Detection of *Bartonella* spp. in wild carnivores, hyraxes, hedgehog and rodents from Israel

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SUMMARY

Bartonella infection was explored in wild animals from Israel. Golden jackals (*Canis aureus*), red foxes (*Vulpes vulpes*), rock hyraxes (*Procavia capensis*), southern white-breasted hedgehogs (*Erinaceus concolor*), social voles (*Microtus socialis*), Tristram's jirds (*Meriones tristrami*), Cairo spiny mice (*Acomys cahirinus*), house mice (*Mus musculus*) and Indian crested porcupines (*Hystrix indica*) were sampled and screened by molecular and isolation methods. *Bartonella*-DNA was detected in 46 animals: 9/70 (13%) golden jackals, 2/11 (18%) red foxes, 3/35 (9%) rock hyraxes, 1/3 (33%) southern white-breasted hedgehogs, 5/57 (9%) Cairo spiny mice, 25/43 (58%) Tristram's jirds and 1/6 (16%) house mice. *Bartonella rochalimae* and *B. rochalimae*-like were widespread among jackals, foxes, hyraxes and jirds. This report represents the first detection of this zoonotic *Bartonella* sp. in rock hyraxes and golden jackals. Moreover, DNA of *Bartonella vinsonii* subsp. *berkhoffii, Bartonella acomydis, Candidatus* Bartonella merieuxii and other uncharacterized genotypes were identified. Three different *Bartonella* strains were isolated from Tristram's jirds, and several genotypes were molecularly detected from these animals. Furthermore, this study reports the first detection of *Bartonella* infection in a southern hedgehog. Our study indicates that infection with zoonotic and other *Bartonella* species is widespread among wild animals and stresses their potential threat to public health.

Key words: *Bartonella rochalimae*, wildlife, carnivores, hyraxes, hedgehog, rodents.

INTRODUCTION

Bartonellae are gram-negative, facultative intracellular, vector-borne bacteria, widely distributed among animal reservoirs, worldwide (Chomel *et al*. 2009). Most *Bartonella* species establish long-term and subclinical infections in their associated reservoir host (Chomel *et al*. 2009). *Bartonella* species have been associated with a wide range of domesticated and wild animals, and to date more than 33 known *Bartonella* species and subspecies have been described, and many other *Candidatus* species and uncharacterized genotypes have been documented (Kosoy *et al*. 2012). Among their host reservoirs predominate mammals, including rodents, carnivores, lagomorphs, insectivorous, marine mammals, bats and primates (Vayssier-Taussat *et al*. 2009). Only a single report of a non-mammalian host (sea turtles) has been reported (Valentine *et al*. 2007). Notably, several *Bartonella* species have been recognized as emerging pathogens for incidental hosts, such as humans and other animals (Chomel and Kasten, 2010). Among them, *Bartonella rochalimae* was isolated from a bacteremic patient, who presented fever and splenomegaly (Eremeeva *et al*. 2007). In addition, *B. rochalimae* was later associated with a fatal case of endocarditis in a dog (Henn *et al*. 2009*b*), revealing its interspecies pathogenic potential. *Bartonella rochalimae* has been associated with wild carnivores, including coyotes (*Canis latrans*), grey foxes (*Urocyon cinereoargenteus*), red foxes (*Vulpes vulpes*) and raccoons (*Procyon lotor*), which are considered to be reservoirs of this species (Henn *et al*. 2009*a*).

Rodents have been reported as reservoirs of several *Bartonella* spp., including the zoonotic species *Bartonella elizabethae* (Daly *et al*. 1993), *Bartonella grahamii* (Birtles *et al*. 1995) and *Bartonella vinsonii* subsp. *arupensis* (Welch *et al*. 1999). Interestingly, many novel and uncharacterized *Bartonella* strains and genotypes are continuously detected in many rodent species (Inoue *et al*. 2009). Furthermore, rodent hosts are easily found to be co-infected with more than one *Bartonella* sp. or variant (Buffet *et al*. 2012; Gutiérrez *et al*. 2014*b*).

In Israel, a diverse range of *Bartonella* species has been detected in various animals and their associated ectoparasites. These include stray and domestic cats (Avidor *et al*. 2004; Gutiérrez *et al*. 2013) and cat fleas (Gutiérrez *et al*. 2015*b*), dogs (Ohad *et al*. 2010) and dog fleas (Sofer *et al*. 2015), cattle and their lice (Gutiérrez *et al*. 2014*a*; Rudoler *et al*. 2014), domestic camels (*Camelus dromedarius*)

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(Rasis *et al*. 2014), rodents, including black rats (*Rattus rattus*), Cairo spiny mice (*Acomys cahirinus*), Sundevall's jirds (*Meriones crassus*), Balochistan gerbils (*Gerbillus nanus*), Anderson's gerbils (*Gerbillus andersoni*) and their associated-fleas (Harrus *et al*. 2009; Morick *et al*. 2009, 2011; Gutiérrez *et al*. 2014*b*) and red foxes (Henn *et al*. 2009*a*). Hence, as the ongoing interaction between pets, farm animals and humans as well as the gradual geographic extension of urban areas to the wild, the risk of transmission of emerging zoonoses is highlighted. The aim of this study was to detect and characterize the *Bartonella* spp. circulating in wild animals in Israel.

MATERIALS AND METHODS

Animal blood and tissue sampling

Spleen samples, EDTA blood, blood spotted onto filter paper and heart clot samples were collected from wild animals during 2008–2011, in 82 locations in Israel, from Rosh Hanikra in the north to Nitzana in the south. A total of 365 samples were collected from 275 animals belonging to the following orders: Rodentia (156 animals), Carnivora (81 animals), Hyracoidea (35 animals) and Erinaceomorpha (three animals). Accordingly, 73 samples from 46 Social voles (*Microtus socialis*), 62 from 43 Tristram's jirds (*Meriones tristrami*), 62 from 57 Cairo spiny mice (*A. cahirinus*), ten from six house mice (*M. musculus*), four from four Indian crested porcupines (*Hystrix indica*), 76 from 70 Golden jackals (*Canis aureus*), 15 from 11 red foxes (*V. vulpes*), 58 from 35 rock hyraxes (*Procavia capensis*) and five samples from three southern white-breasted hedgehogs (*Erinaceus concolor*) were screened. The animals were captured and sampled by the Nature and Parks Authorities of Israel as part of epidemiological and diagnostic studies on leishmaniasis, conducted by the Ministry of Health and the ministry of Environment of Israel. Maps, indicating the collection sites were constructed using their coordinates in AcrMap 10.0 software (Esri, Redlands, CA, USA).

DNA extraction

DNA was extracted from the tissue samples by guanidine thiocyanate technique (Hoss and Paabo, 1993), with the following modifications. Each sample was cut and a portion of 0·5–1 g was placed in a 1·5 mL sterile Eppendorf tube containing 500 μL solution of 4 M Guanidinium thiocyanate (GuSCN), 0·1 M Tris–HCl (pH 6·4), EDTA 0·02 M (pH 6·4) and 1·3% Triton X-100. All tubes, including a control (with all the reagents except a sample), were incubated overnight at 56 °C with constant agitation (50 rpm), followed by a second incubation at 94 °C for 10 min. Then, samples were centrifuged at 14 000 rpm for 5 min. The supernatants were recovered into new sterile 1·5 mL tubes, and 900 μL sodium iodide (NaI, Sigma-Aldrich, MO, USA), 15 μL silica beads (Sigma-Aldrich, MO, USA) and 15 μL of linear acrylamide were added and placed on ice for 1 h, stirring frequently by vortex. The extraction solutions were centrifuged at 5000 rpm for 30 s. The supernatants were discarded and the silica pellets were washed with 500 μL of washing buffer (10 M GuSCN and 0·1 M Tris–HCI, pH 6·4), and were centrifuged at 5000 rpm for 30 s. The supernatants were discarded and the beads were washed with 200 μL of ethanol absolute and centrifuged at 5000 rpm. The ethanol was removed by pipetting, and the tubes were set to dry at room temperature for 2 h. Silica beads were then treated with 90 μL of ultra-pure water (UPW), 10 μL of TE buffer (10 mM Tris pH 7·5, EDTA pH 7·5–8·0, UPW). Purified DNA was obtained in 100 μL of elution buffer. The samples were incubated at 56 °C for 1 h, and finally stored at 20 °C.

DNA from EDTA blood samples (200 μL) was extracted using the Illustra Tissue and Cells genomicPrep Mini Spin kit (GE Healthcare, Buckinghamshire, UK), following the manufacturer's recommendations.

DNA from blood samples spotted onto a filter paper was extracted using the phenol–chloroform–isoamyl alcohol method following the modifications described elsewhere (Strauss-Ayali *et al*. 2004).

HRM real-time PCR analysis

Screening for *Bartonella* spp. DNA was performed by HRM real-time PCR analysis targeting the 16S–23S internal transcribed spacer (ITS), following procedures and protocols described earlier (Gutiérrez *et al*. 2013). In brief, an approximately 190 bp fragment was amplified using primers 321 s and H493 as, described elsewhere (Maggi and Breitschwerdt, 2005). The ITS real-time PCR reactions were carried out in a 20 μL final volumes containing 1 μL of 10 μM solution of each primer, 0·6 μL of 50 μM Syto9 solution (Invitrogen, CA, USA), 5·4 μL of UPW, 10 μL of MAXIMA Hot-Start PCR Master Mix 2X (Thermo Scientific, Surrey, UK) and 2 μL of each genomic DNA. A *Bartonella*-positive DNA (*Bartonella henselae* and *Bartonella* sp. FG 4-1 strains), a *Bartonella*-negative DNA and a non-template DNA (NTC) were used as controls in each run. All reactions carried out using the rotor gene 6000 cycler (Corbett Research, Sydney, Australia).

All samples positive for the ITS *Bartonella*-DNA were later screened for other loci by targeting partial fragments of the transfer-mRNA (*ssrA*), RNA polymerase β-subunit (*rpoB*) and citrate synthase (*gltA*) genes by HRM real-time PCR assays. Accordingly, an approximately 300 bp fragment of the *ssrA* locus

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was amplified using ssrAF and ssrAR primers, as previously described (Diaz *et al*. 2012), and following reaction protocols described earlier (Gutiérrez *et al*. 2014*a*). An approximately 200 bp *rpoB* gene fragment was amplified using primers 600f and 800r, according to previously published conditions and reagent volumes (Morick *et al*. 2009). Finally, an approximately 340 bp *gltA* gene fragment was amplified using primers 443F (Birtles and Raoult, 1996) and 781R, according to previously published conditions (Sofer *et al*. 2015).

Bartonella isolation from DNA-positive EDTA blood samples

Culture isolation from EDTA blood samples of hosts with *Bartonella*-positive DNA was attempted (when adequate blood samples were available). Accordingly, the samples were diluted 1:2 in Schneider's Insect supplemented with 10% fetal bovine serum (Biological Industries, Israel), 5% sucrose (Riess *et al*. 2008), and 2·0 μg mL^{-1} amphotericin B, to enhance the *Bartonella* isolation and reduce fungal contamination, respectively, as previously recommended (Kosoy *et al*. 1997). Briefly, 100 μL of each diluted sample was directly plated on chocolate agar and incubated at 37 °C with 5% CO_2 atmosphere for up to 8 weeks. In addition, a pre-enrichment of the samples in liquid medium was attempted to increase the chances of *Bartonella* isolation. Thus, 200 μL of the sample solution described above was inoculated in 5 mL fresh Schneider's Insect Medium with additives (as mentioned above) and incubated at 37 °C with constant agitation (100 rpm) for 10 days. After the incubation, the tubes were centrifuged at 3000 rpm for 10 min and the supernatant was discarded. The cellular pellet was washed with 1 mL sterile PBS, twice. Finally, a bacteriological loop was used to inoculate the pellet in new chocolate agar and incubated at 37 °C with 5% CO_2 atmosphere for 8 weeks. Any small, round and *Bartonella*-like colony was re-isolated in a new chocolate agar plate. DNA was extracted from the colonies in 200 μL of PBS by a thermal protocol (i.e. 95 °C for 12 min). DNA was collected from the supernatant after centrifugation at 4 °C at 8500 rpm for 5 min.

Sequencing

All positive PCR products were purified and cleaned by NEB Exo-SAP PCR purification kit (New England Biolabs, Inc., Ipswich, MA, USA) and subsequently sequenced with sense and antisense primers using BigDye Terminator cycle sequencing chemistry from Applied Biosystems ABI PRISM 3730xl DNA Analyser and the ABI's Data collection and Sequence Analysis software (ABI, Carlsbad, CA, USA). Further analyses were done by MEGA 5 (Tamura *et al*. 2011). Then, the clean sequences were identified using BLASTn against the GenBank database (http://www.ncbi.nlm.nih.gov). All sequences with a length ⩾200 bp were deposited in the GenBank database. All sequences detected were classified in clones according to the host source and/or to similar characterized *Bartonella* spp. A numeric code was assigned to distinguished clone sequences with identities <99%. In addition, clones with similarities between 99 and 100% were identified with the same number and differentiated with additional alphabetic characters. Co-infected hosts were detected when different sequences were obtained from the different tissues of the same animal (e.g. blood and spleen samples) or by the isolation of different *Bartonella* strains from the same host.

Table 1. *Bartonella* infection rates of wild animals from Israel

Host species	Common name	Number of *Bartonella*-DNA-positive animals, out of tested animals per each species (%)
Carnivora		
Canis aureus	Golden Jackal	9/70 (13%)
Vulpes vulpes	Red fox	2/11 (18%)
Hyracoidea		
Procavia capensis	Rock hyrax	3/35 (9%)
Erinaceomorpha		
Erinaceus concolor	Southern white-breasted hedgehog	1/3 (33%)
Rodentia		
Acomys cahirinus	Cairo spiny mouse	5/57 (9%)
Meriones tristrami	Tristram's jird	25/43 (58%)
Mus musculus	House mouse	1/6 (16%)
Microtus socialis	Social vole	0/46 (0%)
Hystrix indica	Indian crested porcupine	0/4 (0%)

RESULTS

Bartonella-DNA detection

Bartonella-DNA was detected in a total of 58 samples (spleen and/or blood) obtained from 46 animals. The infection rates and the geographical distribution of the positive animal hosts are shown in Table 1 and Fig. 1, respectively. Table 2 shows the characterization of the *Bartonella* sequences detected and obtained from the animals. Further information on the positive animals and the details of the sequence clones detected in this study are included as online supplementary material (Tables S1 and S2).

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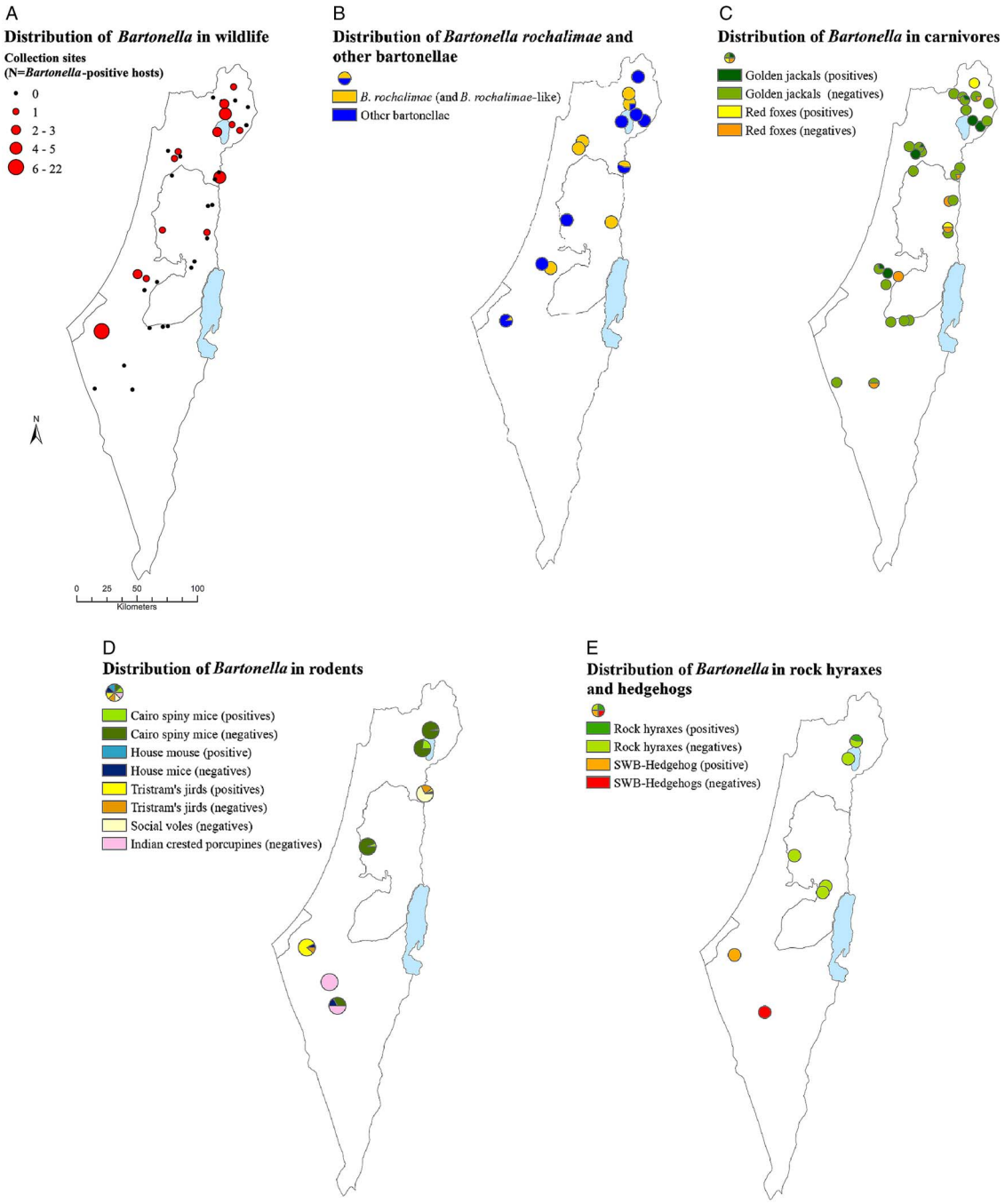

Fig. 1. Maps of Israel and the Palestinian Authority indicating the geographical localization of the collected samples according their *Bartonella*-infection status and host species. (A) Total distribution of *Bartonella*-positive animals in all the collected sites; (B) Distribution of *B. rochalimae* and other *Bartonella* spp. among positive hosts; (C–E) Distribution *Bartonella*-infection according to the different host species collected in the study.

Canis aureus (Golden jackal): Nine golden jackals were found positive for *Bartonella*-DNA. *Bartonella rochalimae*-DNA sequences (1–3 loci detected per sample, 100% identical to *B. rochalimae* sequences) were detected in 55% (5/9) of the positive jackals (Table 2, Fig. 2). Three other animals (33·3%; 3/9) contained DNA sequences closely related to *Bartonella* sp. HMD strains, recently re-named as *Candidatus* Bartonella merieuxii (Chomel *et al.* 2012), which were clustered phylogenetically with ruminant-associated *Bartonella* spp. (Fig. 2). The ITS amplicons from these samples were 100% identical to the *Bartonella* HMD clones (accession number FJ177635.5 and EF614393). In addition, the *ssrA* sequences were closely related to the ruminant *bartonellae, B. bovis* and *B. chomelii* (97 and 96% identity; KF218228 and KM215712 GenBank accession numbers, respectively). Lastly, one jackal harboured sequences of mixed-origin, with an ITS sequence 100% identical to *B. vinsonii* subsp. *berkhoffii* (HQ185695.2) and an *ssrA* sequence identical to the ruminant-like clone described above.

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Table 2. Characterization of *Bartonella* species and strains detected in wild animals from Israel

Host	*Bartonella* infection	Number of hosts infected	Loci obtained (clone/s ID)[a]
Carnivora			
Canis aureus	*Bartonella rochalimae*	5	ITS (clone Ca-Br), *rpoB* (clone Ca-Br), *ssrA* (clone Ca-Br)
C. aureus	Uncultured *Bartonella* sp. clone Ca-1	3	ITS (clone Ca-HMD), *ssrA* (clone Ca-1)
C. aureus	*Bartonella vinsonii* subsp. *berkhoffii*-like and uncultured *Bartonella* sp. clone	1	ITS (clone Ca-Bvb), *ssrA* (clone Ca-1)
Vulpes vulpes	*Bartonella rochalimae*	1	ITS (clone Vv-Br), *ssrA* (clone Vv-Br)
V. vulpes	Uncultured *Bartonella* sp. clone Vv-1	1	ITS (clone Vv-1), *ssrA* (clone Vv-1)
Erinaceomorpha			
Erinaceus concolor	Uncultured *Bartonella* sp. clone Ec	1	ITS (clone Ec-1)
Hyracoidea			
Procavia capensis	*Bartonella rochalimae*	3	ITS (clone Pc-Br), *rpoB* (clone Pc-Br), *ssrA* (clone Pc-Br)
Rodentia			
Acomys cahirinus	*Bartonella acomydis*	2	ITS (clone Ac-Bac), *gltA* (clone Ac-Bac)
A. cahirinus	Uncultured *Bartonella* sp. clones Ac	3	ITS (clone Ac-1A, Ac-1B or Ac-1C), *rpoB* (clone Ac-1),
Meriones tristrami	*Bartonella* sp. strain Mt-2290 (isolate) and uncultured *Bartonella* sp. clones Mt-1 and a *B. rochalimae*-like clone (co-infection)	1	ITS (clone Mt-1A), *rpoB* (clone Mt-1B), *gltA* (clones Mt-2290 and Mt-3), *ssrA* (clone Mt-1A)
Meriones tristrami	*Bartonella* sp. strains Mt-2286.1 and strain Mt-2286.3 and uncultured *Bartonella* sp. clones Mt (co-infection)	1	ITS (clone Mt-1A and strain 2286.3), *rpoB* (clones Mt-4, and colony strains 2286.1 and 2286.3), *gltA* (clones Mt-1A and 2286.1 and 2286·3, *ssrA* (clones Mt-1B and Mt-3)
Meriones tristrami	Uncultured *Bartonella* sp. clones Mt[b]	3	ITS (clone Mt-1A and Mt-1B), *rpoB* (clone Mt-1A and Mt-1C), *gltA* (clone Mt-1B and Mt-1C), *ssrA* (clones Mt-1A, Mt-1B and Mt-2 and Mt-3)
Meriones tristrami	Uncultured *Bartonella* sp. clones and *B. rochalimae*-like clones	4	ITS (Mt-1A or Mt-2), *rpoB* (clone Mt-1B, Mt-1C and Mt-3), *gltA* (clone Mt-2 and Mt-3), *ssrA* (clone Mt-Br1A, Mt-Br1B and Mt-1A)
Meriones tristrami	Uncultured *Bartonella* sp. clone(s) Mt	15	ITS (clone Mt-1A or Mt-1B), *rpoB* (clone Mt-1A, Mt-1B, MT-1C, Mt-2 or Mt-3), *gltA* (clone Mt-1A or Mt-2), *ssrA* (clone Mt-1A, Mt-1B and Mt-2)
Meriones tristrami	*Bartonella rochalimae*-like clone only	1	ITS (clone Mt-2)
Mus musculus	Uncultured *Bartonella* sp. clone Mm	1	ITS (clone Mm-1)

[a] Characterization of the loci obtained from the samples is found in the online supplemental material.
[b] Co-infection with different variants detected in the blood and spleen samples.

Vulpes vulpes (Red fox): Two red fox samples were detected positive for *Bartonella*-DNA, one harbored DNA sequences (ITS and *ssrA* loci) 100% identical to *B. rochalimae* and the other was positive for a *Bartonella* sp. closely related to the HMD strains/ *Candidatus* B. merieuxii (Table 2, Fig. 2). The latter sample contained ITS sequences closely related to the *Candidatus* B. merieuxii detected in the golden jackals, but with a deletion-gap of 22 nucleotides, and the *ssrA* amplicon was 100% identical to the clone detected in the golden jackals (Table 2).

Procavia capensis (Rock hyrax): Three hyraxes were positive for at least two *Bartonella*-DNA loci. All sequences obtained (ITS, *rpoB* and *ssrA* loci) were 100% identical to *B. rochalimae* (Table 2, Fig. 2). All positive animals were collected from the same geographical area (Table 1) and were positive only in their spleen samples.

Erinaceus concolor (Southern white-breasted hedgehog): A single positive blood sample was detected in a hedgehog. The ITS sequence obtained was distant from any previously described *Bartonella* species or strain, with 91% identity to *Bartonella* strain JB-15 (GenBank accession number AB674235.1). This sequence clustered in one ITS clade together with *Bartonella clarridgeiae*

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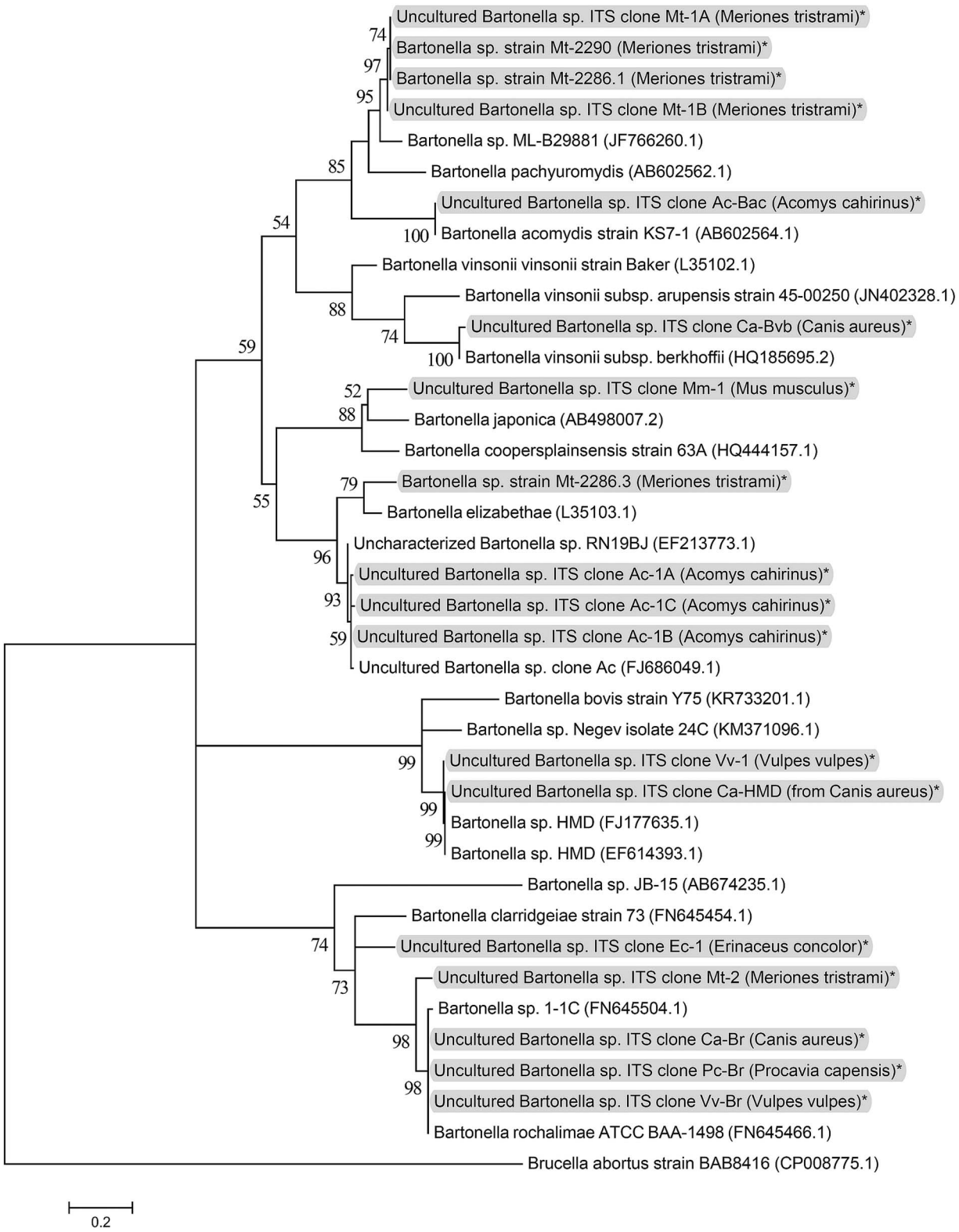

Fig. 2. Maximum-likelihood phylogenetic tree based on the partial ITS locus sequences (~200 bp). Phylogenetic tree was constructed using the MEGA software version 5. Bootstrap replicates were performed to estimate the node reliability, and values were obtained from 1000 randomly selected samples of the aligned sequence data. Bootstrap values higher than 50% are indicated. The host sources of the sequences obtained in this study are indicated in parentheses, highlighted with light grey and marked with an asterisk (*). GenBank reference sequences were used with their accession numbers in parentheses.

and *B. rochalimae* (Fig. 2). No other locus could be amplified from this blood sample.

Acomys cahirinus (Cairo spiny mouse): Five Cairo spiny mice were found positive for *Bartonella*-ITS. All ITS sequences obtained were closely related to *Bartonella* strains previously detected in this host species. Two mice carried ITS sequences 100% identical to *Bartonella acomydis* (GenBank accession

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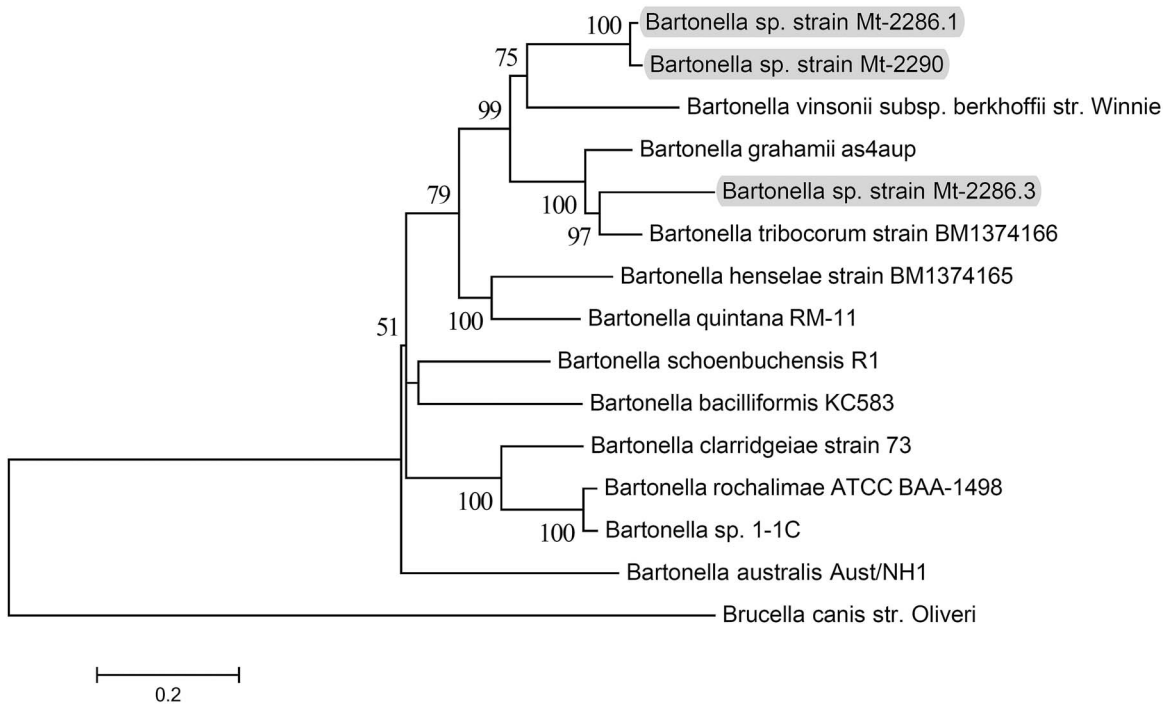

Fig. 3. Maximum-likelihood phylogenetic tree based on the concatenation of four loci (ITS, *gltA*, *rpoB* and *ssrA*) representing ~2400 bp. Phylogenetic tree was constructed using the MEGA software version 4. Bootstrap replicates were performed to estimate the node reliability, and values were obtained from 1000 randomly selected samples of the aligned sequence data. Bootstrap values higher than 50% are indicated. Strains isolated from *M. tristrami* jirds in this study are highlight with light grey. Reference sequences were obtained from whole-sequenced strains deposited in GenBank database.

number AB602564.1; Fig. 2). One of these mice was found positive for a *gltA* fragment (Table 2; deposited in GenBank under accession number KU316220). However, no *gltA* sequences from *B. acomydis* have been deposited in the GenBank database to date, thus the association of the obtained *gltA* sequence with *B. acomydis* could not be confirmed. The other three positive animals carried *Bartonella* genotypes closely related to an uncultured clone previously detected in *A. cahirinus* mice (GenBank accession number FJ686049.1).

Meriones tristrami (Tristram's jird): Tristram's jird was the animal species with the highest *Bartonella* prevalence in this study (Table 1). The sequenced loci revealed a great diversity of infecting genotypes within the two *M. tristrami* populations (Fig. 1), including three clones of ITS, six of *rpoB*, five of *gltA* and five of the *ssrA* fragments. From the 25 positive Tristram's jirds detected, sequence clones were closely related to bartonellae previously detected from rodents and/or their fleas, as well as *B. rochalimae*-like sequences (Table 2, Fig. 2). Moreover, three jirds were confirmed to be co-infected with different *Bartonella* genotypes, since different *Bartonella* sequences were detected in their blood and spleens. Four additional jirds contained loci from different *Bartonella* origin, also suggesting co-infections (online Table S1).

Mus musculus (house mouse): One house mouse was found positive for *Bartonella*-DNA. The ITS amplicon obtained was only distantly related to all known *Bartonella* species or strains, with *Bartonella coopersplainsensis* (GenBank accession number HQ444157.1) and *Bartonella japonica* (GenBank accession number AB498007.2) being the closest matches, with 91 and 90% sequence identities, respectively (Fig. 2).

Microtus socialis (social vole): No *Bartonella*-DNA was detected in any of the social voles screened.

Hystrix indica (Indian crested porcupine): No *Bartonella*-DNA was detected in any of the Indian crested porcupines screened.

Bartonella *culture isolation*

Bartonella isolation was attempted from the blood of 18 of 46 PCR-positive animals, including seven golden jackals, one red fox, three positive hyraxes, six Tristram's jirds and one southern white-breasted hedgehog. Six individual colonies per positive sample were characterized by conventional PCRs and sequencing targeting the same loci used for the molecular detection of all samples. Three *Bartonella* strains were successfully isolated from only two Tristram's jirds (Mt-2290 and Mt-2286, Fig. 3). All isolated colonies from rodent Mt-2290

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were genetically identical (e.g. same ITS, *ssrA*, *gltA* and *rpoB* sequences). Nevertheless, the *gltA* sequence detected in the blood extracted DNA (*B. rochalimae*-like clone, online Table S1) was different from the *gltA* fragment detected in the isolated colonies, confirming the circulation of various genotypes in this jird. Similarly, two different *Bartonella* strains were isolated from rodent Mt-2286 (Mt-2286.1 and Mt-2286.3). The phylogenetic analyses of those isolates demonstrated that the two strains represent distantly related spp., with an identity of 84·5% (Fig. 3). Moreover, the *gltA* and *rpoB* DNA sequences detected from the blood were different from those detected in the isolated strains, demonstrating the presence of at least a third co-infecting *Bartonella* genotype in this host (online Tables S1). The isolated strains Mt-2286.1 and Mt-2290 were closely related with 97·7% sequence identity, demonstrating that similar but not identical genotypes circulate among the same host species (Fig. 3). No *Bartonella* isolates were obtained by the pre-enrichment liquid medium. On the other hand, the overgrowth of other co-infecting bacterial genera in the sub-cultures was a common finding.

Nucleotide sequence accession numbers

Newly identified sequences, longer than 200 bp, obtained directly from the samples were deposited in GenBank database under ITS accession numbers: KU316206-KU316209; *ssrA*: KU316210-KU316219; and *gltA*: KU316220- KU316225.

Sequences (ITS, *rpoB, gltA* and *ssrA*) from isolated strains from *M. tristrami* jirds were deposited under the accession numbers: Mt-2290 strain: KU316226- KU316229; Mt-2286.1 strain: KU316230, KU316232, KU316234, KU316236; and Mt-2286.3 strain: KU316231, KU316233, KU316235 and KU316237.

DISCUSSION

This study reports the detection of *Bartonella* species in wild animals from Israel. DNA sequences of several recognized *Bartonella* spp., such as *B. rochalimae, B. vinsonii* subsp. *berkhoffii* and *B. acomydis, Candidatus* Bartonella species and several uncharacterized genotypes were detected among seven different animal species. Notably, the widespread distribution of the zoonotic *B. rochalimae*, and closely related strains, among different host species and across all the sampling areas is highlighted. This study reports infection with *Bartonella* for the first time in three animal species: the rock hyrax, Tristam's jird and the southern hedgehog. Moreover, the great diversity of *Bartonella* genotypes in Tristram's jirds is emphasized.

Infections with *Bartonella* spp. in wild canids have been reported worldwide (Henn *et al*. 2007, 2009*a*, Schaefer *et al*. 2011; Chomel *et al*. 2012). In this study, DNA sequences from three *Bartonella* spp. were detected in golden jackals (*C. aureus*) and in red foxes (*V. vulpes*), including the zoonotic *B. rochalimae* (in both canids), *B. vinsonii* subsp. *berkhoffii*-like organism (in a golden jackal) and *Bartonella* clones closely related to the proposed *Candidatus* B. merieuxii (in both canids) (Chomel *et al*. 2012). To the best of our knowledge, this study represents the first description of *B. rochalimae* in golden jackals. This zoonotic *Bartonella* species has been isolated from domestic dogs (*Canis lupus familiaris*), coyotes (*C. latrans*), wolfs (*Canis lupus*), island foxes (*Urocyon littoralis*), grey foxes (*U. cinereoargenteus*), red foxes (*V. vulpes*) and raccoons (*P. lotor*) (Henn *et al*. 2007, 2009*a*, Schaefer *et al*. 2011; Gerrikagoitia *et al*. 2012). Thus, our results expand the list of potential reservoirs of *B. rochalimae* in wild carnivores. Moreover, the detection of clones closely related to the *Bartonella* sp. HMD strains (*Candidatus* B. merieuxii) in both canids is notable. These strains were first detected from dogs and *Rhipicephalus sanguineus* ticks collected in Italy and Greece (Diniz *et al*. 2009). In the latter study, the sequences obtained (i.e. ITS locus, the *16S* and the *rpoB* genes) were closely related to ruminant-associated bartonellae. Interestingly, the *ssrA* fragments detected from the canids in our study were also closely related to ruminant bartonellae, suggesting that this *Bartonella* locus belongs to the same species. In addition, as reported previously, we detected differences between the ITS amplicons from the golden jackal (100% identical to those reported from HMD strains) and the red fox (92% identical due to a deletion-gap of 22 nucleotides). Chomel and others (2012), compared the *Bartonella* strain detected from Iraqi dogs and jackals with the original HMD strains, and concluded that they were the same *Bartonella* species, and therefore renamed them as *Candidatus* B. merieuxii. Furthermore, another study reported the infection of dogs from Sri Lanka with the HMD strain (Brenner *et al*. 2013). In summary, the detection of closely related strains from the jackals and a fox in this study reflects the widespread distribution of this newly canid *Candidatus* Bartonella species. Finally, one golden jackal was found to carry a genotype closely related to two different *Bartonella* species. The ITS amplicon was 100% similar to *B. vinsonii* subsp. *berkhoffii* and the *ssrA* fragment was 100% identical to the one detected in the other jackals, suggesting a co-infection with two different *Bartonella* species in this animal. The former finding is surprising, since *B. vinsonii* subsp. *berkhoffii* has not been found in Israel, neither in a dog or human or any other

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animal, to date. However, the short length of the ITS fragment sequenced (213 bp) and the different *Bartonella*-origin of the *ssrA* fragment, prevent confirming that this animal was infected with *B. vinsonii* subsp. *berkhoffii* and not with a closely related genotype. Interestingly, Chomel *et al.* (2012) detected this *Bartonella* sp. in jackals from Iraq. Hence, the capability of golden jackals to be reservoirs of *B. vinsonii* subsp. *berkhoffii* or closely related strains need to be further evaluated in future studies.

In the present study *B. rochalimae* DNA was detected from rock hyraxes (*P. capensis*). All samples were positive for at least two genomic loci. To the best of our knowledge, this represents the first report of *Bartonella* infection in rock hyraxes and adds one non-carnivore animal species as a potential reservoir for *B. rochalimae*. The positive animals were trapped in the same geographical area and only their spleen samples were positive, suggesting that these bacteria are harboured in the spleen in greater concentrations than the blood, allowing their molecular detection. It should be noted that in a recent study conducted in the Palestinian Authority, the authors screened *Bartonella*-DNA from fleas collected from various animals including hyraxes, and no *Bartonella*-DNA was detected in the hyraxes associated-fleas (Nasereddin *et al.* 2014), suggesting a low exposure to *Bartonella* from these hosts in these regions. Hyraxes serve as a reservoir host for *Leishmania tropica*, transmitted by sand flies *Phlebotomus sergenti* (Jaffe *et al.* 2004) and *Phlebotomus arabicus* (Svobodova *et al.* 2006). Our samples were derived from epidemiological and diagnostic studies on leishmaniasis, and none of the *Bartonella*-infected hyraxes were infected with *Leishmania* (data not shown). Thus, the potential role of rock hyraxes as reservoirs for *Bartonella* spp. and the potential vectors involved in their transmission require further investigation.

Rodents have been considered to be important reservoirs of *Bartonella* spp. (Buffet *et al.* 2013; Gutiérrez *et al.* 2015*a*). Wild rodents and their associated fleas, collected from suburban areas and the Negev desert of Israel, have been widely reported as *Bartonella* carriers (Morick *et al.* 2009, 2010, 2011; Gutiérrez *et al.* 2014*b*). In this study, five wild rodent species were screened for *Bartonella* infection, including two species whose *Bartonella* infection status was unexplored previously in Israel. The highest prevalence of *Bartonella* infection was detected in Tristram's jirds (*M. tristrami*) (58% of the total animals sampled). Tristram's jirds carried *Bartonella* genotypes closely related to previously reported bartonellae genotypes and to *B. rochalimae*-like organisms. *Bartonella rochalimae*-like loci amplified from these jirds were 98–100% similar to *B. rochalimae* ATCC BAA-1498 and to the *Bartonella* sp. 1-1C, a *B. rochalimae*-like organism isolated from *Rattus norvegicus* from Taiwan (Lin *et al.* 2008). Isolation of *Bartonella* spp. was obtained from two out of six animals tested. These isolations confirmed a co-infection status of the samples with different *Bartonella* spp. and genotypes. Additionally, the detection of up to three different genotypes, including two distantly related *Bartonella* spp., emphasizes the complex infection composition that these jirds harboured. A previous study performed by our group, detected *Bartonella*-DNA in flea pools collected from this jird species, and identified several genotypes based on the *gltA* and *rpoB* genes (Morick *et al.* 2010). However, in the latter study, no mammalian samples were collected. In the present study, two jird populations from two geographically distant regions were screened. Interestingly, both populations contained multiple *Bartonella* genotypes, showing a remarkable *Bartonella* diversity among these jirds. Thus, the present study confirmed the role of these jirds as carriers and potential reservoirs of *Bartonella* strains.

Cairo spiny mice (*A. cahirinus*) were positive for *B. acomydis*-DNA and two other uncharacterized *Bartonella* genotypes. This is the first detection of *B. acomydis* in this mouse species. This *Bartonella* species was previously isolated and described from the golden spiny mouse, *Acomys russatus* (Sato *et al.* 2013). The other two *Bartonella* genotypes were closely related to a previously identified clone detected in *A. cahirinus* collected from Israel (Morick *et al.* 2009).

One house mouse (*M. musculus*) was detected positive for an uncharacterized *Bartonella* genotype. The genotype was distantly related (~91% sequence identity) to *B. coopersplainsensis* and *B. japonica*, which were first isolated from Australian rats (Gundi *et al.* 2009) and *Apodemus argenteus* from Japan (Inoue *et al.* 2010), respectively. A previous study detected *Bartonella* genotypes in fleas collected from *M. musculus* from Israel (Morick *et al.* 2010). The role of *M. musculus* as *Bartonella*-reservoir seems apparently minor, since epidemiological studies have reported none or very low infection rates in this rodent species (Holmberg *et al.* 2003; Morick *et al.* 2009).

Another interesting finding in this study was the first detection of *Bartonella*-DNA in a Southern white-breasted hedgehog. The sequence detected from this host showed low similarity with other *Bartonella* spp., being the closest match to a genotype detected from a badger (*Meles anakuma*) in Japan (Sato *et al.* 2012). Both genotypes appear to be different from other known *Bartonella* species and cluster together. However, only the ITS fragment was amplified from this sample. This could be attributed to the possibility that as a newly uncharacterized genotype, the primers used were unable to amplify other genetic loci. Another possibility is that the bacterial loads were below the detection level of the assays used, however could be

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amplified by the ITS only, which is known to be a double-copy locus and thus more sensitive than the other targets. This argument applies for other samples that could be amplified only when this locus was targeted.

No *Bartonella* infection was identified in *M. socialis* (social vole) or *H. indica* (Indian crested porcupine), in this study. In a previous study, social voles trapped from suburban areas from Israel also tested negative for *Bartonella*-DNA (Morick *et al.* 2009). Thus, the ecological factors that may limit *Bartonella* infection of *M. socialis*, in areas where other reservoirs co-habit, need to be further explored.

In conclusion, this study reports the identification and genetic characterization of several *Bartonella* species and genotypes in wildlife from Israel. Several zoonotic *Bartonella* spp., especially the widespread distribution of *B. rochalimae* and *B. rochalimae*-like bacteria across wildlife hosts, deserve special attention. Our study indicates that infection with zoonotic and other *Bartonella* species is likely to be widely prevalent among wild animals and stresses their potential threat to public health. It also suggests further exploration of *Bartonella* transmission and its relationship to infection of humans, domestic and wild animals.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0031182016000603

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CONFLICT OF INTEREST

None.

REFERENCES

Avidor, B., Graidy, M., Efrat, G., Leibowitz, C., Shapira, G., Schattner, A., Zimhony, O. and Giladi, M. (2004). *Bartonella koehlerae*, a new cat-associated agent of culture-negative human endocarditis. *Journal of Clinical Microbiology* **42**, 3462–3468.

Birtles, R. J. and Raoult, D. (1996). Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. *International Journal of Systematic Bacteriology* **46**, 891–897.

Birtles, R. J., Harrison, T. G., Saunders, N. A. and Molyneux, D. H. (1995). Proposals to unify the genera *Grahamella* and *Bartonella*, with descriptions of *Bartonella talpae* comb. nov., *Bartonella peromysci* comb. nov., and three new species, *Bartonella grahamii* sp. nov., *Bartonella taylorii* sp. nov., and *Bartonella doshiae* sp. nov. *International Journal of Systematic Bacteriology* **45**, 1–8.

Brenner, E. C., Chomel, B. B., Singhasivanon, O. U., Namekata, D. Y., Kasten, R. W., Kass, P. H., Cortes-Vecino, J. A., Gennari, S. M., Rajapakse, R. P., Huong, L. T. and Dubey, J. P. (2013). *Bartonella* infection in urban and rural dogs from the tropics: Brazil, Colombia, Sri Lanka and Vietnam. *Epidemiology and Infection* **141**, 54–61.

Buffet, J. P., Marsot, M., Vaumourin, E., Gasqui, P., Masseglia, S., Marcheteau, E., Huet, D., Chapuis, J. L., Pisanu, B., Ferquel, E., Halos, L., Vourc'h, G. and Vayssier-Taussat, M. (2012). Co-infection of *Borrelia afzelii* and *Bartonella* spp. in bank voles from a suburban forest. *Comparative Immunology, Microbiology and Infectious Diseases* **35**, 583–589.

Buffet, J. P., Kosoy, M. and Vayssier-Taussat, M. (2013). Natural history of *Bartonella*-infecting rodents in light of new knowledge on genomics, diversity and evolution. *Future Microbiology* **8**, 1117–1128.

Chomel, B. B. and Kasten, R. W. (2010). Bartonellosis, an increasingly recognized zoonosis. *Journal of Applied Microbiology* **109**, 743–750.

Chomel, B. B., Boulouis, H.-J., Breitschwerdt, E. B., Kasten, R. W., Vayssier-Taussat, M., Birtles, R. J., Koehler, J. E. and Dehio, C. (2009). Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. *Veterinary Research* **40**, 29.

Chomel, B. B., McMillan-Cole, A. C., Kasten, R. W., Stuckey, M. J., Sato, S., Maruyama, S., Diniz, P. P. and Breitschwerdt, E. B. (2012). *Candidatus* Bartonella merieuxii, a potential new zoonotic *Bartonella* species in Canids from Iraq. *PLoS Neglected Tropical Diseases* **6**, e1843.

Daly, J. S., Worthington, M. G., Brenner, D. J., Moss, C. W., Hollis, D. G., Weyant, R. S., Steigerwalt, A. G., Weaver, R. E., Daneshvar, M. I. and O'Connor, S. P. (1993). *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. *Journal of Clinical Microbiology* **31**, 872–881.

Diaz, M. H., Bai, Y., Malania, L., Winchell, J. M. and Kosoy, M. Y. (2012). Development of a novel genus-specific real-time PCR assay for detection and differentiation of *Bartonella* species and genotypes. *Journal of Clinical Microbiology* **50**, 1645–1649.

Diniz, P. P., Billeter, S. A., Otranto, D., De Caprariis, D., Petanides, T., Mylonakis, M. E., Koutinas, A. F. and Breitschwerdt, E. B. (2009). Molecular documentation of *Bartonella* infection in dogs in Greece and Italy. *Journal of Clinical Microbiology* **47**, 1565–1567.

Eremeeva, M. E., Gerns, H. L., Lydy, S. L., Goo, J. S., Ryan, E. T., Mathew, S. S., Ferraro, M. J., Holden, J. M., Nicholson, W. L., Dasch, G. A. and Koehler, J. E. (2007). Bacteremia, fever, and splenomegaly caused by a newly recognized *Bartonella* species. *New England Journal of Medicine* **356**, 2381–2387.

Gerrikagoitia, X., Gil, H., Garcia-Esteban, C., Anda, P., Juste, R. A. and Barral, M. (2012). Presence of *Bartonella* species in wild carnivores of northern Spain. *Applied and Environmental Microbiology* **78**, 885–888.

Gundi, V. A., Taylor, C., Raoult, D. and La Scola, B. (2009). *Bartonella rattaustraliani* sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. *International Journal of Systematic and Evolutionary Microbiology* **59**, 2956–2961.

Gutiérrez, R., Morick, D., Gross, I., Winkler, R., Abdeen, Z. and Harrus, S. (2013). Bartonellae in domestic and stray cats from Israel: comparison of bacterial cultures and high-resolution melt real-time PCR as diagnostic methods. *Vector Borne and Zoonotic Diseases* **13**, 857–864.

Gutiérrez, R., Cohen, L., Morick, D., Mumcuoglu, K. Y., Harrus, S. and Gottlieb, Y. (2014*a*). Identification of different *Bartonella* species in the cattle tail louse (*Haematopinus quadripertusus*) and in cattle blood. *Applied and Environmental Microbiology* **80**, 5477–5483.

Gutiérrez, R., Morick, D., Cohen, C., Hawlena, H. and Harrus, S. (2014*b*). The effect of ecological and temporal factors on the composition of *Bartonella* infection in rodents and their fleas. *ISME Journal* **8**, 1598–1608.

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I. S. and Harrus, S. (2015*a*). *Bartonella* infection in rodents and their flea ectoparasites: an overview. *Vector Borne and Zoonotic Diseases* **15**, 27–39.

Gutiérrez, R., Nachum-Biala, Y. and Harrus, S. (2015*b*). Relationship between the Presence of *Bartonella* Species and Bacterial Loads in Cats and Cat Fleas (*Ctenocephalides felis*) under Natural Conditions. *Applied and Environmental Microbiology* **81**, 5613–5621.

Harrus, S., Bar-Gal, G. K., Golan, A., Elazari-Volcani, R., Kosoy, M. Y., Morick, D., Avidor, B. and Baneth, G. (2009). Isolation and genetic characterization of a *Bartonella* strain closely related to *Bartonella tribocorum* and *Bartonella elizabethae* in Israeli commensal rats. *American Journal of Tropical Medicine and Hygiene* **81**, 55–58.

Henn, J. B., Gabriel, M. W., Kasten, R. W., Brown, R. N., Theis, J. H., Foley, J. E. and Chomel, B. B. (2007). Gray foxes (*Urocyon cinereoargenteus*) as a potential reservoir of a *Bartonella clarridgeiae*-like bacterium and domestic dogs as part of a sentinel system for surveillance of zoonotic

arthropod-borne pathogens in northern California. *Journal of Clinical Microbiology* **45**, 2411–2418.

Henn, J. B., Chomel, B. B., Boulouis, H. J., Kasten, R. W., Murray, W. J., Bar-Gal, G. K., King, R., Courreau, J. F. and Baneth, G. (2009*a*). *Bartonella rochalimae* in raccoons, coyotes, and red foxes. *Emerging Infectious Diseases* **15**, 1984–1987.

Henn, J. B., Gabriel, M. W., Kasten, R. W., Brown, R. N., Koehler, J. E., MacDonald, K. A., Kittleson, M. D., Thomas, W. P. and Chomel, B. B. (2009*b*). Infective endocarditis in a dog and the phylogenetic relationship of the associated '*Bartonella rochalimae*' strain with isolates from dogs, gray foxes, and a human. *Journal of Clinical Microbiology* **47**, 787–790.

Holmberg, M., Mills, J. N., McGill, S., Benjamin, G. and Ellis, B. A. (2003). *Bartonella* infection in sylvatic small mammals of central Sweden. *Epidemiology and Infection* **130**, 149–157.

Hoss, M. and Paabo, S. (1993). DNA extraction from Pleistocene bones by a silica-based purification method. *Nucleic Acids Research* **21**, 3913–3914.

Inoue, K., Maruyama, S., Kabeya, H., Hagiya, K., Izumi, Y., Une, Y. and Yoshikawa, Y. (2009). Exotic small mammals as potential reservoirs of zoonotic *Bartonella* spp. *Emerging Infectious Diseases* **15**, 526–532.

Inoue, K., Kabeya, H., Shiratori, H., Ueda, K., Kosoy, M. Y., Chomel, B. B., Boulouis, H. J. and Maruyama, S. (2010). *Bartonella japonica* sp. nov. and *Bartonella silvatica* sp. nov., isolated from *Apodemus* mice. *International Journal of Systematic and Evolutionary Microbiology* **60**, 759–763.

Jaffe, C. L., Baneth, G., Abdeen, Z. A., Schlein, Y. and Warburg, A. (2004). Leishmaniasis in Israel and the Palestinian Authority. *Trends in Parasitology* **20**, 328–332.

Kosoy, M., Hayman, D. T. S. and Chan, K.-S. (2012). *Bartonella* bacteria in nature: where does population variability end and a species start? *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* **12**, 894–904.

Kosoy, M. Y., Regnery, R. L., Tzianabos, T., Marston, E. L., Jones, D. C., Green, D., Maupin, G. O., Olson, J. G. and Childs, J. E. (1997). Distribution, diversity, and host specificity of *Bartonella* in rodents from the Southeastern United States. *American Journal of Tropical Medicine and Hygiene* **57**, 578–588.

Lin, J. W., Chen, C. Y., Chen, W. C., Chomel, B. B. and Chang, C. C. (2008). Isolation of *Bartonella* species from rodents in Taiwan including a strain closely related to '*Bartonella rochalimae'* from *Rattus norvegicus*. *Journal of Medical Microbiology* **57**, 1496–1501.

Maggi, R. G. and Breitschwerdt, E. B. (2005). Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. *Journal of Clinical Microbiology* **43**, 1171–1176.

Morick, D., Baneth, G., Avidor, B., Kosoy, M. Y., Mumcuoglu, K. Y., Mintz, D., Eyal, O., Goethe, R., Mietze, A., Shpigel, N. and Harrus, S. (2009). Detection of *Bartonella* spp. in wild rodents in Israel using HRM real-time PCR. *Veterinary Microbiology* **139**, 293–297.

Morick, D., Krasnov, B. R., Khokhlova, I. S., Shenbrot, G. I., Kosoy, M. Y. and Harrus, S. (2010). *Bartonella* genotypes in fleas (Insecta: Siphonaptera) collected from rodents in the Negev desert, Israel. *Applied and Environmental Microbiology* **76**, 6864–6869.

Morick, D., Krasnov, B. R., Khokhlova, I. S., Gottlieb, Y. and Harrus, S. (2011). Investigation of *Bartonella* acquisition and transmission in *Xenopsylla ramesis* fleas (Siphonaptera: Pulicidae). *Molecular Ecology* **20**, 2864–2870.

Nasereddin, A., Risheq, A., Harrus, S., Azmi, K., Ereqat, S., Baneth, G., Salant, H., Mumcuoglu, K. Y. and Abdeen, Z. (2014). *Bartonella* species in fleas from Palestinian territories: prevalence and genetic diversity. *Journal of Vector Ecology: Journal of the Society for Vector Ecology* **39**, 261–270.

Ohad, D. G., Morick, D., Avidor, B. and Harrus, S. (2010). Molecular detection of *Bartonella henselae* and *Bartonella koehlerae* from aortic valves of Boxer dogs with infective endocarditis. *Veterinary Microbiology* **141**, 182–185.

Rasis, M., Rudoler, N., Schwartz, D. and Giladi, M. (2014). *Bartonella dromedarii* sp. nov. isolated from domesticated camels (*Camelus dromedarius*) in Israel. *Vector Borne and Zoonotic Diseases* **14**, 775–782.

Riess, T., Dietrich, F., Schmidt, K. V., Kaiser, P. O., Schwarz, H., Schafer, A. and Kempf, V. A. J. (2008). Analysis of a novel insect cell culture medium-based growth medium for *Bartonella* species. *Applied and Environmental Microbiology* **74**, 5224–5227.

Rudoler, N., Rasis, M., Sharir, B., Novikov, A., Shapira, G. and Giladi, M. (2014). First description of *Bartonella bovis* in cattle herds in Israel. *Veterinary Microbiology* **173**, 110–117.

Sato, S., Kabeya, H., Miura, T., Suzuki, K., Bai, Y., Kosoy, M., Sentsui, H., Kariwa, H. and Maruyama, S. (2012). Isolation and phylogenetic analysis of *Bartonella* species from wild carnivores of the suborder Caniformia in Japan. *Veterinary Microbiology* **161**, 130–136.

Sato, S., Kabeya, H., Fujinaga, Y., Inoue, K., Une, Y., Yoshikawa, Y. and Maruyama, S. (2013). *Bartonella jaculi* sp. nov., *Bartonella callosciuri* sp. nov., *Bartonella pachyuromydis* sp. nov. and *Bartonella acomydis* sp. nov., isolated from wild Rodentia. *International Journal of Systematic and Evolutionary Microbiology* **63**, 1734–1740.

Schaefer, J. D., Kasten, R. W., Coonan, T. J., Clifford, D. L. and Chomel, B. B. (2011). Isolation or detection of *Bartonella vinsonii* subspecies *berkhoffii* and *Bartonella rochalimae* in the endangered island foxes (*Urocyon littoralis*). *Veterinary Microbiology* **154**, 135–139.

Sofer, S., Gutiérrez, R., Morick, D., Mumcuoglu, K. Y. and Harrus, S. (2015). Molecular detection of zoonotic bartonellae (*B. henselae, B. elizabethae* and *B. rochalimae*) in fleas collected from dogs in Israel. *Medical and Veterinary Entomology* **29**, 344–348.

Strauss-Ayali, D., Jaffe, C. L., Burshtain, O., Gonen, L. and Baneth, G. (2004). Polymerase chain reaction using noninvasively obtained samples, for the detection of *Leishmania infantum* DNA in dogs. *Journal of Infectious Diseases* **189**, 1729–1733.

Svobodova, M., Votypka, J., Peckova, J., Dvorak, V., Nasereddin, A., Baneth, G., Sztern, J., Kravchenko, V., Orr, A., Meir, D., Schnur, L. F., Volf, P. and Warburg, A. (2006). Distinct transmission cycles of *Leishmania tropica* in 2 adjacent foci, Northern Israel. *Emerging Infectious Diseases* **12**, 1860–1868.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Valentine, K. H., Harms, C. A., Cadenas, M. B., Birkenheuer, A. J., Marr, H. S., Braun-McNeill, J., Maggi, R. G. and Breitschwerdt, E. B. (2007). *Bartonella* DNA in loggerhead sea turtles. *Emerging Infectious Diseases* **13**, 949–950.

Vayssier-Taussat, M., Le Rhun, D., Bonnet, S. and Cotte, V. (2009). Insights in *Bartonella* host specificity. *Annals of the New York Academy of Sciences* **1166**, 127–132.

Welch, D. F., Carroll, K. C., Hofmeister, E. K., Persing, D. H., Robison, D. A., Steigerwalt, A. G. and Brenner, D. J. (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. *Journal of Clinical Microbiology* **37**, 2598–2601.