region is characterized by hot and dry summers (mean daily temperature in July is 25 to 34°C) and relatively cool winters (mean daily temperature in January is 11 to 14°C). Annual rainfall ranges from 108 mm per annum in the northern part of the region to 25.8 mm per annum in its southern part. The dominant vegetation consists of *Retama raetam* (white weeping broom), *Zygo-*

* Corresponding author. Mailing address: Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel. Phone: 972-3-9688546. Fax: 972-8-9489956. E-mail: harrus@agri.huji.ac.il.

▽ Published ahead of print on 27 August 2010.

phyllum dumosum (desert shrub), *Artemisia monosperma* (bushy leafy shrub), *Anabasis articulata* (jointed anabis), *Atriplex halimus* (Mediterranean saltbush), *Echiochilon fruticosum* (North African shrub), *Haloxylon persicum* (white saxaul), and *Calligonum comosum* (abal) (18). Sherman live traps baited with millet seeds were arranged across each sampling plot in a 100- × 100-m grid using 25 or 50 traps per grid. Fleas were collected from a captured individual rodent by brushing the animal's fur thoroughly, using a toothbrush over a white plastic pan. Collected fleas were placed in Eppendorf vials with 70% alcohol and delivered to the laboratory for identification. Each rodent was identified by species, sexed, weighed (with a Pesola spring scale), marked by subcutaneously implanting a microtransponder (Trovan ID-100; United Kingdom), and released at the capture site. After counting and identification, fleas were grouped in pools ranging from 1 to 10 fleas based on their species, sex, and rodent host species. The study was approved by the Nature and National Parks Protection Authority (2007/27779).

DNA extraction. Fleas were minced on a slide with a scalpel into minor pieces. DNA was extracted using the DNA extraction kit (Illustra Tissue Mini Spin kit; GE Healthcare, Buckinghamshire, United Kingdom) according to the manufacturer's instructions.

PCR analysis and sequencing. Molecular diagnosis of *Bartonella* DNA was carried out by conventional PCR targeting the 313-bp fragment of the citrate synthase gene (*gltA*) using primers Bhcs.781p and Bhcs.1137n (22, 26) and targeting the 16S-23S internal transcribed spacer (ITS) region (496 to 549 bp) using primers 321s and 983 (20) and by the high-resolution melt (HRM) real-time PCR analysis targeting a 194-bp fragment of the RNA polymerase B (*rpoB*) gene using primers 600f and 800r (21). Positive amplicons were purified using the PCR purification kit (ExoSAP-IT; USB, Ohio). DNA sequencing was carried out utilizing the BigDye Terminator cycle sequencing chemistry from Applied Biosystems (ABI), the 3700 DNA analyzer, and the ABI data collection and sequence analysis software. Further analysis was done using Sequencher software, version 4.8 for Mac (Gene Codes Corporation, Michigan). The obtained sequences were analyzed initially by BLAST through the NCBI's Mega-BLAST algorithm and further aligned with other *Bartonella* sequences.

Phylogenetic analysis. Multiple alignments of the DNA fragments were generated using ClustalW 1.83 (27). Phylogenetic trees obtained from the alignments were compared by several programs from Phylip package v.3.68 (23). The Seqboot program was initially run with 100 data sets. Then, the DNAML (maximum likelihood) program was run on the Seqboot data sets using the default parameters of the DNAML program and 3-time jumbles. The Concense program was run to build the consensus tree. The NJplot program was used to draw the phylogenetic trees. All representative sequences of *gltA* from positive flea pools were translated to amino acid sequences and compared with each other and with closely related *Bartonella* species.

Nucleotide sequence accession numbers. Newly identified sequences were deposited in GenBank under accession numbers GU354260, GU354263, GU354264,GU354268, GU354269, GU354270, GU354272 to GU354281, GU354282 to GU354306, GU937230, GU937231, GU937232, and GU937233.

RESULTS

Rodents and fleas. A total of 245 flea pools were collected from 122 rodents: *Leptopsylla algira* fleas were collected from 11 *Mus musculus* (house mouse) mice; *Synosternus cleopatrae* fleas were collected from 63 *Gerbillus andersoni allenbyi* (Anderson's gerbil) gerbils, 7 *Gerbillus pyramidum* (greater Egyptian gerbil) gerbils, and 5 *Meriones sacramenti* (Buxton's jird) jirds; and *Xenopsylla ramesis* fleas were collected from 33 *Meriones tristrami* (Tristram's jird) jirds and 3 *Dipodillus dasyurus* (Wagner's gerbil) gerbils.

***gltA* analysis.** Ninety-four of 245 (38.3%) flea pools collected from 62/122 (50.8%) rodents contained *Bartonella gltA* fragments. Sixteen different *Bartonella* genotypes were identified in fleas collected from 5 different rodent species. Figure 1 describes the phylogenetic analysis of the identified *Bartonella* genotypes.

In 4 of 12 pools of *L. algira* fleas collected from 2 *M. musculus* mice (one male and one female flea pool from each rodent), identified *Bartonella* genotypes (deposited as GenBank accession number GU354260) were 96% similar to *Bartonella* sp. OE 1-1 (AB445001) (Fig. 1).

In 4 of 8 pools of *S. cleopatrae* fleas collected from 2 *M. sacramenti* jirds, *Bartonella* genotypes (deposited as GenBank accession number GU354270) were 96% similar to *Bartonella* sp. TL-sv4 (AJ583130). Both male and female flea pools collected from two *M. sacramenti* were found positive (Fig. 1).

In 55 of 116 pools of *S. cleopatrae* fleas collected from 35 *G. andersoni allenbyi* gerbils, nine different *Bartonella* genotypes were detected (Fig. 1). The most common *Bartonella* strain detected was 100% similar to *Bartonella* sp. OE 1-1, detected in 26/55 positive flea pools. Male and female flea pools collected from the same individual of *G. andersoni allenbyi* contained the *gltA* gene fragment (deposited as GenBank accession number GU354264) 98% similar to *Bartonella henselae* (Fig. 1).

In 6 of 14 pools of *S. cleopatrae* fleas collected from 3 *G. pyramidum* gerbils, two different *Bartonella* genotypes were detected. In two *G. pyramidum* individuals, male and female flea pools contained a *gltA* fragment 100% similar to *Bartonella* sp. OE 1-1. In another *G. pyramidum* individual, the male flea pool contained a *gltA* fragment (deposited as GenBank accession number GU354263) that was 95% similar to *Bartonella* sp. OE 5-1 (AB444992) (Fig. 1).

In 31 of 76 pools of *X. ramesis* fleas collected from 20 *M. tristrami* jirds, six different *Bartonella* strains were identified. In one female flea pool from *M. tristrami*, *B. henselae* DNA was detected (313 bp; 100% identity to GenBank accession number GU056191) (Fig. 1).

16S-23S internal transcribed spacer (ITS). Sixty-six out of 245 (26.9%) flea pools collected from 59/122 (48.3%) rodents were found positive for the *Bartonella* 16S-23S ITS. All the ITS-positive samples were positive for *gltA* as well. The ITS sequences were grouped into two distinct phylogenetic clusters (Fig. 2): one cluster of *Bartonella* genotypes was identified from 28 flea pools collected from 26 gerbils (*Gerbillus*) only, and the second cluster of genotypes was identified from 37 flea pools collected from 33 jirds (*Meriones*) together with a single sample that was collected from a gerbil. The ITS sequences were deposited in GenBank with accession numbers GU354272 to GU354281, GU937232, GU937233, GU937231, and GU937230.

***rpoB*.** Seventy-four out of 245 (30.2%) flea pools collected from 54/122 (44.2%) rodents were found positive for the *Bartonella rpoB* gene fragment. All these pools were positive for the *gltA* fragment. Eleven different genotypes were identified in *G. andersoni allenbyi*, and 14 different genotypes were identified in *M. tristrami* (deposited as GenBank accession numbers GU354282 to GU354306).

The DNA sample detected in fleas collected from *M. tristrami* which contained a *Bartonella gltA* fragment that was identical to *Bartonella henselae* (GenBank accession number GU056191) contained an *rpoB* fragment (194 bp) that was 95% similar to *B. henselae* (new GenBank accession number GU354283). *rpoB* could not be amplified in the other DNA samples that contained *gltA* fragments that were 98% similar to *B. henselae*.

Predicted amino acid analysis. The *gltA*-based amino acid analysis indicated that most of the genomic substitutions between the sequences detected in this study were proteomically synonymous with a few nonsynonymous substitutions. In two genotypes (deposited as GenBank accession numbers GU354263 and GU354268), there were two amino acid substitutions compared to their closely related reference strains

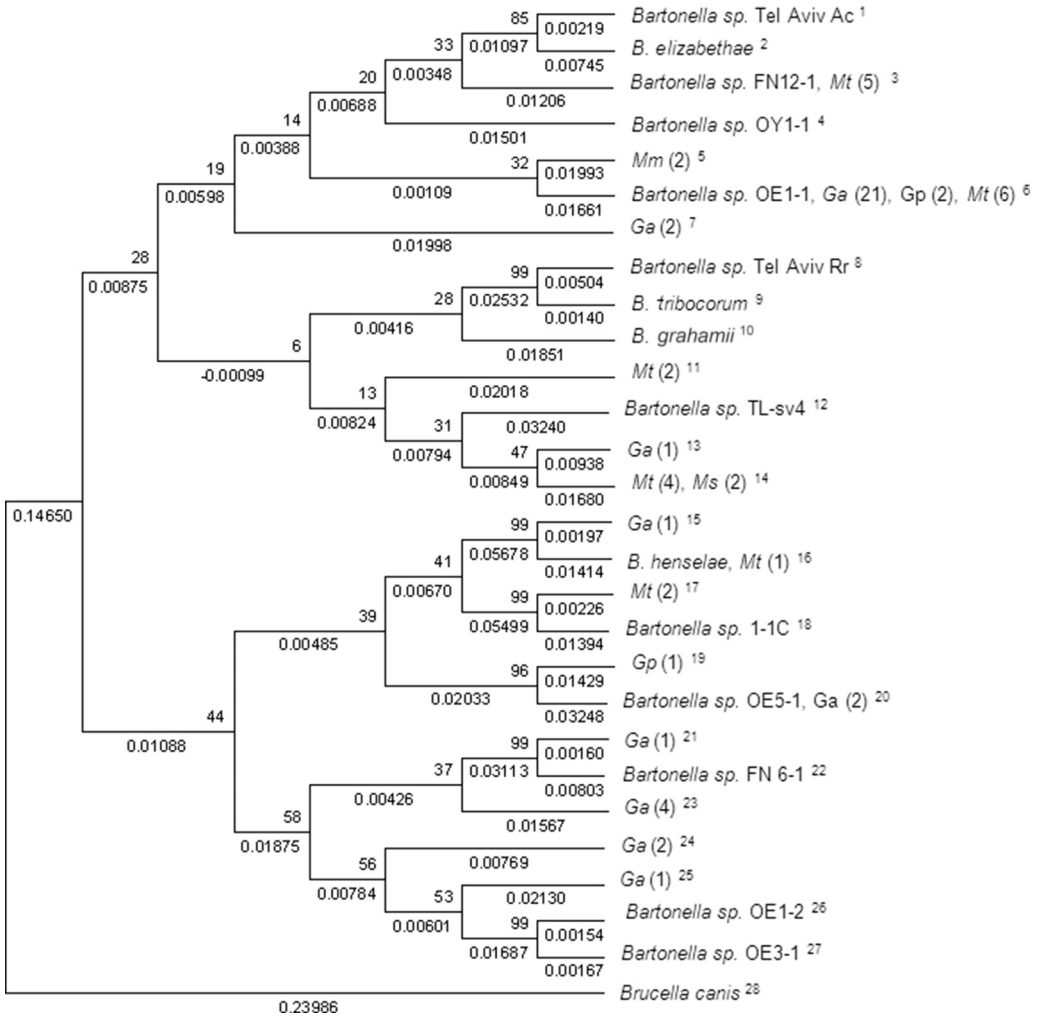

FIG. 1. *gltA*-based phylogenetic analysis of *Bartonella* genotypes detected in fleas collected from wild rodents according to the rodent host. The numbers of rodents detected as *Bartonella* positive in this study are indicated in parentheses. Reference strains are presented with no parentheses. Rodent hosts: Mt, *Meriones tristrami*; Ms, *Meriones sacramenti*; Ga, *Gerbillus andersoni allenbyi*; Gp, *Gerbillus pyramidum*; Mm, *Mus musculus*. *Leptopsylla algira* fleas were collected from *M. musculus*. *Synosternus cleopatrae* fleas were collected from *M. sacramenti*, *G. andersoni allenbyi*, and *G. pyramidum*. *Xenopsylla ramesis* fleas were collected from *M. tristrami*. GenBank accession numbers are represented by superscript numbers: 1, FJ686050; 2, GQ225710; 3, AB445003; 4, AB445005; 5, GU354260; 6, AB445001; 7, GU354267; 8, FJ686053; 9, AM260525; 10, GU056195; 11, GU354269; 12, AJ583130; 13, GU354262; 14, GU354270; 15, GU354264; 16, GU056191; 17, GU354271; 18, EU551154; 19, GU354263; 20, AB444992; 21, GU354261; 22, AB444983.1; 23, GU354265; 24, GU354266; 25, GU354268; 26, AB444981; 27, AB444982; 28, CP000872.

(*Bartonella* sp. OE 5-1, GenBank accession number AB444992, and *Bartonella* sp. OE 3-1, GenBank accession number AB444982, respectively). In the former, arginine substituted for lysine in position 108 and serine substituted for arginine in position 85, while in the latter asparagine substituted for serine in position 44 and asparagine substituted for aspartate in position 27. In two genotypes (new GenBank accession numbers GU354260 and GU354269), there was one amino acid substitution compared to their reference strains (*Bartonella* sp. OE 1-1, GenBank accession number AB445001, and *Bartonella grahamii*, GenBank accession number AY584857). In the former isoleucine substituted for asparagine in position 97 and lysine substituted for isoleucine in position 10.

Comparison of *Bartonella* DNA prevalence and diversity values between flea sexes. Sixty-two out of the 122 (50.8%) rodents carried *Bartonella* DNA-positive fleas. In 62 out of the 94 (65%) flea pools which were found positive for *Bartonella gltA*, similar *Bartonella* DNAs were identified in both female and male fleas collected from the same rodent. In three cases, *Bartonella* DNA identified in male fleas was different from *Bartonella* DNA identified in female fleas collected from the same rodent host. Fourteen positive male flea pools were identified when the female pools collected from the same rodent were negative, and 12 positive female flea pools were identified when the male pools collected from the same rodent were negative.

DISCUSSION

In the current study, *Bartonella* DNA was detected in a high percentage of the flea pools (collected from individual rodents), suggesting a high prevalence of *Bartonella* spp. in ro-

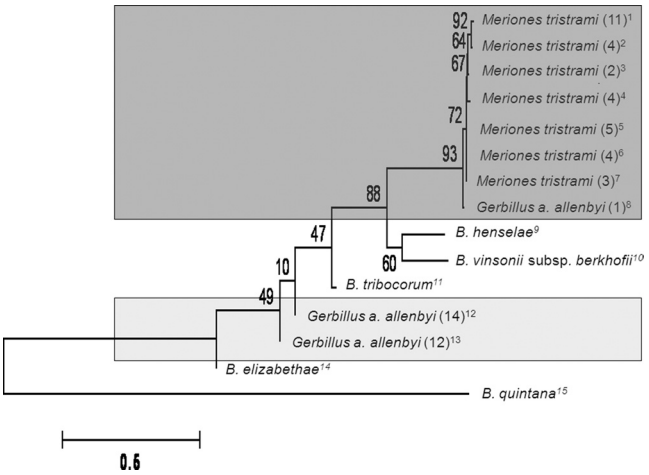

FIG. 2. Phylogenetic analysis of *Bartonella* strains detected in fleas collected from wild rodents according to the rodent host, targeting a fragment of the 16S-23S intergenic spacer (ITS) locus. Gray shading indicates two different *Bartonella* clusters. The numbers of animals detected as *Bartonella* positive in this study are indicated in parentheses. Reference strains are presented with no parentheses. GenBank accession numbers are represented by superscript numbers: 1, GU354278; 2, GU354277; 3, GU937232; 4, GU937233; 5, GU354281; 6, GU354280; 7, GU937231; 8, GU354274; 9, DQ529247; 10, DQ360834; 11, AM260525; 12, GU937230; 13, GU354273; 14, L35103; 15, DQ648598.

dent fleas in the Negev Desert in Israel. Moreover, about half of the rodents that were captured were infested with *Bartonella*-positive fleas, suggesting the high prevalence of *Bartonella* spp. in rodents in this region. Sixteen different *Bartonella* genotypes were identified in fleas collected from 5 different rodent species, suggesting that some rodent species possibly can harbor more than one *Bartonella* genotype. The clinical significance of these findings has yet to be elucidated.

The 313-bp *gltA* fragment used in this study was found to be a good target for screening fleas for *Bartonella* infection and for identification to species level. All flea pools that were found positive by *rpoB* or ITS screening were also positive by *gltA*. The *Bartonella gltA* sequence database in GenBank is the largest and most frequently updated among the different collections of *Bartonella* deposited sequences and therefore allows a more accurate differentiation between *Bartonella* species and strains. The *gltA*-based proteomic analysis indicated that most amino acid substitutions were synonymous, highlighting the important and critical function of the citrate synthase (*gltA*) enzyme in bacteria. Whether the substitutions detected in this study have any significance for the function of this enzyme warrants further investigation.

Interesting information was gained by analyzing the ITS locus among detected *Bartonella* genotypes. Except for one *Bartonella* genotype (which was found in one pool collected from a gerbil), the *Bartonella* ITS genotypes collected from *M. tristrami* grouped in one cluster, while *Bartonella* genotypes from flea pools collected from *G. andersoni allenbyi* grouped in a different cluster, suggesting that the ITS locus can assist in defining a group of *Bartonella* genotypes according to their rodent host. There may be at least three, not mutually exclusive reasons for the occurrence of two phylogenetic clusters. This can be related to differences in (i) rodent host species (*Gerbillus* and *Dipodillus* versus *Meriones*), (ii) flea vector species (*S. cleopatrae* versus *X. ramesis*), and (iii) habitat type (sand dunes versus loess plains). Indeed, in the study area, *G. andersoni allenbyi* is a sand dweller whereas *M. tristrami* inhabits loess plains. The characteristic flea of the former is *S. cleopatrae*, whereas the characteristic flea of the latter is *X. ramesis* (16).

In this study, a *gltA* fragment, identical to *Bartonella henselae*, was detected in one flea pool. The *rpoB* fragment of this sample was 95% similar to *B. henselae* strain Houston-1 (BX897699; AF171070) and to *B. henselae* that was previously detected in the aortic valve of a boxer dog (FJ832095). Another *gltA* fragment, highly similar to *B. henselae*, was detected in two other flea pools. Based on the 16S rRNA analysis, *B. henselae* was once previously reported in rodents (wood mice, *Apodemus sylvaticus*), in Denmark (6). The detection of *Bartonella* strains closely related to *B. henselae*, the agent of cat scratch disease, in rodent fleas suggests the zoonotic potential of these pathogens and warrants further investigation of their pathogenic capability.

Male and female fleas were found to be highly similar in their capacities to carry *Bartonella* organisms. In several flea pools, collected from the same rodent, male flea pools were positive while the female flea pools were negative and vice versa. As the frequencies of the latter findings were almost the same for male and female pools and were found in a relatively small number of pools compared to those for the rodents that carried positive male and female flea pools, these findings are probably incidental. However, as no characterization of *Bartonella* in the hosting rodent was carried out and no information on whether the fleas were hosting more than one host was available due to the characteristics of this survey, this enigma will remain unsolved. Previous studies have shown that the absolute amount of a blood meal taken by a male flea is smaller than that of a female flea meal (15, 17). Our results suggest that the difference in the amount of a blood meal between male and female fleas is not a critical factor for acquiring *Bartonella* bacteria. In three cases, different *Bartonella* strains were found in male and female flea pools collected from the same rodent. These observations could be explained by coinfection of multiple *Bartonella* strains in the same rodent host rather than by different associations between *Bartonella* genotypes and male or female fleas.

Bartonella genotypes that were detected in this study were similar or identical to *Bartonella* strains that were previously detected in different rodent species from a worldwide geographic distribution: *Bartonella* sp. OE 1-1 (AB445001), *Bartonella* sp. OE 5-1 (AB444992), *Bartonella* sp. OE 3-1 (AB444982), and *Bartonella* sp. OE 1-2 (AB444981) were previously detected in *G. pyramidum* in Egypt (9); *Bartonella* sp. FN 12-1 (AB445003) and *Bartonella* sp. FN 6-1 (AB444983) were previously detected in *Pachyuromys duprasi* rodents that were imported to Japan from a breeding farm in the Netherlands (9); *Bartonella* sp. 1-1C (EU551154) was previously detected in *Rattus norvegicus* in Taiwan (19); and *Bartonella* sp. TL-sv4 (AJ583130) was previously detected in *Tatera leucogaster* from South Africa (24). The explanation for the worldwide existence of similar genotypes is unknown and yet to be defined.

The most common *Bartonella* strain identified in this study was 100% similar to *Bartonella* sp. OE 1-1, which was previously detected in *G. pyramidum* from Egypt (9). In this study it was detected in fleas collected from 3 different rodent species: *G. andersoni allenbyi*, *M. tristrami*, and *G. pyramidum*. The close geographic proximity between Egypt and the Negev Desert in Israel suggests that these rodents share similar habitats and therefore similar fleas and *Bartonella* strains.

A study investigating the diversity of *Bartonella* spp. in *Spermophilus richardsonii* ground squirrels at multiple sites in Saskatchewan, Canada, suggested that in sites where dispersal is limited and rodent hosts are separated from their neighbors by barriers, a lower diversity of *Bartonella* genotypes might be expected, whereas in locations where a few rodent hosts share the same area, spillover and establishment of *Bartonella* genotypes from one rodent host to another are possible (10). The rodents included in this study live in two different habitats. *G. andersoni allenbyi*, *G. pyramidum*, and *M. sacramenti* are sand dwellers while *M. tristrami*, *M. musculus*, and *D. dasyurus* inhabit loess plains. The dispersal within each habitat is high and might be the reason for the great diversity of *Bartonella* spp. found in the current study.

Field and laboratory studies have suggested that some host specificity occurs among *Bartonella* spp. (13, 14). There is also evidence that a single species of *Bartonella* might infect more than one host species at a given site (2). In a recent study performed by our group, a single strain of *Bartonella* species closely related to both *B. tribocorum* and *B. elizabethae* was identified in rat spleens and in fleas collected from black rats (*Rattus rattus*) captured in different geographical locations in Israel (21). Our current study indicates a great diversity of *Bartonella* strains circulating among wild rodent fleas in the Negev Desert compared to *Bartonella* strains detected in fleas collected from commensal black rats in urban areas in Israel (10, 11). Recent studies have shown that fleas might carry a broader range of *Bartonella* spp. than do their hosts (1, 4). As no attempts were made in this study to isolate and characterize *Bartonella* spp. in the hosting rodents, we cannot determine their *Bartonella* hosting capacities in comparison to those of their fleas. The relation between the proximity to urban centers and the possible effect on *Bartonella* diversity needs further investigation, as wild rodents and their parasitizing fleas might be found to play a role in public health issues.

In conclusion, this study adds to the knowledge of the diversity of *Bartonella* spp. and strains in wild rodents in the Negev Desert in Israel. More research is required to better understand the diversity of *Bartonella* species in rodent communities and its relation to different geographical and climate conditions, as well as proximity to urban centers and populated areas. The finding of *Bartonella* spp. closely related to *B. henselae*, the agent of cat scratch disease, should warrant further investigation of these species.

ACKNOWLEDGMENTS

This study was sponsored by the Niedersachsen-Israel cooperation fund (VWZN2176) and partly supported by ISF grants 297/07 (to G.I.S.) and 27/08 (to B.R.K. and I.S.K.).

We thank the Center for Absorption in Science, Ministry of Immigrant Absorption, for their financial support.

This is publication no. 687 of the Mitrani Department of Desert Ecology.

REFERENCES

1. **Abbot, P., A. E. Aviles, L. Eller, and L. A. Durden.** 2007. Mixed infections, cryptic diversity, and vector-borne pathogens: evidence from Polygenis fleas and *Bartonella* species. Appl. Environ. Microbiol. **73:**6045–6052.
2. **Billeter, S. A., M. G. Levy, B. B. Chomel, and E. B. Breitschwerdt.** 2008. Vector transmission of *Bartonella* species with emphasis on the potential for tick transmission. Med. Vet. Entomol. **22:**1–15.
3. **Birtles, R. J., S. M. Hazel, M. Bennett, K. Bown, D. Raoult, and M. Begon.** 2001. Longitudinal monitoring of the dynamics of infections due to *Bartonella* species in UK woodland rodents. Epidemiol. Infect. **126:**323–329.
4. **Brinkerhoff, R. J., H. Kabeya, K. Inoue, Y. Bai, and S. Maruyama.** 2010. Detection of multiple *Bartonella* species in digestive and reproductive tissues of fleas collected from sympatric mammals. ISME J. **4:**955–958.
5. **Ellis, B. A., R. L. Regnery, L. Beati, F. Bacellar, M. Rood, G. G. Glass, E. Marston, T. G. Ksiazek, D. Jones, and J. E. Childs.** 1999. Rats of the genus Rattus are reservoir hosts for pathogenic *Bartonella* species: an Old World origin for a New World disease? J. Infect. Dis. **180:**220–224.
6. **Engbaek, K., and P. A. Lawson.** 2004. Identification of *Bartonella* species in rodents, shrews and cats in Denmark: detection of two *B. henselae* variants, one in cats and the other in the long-tailed field mouse. APMIS **112:**336–341.
7. **Guptill, L.** 2010. Bartonellosis. Vet. Microbiol. **140:**347–359.
8. **Harrus, S., G. K. Bar-Gal, A. Golan, R. Elazari-Volcani, M. Y. Kosoy, D. Morick, B. Avidor, and G. Baneth.** 2009. Isolation and genetic characterization of a *Bartonella* strain closely related to *Bartonella tribocorum* and *Bartonella elizabethae* in Israeli commensal rats. Am. J. Trop. Med. Hyg. **81:**55–58.
9. **Inoue, K., S. Maruyama, H. Kabeya, K. Hagiya, Y. Izumi, Y. Une, and Y. Yoshikawa.** 2009. Exotic small mammals as potential reservoirs of zoonotic *Bartonella* spp. Emerg. Infect. Dis. **15:**526–532.
10. **Jardine, C., D. McColl, G. Wobeser, and F. A. Leighton.** 2006. Diversity of *Bartonella* genotypes in Richardson's ground squirrel populations. Vector Borne Zoonotic Dis. **6:**395–403.
11. **Kerkhoff, F. T., A. M. Bergmans, A. van Der Zee, and A. Rothova.** 1999. Demonstration of *Bartonella grahamii* DNA in ocular fluids of a patient with neuroretinitis. J. Clin. Microbiol. **37:**4034–4038.
12. **Kosoy, M., M. Murray, R. D. Gilmore, Jr., Y. Bai, and K. L. Gage.** 2003. *Bartonella* strains from ground squirrels are identical to *Bartonella washoensis* isolated from a human patient. J. Clin. Microbiol. **41:**645–650.
13. **Kosoy, M. Y., R. L. Regnery, T. Tzianabos, E. L. Marston, D. C. Jones, D. Green, G. O. Maupin, J. G. Olson, and J. E. Childs.** 1997. Distribution, diversity, and host specificity of *Bartonella* in rodents from the southeastern United States. Am. J. Trop. Med. Hyg. **57:**578–588.
14. **Kosoy, M. Y., E. K. Saito, D. Green, E. L. Marston, D. C. Jones, and J. E. Childs.** 2000. Experimental evidence of host specificity of *Bartonella* infection in rodents. Comp. Immunol. Microbiol. Infect. Dis. **23:**221–238.
15. **Krasnov, B. R.** 2008. Functional and evolutionary ecology of fleas: a model for ecological parasitology. Cambridge University Press, Cambridge, United Kingdom.
16. **Krasnov, B. R., M. Hastriter, S. G. Medvedev, G. I. Shenbrot, I. S. Khokhlova, and V. S. Vaschenok.** 1999. Additional records of fleas (*Siphonaptera*) on wild rodents in the southern part of Israel. Isr. J. Zool. **45:**333–340.
17. **Krasnov, B. R., G. I. Shenbrot, I. S. Khokhlova, H. Hawlena, and A. A. Degen.** 2008. Sex ratio in flea infrapopulations: number of fleas, host gender and host age do not have an effect. Parasitology **135:**1133–1141.
18. **Krasnov, B. R., G. I. Shenbrot, S. G. Medvedev, V. S. Vatschenok, and I. S. Khokhlova.** 1997. Host-habitat relations as an important determinant of spatial distribution of flea assemblages (*Siphonaptera*) on rodents in the Negev Desert. Parasitology **114:**159–173.
19. **Lin, J. W., C. Y. Chen, W. C. Chen, B. B. Chomel, and C. C. Chang.** 2008. Isolation of *Bartonella* species from rodents in Taiwan including a strain closely related to '*Bartonella rochalimae*' from *Rattus norvegicus*. J. Med. Microbiol. **57:**1496–1501.
20. **Maggi, R. G., and E. B. Breitschwerdt.** 2005. Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. J. Clin. Microbiol. **43:**1171–1176.
21. **Morick, D., G. Baneth, B. Avidor, M. Y. Kosoy, K. Y. Mumcuoglu, D. Mintz, O. Eyal, R. Goethe, A. Mietze, N. Shpigel, and S. Harrus.** 2009. Detection of *Bartonella* spp. in wild rodents in Israel using HRM real-time PCR. Vet. Microbiol. **139:**293–297.
22. **Norman, A. F., R. Regnery, P. Jameson, C. Greene, and D. C. Krause.** 1995. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J. Clin. Microbiol. **33:**1797–1803.
23. **Persson, B.** 2000. Bioinformatics in protein analysis. EXS **88:**215–231.
24. **Pretorius, A. M., L. Beati, and R. J. Birtles.** 2004. Diversity of *Bartonellae* associated with small mammals inhabiting Free State province, South Africa. Int. J. Syst. Evol. Microbiol. **54:**1959–1967.
25. **Probert, W., J. K. Louie, J. R. Tucker, R. Longoria, R. Hogue, S. Moler, M. Graves, H. J. Palmer, J. Cassady, and C. L. Fritz.** 2009. Meningitis due to a

Bartonella washoensis-like human pathogen. J. Clin. Microbiol. **47:**2332–2335.

26. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N. Engl. J. Med. **323:**1573–1580.
27. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
28. **Welc-Faleciak, R., A. Paziewska, A. Bajer, J. M. Behnke, and E. Sinski.** 2008. *Bartonella* spp. infection in rodents from different habitats in the Mazury Lake District, Northeast Poland. Vector Borne Zoonotic Dis. **8:**467–474.
29. **Welch, D. F., K. C. Carroll, E. K. Hofmeister, D. H. Persing, D. A. Robison, A. G. Steigerwalt, and D. J. Brenner.** 1999. Isolation of a new subspecies, *Bartonella vinsonii* subsp. *arupensis*, from a cattle rancher: identity with isolates found in conjunction with *Borrelia burgdorferi* and *Babesia microti* among naturally infected mice. J. Clin. Microbiol. **37:** 2598–2601.