Ectoparasites of Gray Squirrels in Two Different Habitats and Screening of Selected Ectoparasites for Bartonellae

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ECTOPARASITES OF GRAY SQUIRRELS IN TWO DIFFERENT HABITATS AND SCREENING OF SELECTED ECTOPARASITES FOR BARTONELLAE

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ABSTRACT: Gray squirrels, *Sciurus carolinensis*, were livetrapped in 2 different habitat types, woodland (67 squirrels) and parkland (53 squirrels), in southeastern Georgia. Ectoparasites were recovered from anesthetized squirrels and compared between hosts from the 2 habitats. Because of the absence of low vegetation in parkland habitats, it was hypothesized that the ectoparasite fauna, especially ticks and chiggers, would be more diverse on woodland squirrels. The results were generally in agreement with this hypothesis. Seventeen species of ectoparasites were recovered from woodland squirrels, compared with 6 species from parkland squirrels. Five species of ticks and 3 species of chiggers parasitized the woodland squirrels compared with no ticks or chiggers on the parkland squirrels. Significantly higher infestation prevalences were recorded on woodland compared with parkland squirrels for the flea *Orchopeas howardi*, the tick *Amblyomma americanum*, and the mesostigmatid mite *Androlaelaps fahrenholzi*. The mean intensity for *O. howardi* also was significantly higher on woodland than on parkland squirrels. Because a new strain of *Bartonella* sp. was isolated recently from *S. carolinensis* in Georgia, selected ectoparasites from this study were screened for bartonellae by polymerase chain reaction (PCR). Some of the fleas and lice, but none of the mites tested, were PCR positive, suggesting that fleas, or lice, or both, might be vectors of bartonellae between squirrels. Six distinct strains of *Bartonella* sp. were detected, 2 in fleas and 4 in lice.

Several authors have documented ectoparasite faunas associated with the gray squirrel, *Sciurus carolinensis* Gmelin, in various parts of the eastern United States. Katz (1938) reported ectoparasites from this rodent in Ohio; Wilson (1961), Whitaker et al. (1976), and Whitaker (1982) in Indiana; Durden (1980) in Tennessee; and Wilson et al. (1991) in Florida. However, there has been no previous study in which the ectoparasite faunas of this squirrel in different habitats have been compared. We hypothesized that ectoparasites that quest for hosts from vegetation (ticks and chiggers) would be less abundant, and possibly absent, in mowed areas compared with woodland sites with understory vegetation.

Some blood-feeding arthropods are known to be vectors of various species of *Bartonella* (Krampitz, 1962; Chomel et al., 1996; Maurin et al., 1997; Karem et al., 2000; Chang et al., 2001; La Scola et al., 2001). These include certain sandflies (Psychodidae) that transmit *Bartonella bacilliformis* (Strong, Tyzzer, Brues, Sellards, and Gastiaburu), the agent of Carrión's disease in the neotropics; human body lice (Pediculidae) that transmit *B. quintana* (Schmincke), the agent of trench fever (which sometimes manifests as bacillary angiomatosis or endocarditis); and the cat flea *Ctenocephalides felis* (Bouché) (Pulicidae), a vector of *B. henselae* (Regnery, Anderson, Clarridge, Rodriguezbarrados, Jones, and Carr), the agent of cat scratch disease. More than 40 yr ago, Krampitz (1962) demonstrated that the Oriental rat flea, *Xenopsylla cheopis* (Rothschild), can transmit bartonellae to European voles through its infectious feces.

Recently, bartonellae have been detected in several North American rodents (Kosoy et al., 1997, 1998; Ellis et al., 1999; Bown et al., 2002), but it is not known how transmission occurs between individual mammals. Because some blood-feeding arthropods are known to transmit other bartonellae, it is possible that ectoparasites of these rodents could potentially transmit these recently detected strains. A novel strain of *Bartonella* sp. was documented recently from gray squirrels in eastern North America (including our study sites in Georgia) and the U.K. (Bown et al., 2002). We, therefore, decided to screen selected ectoparasites from this study for bartonellae using a molecular methodology. Any *Bartonella* sp.–positive ectoparasite species could then be investigated in future studies to determine their vector potential.

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MATERIALS AND METHODS

Animal collection

From 1994 to 2001, Gray squirrels (*S. carolinensis*) were sampled in 7 localities in southeastern Georgia (Bulloch, Chatham, Coffee, Liberty, and Screven counties). Traps were set at each locality for 2–3 days for each trapping event. Squirrels were captured using Tomahawk live traps (4 × 14 × 40 cm; Tomahawk Trap Co., Tomahawk, Wisconsin) baited with sunflower seeds and sliced apple, near sites of observed squirrel activity in 2 distinct habitat types (parkland or woodland). Parkland habitats included mowed residential areas and mowed, open sites on the campus of Georgia Southern University with few trees and no understory vegetation and little or no leaf litter. Woodland habitat included natural and secondary growth sites with abundant trees, understory vegetation, and leaf litter. To avoid seasonal bias, both habitats were sampled during each trapping event.

Traps were visited each morning and afternoon and those with captured squirrels were double bagged and taken to Georgia Southern University. As a biosafety precaution, squirrels were examined in a designated quarantine room and handlers wore latex gloves during all procedures; blood and ectoparasite samples were confined in sealed tubes immediately after collection. Squirrels were lightly anesthetized with a 1:10 mixture of xylazene sulfate and ketamine hydrochloride administered intramuscularly using a 26-G tuberculin syringe. Each animal was bled from the femoral vein, ear-tagged, and later released at the site of capture after recovery from anesthesia. Bloods were frozen at −70 C and later used in hantavirus and *Bartonella* sp. studies.

Ectoparasite collection and identification

Ectoparasites were collected from anesthetized squirrels by combing each animal with a flea comb over a large white pan and then systematically examining the pelage and skin of the entire body surface area. Ectoparasites were collected and retained in labeled vials containing 70% ethanol for subsequent identification using a high-power binocular microscope. Some specimens were cleared in 10% potassium hydroxide (certain fleas and lice) or lactophenol (certain mites) and slide mounted before identification at higher magnifications. Voucher ectoparasites are deposited in the Department of Biology or the U.S. National Tick Col-

lection at Georgia Southern University; representative accession numbers (from 102 accessioned collections) include L1159 through L1161, L1290 through L1300, L1341, L2817 through L2823, and L2855.

DNA extraction from ectoparasites

In a previous study, 4 of 15 gray squirrels in the study area were infected with *Bartonella* sp. (Bown et al., 2002). In the present study, DNA was extracted from ectoparasites collected from these 4 infected squirrels using the QIAamp Tissue Kit (Qiagen Inc., Chatsworth, California). Most of the ectoparasites were pooled by arthropod species and host individual (Table II). Using aseptic technique, arthropods were first vacuum dried for 2 hr. Specimens were then removed from the vials individually with sterile forceps and placed in another vial with 180 µl of Buffer ATL followed by 20 µl of proteinase K (Qiagen Inc.). Arthropods were cut into small fragments with a sterile scalpel blade (number 11), incubated in a 55 C water bath overnight, and 200 µl of Buffer AL was then added to each vial. Vials were then placed in a 72 C water bath for 10 min. The remaining protocol followed manufacturer's directions.

Detection of bartonellae in ectoparasties

Polymerase chain reaction (PCR) and sequencing of bartonellae from ectoparasites followed techniques described previously (Ellis et al., 1999). Briefly, the PCR used primers (BhCS781.p and BhCS1137.n) described by Norman et al. (1995) to produce a 379-bp amplicon of the citrate synthase (*glt*A) gene. Negative and positive controls (double-distilled H_2O and DNA from cultures of *B. henselae* [Houston-1 strain] obtained from experimentally infected cats, respectively) were used in each PCR run. PCR products were then electrophoresed in a 2% agarose gel.

After purification (Wizard PCR Prep, Promega, Madison, Wisconsin), products of the correct size were sequenced in both directions with the primers listed above as sequencing primers and the Big Dye terminator sequencing kit on an ABI Prism 310 capillary-automated sequencing machine (Applied Biosystems, Foster City, California). Novel sequences were submitted to GenBank.

A consensus sequence was obtained by using the GAP4 program (Staden, 1996) and sequences from ectoparasites were compared with those in GenBank using the BLASTN program of GCG software (Wisconsin Sequence Analysis Package, Genetics Computer Group, version 8.1). Sequences of all published species of *Bartonella*, as well as sequences from unpublished bartonellae that were identified from the BLASTN output as being closest to the ectoparasite samples, were aligned using PILEUP (GCG). Similarity values of sequences were calculated using length of the shorter sequences (257 bp) without gaps in the program OLDDISTANCES (GCG).

Bartonella spp. GenBank sequence accession numbers used for comparisons included the following: *B. bacilliformis* (U28076); *B. doshiae* (Z70017); *B. alsatica* (AF204273); *B. birtlesii* (AF204272); *B. vinsonii* (U28074); *B. vinsonii berkhoffii* (Z70015); *B. taylorii* (Z70013); *B. quintana* (Z70014); *B. henselae* (L38987); *B. elizabethae* (Z70009); *B. grahamii* (Z70016); *B. tribocorum* (AJ005494); *B. clarridgeae* (U84386); *B. bovis* (AF293394); *B. capreoli* (AF293392); *B. shoenbuchii* (AJ278186); SV06UK (AF449761); SB944NV (AF470616); TM1950NV (AF451161).

Statistical analysis

Prevalence (percent of squirrels infested) and mean intensity (mean per infested squirrel) for the recorded ectoparasite species were compared between squirrels from the 2 habitat types. Prevalences were compared using chi-squared analysis with Bonferroni adjustment; mean intensities were compared using the Kruskal–Wallis test; JMP software (SAS Institute, Cary, North Carolina) for Windows/Mac was used for both tests.

RESULTS

Trap success in woodland and parkland habitats

In total, 410 trap nights from 95 nights were completed; 120 gray squirrels were captured for an overall trap success of 29%. Trap success was 24% in woodland habitats (67 captures in 275 trap nights) and 39% in parkland habitats (53 captures in 135 trap nights). Other mammals captured incidentally during the study included Virginia opossum *Didelphis virginiana* Kerr (6 captures), eastern woodrat *Neotoma floridana* (Ord) (5 captures), and hispid cotton rat *Sigmodon hispidus* Say and Ord (5 captures).

Ectoparasite diversity

Seventeen species of ectoparasites were recovered from woodland squirrels compared with 6 species from parkland squirrels (Table I). The main differences between the ectoparasite faunas of the squirrels between the 2 habitats involved ticks and chiggers. Five species of ticks and 3 species of chiggers parasitized the woodland squirrels, whereas no species of either of these groups was found on parkland squirrels. Statistically significant differences were noted between the 2 habitats for infestation prevalences of the flea *Orchopeas howardi* (Baker), the lone star tick *Amblyomma americanum* (L.), and the mesostigmatid mite *Androlaelaps fahrenholzi* (Berlese) (Table I). In addition, statistically significant differences were noted for mean intensities of *O. howardi* between habitats (Table I). In all these cases, the higher infestation parameters were recorded for woodland squirrels.

Bartonellae in ectoparasites

Partial *glt*A sequences were obtained from ectoparasites from 2 of the 4 gray squirrels that were known to be infected with *Bartonella* sp. Ectoparasites tested from the other 2 infected squirrels included the sucking louse *Neohaematopinus sciuri* Jancke from 1 animal and *N. sciuri*, *O. howardi*, and the mesostigmatid mite *A. casalis* (Berlese) from another animal. Although both *N. sciuri* and *O. howardi* from the other 2 animals were PCR positive, it was not possible to obtain clean sequence results on these specimens, and additional attempts to sequence them failed.

Partial *glt*A sequencing results from the ectoparasites showed that 1 of the *O. howardi* sequences (SC260GA) was 98% similar to the *Bartonella* sp. previously shown to infect eastern gray squirrels (SV06UK; Bown et al., 2002; Table II). The other *O. howardi* sequence was distinct from other known species of *Bartonella* but most similar to *B. quintana* (94.5%). Sequences from *N. sciuri* taken from the same squirrel were dissimilar. One was very similar to a *Bartonella* sp. isolated from a California ground squirrel, *Spermophilus beecheyi* (Richardson), in Nevada (SC262GA), whereas the other was most similar to that from another *N. sciuri* taken from a different animal (SC272GA). The only sequence from a pool of the sucking louse *Hoplopleura sciuricola* (SC272GA) was most similar to that of a *Bartonella* sp. isolated from a least chipmunk *Tamias minimus* Bachman (TM1950NV). Two of the 3 sequences (SC264GA and SC272GA) from *N. sciuri* were similar to those of *B. henselae*.

DISCUSSION

The combined ectoparasite fauna on gray squirrels that we recorded in southeastern Georgia is relatively similar to that reported from this host previously. For example, Whitaker et

TABLE I. Ectoparasites recovered from gray squirrels (*Sciurus carolinensis*) in woodland and parkland habitats in southeastern Georgia, 1994–2001.

Ectoparasite species	Woodland (n = 67)		Parkland (n = 53)		*P* value (prevalence)	*P* value (mean intensity)
	% Prevalence	Mean intensity	% Prevalence	Mean intensity		
Sucking lice						
Enderleinellus longiceps	1.5	13.0	—	—	0.3718	ND*
Hoplopleura sciuricola	20.9	11.3	11.3	6.2	0.2710	0.2724
Neohaematopinus sciuri	32.8	26.0	45.3	8.4	0.1637	0.2555
Flea						
Orchopeas howardi	76.1	8.8	50.9	4.8	0.0041†	0.0285†
Ticks						
Amblyomma americanum	22.4	3.5	—	—	0.0002†	ND
Amblyomma maculatum	1.5	1.0	—	—	0.3718	ND
Dermacentor variabilis	4.5	1.7	—	—	0.1187	ND
Ixodes affinis	3.0	1.0	—	—	0.2046	ND
Ixodes scapularis	1.5	1.0	—	—	0.3718	ND
Mesostigmatid mites						
Androlaelaps casalis	7.5	3.2	4.5	1.3	0.6943	0.6084
Androlaelaps fahrenholzi	13.4	3.0	1.9	3.0	0.0231†	0.3074
Eulaelaps stabularis	3.0	5.0	—	—	0.2046	ND
Haemogamasus reidi	1.5	1.0	1.9	1.0	0.8670	1.000
Actinedid mite						
Cheyletus eruditus	1.5	1.0	—	—	0.3718	ND
Chiggers						
Eutrombicula splendens	3.0	5.0	—	—	0.2046	ND
Myiatrombicula cynos	4.5	3.3	—	—	0.1187	ND
Neotrombicula whartoni	1.5	2.0	—	—	0.3718	ND

* ND, test not done.
† Denotes significant differences with 95% confidence limits.

al. (1976) and Whitaker (1982) recorded 23 species of ectoparasites from *S. carolinensis* in Indiana, including 4 species of sucking lice, 2 species of fleas, 3 species of ticks, 7 species of chiggers, and 6 species of mites belonging to other families. They recorded the same 3 species of sucking lice that we recorded, which are typical associates of this rodent (Durden and Musser, 1994), and also found the flea *O. howardi* to be common, as we did. They also recorded 2 of the same species of ticks that we collected, i.e., *A. americanum* and *Dermacentor variabilis* (Say), and the same 3 species of mesostigmatid mites, i.e., *A. casalis*, *A. fahrenholzi*, and *Haemogamasus reidi* Ewing. However, they recorded a more diverse chigger fauna than we did, as well as specimens of the squirrel tick *Ixodes marxi* Banks, which has not been recorded in Georgia. Also, in Indiana, Wilson et al. (1961) recorded 6 species of ectoparasites from *S. carolinensis* consisting of 2 species of sucking lice (both of which we collected), 3 species of fleas (1 of which we collected), and 1 species of tick (*I. marxi*). In Tennessee, Durden (1980) recorded the same 3 species of sucking lice and the same species of flea that we recorded in this survey. In Florida, Wilson et al. (1991) recorded 11 species of ectoparasites from an urban population of *S. carolinensis*, including the same species of sucking lice and flea and the same 2 species of *Androlaelaps* mites that we recorded. Katz (1938) reported 5 arthropod groups from gray squirrels in Ohio identified as a sucking louse (*H. sciuricola* Ferris), a bot (*Cuterebra* sp.), a flea (*Cer-*

TABLE II. Results of partial *glt*A sequencing analyses from ectoparasites from *Bartonella*-infected gray squirrels (percent similarity values).

Host no.	Ectoparasite species	No.	Stage(s)	Sequence ID	Most similar *Bartonella* (% similarity)
1	*Orchopeas howardi*	1	Adult	SC260GA	SV06UK (97.7)
1	*Neohaematopinus sciuri*	1	Adult	SC262GA	SB944NV (97.3)
1	*Neohaematopinus sciuri*	1	Nymph	SC264GA	SC272GA (97.7); *Bartonella henselae* (97.3)
14	*Orchopeas howardi*	3	Adult	SC267GA	*Bartonella quintana* (94.5)
14	*Hoplopleura sciuricola*	7	Adult/nymphs	SC270GA	TM1950NV (93.0)
14	*Neohaematopinus sciuri*	4	Nymph	SC272GA	*Bartonella henselae* (99.6)

atophyllus fasciatus, which is now treated as *Nosopsyllus fasciatus* [Bosc]), unidentified immature ticks, and unidentified mites.

Clearly, there are major differences between the ectoparasite faunas of gray squirrels inhabiting the 2 different habitat types. Squirrels inhabiting parkland supported far fewer ectoparasite species (6) than those inhabiting woodland (17) in southeastern Georgia (Table I). As predicted, the main difference between these 2 faunas involved ectoparasites that quest for hosts from vegetation, i.e., chiggers and ticks. The absence of understory vegetation, and also of leaf litter in most cases, in the parkland habitat presumably made these sites too dry to promote long-term survival of chiggers and ticks. For long-term survival, representatives of both these ectoparasite groups require high humidity in the leaf litter or ground-level vegetation (or both) (Needham and Teel, 1991; Mullen and OConnor, 2002).

Similarly, the infestation prevalences for 2 species of ectoparasites (*O. howardi* and *A. fahrenholzi*) that parasitized squirrels in both habitats were significantly higher in woodland, as was the mean intensity of 1 of these species (*O. howardi*). These differences are harder to rationalize because the immature stages of both these ectoparasite species develop in host nest material (Durden et al., 2000) that appeared to be similar in both habitats. However, the absence of many kinds of understory vegetation for use as potential nesting materials in parkland habitat may have adversely affected these 2 ectoparasites in some of those sites. Also, dreys (squirrel nests) in parkland trees may have been more prone to desiccation because these were in a more open environment with few surrounding trees or attached vines. Another factor that could have theoretically influenced these data is that the parkland habitats may have been subjected to pesticides that could have reduced ectoparasite numbers on squirrels and in their nests. No homeowner with yards in which we trapped squirrels used outside pesticides. However, building perimeters and fire ant mounds on the campus of Georgia Southern University are sometimes treated with insecticides. Nevertheless, we do not believe that these low-level treatments would significantly affect ectoparasites on squirrels or in their dreys.

Six unique *Bartonella* genotypes were obtained from 3 species of ectoparasites feeding on 2 *Bartonella* sp.–infected animals. An expected finding was that 1 sequence was most similar to the genotype of a *Bartonella* sp. isolate obtained previously from gray squirrels (SC260GA; Bown et al., 2002). An interesting find was the 2 sequences that were most similar to *Bartonella* sp. isolated from other sciurid rodents (1 from *T. minimus* [TM1950NV] and 1 from *S. beecheyi* [SB944NV]). The *Bartonella* sp. sequence from *S. beecheyi* is identical to that of a *Bartonella* sp. isolated from a human patient with cardiac disease (GenBank AF050108; strain designation NVH1; R. Regnery, pers. comm.). Sequences from other ectoparasites were most similar to another human pathogen, *B. henselae*.

The *glt*A sequence used in this study was short. However, this segment of the *glt*A gene has been shown to be highly variable and, as such, it is an effective tool for delineating among *Bartonella* species (Birtles and Raoult, 1996). Nonetheless, this study should be viewed as a preliminary survey for bartonellae in ectoparasites infesting the gray squirrel. Sequencing of additional genes, as well as further molecular and biochemical characterization, of the bartonellae found in ectoparasites is warranted.

Some of the ectoparasites we recorded are known to be vectors of zoonotic pathogens. These include the tick species *A. americanum*, a vector of at least 2 species of ehrlichiae, which can cause disease in humans; *D. variabilis*, a vector of the agent of Rocky Mountain spotted fever; and the blacklegged tick *I. scapularis* Say, a vector of Lyme disease spirochetes, the agent of human granulocytic ehrlichiosis, and other human pathogens (Sonenshine et al., 2002).

This is the first report of sequence-confirmed *Bartonella* sp. from 3 species of ectoparasites—1 flea and 2 sucking lice. However, the development of *Bartonella* sp. in these ectoparasites has not been studied. Laboratory studies are needed to show whether these species may be competent vectors of *Bartonella* sp., and these results do not necessarily incriminate these ectoparasites in the transmission of *Bartonella* sp. to humans. However, Krampitz (1962) has implicated fleas as vectors of bartonellae (reported as *Grahamella*) between wild rodents, and Chomel et al. (1996) have demonstrated laboratory transmission of *B. henselae* between cats by cat fleas. These authors, as well as Higgins et al. (1996), Foil et al. (1998), and Finkelstein et al. (2002), all implicated a fecal rather than a salivary transmission route by fleas for infectious bartonellae. Some other zoonotic bartonellae, such as *B. clarridgeiae* Lawson and Collins, have also been detected in fleas (La Scola et al., 2002).

Additional studies with respect to *Bartonella* sp. infection in the gray squirrel and its ectoparasites are needed. Given the wide distribution of this rodent in North America, its peridomestic affinities, and its introduction into some other countries, e.g., England, infection with bartonellae may represent a potential public health concern, particularly in urban areas.

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