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Bartonella infection in sylvatic small mammals of central Sweden

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SUMMARY

Sylvatic small mammals were captured in rural habitats near Uppsala, Sweden, to measure the prevalence of bartonella infections, characterize bacterial isolates and identify their host range, and increase our understanding of host–pathogen ecology. During 7 nights of trapping at 3 localities, 236 small mammals were captured (trap success 30 %). Bartonella were isolated from bloods of *Apodemus flavicollis* (19 of 110 tested), *Apodemus sylvaticus* (6/25), *Clethrionomys glareolus* (9/60), *Microtus agrestis* (1/3), *Mus musculus* (1/18), and *Sorex araneus* (3/20). Nucleotide sequencing (a 338 bp fragment of the *glt*A gene) of 40 isolates yielded 6 unique genotypes. Five of the 6 genotypes were most similar to other known bartonella isolated from Old World small-mammal hosts. The most frequent genotype (83 %) was isolated from *A. flavicollis* and *M. musculus* and was identical to *Bartonella grahamii*, a recently demonstrated human pathogen. These two hosts were most frequently captured in and around human structures and work places, thus providing conditions that could potentially lead to frequent human infections.

INTRODUCTION

Members of the genus *Bartonella* are fastidious, Gram-negative bacteria that may be grown *in vitro* on blood-rich media. Thirteen species are currently recognized in the genus, including seven known or suspected to be pathogenic to humans. Recognition of a wide range of clinical diseases caused by bartonella has prompted studies focused on the identification of the non-human reservoirs and vectors for these bacteria.

Since the beginning of the last century intracellular bacteria have been observed in a wide range of animals [1]. These bacteria formerly identified as *Grahamella* were reclassified as *Bartonella* in 1995 [2]. Studies in Europe and the United States have shown that numerous genotypes of bartonellae circulate in wild animals. Four new species of *Bartonella* were described from small woodland mammals in the United Kingdom [3, 4], including *Bartonella grahamii* and *B. taylorii* from voles and field mice (*Clethrionomys glareolus*, *Microtus agrestis* and *Apodemus* species), *B. doshiae* from a field vole (*M. agrestis*), and *B. birtlesii* from *Apodemus* spp. Two novel *Bartonella* species were isolated in eastern France: *B. tribocorum* from the blood of wild *Rattus norvegicus*, and *B. alsatica* from wild rabbits (*Oryctolagus cuniculus*) [5, 6].

A study of rodents in the southeastern United States identified new genotypes of bartonella that clustered in four phylogenetic groups and were distinct from those described from Old World animals [7]. Wild-captured *R. norvegicus* in the United States were infected with several genotypes of bartonellae genetically similar to

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those found in the Old World, including one identical to the human pathogen, *Bartonella elizabethae* [8]. In contrast, most of the bartonella that infected *Rattus rattus* were similar to that found in North American indigenous rodents [8]. Although our understanding of both species diversity and intra-species variation of bartonellae is increasing in some parts of the world, no studies have been conducted in Scandinavia. Many of the small mammals that act as reservoirs for bartonellae in Europe are known to occur in central Sweden (e.g. *Apodemus flavicollis*, *Clethrionomys glareolus*, *M. agrestis*; [9]), so it was anticipated that bartonellae-infected small mammals would be present in this area of Scandinavia. Identification and characterization of bartonellae from mammals in this region may provide public health officials with the ability to diagnose previously undescribed zoonotic illnesses. Studies that increase our understanding of reservoir ecology have important public health implications, including identification of disease-endemic areas and mechanisms of human infection, and the development of risk-reduction intervention strategies [10]. Initial steps toward these goals include the identification and characterization of the specific pathogens, identification of their associated reservoir hosts and the estimation of prevalence in these hosts, estimation of the relative abundance of hosts, identification of the geographic distribution and more specific habitat distribution of host species, and attempts to understand the mechanisms of transmission of bartonellae within host populations [9].

The purpose of the present study was to characterize bartonella isolates; determine their relationships to known bartonellae, including recognized human pathogens; identify their hosts and associated habitats; measure prevalences of infection; and examine capture data for evidence of specific transmission mechanisms within a small mammal assemblage in a rural area of central Sweden near the city of Uppsala.

MATERIALS AND METHODS

Sample sites

Small mammals were captured at three sites 30–50 km from Uppsala, including Håtunaholm (59° 37·5′ N, 17° 37·2′ E), Kumla (59° 35·8′ N, 17° 13·2′ E), and Ålbo (59° 52·3′ N, 17° 20·6′ E). Sites were approximately equal distances from each other, with a maximum distance of 32 km between Håtunaholm and Ålbo and the closest distance (23 km) between Håtunaholm and Kumla. Trapping occurred from 10 September to 18 September, 1999. Håtunaholm was trapped for 4 nights; the other two sites were visited for 2 nights each.

Specimen collection

Small mammals were trapped using Sherman (9 × 9 × 23 cm; H. B. Sherman Live Trap Co., Tallahassee, FL, USA) and Tomahawk (14 × 14 × 40 cm; Tomahawk Trap Co., Tomahawk, WI, USA) live-capture traps. Each night approximately 100–130 Sherman traps and 15 Tomahawk traps were baited with peanut butter mixed with rolled oats, and placed in lines of 10–20 traps each, at approximately 5 m intervals, in available sylvatic and peridomestic habitat types. The most commonly sampled habitats included forest, peridomestic (e.g. barns, sheds, rubbish piles), hedgerows, rock walls, and disturbed habitats (roadside, fence lines, disturbed forest, etc.). Traps containing captured small mammals were collected each morning and transported in double plastic bags to a central outdoor processing site where they were necropsied the same day of collection.

To reduce the chance of human infections with highly virulent rodent-borne agents such as hantaviruses, trapping and sampling protocols followed established safety guidelines [11]. Briefly, investigators wore protective clothing, including disposable gloves and gowns, and wore respirators fitted with high-efficiency particulate air (HEPA) filters. Animals were anaesthetized with methoxyflurane, bled from the retro-orbital capillary plexus, and then sacrificed. The following items were recorded for each capture: species identification, standard morphometric measurements (mass; lengths of head, body, tail, hind foot, and ear), gender, reproductive condition (testes abdominal or scrotal; vagina closed or perforate), and the presence of scars (or wounds) and ectoparasites (fleas and ticks). Lungs, kidneys, liver, spleen and heart were collected. All samples were frozen on dry ice in the field, and then stored at −70 °C until analysed. Formalin-preserved voucher specimens (carcasses) were catalogued and archived at the Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico.

Bacterial isolation and characterization

Isolation of bartonella was performed as described earlier [8]. Briefly, 100 μl of whole blood (or

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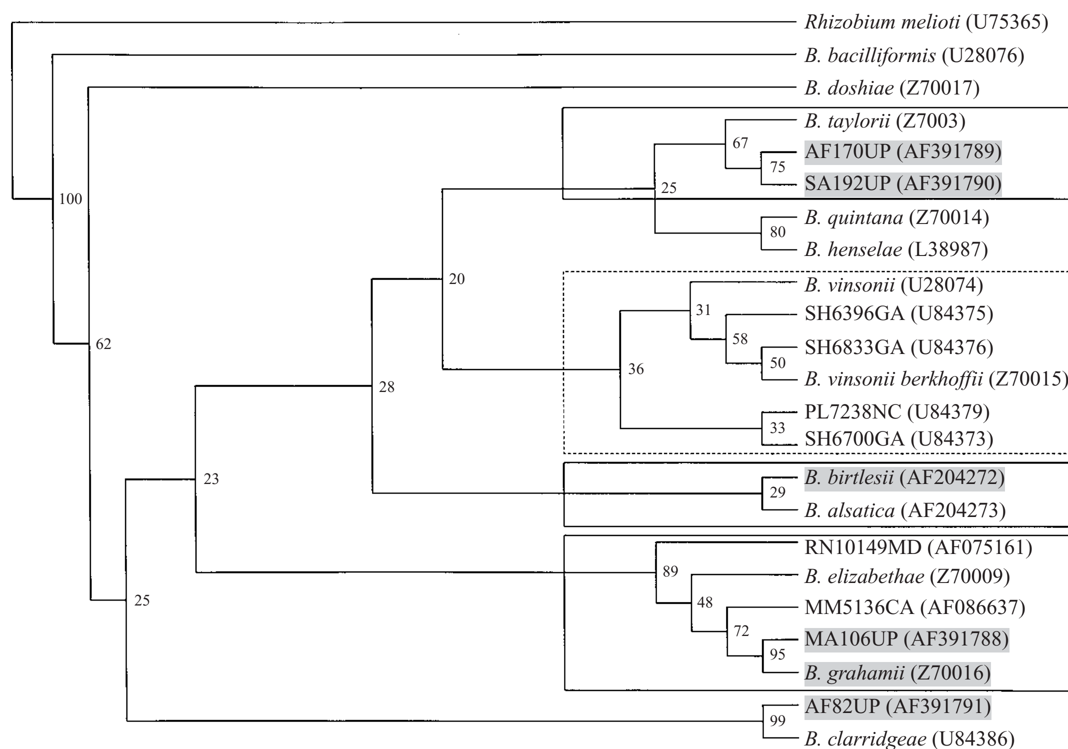

Fig. 1. Phylogram using parsimony method (338 bp fragment of *glt*A gene with 100 bootstraps). Genbank accession number given in parentheses after each strain designation. Novel genotypes obtained from this study are highlighted. The New World bartonellae clades associated primarily with sigmodontine rodent hosts are identified within the dotted box; clades identified from genotypes from Old World hosts in solid lines.

alternatively 25 μl diluted 1:4 in *Brucella* broth when sample size was limited) was plated onto brain–heart infusion agar supplemented with 5% rabbit blood. The plates were incubated at 32° C in 5% CO_2 for up to 3 weeks. Colonies tentatively identified as bartonella were sub-cultured in two steps. Bacterial colonies were picked, suspended in 50 μl distilled H_2O, and heated to 95° C for 3 min. One microlitre of this suspension was used as a template in PCR reactions, using primers amplifying 379 bp from the citrate synthase gene of *Bartonella* spp. [12].

Analysis of sequencing data

PCR products of the correct size were purified (QIAQuick Purification Kit, Qiagen, Inc.), then sequenced in both directions using the above-mentioned oligonucleotides as sequencing primers with the Big Dye terminator sequencing kit on an ABI Prism 310 capillary-automated sequencing machine (Applied Biosystems, Foster City, CA, USA). Sequences were assembled with the GAP4 program of the Staden package [13], aligned with PILEUP (GCG; Wisconsin Sequence Analysis Package, Genetic Computer Group (GCG), version 8.1) and similarity values calculated using OLDDISTANCES (GCG). Maximum parsimony and nearest-neighbour analyses were performed with DNAPARS (PHYLIP 3.50; [14]) on 100 bootstrap replicas using *Rhizobium melioti* as an outgroup. The consensus tree was drawn using TreeView [15].

Sequences from the novel genotypes obtained were submitted to Genbank. Accession numbers from the novel genotypes from this study, as well as those used from Genbank in the phylogenetic analyses, are given in Figure 1.

Statistical analysis

Data were analysed using SPSS [16] and Epi-Info version 6 [17] software. Trap success was used as a measure of relative rodent population density, and was calculated as the number of captures per 100 trap nights (trap nights were the number of traps set per night × the number of nights minus one half the number of sprung-but-empty traps). Associations of rodent species with habitats were assessed by the χ^2 test. Expected numbers of captures for each species in each habitat were derived by multiplying the proportion of

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Table 1. *Prevalence of infection of small mammals captured at three localities in central Sweden, September 1999. Number of infected individuals and number tested by species and locality are given with prevalence (%) in parentheses*

Species	Ålbo	Håtunaholm	Kumla	Total
Apodemus flavicollis	2/26 (7·7)	10/64 (15·6)	7/20 (35·0)	19/110 (17·3)
Apodemus sylvaticus	2/12 (16·7)	3/10 (30·0)	1/3 (33·3)	6/25 (24·0)
Clethrionomys glareolus	0/4 (0·0)	6/22 (27·3)	3/34 (8·8)	9/60 (15·0)
Microtus agrestis	NC*	NC	1/3 (33·3)	1/3 (33·3)
Mus musculus	0/1 (0·0)	1/15 (6·7)	0/2 (0·0)	1/18 (5·6)
Sorex araneus	3/10 (30·0)	0/5 (0·0)	0/5 (0·0)	3/20 (15·0)
Total	7/53 (13·2)	20/116 (17·2)	12/67 (17·9)	39/236 (16·5)

* NC, no captures.

trap nights in a specific habitat by the total number of individuals of each species captured.

Relative age of the rodents was estimated by using body mass classes. For the three most frequently infected species, mass classes were defined by dividing the body weight data at the 33rd and 66th percentiles. Mass classes for *A. flavicollis* were 13–24 g, 25–31 g, and 32–48 g. Those for *C. glareolus* were 11–13 g, 14–15 g, and 16–26 g; *A. sylvaticus* mass classes were 12–14 g, 15–17 g, and 18–30 g.

Focality of infection was examined using exact probabilities from the binomial distribution computed by comparing numbers of infected animals at each site to an expected distribution based on the overall observed prevalence [18]. The association between prevalence of infection and mass class was evaluated for the most commonly infected species (*A. flavicollis*, *C. glareolus* and *A. sylvaticus*) using the χ^2 for linear trend controlling for gender as a confounder.

RESULTS

Trap success and species collected

A total of 77 trap nights in Tomahawk traps yielded no captures; thus, only results from the trapping effort with Sherman traps are reported here. During 7 nights of trapping (816 Sherman trap nights), 236 small mammals were captured, for an overall trap success of 30%. Overall small-mammal abundance (as indicated by trap success) were similar among the three sites: 24% at Ålbo (53 captures in 217 trap nights), 33% at Håtunaholm (116 in 348 trap nights), and 30% at Kumla (67 in 224 trap nights). Six species were captured in the following frequencies: *A. flavicollis* (110; 47% of captures), *C. glareolus* (60; 25%), *Apodemus sylvaticus* (25; 11%), *Sorex araneus* (20; 8%); *Mus musculus* (18; 8%), and *M. agrestis* (3; 1%).

Relative contribution of species to the small-mammal assemblage varied among sites and major habitat types (Table 1, Fig. 2). About half of the captures came from Håtunaholm (116 of 236), while similar numbers were trapped at Kumla and Ålbo (67 and 53, respectively for 28 and 22%). *A. flavicollis* was the numerically dominant species at two of the sites (Håtunaholm and Ålbo), while *C. glareolus* was the dominant species in Kumla (Table 1). Three *M. agrestis* were captured from Kumla. Capture frequencies among habitat types varied significantly from expected values based on total trap success for *A. flavicollis* ($\chi^2 = 19{\cdot}8$; $P < 0{\cdot}001$), *C. glareolus* ($\chi^2 = 38{\cdot}2$; $P < 0{\cdot}0001$), and *A. sylvaticus* ($\chi^2 = 10{\cdot}2$; $P < 0{\cdot}05$). Captures of *A. flavicollis* were 30% greater than expected in peridomestic areas (Fig. 2). *C. glareolus* were captured more frequently than expected in rock wall (19 captures *vs.* 6 expected). *A. flavicollis*, *C. glareolus* and *A. sylvaticus* were captured 32, 35 and 57%, respectively, more frequently than expected in forest habitats. More than half of the captures of the insectivore *S. araneus* were from forest, while 78% of the *M. musculus* came from peridomestic environs (sheds and barns) (Fig. 2).

Almost equal numbers of males and females were captured for *A. flavicollis* and *C. glareolus* (56 *vs.* 53 and 29 *vs.* 31, respectively). In contrast, males were the dominant gender among *A. sylvaticus* captured (18 *vs.* 6). Overall, 18% of *A. flavicollis* (20 of 110), 8% of *C. glareolus* (5 of 55), and 4% of *A. sylvaticus* (1 of 25) had scars. Frequencies of animals with scars (an indicator of frequency of aggressive encounters) were not significantly different for female and male *A. flavicollis* (17 *vs.* 20%), *A. sylvaticus* (0 *vs.* 6%), or *C. glareolus* (3 *vs.* 14%; Fisher's exact test, $P > 0{\cdot}05$ for all species). Scars were more frequent in *A. flavicollis* than in *C. glareolus* and *A. sylvaticus* ($\chi^2 = 5{\cdot}42$; $P = 0{\cdot}06$).

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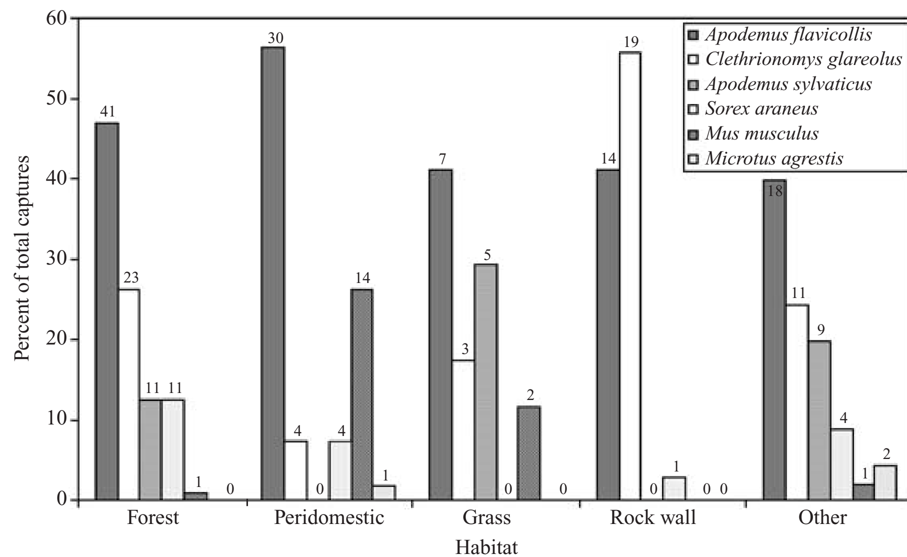

Fig. 2. Percent total captures of small mammals by species for each of the major habitats sampled in central Sweden. Numbers above each bar are frequencies of captures. Other habitat includes edge of woodland, hedgerow, canal, willows, pond, and hay bales.

Significantly more scarring was observed in older *A. flavicollis*: 37% (14 of 24) in the oldest mass class had scars compared to 8% in the other two mass classes ($\chi^2 = 13{\cdot}32$; $P = 0{\cdot}001$). Differences in scarring trends with age may have been difficult to detect for *C. glareolus* and *A. sylvaticus* due to small sample sizes of animals with scars in these species ($n = 5$ and 1, respectively).

Prevalence of infection

Overall prevalences of infection with bartonella were similar from the three locations sampled (13–18%, Table 1). Statistical evidence for spatial focality of infection with bartonella was found only for *A. flavicollis* where prevalence at Kumla was significantly higher than the overall prevalence ($P = 0{\cdot}02$, binomial test). In general, the most frequently infected host at a given site was not the numerically dominant species. At Håtunaholm where *A. flavicollis* was the dominant species, the prevalence was higher in *C. glareolus* (27·3 *vs.* 15·6%), while in Kumla, where *C. glareolus* was the dominant species, *A. flavicollis* had a higher prevalence of infection (40 *vs.* 8·8%; Table 1). At Ålbo the dominant species (*A. flavicollis*) had a relatively lower prevalence of infection (7·7%), and very few *C. glareolus* were captured ($n = 4$; all negative).

Prevalence of infection was similar for males and females for *A. flavicollis*, *C. glareolus* and *A. sylvaticus* ($P \geqslant 0{\cdot}05$ for all tests). However, younger *A. flavicollis* tended to have a higher prevalence of infection than older animals (40 *vs.* 16% for males, and 19 *vs.* 8% for females, respectively; χ^2 for linear trend $= 2{\cdot}76$; $P = 0{\cdot}09$; controlling for sex as a confounder). In contrast, proportions of *A. flavicollis* with scars increased with age (28 and 20% for oldest and youngest males, and 54 *vs.* 4% for oldest and youngest females; χ^2 for linear trend $= 10{\cdot}39$; $P = 0{\cdot}001$; controlling for sex as a confounder). Ticks and fleas were found on 9 and 6 animals, respectively, however none of these small mammals were infected with bartonella.

Phylogenetic relationships

Six unique genotypes were obtained from the 40 isolates sequenced, including four novel genotypes not present in Genbank. Most of the isolates (82·5%; $n = 33$) corresponded to a single genotype identical to the type strain for *B. grahamii*. These 33 isolates were from 18 *A. flavicollis*, 5 *A. sylvaticus*, 9 *C. glareolus* and 1 *M. musculus*. One novel genotype (MA106UP) was isolated from a single *M. agrestis* and was 99% similar to *B. grahamii* (Table 2). A single isolate obtained from one *A. sylvaticus* was identical to the newly described species *B. birtlesii* (AS63UP; Genbank accession no. AF204272) that was originally isolated from the same rodent species in the UK. One genotype (AF170UP from one *A. flavicollis*) was most similar to *Bartonella taylorii* (94% similarity) and another unique genotype obtained in this study (SA192UP, obtained from three

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Table 2. *Percent similarity values of bartonella isolates from small mammals captured in central Sweden with other bartonellae*

	CLAR	*AF82UP*	HENS	QUINT	GRAH	*MA106UP*	MM5136CA	RN10149MD	ELIZ	BIRT	ALS	*AF170UP*	*SA192UP*	TAYL	SH6396GA	SH6833GA	VINSBERK	VINS	PL7238NC	SH6700GA	DOSH	BACIL	RMEL
CLAR	100	93·8	86·1	87·6	87·3	86·7	87·3	87·0	86·7	86·7	85·2	86·1	85·2	86·7	87·6	87·6	87·0	87·0	87·6	87·6	86·7	84·9	66·0
AF82UP		100	86·1	87·9	88·2	87·0	88·8	88·2	87·0	87·0	84·3	84·3	84·6	86·1	87·9	87·9	86·7	87·3	87·9	86·7	84·6	85·8	66·0
HENS			100	91·7	88·8	88·5	88·2	88·8	86·4	90·5	88·5	88·2	89·1	89·1	90·2	89·9	90·5	89·6	89·4	88·2	86·7	84·3	64·5
QUINT				100	87·6	87·0	88·2	87·6	85·8	88·5	87·6	87·3	87·3	89·1	89·4	89·1	89·1	89·1	89·4	87·6	84·6	83·4	65·7
GRAH					100	98·8	97·6	95·3	94·4	90·5	88·8	89·4	90·5	88·8	90·8	90·8	90·2	90·5	91·4	90·2	87·3	84·9	67·2
MA106UP						100	96·5	94·1	93·5	89·6	87·9	89·1	90·2	88·5	89·9	89·9	89·4	89·6	90·5	89·1	87·3	84·6	66·3
MM5136CA							100	94·4	94·4	90·5	87·9	88·2	89·6	88·8	90·2	89·6	89·6	89·9	90·8	90·5	86·7	84·6	67·8
RN10149MD								100	94·4	91·4	89·6	87·6	88·5	89·1	91·1	90·8	90·8	90·5	90·8	89·1	86·4	85·5	68·9
ELIZ									100	89·1	87·3	86·7	87·6	87·3	89·9	89·6	89·4	89·9	90·5	89·4	86·4	83·1	68·1
BIRT										100	92·3	89·6	89·1	90·8	91·7	92·6	92·0	91·7	92·0	89·6	88·2	85·5	66·9
ALS											100	90·2	90·2	89·1	92·3	91·7	92·0	92·0	91·1	87·0	85·8	84·0	68·6
AF170UP												100	95·0	94·1	92·3	92·6	92·0	93·2	92·9	89·6	86·7	84·9	67·5
SA192UP													100	92·0	91·1	91·1	90·5	91·7	92·0	89·1	86·7	85·8	66·9
TAYL														100	92·3	92·0	91·4	93·2	92·9	89·6	86·1	85·2	68·9
SH6396GA															100	97·9	97·6	97·3	96·5	91·7	86·7	86·4	68·6
SH6833GA																100	97·6	96·5	96·2	92·3	87·3	85·8	68·1
VINSBERK																	100	96·2	95·6	91·4	86·4	85·2	68·9
VINS																		100	96·2	92·0	87·0	86·7	68·1
PL7238NC																			100	93·2	87·6	84·9	67·5
SH6700GA																				100	85·8	81·7	67·8
DOSH																					100	83·7	67·2
BACIL																						100	66·9
RMEL																							100

gltA; 328 bp; CLAR, *B. clarridgeae*; HENS, *B. henselae*; QUINT, *B. quintana*; GRAH, *B. grahamii*; ELIZ, *B. elizabethae*; BIRT, *B. birtlesii*; ALS, *B. alsatica*; TAYL, *B. taylorii*; VINSBERK, *B. vinsonii berkhoffii*; VINS, *B. vinsonii*; DOSH, *B. doshiae*; BACIL, *B. bacilliformis*; RMEL, *Rhizobium melioti*. Genotypes in italics indicate those from the current study. Genbank accession numbers are given in Figure 1.

S. Araneus, 95% similarity to AF170UP). Genotype AF82UP was isolated from a single *A. flavicollis* and was distinct from all other unique genotypes obtained in this study (84–88% similarity with other genotypes obtained in this study; Table 2). It was found to be most similar to *B. clarridgeae* (94%).

Both neighbour joining and parsimony methods yielded phylograms of similar topologies; results of the parsimony analysis are given in Figure 1. New World and Old World clades were apparent, although not always well supported by bootstrap values. The genotypes identified from this study appeared in four distinct clades, three of which contained other bartonellae isolated from small mammals of Old World origin. *A. flavicollis* hosted genotypes in all four clades. There was more than one rodent host represented in two of the clades. One clade contained bartonellae from *C. glareolus* and *M. agrestis* (*B. taylorii*), *A. flavicollis* (AF170UP), and *S. araneus* (SA192UP). The other clade contained bartonellae isolated from *R. norvegicus* (*B. elizabethae* and RN10149MD), *C. glareolus*, *M. agrestis* and *Apodemus* species (*B. grahamii*), and *M. musculus* (MM5136CA). The other two clades with Swedish bartonellae did not contain genotypes from other rodent species. One clade included *B. birtlesii* and *B. alsatica* (the latter from a lagomorph, the European wild rabbit, *O. cuniculus* [6]). The other clade included the Swedish bartonella genotype AF82UP with bartonellae from cats [*B. clarridgeae* (a human pathogen which, unlike most bartonellae, is flagellated)]. The human pathogens, *B. quintana* and *B. henselae* grouped together (Fig. 1).

DISCUSSION

Except for a few important studies [3, 19], the occurrence and distribution of bartonella in European hosts is largely unknown. This is the first study to identify bartonella associated with small mammals in Scandinavia. We have identified six novel bartonella genotypes (including four previously undescribed genotypes) and demonstrated their common association with several widespread and abundant small mammals in Scandinavia. The most common bartonella genotype isolated in our study was identical to *B. grahamii*, which has been implicated recently as a human pathogen [20]. The most common host for *B. grahamii* was *A. flavicollis*, a species that we found to be associated frequently with peridomestic habitats that may bring them into contact with humans in rural areas of Scandinavia. In addition, one isolate from the house mouse, *M. musculus* (also notoriously associated with human habitations) was identical to *B. grahamii*. The possible public health implications of these findings merit further investigation.

An understanding of habitat associations of reservoir species can help to identify at risk areas and may contribute to the development of specific intervention strategies [21]. The demonstration of a lack of tight host specificity for *B. grahamii* in Sweden would suggest that targeting any one species for control efforts would not be effective in preventing human infection. Nevertheless, the finding that *B. grahamii* frequently infects the two species that were most commonly associated with peridomestic environments suggests that rodent control in and around the home would help to decrease the incidence of any disease that may be associated with *B. grahamii* in Sweden.

The small mammals captured in this study represented most of the known rodent species and one common insectivore in the study area [9]. Notably absent was *R. norvegicus* and *Arvicola terretris*, even though we included habitat types where these small mammals are found (e.g. peridomestic and near water). Since other investigators have isolated bartonellae from *R. norvegicus* (*B. elizabethae* [8, 22]; *B. tribicorum* [5]), it would be of interest to examine them in Scandinavia.

More than 80% of the bartonella isolates from our study were identical to *B. grahamii*. Other investigators have isolated *B. grahamii* from the same rodent species found in this study (*Apodemus* spp., *C. glareolus*, and *M. agrestis* [3]). Our results show that in addition to these hosts, *B. grahamii* can also infect *M. musculus*. We isolated one bartonella identical to that described as *B. birtlesii* in Genbank [4]. In a parsimony analysis, three genotypes from Swedish rodents grouped with bartonellae previously described from other Old World hosts. These included *B. taylorii* and *B. grahamii* (from *C. glareolus*, *M. agrestis* and *Apodemus* species), *B. elizabethae* (from *R. norvegicus*), a bartonella from *R. norvegicus* collected in Baltimore, MD, USA (RN10149MD), a bartonella genotype from a house mouse (*M. musculus*; MM5136CA) from California, USA, and *B. alsatica* (from wild rabbits, *O. cuniculus*). Our very small sample of *M. agrestis* ($n=3$) did not yield an isolate of *B. doshiae*, a species of *Bartonella* isolated from *M. agrestis* in Great Britain [2].

The wide host range of *B. grahamii* demonstrated in our study corroborates the findings of low host specificity by Birtles et al. [3, 19]. Since the genotypes

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in this study and that of Birtles et al. [3] were based on characterization of a partial sequence of one gene, or other methodologies such as RFLP, these patterns should be explored by additional genetic characterization using methods such as fingerprinting by pulsed-field gel electrophoresis (PFGE) or IRS–PCR. In addition, the methods of characterization used in this study and that of Birtles et al. [3] were not designed to detect co-infection of mammals with several genotypes of bartonellae. Recently Birtles et al. [19] showed that British woodland rodents are infected with different bartonella genotypes over time. In addition, co-infection of an individual rodent with several genotypes of bartonella occurs. Host specificity occurs in some New World bartonella-rodent host associations (e.g. *Peromyscus* [7]). In this study the three isolates from *S. araneus* were of a single genotype (SA192UP) that was not isolated from any other species. Although suggestive of a specific host–pathogen relationship, much larger sample sizes would be required to confirm specificity.

Our study lends support to the hypothesis that bartonellae from Old and New World hosts are phylogenetically distinguishable. Additional isolates of bartonellae from small mammals captured throughout Europe and Asia should be characterized to see if the observations from this study are corroborated.

Our study contributes toward the understanding of several aspects of the natural history of bartonella infection in host populations. The mechanisms of transmission of bartonella infection among small-mammal hosts are unknown. A correlation between density and seroprevalence would implicate some density-dependent mechanism of horizontal transmission. We found the lowest prevalence at the site with the lowest trap success. However, with only three data points (three sites sampled), a rigorous analysis of this question was not possible and will have to await larger studies. Similarly, our results based on three sites did not support the observation [6] that sites where a given host species was most dominant had the highest prevalence of infection. Experimental evidence for vector-borne transmission between small-mammal hosts is very limited, but data indicate the cat flea, *Ctenocephalides felis*, may be a competent vector for *B. henselae* [23]. We found no positive association between infection rates and the presence of ectoparasites (fleas or ticks). Experimental laboratory studies, as well as field studies based on larger sample sizes and covering several seasons, are needed to provide definitive data.

Differences in infection prevalence between genders would suggest a specific transmission mechanism that favours males or females. For example, the demonstration of infection prevalence in male rodents, coupled with a correlation between infection and the presence of scars, helped to implicate fighting among more aggressive males as a mechanism of transmission of hantaviruses among reservoir hosts [24]. Our finding of equal prevalence among male and female hosts implies a transmission mechanism that affects the sexes equally. The lack of a correlation between infection prevalence and presence of scars also speaks against fighting as a transmission mechanism.

The trend toward higher infection rates in younger *A. flavicollis* suggests that infection may be self-limiting and immunizing, such that older animals have cleared their earlier infections and remain immune to further infection. The resulting immunity of older individuals would result in the observed pattern of more frequent infection in younger mice. Observations of a pattern of higher prevalence do not help us to distinguish between horizontal and vertical infection, since the same pattern would result whether exposure is greater in younger animals or is equal across age classes. However, if infection is transient and immunizing, the presence of infection in older individuals indicates that horizontal infection has occurred at least occasionally. The higher prevalence in younger individuals may be due to more frequent horizontal transmission among non-immune young, or it may be due to vertical transmission (*in utero* or in the nest). Laboratory studies and long-term field studies are required to distinguish among these options.

There were several limitations to this study. Trapping of small mammals only occurred during one season, so seasonal variation of infection with bartonellae could not be assessed in the small mammal population. In addition, only one gene was used to quantify the genetic variability in the isolates obtained. By including several genes such as *groEL* and 16S in the analyses as well as other methodologies (e.g. infrequent restriction site PCR [25]), other patterns regarding the phylogeny of these bacteria may emerge. Additional characterization of these isolates including biochemical testing and electron micrography is warranted. Despite these limitations, we have demonstrated that some species of common small mammals from central Sweden are frequently infected with bartonellae.

A recently published report on *B. grahamii* (diagnosed by PCR) in the ocular fluids of a patient with

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neuroretinitis indicates that this species can cause disease in humans [20]. *A. flavicollis* was one of the most commonly captured rodents in peridomestic habitats (Fig. 2), and this rodent species was infected frequently with *B. grahamii*. Considering the high likelihood of interactions between humans and these small mammals, *B. grahamii* has the potential to emerge as a new zoonotic agent.

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