Epidemiol. Infect. (2016), **144**, 1837–1844. © Cambridge University Press 2016
doi:10.1017/S0950268816000108

Molecular epidemiology of *Bartonella* species isolated from ground squirrels and other rodents in northern California

A. C. ZIEDINS[1], B. B. CHOMEL[1]*, R. W. KASTEN[1], A. M. KJEMTRUP[2] AND C.-C. CHANG[3]

[1] *Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, USA*
[2] *California Department of Public Health, Vector-Borne Disease Section, Sacramento, CA, USA*
[3] *Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taichung, Taiwan*

Received 16 October 2015; Final revision 3 December 2015; Accepted 10 January 2016; first published online 15 February 2016

SUMMARY

Bartonella spp. are endemic in wild rodents in many parts of the world. A study conducted in two northern California counties (Sonoma and Yolo) sampling California ground squirrels (*Otospermophilus beecheyi*) and four other rodent species (*Peromyscus maniculatus, P. boylii, P. truei* and *Neotoma fuscipes*) led to the isolation of small Gram-negative bacilli which were identified as *Bartonella* spp. based on colony morphology, polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and partial gene sequencing. Overall, *Bartonella* spp. were isolated from the blood of 71% (32/45) of the ground squirrels and one third (22/66) of the other rodents. PCR–RFLP analysis of the *glt*A and 16S rRNA genes yielded seven unique profiles, four for the ground squirrels and three for the other rodents. Isolates from each PCR–RFLP profiles were submitted for partial sequencing. Ground squirrel isolates were most closely related to *B. washoensis*, whereas the other rodent isolates were closest to *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *arupensis*. Two of these three species or subspecies are known zoonotic agents.

Key words: Bacterial infections, *Bartonella*, deer mouse, dusky-footed woodrat, ground squirrel.

INTRODUCTION

Bartonella are emerging pathogens of increasing human and veterinary medical importance. Members of this genus are small, curved, Gram-negative, rod-shaped bacteria that parasitize erythrocytes of their hosts [1]. *Bartonella* are isolated from a wide range of mammalian species, including humans. New species, many of them not yet fully described have been isolated from a wide range of rodents [2–4]. A common feature of most of these bacteria is transmission by arthropod vectors, especially fleas, as shown for cats and various rodent species [5–7]. Several rodent-borne *Bartonella* spp. are zoonotic causing a wide range of clinical manifestations from prolonged fever to endocarditis, such as *B. elizabethae, B. grahamii, B. washoensis, B. vinsonii* subsp. *arupensis* or *Candidatus Bartonella volans* [8–14].

Little is known on the distribution of *Bartonella* spp. in wild rodents, including ground squirrels (*Otospermophilus becheeyi*) in California, as only a limited number of studies were conducted in that state or neighbouring states [13, 15]. The objective of the present study was to evaluate the prevalence of *Bartonella* spp. in ground squirrels and other

* Author for correspondence: Dr B. B. Chomel, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Ca 95 616, USA.
(Email: bbchomel@ucdavis.edu)

small rodents from northern central California and determine which *Bartonella* spp. were infecting these small mammals.

MATERIALS AND METHODS

Study area and sampling procedures

One of the study areas was located in a semi-rural residential subdivision on the eastern slope of the foothills in southern Sonoma County, California (122° 46′ W, 38° 26′ N). The specific trapping sites were two non-adjoining properties of ~2 ha each, at 420–450 m elevation, with a creek flowing through both properties year round [16]. The predominant vegetation included California live oak (*Quercus agrifolia*) and California bay trees (*Umbellularia californica*) that provided a dense leaf litter. The climate is Mediterranean, with cool, moist winters and hot, dry summers. The other study area was located in Davis, CA at two sites, one in south Davis and the other in west Davis, Yolo County (121° 44′ W, 38° 32′ N).

Rodents

From 28 October to 2 February 1998, 110 Sherman traps (H. B. Sherman Traps, USA) were placed once a month in five series of trap lines, each ~200 m long with a trap placed every ~8·5 m. Traps were set in the evening. The following morning, trapped animals were processed at the field site. Animals were anesthetized with methoxyflurane (Metofane; Schering-Plough, USA), weighed, measured and species, sex and age (adult, juvenile) were recorded. A minimum of 500 µl blood was collected via retro-orbital puncture in a Monoject EDTA samplette™ tube (Medtronic, USA). The animals were ear-tagged, then returned to the original trap location. Procedures followed an approved animal use and care protocol through the University of California, Davis [16].

Ground squirrels

Ground squirrels were trapped using Tomahawk live traps baited with oats at two sites on the Davis Campus of the University of California over a 2-week period in June 1999. Both trap sites were located in open fields along roadways, and the predominant vegetation was dry grassland, typical of the Central Valley region of California in the summer. Traps were set at night and checked daily. Captured ground squirrels were brought to a central processing location for examination and blood collection. Prior to euthanasia, animals were anaesthetized with CO_2, information on age and sex was recorded and blood was drawn by intra-cardiac puncture into 2 ml plastic EDTA tubes. This study was conducted under protocol 9275 approved by the University of California, Davis Institutional Animal Care and Use Committee (IACUC) [17].

Culture and isolation of *Bartonella*

Isolation of *Bartonella* spp. was performed for all samples. Frozen blood was thawed at room temperature and spun for 30 min at 5000 ***g***. Supernatant was removed and 125 µl M199 isolation medium was added to the pellet, as described previously [18]. Blood was plated on fresh heart infusion agar supplemented with 5% fresh rabbit blood. For ground squirrel blood, two plates were inoculated, one with 250 µl and the other one with the rest of the volume ranging from 25 to 350 µl. Rodent samples were inoculated onto one agar plate using a 50:50 buffer/blood volume mixture) due to the small blood volume available. Agar plates were incubated at 35 °C in an aerobic atmosphere of 5% CO_2 for 3–4 weeks and checked regularly (at least twice a week) for bacterial growth. The typically observed *Bartonella* colonies became visible on the primary plates within 3–7 days. Colonies tentatively identified as *Bartonella*-like were streaked for isolation onto new agar plates. Most of the isolates appeared small, circular, raised, greyish-white or translucent, and slightly adherent to the surface of the agar. For DNA extraction, single colonies were picked and placed in sterile 1·5 ml vials containing 100 µl sterile double deionized water and frozen at −80 °C. The remaining colonies were harvested and placed in screw-cap vials with 0·5 ml brain heart infusion medium and stored at −80 °C.

DNA extraction

Crude DNA extracts suitable for use in polymerase chain reactions (PCRs) were obtained from the culture isolates. Samples frozen in water were allowed to thaw at room temperature before heating at 100 °C for 15 min. Vials were vortexed for 10 s while still hot, then spun in a micro-centrifuge for 10 min at 16 000 ***g*** at 4 °C. Five microlitres of the supernatant was placed into a sterile 500 µl vial containing 45 µl sterile double deionized water. This 1:10 dilution was used as template in all subsequent PCR reactions. The remaining supernatant was transferred to sterile vials and stored at −80 °C.

https://doi.org/10.1017/S0950268816000108 Published online by Cambridge University Press

PCR–restriction fragment length polymorphism (PCR–RFLP)

Rodent and ground squirrel isolates were identified using PCR–RFLP analysis of the citrate synthase (*glt*A) and 16S rRNA genes, as described previously [19–21]. A 381 base-pair (bp) fragment of the *glt*A gene was amplified using two oligonucleotides homologous to the *glt*A gene of *B. henselae* Houston I (BhCS781.p and BhCS1137.n) [19, 21]. All PCR amplifications were performed with a PTC-200 DNA Engine (Bio-Rad, USA). PCR amplifications were performed with 5 µl sample in a mixture containing 50 mM KCl, 10 mM Tris–HCl, 1·5 mM $MgCl_2$, 0·001% gelatin, 0·1% Brij-35, 200 µM of each deoxynucleotide triphosphate, 0·5 µM of each primer, and 0·2 U thermostable Ampli-Taq DNA polymerase (ThermoFisher Scientific, USA). This mixture was incubated at 95 °C for 2 min and amplified for 40 cycles at 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, and subsequently at 72 °C for 5 min. Amplified products were verified by gel electrophoresis on a 2% agarose gel in 0·5 × Tris-borate EDTA buffer followed by staining with ethidium bromide and visualization by a UV transilluminator.

The amplified product of the *glt*A gene obtained with the set of primers suggested by Norman *et al.* [19] was digested with *Taq*I (Promega, USA), *Hha*I, *Mse*I and *Aci*I (New England BioLabs, USA) restriction endonucleases. A 1400 bp segment was amplified using two eubacterial universal primers specific for the 16S rRNA gene: P8 (5'-AGAGTTTGATCCTGGCTCAG-3') and Pc1544 (5'-AAGGAGGTGATCCAGCCGCA-3') [22]. The amplified product of the 16S rRNA gene was digested with *Dde*I (Boehringer GmbH, Germany) restriction endonuclease. The digestion conditions used were those recommended by the enzymes' manufacturer. Banding patterns were compared with those of *B. henselae* (ATCC no. 49 882) and *B. clarridgeiae* (ATCC no. 51 734).

Species identification

The PCR products of the eight selected isolates used for DNA sequencing were purified with Microcon centrifugal filter devices (Millipore Corp., USA) and sequenced with a fluorescence-based automated sequencing system (Davis Sequencing, USA). The BLASTN program of GCG software (Wisconsin Sequence Analysis Package, v. 10; Genetics Computer Group, USA) was applied to determine the bacterial species and subspecies closest to the sequencing results of the *Bartonella*-positive samples, based on the DNA sequence similarity of 273 bp of the *glt*A gene. Then, the GAP program was used for sequence alignments and determination of the percentage of DNA similarity between the sequences of the *glt*A gene for each *Bartonella*-positive sample and the closest bacterial species and subspecies.

Statistical analysis

Univariate analysis by χ^2 and Fisher's exact tests was applied to evaluate risk factors associated with *Bartonella* bacteraemia in rodents and ground squirrels. χ^2 for trend was used to compare groups of rodents and ground squirrels by location, age and sex. Two-tailed Fisher's exact test was used for cell values of <5 when calculating odds ratios. The level of significance was established at $P < 0{\cdot}05$.

RESULTS

One hundred and thirty-seven ground squirrels and other rodents, representing five different species, were captured from two main sites in northern central California (Table 1). The 76 rodents captured in Sonoma County included 35 deer mice (*Peromyscus maniculatus*), including 18 males and 26 adults, 32 dusky-footed woodrats (*Neotoma fuscipes*), including nine males and 21 adults, six brush mice (*Peromyscus boylii*) including three males and five adults and three pinyon mice (*Peromyscus truei*) including two males and one adult. Blood samples were also collected from 61 ground squirrels including 24 males and 29 adults, with 43 animals (21 males and 18 adults) captured in South Davis and 18 animals (three males and 11 adults) captured in West Davis, Yolo County.

Blood cultures were performed from all 137 ground squirrels and other rodents. However, major bacterial or fungal contamination led to discarding of 10 rodent samples (7 *N. fuscipes*, 3 *P. maniculatus*) and 16 ground squirrel samples (14 from South Davis, 2 from West Davis). Isolates identified as possible *Bartonella* organisms, were recovered from 22 (33·3%) of the 66 non-contaminated rodent blood cultures and from 37 (82·2%) of the 45 non-contaminated ground squirrel cultures (Table 2). Colonies tentatively identified as *Bartonella* were slow growing, generally visible within 3–7 days of plating. Most appeared small (0·5–1·0 mm), raised,

https://doi.org/10.1017/S0950268816000108 Published online by Cambridge University Press

Table 1. *Rodents tested for* Bartonella *by geographical location, species, age and sex, northern California, 1996–1999*

		Location (county)							
		Sonoma				Yolo (Davis)			
		Sex		Age		Sex		Age	
Species	**Total**	Male	Female	Adult	Juv.	Male	Female	Adult	Juv.
Peromyscus maniculatus	**35**	18*	16*	26†	8†	0	0	0	0
P. boylii	**6**	3	3	5	1	0	0	0	0
P. truei	**3**	2	1	1	2	0	0	0	0
Neotoma fuscipes	**32**	9	23	21	11	0	0	0	0
Otospermophilus becheeyi	**61**	0	0	0	0	24‡	35‡	29	32

* Sex not recorded for one individual.
† Age not recorded for one individual.
‡ Sex not recorded for two individuals.

Table 2. Bartonella *infection in wild rodents from northern central California, 1996–1999*

Location	Rodent species	No. of non-contaminated cultures	No. positive (%)
Sonoma	*Peromyscus maniculatus*	32*	15 (46·9)
	P. boylii	6	2 (33·3)
	P. boylii	3	2 (66·6)
	Neotoma fuscipes	25†	3 (12·0)
Yolo (Davis)	*Otospermophilus becheeyi*	45‡	32 (71·1)

* Three cultures contaminated were excluded.
† Seven cultures contaminated.
‡ Sixteen cultures contaminated.

circular, greyish-white or translucent and were slightly adherent to the agar surface.

The 22 rodent isolates were confirmed to be *Bartonella* by PCR of a fragment of the *glt*A gene. *Bartonella* isolates were obtained from four different rodent species: 15 (47%) *P. maniculatus*, three (12%) *N. fuscipes*, two (33·3%), *P. boylii* and two (66·6%) *P. truei*. The level of bacteraemia ranged from 10 to >8000 colony-forming units (c.f.u.)/ml. The median level of bacteraemia was 150 c.f.u./ml. After excluding the 10 animals for which blood cultures were contaminated, 16 (33·3%) of the 48 adults and four (22·2%) of the 18 juveniles were *Bartonella* bacteraemic. There was no statistically significant difference in age or sex in those rodents detected to be *Bartonella* bacteraemic ($P > 0{\cdot}05$).

For the ground squirrels, 32 of the 37 culture-positive animals were confirmed to be *Bartonella* spp. by PCR of a fragment of the *glt*A gene. Overall, prevalence was 71% (32/45). Bacteraemia levels ranged from 4 to 1728 c.f.u./ml with a median of 228 c.f.u./ml. Bacteraemia prevalence did not differ significantly by trapping site, age or sex.

To assess the genotypic diversity in the 22 rodents and 32 ground squirrel isolates, a portion (381 bp fragment) of the citrate synthase (*glt*A) and 16S rRNA (1400 bp fragment) genes were amplified by PCR and analysed by RFLP [22]. For the *glt*A gene, digestion was performed using *Taq*I, *Hha*I, *Mse*I and *Aci*I restriction endonucleases, which allowed identification of six distinct profiles, four for ground squirrels and two for other rodents (data not shown). Similarly, analysis of the 16S rRNA gene revealed three unique profiles (one for ground squirrels and two for other rodents) following digestion with *Dde*I restriction endonuclease. Combining PCR–RFLP data for both genes led to seven different profiles (four for ground squirrels and three for other rodents).

https://doi.org/10.1017/S0950268816000108 Published online by Cambridge University Press

Table 3. *DNA similarity values based on 289 bp of the citrate synthase gene (gltA) of the four selected other rodent strains compared to those of* Bartonella *strains in GenBank (bold >97%)*

		Similarity (%)			
Bartonella species or strain	GenBank no.	Nf9730	Pm9804	Pt9809	Pt9811
B. v. vinsonii	Z70015	95·95	95·95	**97·58**	**97·23**
Strain B-1	U84375	95·50	95·85	**98·62**	**98·27**
Strain B-2	U84376	95·16	95·50	**97·58**	**97·23**
Strain SH9282GA	AF082323	96·19	96·19	**98·62**	**98·27**
B. v. arupensis	U77057	**97·23**	**99·31**	96·89	96·54
Strain D-1	U84379	**97·58**	**99·65**	**97·23**	96·89
Strain D-2	U84380	96·89	**99·65**	96·54	96·19
Strain D-3	U84381	96·54	**98·96**	96·89	96·54
Strain D-4	U84382	**97·23**	**99·31**	96·89	96·54
Strain D-5	U84383	96·54	**98·62**	96·19	95·85
Strain D-6	U84384	96·54	**98·96**	96·89	96·54
Strain D-7	U84385	96·89	**99·65**	96·54	96·19
B. v. berkhoffii	U28075	94·12	94·46	96·89	96·54
B. washoensis	AF050108	92·04	93·43	93·08	92·73
B. taylorii	Z70013	93·43	93·08	93·77	93·43
B. grahamii	Z70016	92·73	92·04	92·39	92·04
B. tribocorum	AJ005494	91·70	91·00	92·73	92·39
B. elizabethae	U28072	90·66	89·97	90·66	90·31
B. doshiae	Z70017	90·31	89·62	88·58	88·24
B. henselae H-1	L38987	88·93	89·62	90·66	90·31
B. koehlerae	AF176091	89·62	89·27	90·66	90·31
B. quintana	Z70014	88·93	89·97	89·97	89·62
B. bovis (*weissii*)	AF071190	89·62	89·62	89·27	88·93
B. clarridgeiae	U84386	89·27	88·58	88·93	88·58
B. bacilliformis	U28076	85·12	84·08	84·78	85·12
Strain A-1	U84372	92·73	93·08	93·08	92·73
Strain A-2	U84373	92·39	92·39	92·73	92·39
Strain A-3	U84374	92·73	94·12	93·08	92·73
Strain C-1	U84377	90·31	90·66	90·66	90·31
Strain C-2	U84378	90·31	90·66	90·66	90·31
Strain C7-rat	Z70020	90·66	89·97	90·66	90·31
Strain C4-phy	Z70019	91·35	92·04	91·70	91·35
Strain C1-phy	Z70022	90·66	91·35	91·00	90·66
Strain R-phy1	Z70010	93·08	93·43	93·43	93·08
Strain R-phy2	Z70011	91·35	92·04	91·70	91·35
Strain N40	Z70012	91·35	91·70	92·39	92·04
Strain AF0755167	AF075167	92·73	93·08	93·08	92·73
Strain MM5136CA	AF086637	91·70	91·00	91·35	91·00
Strain SH8200GA	AF082321	92·04	92·39	92·39	92·04
Strain SH8776GA	AF082322	92·73	93·43	93·08	92·73
Strain RR13863PO	AF086636	91·70	91·00	92·73	92·39
Strain RN10623LA	AF075164	92·04	91·35	93·08	92·73
Strain RN10149MD	AF075161	91·35	90·66	92·39	92·04
Strain RN10616LA	AF075162	88·93	88·58	89·27	88·93
Strain RN10617LA	AF075163	88·93	87·89	88·58	88·24
Strain RN10627LA	AF075165	90·31	89·97	91·00	90·66
Strain RN10631LA	AF075166	90·66	90·31	91·35	91·00

To fully identify the *Bartonella* spp. or subspecies isolated, 1–2 isolates of each of the seven PCR–RFLP profile groupings were selected for partial sequencing of the *glt*A and 16S rRNA genes based on the strength and clarity of the gel banding pattern. The eight isolates selected included four obtained

https://doi.org/10.1017/S0950268816000108 Published online by Cambridge University Press

Table 4. *DNA similarity values based on 289 bp of the citrate synthase gene of the four selected ground squirrel strains compared to those of* Bartonella *strains in GenBank (bold >96%)*

		Similarity (%)			
Bartonella strain	GenBank number	Sb20	Sb19	Sb57	Sb9
B. vinsonii subsp. *vinsonii*	Z70015	91·35	91·70	89·62	89·97
B. vinsonii subsp. *arupensis*	U77057	92·73	93·08	91·70	92·04
B. vinsonii subsp. *berkhoffii*	U28075	91·70	92·04	89·97	90·31
B. washoensis	AF050108	**100·00**	**99·65**	**97·92**	**96·19**
B. taylorii	Z70013	90·31	90·66	88·58	88·93
B. grahamii	Z70016	89·27	89·62	89·27	90·31
B. tribocorum	AJ005494	89·62	89·97	88·58	89·62
B. elizabethae	U28072	87·89	88·24	88·24	88·93
B. doshiae	Z70017	87·89	88·24	87·20	86·51
B. henselae H-1	L38987	92·39	92·04	90·31	90·31
B. koehlerae	AF176091	91·35	91·70	91·00	89·62
B. quintana	Z70014	91·70	92·04	89·62	89·62
B. bovis/weissii	AF071190	88·58	88·93	87·89	89·27
B. clarridgeiae	U84386	87·89	88·24	88·24	88·93
B. bacilliformis	U28076	83·39	83·74	83·05	83·05

All other strains described by Kosoy *et al.* [2], Ellis *et al.* [3] and Birtles & Raoult [4] presented in Table 3 had values of <95% similarity.

from ground squirrels (isolate nos. Sb20, Sb19, Sb9, Sb57), one from a deer mouse (Pm9804), one from a dusky-footed woodrat (Nf9730) and the two pinyon mouse isolates (Pt9809 and Pt9811). A 289 bp fragment of the *glt*A gene from these eight isolates was compared to *Bartonella* sequence data available in GenBank. The mice isolates were most similar to *B. vinsonii* spp., including *B. vinsonii* subsp. *arupensis* for the deer mouse (99·31% homology) and the dusky-footed woodrat (97·23% homology) and *B. vinsonii* subsp. *vinsonii* for the two pinyon mice (97·58% and 97·23% homology) (Table 3). For the four ground squirrel isolates, the *glt*A sequences were closest to *B. washoensis* (homology range: 96·2–100%) (Table 4). These data were also confirmed by the 16S rRNA sequences on 515 bp (99·2–100% homology with *B. washoensis* for the ground squirrels, but was less discriminative for the three *B. vinsonii* subspecies for the rodents). They were still the closest to *B. vinsonii* subsp. *arupensis* or *B. vinsonii* subsp. *vinsonii* (homology range: 99·2–99·8%) compared to *B. vinsonii* subsp. *berkhoffii* (homology range: 99–99·4%). In addition to known *Bartonella* spp., the rodent isolates were also closely related to isolates previously described from rodents of the same species [2]. The deer mouse isolate (Pm9804) was closest to strains D1, D2, and D7 also isolated from *P. maniculatus P. leucopus* and *P. gossypinus* in southeastern USA and the two pinyon mice isolates were closest to an isolate from a cotton rat (*Sigmodon hispidus*) B1 and a *Peromyscus* spp. D1 [2].

DISCUSSION

The present study reports the identification of *B. washoensis* in California ground squirrels, *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *arupensis* from four other rodent species from northern California. To our knowledge, this is the first report of the presence of a *Bartonella* species in pinyon mice (*P. truei*). The overall prevalence of *Bartonella* in rodents from Sonoma (33·3%) was comparable with reports from other regions of North America [2, 23, 24] and varied significantly among host species, which is similar to the pattern observed in other areas. For example, the high prevalence reported in *Peromyscus* spp. (33–67%) falls within the range observed in the United States [2, 24, 25]. More specifically, the strains isolated from rodents were quite similar to strains isolated from rodents in western USA [24, 25] and northern Mexico [26], and from ground squirrels and prairie dogs [13, 23, 24]. The isolates from rodents from Sonoma were also closely related to strains isolated from small rodents from the southeast of the United States including strains of group D described by Kosoy *et al.* [2], isolated from *P. maniculatus* and *P. leucopus*, suggestive of adaptation of these

https://doi.org/10.1017/S0950268816000108 Published online by Cambridge University Press

Bartonella spp. to their specific hosts. In contrast to the identification of *B. vinsonii* subsp. *vinsonii* in woodrats from New Mexico [27], our *N. fuscipes* isolate was closer to *B. vinsonii* subsp. *arupensis*, indicating that woodrats can be reservoirs for these two *B. vinsonii* subspecies. Two of the species isolated in northern California (*B. vinsonii* subsp. *arupensis* and *B. washoensis*) have been described as zoonotic pathogens [13, 14]. This warrants further studies to understand their potential role in causing human diseases in northern California, similar to what has been reported for urban rats in Los Angeles [15], where *B. rochalimae*, a known human and canine pathogen [28, 29], was isolated from 37 (18·5%) rats. Most rodent-borne *Bartonella* spp. are flea-borne [30], and several of the ground squirrels were flea infested at time of capture (data not shown). For instance, *B. vinsonii* subsp. *vinsonii* DNA was detected from *Ctenophthalmus pseudagyrtes*, a flea of insectivores and small rodents [30]. Similarly, in a human case of *B. washoensis* meningitis and early sepsis, *Oropsylla montana* fleas were implicated as the vector for disease transmission, as *O. montana* is the most common flea species to parasitize California ground squirrels and 8/11 flea pools were PCR positive for *B. washoensis* [31].

Based on our *glt*A results, it is likely that phylogenic group D described by Kosoy *et al*. [2] corresponds to *B. vinsonii* subsp. *arupensis*, with similar rodent species of the genus *Peromyscus* being infected. Similarly, Kosoy's phylogenic group B infecting cotton rats (*Sigmodon hispidus*), including strain Sh9282GH, is more likely to correspond to *B. vinsonii* subsp. *vinsonii*, as detected in our pinyon mice. It also suggests that strain SH9282 GA isolated from a rat (*Rattus rattus*) is closest to *B. vinsonii* subsp. *vinsonii* rather than to *B. vinsonii* subsp. *berkhoffii* [3]. However, our isolates are quite distinct from *Bartonella* isolates described either in rodents from Peru or in a wood mouse (*Apodemus sylvaticus*) from the UK [4]. In contrast to other reports [32], none of the rodents were co-infected with various *Bartonella* strains, as previously shown for ground squirrels, which appear to be an exclusive host of *B. washoensis* [13, 24] or *P. maniculatus*, usually infected with variants of *B. vinsonii* subsp. *arupensis* [25].

ACKNOWLEDGEMENTS

The authors thank Desley A. Whisson for allowing us to take part in her collection of ground squirrels of her specific study [17].

DECLARATION OF INTEREST

None.

REFERENCES

1. **Chomel BB, Kasten RW.** Bartonellosis, an increasingly recognized zoonosis. *Journal of Applied Microbiology* 2010; **109**: 743–750.
2. **Kosoy MY, *et al.*** Distribution, diversity, and host specificity of *Bartonella* in rodents from the South-eastern United States. *American Journal of Tropical Medicine and Hygiene* 1997; **57**: 578–588.
3. **Ellis BA, *et al.*** Rats of the genus *Rattus* are reservoir hosts for pathogenic *Bartonella* species: an Old World origin for a New World disease? *Journal of Infectious Diseases* 1999; **180**: 220–224.
4. **Birtles RJ, Raoult D.** Comparison of partial citrate synthase gene (gltA) sequences for phylogenetic analysis of *Bartonella* species. *International Journal of Systematic Bacteriology* 1996; **46**: 891–897.
5. **Chomel BB, *et al.*** Experimental transmission of *Bartonella henselae* by the cat flea. *Journal of Clinical Microbiology* 1996; **34**: 1952–1956.
6. **Bown KJ, Bennet M, Begon M.** Flea-borne *Bartonella grahamii* and *Bartonella taylorii* in bank voles. *Emerging Infectious Diseases* 2004; **10**: 684–687.
7. **Gutiérrez R, *et al.*** *Bartonella* infection in rodents and their flea ectoparasites: an overview. *Vector Borne and Zoonotic Diseases* 2015; **15**: 27–39.
8. **Bai Y, *et al.*** Bartonella vinsonii subsp. *arupensis* in humans, Thailand. *Emerging Infectious Diseases* 2012; **18**: 989–991.
9. **Breitschwerdt EB, *et al.*** A groundhog, a novel *Bartonella* sequence, and my father's death. *Emerging Infectious Diseases* 2009; **15**: 2080–2086.
10. **Daly JS, *et al.*** *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. *Journal of Clinical Microbiology* 1993; **31**: 872–881.
11. **Fenollar F, Sire S, Raoult D.** *Bartonella vinsonii* subsp. *arupensis* as an agent of blood culture-negative endocarditis in a human. *Journal of Clinical Microbiology* 2005; **43**: 945–947.
12. **Kerkhoff FT, *et al.*** Demonstration of *Bartonella grahamii* DNA in ocular fluids of a patient with neuroretinitis. *Journal of Clinical Microbiology* 1999; **37**: 4034–4038.
13. **Kosoy M, *et al.*** *Bartonella* strains from ground squirrels are identical to *Bartonella washoensis* isolated from a human patient. *Journal of Clinical Microbiology* 2003; **41**: 645–650.
14. **Welch DF, *et al.*** 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* 1999; **37**: 2598–2601.
15. **Gundi VA, *et al.*** *Bartonella* spp. in rats and zoonoses, Los Angeles, California, USA. *Emerging Infectious Diseases* 2012; **18**: 631–6333.

16. **Kjemtrup AM, Robinson T, Conrad PA.** Description and epidemiology of *Theileria youngi* n. sp. from a northern Californian dusky-footed woodrat (*Neotoma fuscipes*) population. *Journal of Parasitology* 2001; **87**: 373–378.
17. **Whisson DA, Salmon TP.** Effect of diphacinone on blood coagulation in *Spermophilus beecheyi* as a basis for determining optimal timing of field bait applications. *Pest Management Science* 2002; **58**: 736–738.
18. **Koehler JE, *et al.*** Isolation of *Rochalimaea* species from cutaneous and osseous lesions of bacillary angiomatosis. *New England Journal of Medicine* 1992; **327**: 1625–1631.
19. **Norman AF, *et al.*** Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *Journal of Clinical Microbiology* 1995; **33**: 1797–1803.
20. **Gurfield AN, *et al.*** Co-infection with *Bartonella clarridgeiae* and *Bartonella henselae* and with different *Bartonella henselae* strains in domestic cats. *Journal of Clinical Microbiology* 1997; **35**: 2120–2123.
21. **Molia S, *et al.*** Prevalence of *Bartonella* infection in wild African lions (*Panthera leo*) and cheetahs (*Acinonyx jubatus*). *Veterinary Microbiology* 2004; **100**: 31–41.
22. **Regnery RL, *et al.*** Characterization of a novel *Rochalimaea* species, *R. henselae* sp. nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient. *Journal of Clinical Microbiology* 1992; **30**: 265–274.
23. **Jardine C, *et al.*** Rodent-associated *Bartonella* in Saskatchewan, Canada. *Vector Borne and Zoonotic Diseases* 2005; **5**: 402–409.
24. **Bai Y, *et al.*** Characterization of *Bartonella* strains isolated from black-tailed prairie dogs (*Cynomys ludovicianus*). *Vector Borne and Zoonotic Diseases* 2008; **8**: 1–5.
25. **Bai Y, *et al.*** Persistent infection or successive reinfection of deer mice with *Bartonella vinsonii* subsp. *arupensis*. *Applied Environmental Microbiology* 2011; **77**: 1728–1731.
26. **Rubio AV, *et al.*** Prevalence and genetic diversity of *Bartonella* strains in rodents from northwestern Mexico. *Vector Borne and Zoonotic Diseases* 2014; **14**: 838–845.
27. **Morway C, *et al.*** A longitudinal study of *Bartonella* infection in populations of woodrats and their fleas. *Journal of Vector Ecology* 2008; **33**: 353–364.
28. **Eremeeva ME, *et al.*** Bacteremia, fever, and splenomegaly caused by a newly recognized *Bartonella* species. *New England Journal of Medicine* 2007; **356**: 2381–2387.
29. **Henn JB, *et al.*** 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* 2009; **47**: 787–790.
30. **Reeves WK, *et al.*** Association of *Bartonella* with the fleas (Siphonaptera) of rodents and bats using molecular techniques. *Journal of Vector Ecology* 2007; **32**: 118–122.
31. **Probert W, *et al.*** Meningitis due to a '*Bartonella washoensis*'-like human pathogen. *Journal of Clinical Microbiology* 2009; **47**: 2332–2335.
32. **Telfer S, *et al.*** Parasite interactions in natural populations: insights from longitudinal data. *Parasitology* 2008; **135**: 767–781.