

***Bartonella* and *Rickettsia* in fleas and lice from mammals in South Carolina, U.S.A.**

Will K. Reeves¹✉, Mark P. Nelder², and James A. Korecki²

¹Centers for Disease Control and Prevention, Viral and Rickettsial Zoonoses Branch, Mailstop G-13, 1600 Clifton Rd. NE, Atlanta, GA 30333, U.S.A.

²Clemson University, Department of Entomology, Soils, and Plant Sciences, 114 Long Hall, Clemson, SC 29634, U.S.A.

Received 25 March 2005; Accepted 1 August 2005

ABSTRACT: Species in the genera *Bartonella* and *Rickettsia* are vector-borne pathogens of humans and domestic animals. The natural reservoirs and enzootic transmission cycles of these bacteria are poorly known in South Carolina. Thirteen species of lice and fleas were collected from urban animals and screened for the presence of *Bartonella* and *Rickettsia* by PCR amplification using genus-specific primers. *Bartonella henselae* was present in cat fleas (*Ctenocephalides felis*) from Virginia opossums (*Didelphis virginiana*) and a novel genotype of *Bartonella* was detected in *Orchopeas howardi* from an eastern gray squirrel (*Sciurus carolinensis*). We detected *R. typhi* and three novel genotypes *Rickettsia* in other species of fleas and lice. *Rickettsia typhi*, the causative agent of murine typhus, was detected in two pools of lice (*Enderleinellus marmotae*) from the woodchuck (*Marmota monax*). Cat fleas harbored one of two novel genotypes of *Rickettsia*. A third novel *Rickettsia* was detected in *Orchopeas howardi* from an eastern gray squirrel. **Journal of Vector Ecology 30 (2): 310-315. 2005.**

Keyword Index: *Rickettsia*, *Bartonella*, murine typhus, lice, fleas.

INTRODUCTION

Lice (Phthiraptera) and fleas (Siphonaptera) are obligate, blood-feeding, ectoparasites of mammals and birds on all continents and most oceanic islands (e.g. Kim et al. 1986, Price et al. 2003). Flea-and louse-borne diseases, such as murine typhus, epidemic typhus, trench fever, and plague, have had a significant influence on human history. Modern antibiotics and vector control methods have dramatically reduced the morbidity and mortality associated with these diseases, but the etiologic agents of louse- and flea-borne diseases remain in the U.S.A. Recent cases of urban trench fever in American cities indicate that active transmission of *Bartonella quintana* still occurs (Spach et al. 1995). Sporadic cases of epidemic typhus, caused by *Rickettsia prowazekii*, occur in the United States when humans are exposed to infected southern flying squirrels, *Glaucomys volans* (Linnaeus), or their ectoparasites (Sonenshine et al. 1978). Emerging infectious diseases caused by previously unknown pathogens, such as the flea-borne pathogen *Rickettsia felis*, have been associated with typhus and dengue-like illness in humans worldwide (Williams et al. 1992, Azad et al. 1997). Other pathogens, such as *Bartonella henselae*, cause cat scratch disease in thousands of Americans annually (Kaplan et al. 2002).

Vector-borne diseases have played a monumental role in the history of South Carolina by killing or debilitating thousands of citizens (Adler and Wills 2003). Species in the genera *Rickettsia* and *Bartonella* are primarily zoonotic pathogens with nonhuman, vertebrate reservoirs and arthropod vectors. Ectoparasites must be screened continuously to detect

new and emerging infectious diseases. We examined 13 species of fleas and lice from urban mammals of upstate South Carolina to determine if these ectoparasites harbored rickettsial agents. Previous studies have not focused on rickettsial agents in ectoparasites from this region of South Carolina.

MATERIALS AND METHODS

We collected fleas and lice from 24 animals and four animal nests in upstate South Carolina. The majority of the animals were killed by automobiles and collection techniques were reported by Nelder and Reeves (2005). Additional specimens were obtained from the Clemson University Arthropod Collection (CUAC). Collections were limited to the Piedmont and Foothills ecoregions of South Carolina in Aiken, Anderson, Cherokee, Greenville, Oconee, and Pickens counties (Table 1).

Each ectoparasite was screened for DNA from *Bartonella* and *Rickettsia* by polymerase chain reaction (PCR) amplification. Individual fleas and lice or pools of lice (three per pool) (Table 1) were frozen in liquid nitrogen and crushed with a sterile Teflon pestle. Total DNA was extracted from the pulverized remains with an IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, WA) and resuspended in nuclease-free water. We detected DNA from *Bartonella* and *Rickettsia* spp. by PCR amplification using the BARTON-1 (52'-TAACCGATATTGGTTGTGTTGAAG-32) and BARTON-2 (52'-TAAAGCTAGA AAGTCTGGCAACATAACG-32) and primer-1 (5'-GCTCTTGCAACTTCTATGTT-3') and primer-2

(CATTGTTTCGTCAGGTTGGCG-3') primers to amplify a fragment of the riboflavin synthase gene (*ribC*) of *Bartonella* and the 17 kD antigenic gene of *Rickettsia*, as described by Johnson et al. (2003) and Webb et al. (1990). We amplified the citrate synthase (*gltA*) and 16s rDNA genes from specimens with unidentified *Rickettsia* spp., using primers described by Roux et al. (1997) and the Rick16sF1 (3' GTATGCTTAACACATGCAAGTCGAAC 5') and Rick16sR4 (3' TCCGCGATTACTAGCGATTCC 5') primers. The PCR amplification cycle for the Rick16sF1 and Rick16sR4 primers consisted of an initial DNA degradation at 95° C for 5 min, followed by 30 s denaturation at 95° C, primer annealing at 55° C for 1 min, and extension at 72° C for 2 min. Samples were amplified for 45 consecutive cycles with a 10-min final extension at 72° C. All stock PCR and sequencing primers were at concentrations of 20 µMol. Each PCR tube contained 12.5 µl of Taq PCR Master Mix Kit (Qiagen, Valencia, CA), 7.5 µl of nuclease-free water, 1.25 µl of each primer, and 2.5 µl of DNA extract in water. PCR products were separated by 2% agarose gel electrophoresis and visualized under ultraviolet light with ethidium bromide. Positive and negative controls were used in all screens and consisted of genomic DNA of *B. henselae*, *R. rickettsii*, or distilled water. Products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA). Duplicate sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) using PCR primers, and excess dye was removed with a DyeEx 2.0 column (Qiagen, Valencia, CA). Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA). Primer sequences were removed and sequences assembled with Seqmerge (Accelrys, San Diego, CA). Assembled sequences were compared to those in GenBank using the BLAST 2.0 program (NCBI, Bethesda, MD). Identification of bacterial species was based on sequence similarity to known species. Novel sequences were determined to represent unique taxa.

PCR products from the 17kD antigenic gene were digested with *AluI* for a restriction fragment length polymorphism (RFLP) pattern. Samples (5µl) of amplified PCR products from *Rickettsia* sp. Rf2125 genotype, *Rickettsia* sp. Rf31 genotype, *R. typhi*, *R. rickettsii*, and *R. felis* were subjected to the restriction endonuclease *AluI* (New England Biolabs, Beverly, MA) digestion at 65° C for 6 h in 10 µl volumes, as recommended by the manufacturer. Digested samples were separated by 4.5% agarose gel electrophoresis and visualized under ultraviolet light with ethidium bromide. Voucher specimens of each flea and louse species are deposited in the CUAC. Accession numbers for the DNA sequences are deposited in GenBank, with the following accession numbers *ribC* for *Bartonella* sp. Oh6 (AY953283), *B. henselae* (AY953284), 17 kD antigenic gene for *R. typhi* (AY867871), *Rickettsia* sp. Rf31 (AY953285), *Rickettsia* sp. Rf2125 (AY953286), and *Rickettsia* sp. Oh16 (AY953287), *gltA* from *Rickettsia* sp. Rf31 (AY953288) and *Rickettsia* sp. Rf2125 (AY953289), and the 16s rDNA from *Rickettsia* sp. Oh16 (AY953290).

RESULTS AND DISCUSSION

We collected and screened a total of 42 lice and 38 fleas. DNA from *Bartonella* and *Rickettsia* were detected in 23 individual fleas and two pools of lice (Table 1). The prevalence of rickettsial agents ranged from 100% for three *Rickettsia* spp. in *Ctenocephalides felis* (Bouché) and pools of *Enderleinellus marmotae* Ferris, to no detectable rickettsial DNA in the remaining species of lice. PCR products from positive controls were present during all reactions, and PCR with the negative control amplified nothing.

Species in the genus *Bartonella* are Gram-negative bacteria that infect erythrocytes of vertebrates and are putatively transmitted by hematophagous arthropods. There are at least 16 species or subspecies of *Bartonella* and at least nine of these are associated with disease in humans (Ciervo and Ciceroni 2004, Dehio et al. 2004). The arthropod vectors and transmission cycles of most *Bartonella* spp. are unknown. We detected two *Bartonella* spp. in fleas and none in lice.

Bartonella henselae, the causative agent of cat scratch disease, has a worldwide distribution and is associated with domestic cats, *Felis silvestris* Schreber, and the cat flea, *C. felis* (Chomel et al. 1996). Experimental evidence implicates *C. felis* as the enzootic vector of *B. henselae* to cats (Chomel et al. 1996), but additional routes of transmission and vectors probably exist. Cat scratch disease is rarely fatal but more than 22,000 cases occur in the United States annually (Kaplan et al. 2002), making it one of the most common arthropod-associated diseases in the U.S.A.

DNA from *B. henselae* was detected in 2/19 *C. felis* from Virginia opossums, *Didelphis virginiana* Kerr. The presence of *B. henselae* in wild-caught cat fleas implicates both *C. felis* and opossums in maintaining this pathogen. If cat fleas are competent vectors of *B. henselae*, they could serve as reservoirs and potential bridge vectors between wild mammals and pets or humans. Fleas collected from pets were negative for the *ribC* of *Bartonella* and were determined to be uninfected (Table 1). However, domestic animals such as pet cats and dogs might be exposed to fleas from peridomestic mammals such as the Virginia opossum.

Orchopeas howardi (Baker) is mainly an ectoparasite of tree squirrels but over 53 arboreal animals including raccoons, opossums, squirrels, and eight species of birds have been recorded as hosts of this flea (Lewis 2000). The eastern gray squirrel, *Sciurus carolinensis* Gmelin, is naturally infected with *Bartonella* spp. and the vectors of these bacteria are suspected to be ectoparasitic arthropods (Bown et al. 2002). Kosoy et al. (2003) reported that some *Bartonella* of rodents are infectious to humans, but there is no evidence that the squirrel agents are pathogenic to humans or squirrels. *Bartonella* sp. Oh6 genotype was detected in a squirrel flea, *O. howardi*. The sequence of the PCR amplicon from this bacterium was 97% similar to *Bartonella birtlesii*. *Bartonella birtlesii* naturally infects mice, *Apodemus* spp., (Bermond et al. 2000) and might be closely related to the *Bartonella* spp. of squirrels. Durden et al. (2004) reported two novel *Bartonella* spp. from *O. howardi* collected from squirrels in southern Georgia but sequenced the citrate synthase gene

Table 1. Fleas and lice screened for *Rickettsia* and *Bartonella* species in South Carolina, U.S.A., 1931-2004.

Arthropod species	Number of arthropods examined	Collection data	<i>Rickettsia</i> spp. detected (prevalence)	<i>Bartonella</i> spp. detected (prevalence)
<i>Bovicola caprae</i> (Gurlt)	3 (1 pool)	Pickens Co., Sixmile, 8 February 2003, ex <i>Capra hircus</i> Linnaeus	No	No
<i>Ceratophyllus celsus</i> Jordan	4	Oconee Co., Seneca River Bridge, 12 July 2004, ex <i>Petrochelidon pyrrhonota</i> (Vielot) nest	No	No
<i>Ctenocephalides felis</i> (Bouché)	1	Pickens Co., Clemson, 9 June 2004, ex <i>Didelphis virginiana</i> Kerr	<i>Rickettsia</i> sp. Rf2125 genotype (1/1)	<i>Bartonella henselae</i> (1/1)
<i>Ctenocephalides felis</i>	4	Pickens Co., Clemson, 9 June 2004, ex <i>D. virginiana</i>	<i>Rickettsia</i> sp. Rf2125 genotype (4/4)	No
<i>Ctenocephalides felis</i>	5	Pickens Co., Clemson, 22 June 2004, ex <i>D. virginiana</i>	<i>Rickettsia</i> sp. Rf2125 genotype (5/5)	<i>Bartonella henselae</i> (1/5)
<i>Ctenocephalides felis</i>	1	Pickens Co., Clemson, 13 June 2004, ex <i>Felis silvestris</i> Schreber (domestic cat)	<i>Rickettsia</i> sp. Rf2125 genotype (1/1)	No
<i>Ctenocephalides felis</i>	1	Cherokee Co., Gaffney, 7 November 1993, ex <i>Canis lupus</i> Linnaeus (domestic dog)	<i>Rickettsia</i> sp. Rf31 genotype (1/1)	No
<i>Ctenocephalides felis</i>	4	Pickens Co., Central, 30 November 2004, ex <i>F. silvestris</i>	<i>Rickettsia</i> sp. Rf2125 genotype (4/4)	No
<i>Ctenocephalides felis</i>	3	Pickens Co., Clemson, 22 December 2004, ex <i>C. lupus</i>	<i>Rickettsia</i> sp. Rf2125 genotype (3/3)	No
<i>Enderleinellus marmotae</i> Ferris	6 (2 pools)	Pickens Co., Clemson, 11 May 2004, ex <i>Marmota monax</i> (Linnaeus)	<i>Rickettsia typhi</i> (2/2)	No
<i>Haematopinus suis</i> (Linnaeus)	1	Aiken Co., White Pond, 12 November 1963, ex <i>Sus scrofa</i> Linnaeus	No	No
<i>Haematopinus suis</i>	1	Pickens Co., Clemson, 18 April 1968, ex <i>S. scrofa</i>	No	No
<i>Haematopinus suis</i>	1	Pickens Co., Clemson, 10 January 1984, ex <i>S. scrofa</i>	No	No
<i>Linognathus africanus</i> Kellogg and Paine	3 (1 pool)	Pickens Co., Sixmile, 8 February 2003, ex <i>C. hircus</i>	No	No
<i>Linognathus vituli</i> (Linnaeus)	3 (1 pool)	Pickens Co., Clemson, 1 January 1931, ex <i>Bos taurus</i> Linnaeus	No	No
<i>Neohaematopinus sciuri</i> (Osborn)	6 (2 pools)	Anderson Co., Fants Grove, 10 May 2003, ex <i>Sciurus carolinensis</i> Gmelin	No	No
<i>Neohaematopinus sciuri</i>	1	Anderson Co., Pendelton, 15 May 2004, ex <i>S. carolinensis</i>	No	No
<i>Orchopeas howardi</i> (Baker)	1	Pickens Co., Clemson, 16 June 2004, ex <i>S. carolinensis</i>	No	No
<i>Orchopeas howardi</i>	11	Greenville Co., Greenville, 21 September 2004, ex <i>S. carolinensis</i>	No	<i>Bartonella</i> sp. Oh6 near <i>B. birtlesii</i> (1/11)
<i>Orchopeas howardi</i>	3	Anderson Co., Anderson, 30 June 2004, ex <i>S. carolinensis</i>	<i>Rickettsia</i> sp. Oh16 (1/3)	No
<i>Pediculus humanus capitus</i> De Geer	3 (1 pool)	Greenville Co., Greenville, 12 September 1994, ex <i>Homo sapiens</i> Linnaeus	No	No
<i>Pediculus humanus capitus</i>	3 (1 pool)	Pickens Co., Sixmile, 20 May 1996, ex <i>H. sapiens</i>	No	No
<i>Pediculus humanus humanus</i> Linnaeus	3 (1 pool)	Greenville Co., Ninty Six, 1 January 1975, ex <i>H. sapiens</i>	No	No
<i>Polyplax serrata</i> Burmeister	1	Oconee Co., Seneca, 19 February 2004, ex <i>Mus musculus</i> Linnaeus	No	No
<i>Stachiella octomaculatus</i> (Paine)	6 (2 pools)	Pickens Co., Clemson, 5 July 2004, ex <i>Procyon lotor</i> (Linnaeus)	No	No

rather than the riboflavin synthase genes. The flea harboring the *Bartonella* sp. Oh6 genotype was collected from a dead squirrel at a zoo and might have fed previously on other hosts. This novel agent was detected in 1/15 *O. howardi* and the role of fleas and squirrels in the natural transmission cycle of this agent is unknown.

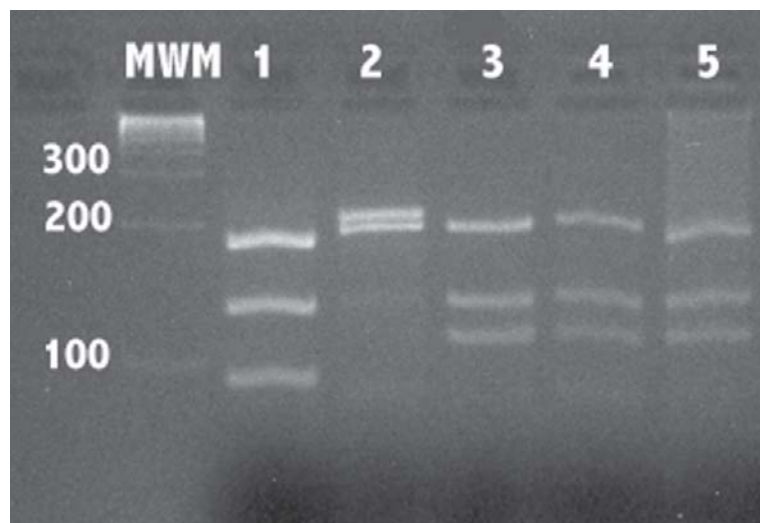
Four species in the genus *Rickettsia*, including *R. typhi*, were detected in lice and fleas. *Rickettsia typhi*, the causative agent of murine or endemic typhus, has a worldwide distribution and is endemic in the United States. Murine typhus was historically a widespread disease in the United States and epidemics of murine typhus in 1944 produced more than 5,338 cases (Love and Smith 1960, Mohr et al. 1953). Endemic foci, such as those in southern Texas, currently produce an average of about 50 cases per year (Boostrom et al. 2002). The oriental rat flea, *Xenopsylla cheopis* Rothschild, was considered the primary vector of *R. typhi* and maintains this pathogen by both vertical and horizontal transmission (Farhang-Azad et al. 1985). Lice are not considered significant vectors of *R. typhi*, but the human body louse, *Pediculus humanus* (Linnaeus), is an experimental vector in the laboratory (Houhamdi et al. 2003) and *Hoplopleura pacifica* Ewing might transmit the pathogen to rats (Traub et al. 1978). Neither woodchucks, *Marmota monax* (Linnaeus), nor their lice, *E. marmotae*, have been associated with *R. typhi*. Serologic data from *M. monax* in the northeastern United States were negative for *R. typhi* antibodies (Nicholson et al. 2003).

DNA from *R. typhi* was detected by PCR in two pools of adult *E. marmotae* from a road-killed woodchuck. Based on a BLAST search, the 412 bp sequence for the 17 kD antigenic gene was a 100% match to that of the Wilmington strain of *R. typhi* (GenBank Accession# AE017197). The presence of DNA from *R. typhi* in lice does not prove that they are vectors

of this agent. The lice were either feeding on an infected host or acquired the pathogen transovarially. *Enderleinellus marmotae* feeds exclusively on *M. monax* and does not bite humans. Several species of fleas, including *Pulex irritans* Baker, *O. howardi*, and *Oropsylla arctomys* (Baker), feed on *M. monax* (Palmer and Wingo 1972, Whitaker and Schmeltz 1973), but their roles in the transmission of *R. typhi* are unknown. Fleas were not reported from *M. monax* in the most recent checklist of fleas from South Carolina (Durden et al. 1999). Our discovery of *R. typhi* in lice demonstrates that this pathogen is present in urban animals in South Carolina.

Three novel genotypes of *Rickettsia* were detected in fleas from South Carolina (Table 1). DNA from a novel *Rickettsia* sp. Oh16 genotype was detected in 1/15 *O. howardi*. Sequences of both the amplicons of the 16s rDNA and 17 kD antigenic genes were unique. The 17 kD gene amplicons were 97% similar to the *Rickettsia* "ARANHA" genotype (GenBank Accession# AY360215). A second genotype of *Rickettsia* was detected in a specimen of *C. felis* from Gaffney (Table 1). The *gltA* amplicon sequence from this *Rickettsia* was identical to the *Rickettsia* sp. Rf31 genotype (GenBank Accession# AF516331) reported from the Thailand-Myanmar border (Parola et al. 2003). All other *C. felis* in our study harbored an undescribed *Rickettsia* sp. with a *gltA* sequence that was identical to that of the *Rickettsia* sp. Rf2125 genotype (GenBank Accession# AF516333) from *Ctenocephalides* spp. collected at the Thailand-Myanmar border and reported by Parola et al. (2003). These *Rickettsia* spp. are not known to cause disease in humans. We are the first to report these bacteria from fleas outside of Asia, but the *Rickettsia* sp. Rf2125 genotype was also detected in fleas from Egypt (unpublished data). Azad et al. (1997) reported *R. felis* from fleas in California, Georgia, Tennessee, and Texas and based their identifications on the RFLP patterns produced by *AluI*

Figure 1. Restriction fragment length polymorphism pattern from the 17kD antigenic gene amplicons digested with *AluI* at 65°C for 6 h, separated by 5% agarose gel electrophoresis, and visualized under ultraviolet light with ethidium bromide. MWM: molecular weight marker with 300, 200, and 100 base pair bands labeled, 1: *Rickettsia rickettsii*, 2: *R. typhi*, 3: *R. felis*, 4: *Rickettsia* sp. Rf31 genotype, and 5: *Rickettsia* sp. Rf2125 genotype.



digestion of the 17 kD antigenic gene amplicons. The RFLP patterns produced by *AluI* digestion of the 17 kD antigenic gene amplicons of *Rickettsia* sp. Rf2125 genotype, *Rickettsia* sp. Rf31 genotype, and *R. felis* are nearly identical and essentially indistinguishable (Figure 1). In addition, the *Rickettsia* detected in *C. felis* might be antigenically similar to *R. felis* and cross react when analyzed by an indirect fluorescent antibody test (IFA). We sequenced the PCR amplicons from the fleas in our study and determined that *R. felis* was not present. All of the cat fleas harbored novel *Rickettsia*. Contamination of the samples was unlikely because fleas from different collections were tested on separate dates, negative controls were never contaminated, and fleas from other locations in SC and GA were not infected with this agent. The public health implications of these new *Rickettsia* spp. are unknown. *Ctenocephalides felis* from pets harbored *Rickettsia* spp. and pet owners in South Carolina are therefore exposed to these agents. If these genotypes of *Rickettsia* are not pathogenic and exclude the establishment of *R. typhi* or *R. felis* in fleas, then the recent paucity of typhus-like illnesses in South Carolina could result from competitive interactions between species of *Rickettsia*.

Rickettsial DNA was not detected in all of the specimens examined (Table 1), however some material from the CUAC harbored Rickettsial DNA indicating that they were appropriately preserved. Some ectoparasites might be refractory to rickettsial infection, could have fed on uninfected hosts, or were poorly preserved and any rickettsial DNA degraded prior to our study. Further research should clarify the roles of fleas and lice in the natural history of rickettsial pathogens in South Carolina.

Acknowledgments

We thank C.E. Beard, K.D. Cobb, M.A. MacCarroll, S.K. Kim, and S. Miller for their assistance with field collections of ectoparasites; P.H. Adler, G.A. Dasch, and J.C. Morse for allowing us to use laboratory space and equipment; A.D. Loftis for assisting with PCR primer development; and L.A. Durden for reviewing this manuscript and verifying identifications of *E. marmotae*.

REFERENCES CITED

- Adler, P. H. and W. Wills. 2003. Legacy of death: the history of arthropod-borne human diseases in South Carolina. *Am. Entomol.* 49: 216-228.
- Azad, A.F., S. Radulovic, J.A. Higgins, B.H. Noden, and J.M. Troyer. 1997. Flea-borne rickettsioses: ecological considerations. *Emerg. Infect. Dis.* 3: 319-327.
- Bermond, D., R. Heller, F. Barrat, G. Delacour, C. Dehio, A. Alliot, H. Monteil, B. Chomel, H.J. Boulouis, and Y. Piemont. 2000. *Bartonella birtlesii* sp. nov., isolated from small mammals (*Apodemus* spp.). *Int. J. Syst. Evol. Microbiol.* 50: 1973-1979.
- Boostrom, A, M.S. Beier, J.A. Macaluso, K.R. Macaluso, D. Sprenger, J. Hayes, S. Radulovic, and A.F. Azad. 2002. Geographic association of *Rickettsia felis*-infected Virginia opossums with human murine typhus, Texas. *Emerg. Infect. Dis.* 8: 549-554.
- Bown K.J., B.A. Ellis, R.J. Birtles, L.A. Durden, J. Lello, M. Begon, and M. Bennett. 2002. New world origins for haemoparasites infecting United Kingdom grey squirrels (*Sciurus carolinensis*), as revealed by phylogenetic analysis of *Bartonella* infecting squirrel populations in England and the United States. *Epidemiol. Infect.* 129: 647-653.
- Chomel, B.B., R.W. Kasten, K. Floyd-Hawkins, B. Chi, K. Yamamoto, J. Roberts-Wilson, A.N. Gurfield, R.C. Abbott, N.C. Pedersen, and J.E. Koehler. 1996. Experimental transmission of *Bartonella henselae* by the cat flea. *J. Clin. Microbiol.* 34: 1952-1965.
- Ciervo, A. and L. Cicconi. 2004. Rapid detection and differentiation of *Bartonella* spp. by a single-run real time PCR. *Mol. Cell. Probes* 18: 307-312.
- Dehio, C., U. Sauder, and R. Hiestand. 2004. Isolation of *Bartonella schoenbuchensis* from *Lipoptena cervi*, a blood-sucking arthropod causing deer ked dermatitis. *J. Clin. Microbiol.* 42: 5320-5323.
- Durden, L.A., W. Wills, and K.L. Clark. 1999. The fleas (Siphonaptera) of South Carolina with an assessment of their vectorial importance. *J. Vector Ecol.* 24: 171-181.
- Durden, L.A., B.A. Ellis, C.W. Bank, J.D. Crowe, and J.H. Oliver, Jr. 2004. Ectoparasites of gray squirrels in two different habitats and screening of selected ectoparasites for bartonellae. *J. Parasitol.* 90: 485-489.
- Farhang-Azad, A., R. Traub, and S. Baqar. 1985. Transovarial transmission of murine typhus rickettsiae in *Xenopsylla cheopis* fleas. *Science* 227: 543-545.
- Houhamdi, L., Fournier P.E., Fang R., and Raoult D. 2003. An experimental model of human body louse infection with *Rickettsia typhi*. *Ann. New York Acad. Sci.* 990: 617-627.
- Johnson G., M. Ayers, S.C. McClure, S.E. Richardson, and R. Tellier. 2003. Detection and identification of *Bartonella* species pathogenic for humans by PCR amplification targeting the riboflavin synthase gene (*ribC*). *J. Clin. Microbiol.* 41: 1069-1072.
- Kaplan, S., J. Rawlings, C. Paddock, J. Childs, R. Regnery, and M. Reynolds. 2002. Cat-scratch disease in children - Texas, September 2000–August 2001. *MMWR* 51: 2647-2649.
- Kim, K.C., H.D. Pratt, and C.J. Stojanovich. 1986. *The sucking lice of North America*. Pennsylvania State University Press. University Park, Pennsylvania. U.S.A. 241 pp.
- Kosoy, M., M. Murray, R.D. Gilmore, Jr., Y. Bai, and K.L. Gage. 2003. *Bartonella* strains from ground squirrels are identical to *Bartonella washoensis* isolated from a human patient. *J. Clin. Microbiol.* 41: 645-650.
- Lewis, R.E. 2000. A taxonomic review of the North American genus *Orchopeas* Jordan, 1933 (Siphonaptera: Ceratophyllidae: Ceratophyllinae). *J. Vector Ecol.* 25: 164-189.
- Love, G.J. and W.W. Smith. 1960. Murine typhus investigations in southwestern Georgia. *Publ. Hlth. Rep.*

- 75: 109-120.
- Mohr, C.O., N.E. Good, and J.H. Schubert. 1953. Status of murine typhus infection in domestic rats in the United States, 1952, and relation of infection by oriental rat fleas. *Am. J. Public Health* 43: 1514-1522.
- Nelder, M.P. and W.K. Reeves. 2005. Ectoparasites of road-killed vertebrates in northwestern South Carolina, U.S.A. *Vet. Parasitol.* 129: 313-322.
- Nicholson, W.L., D.J. Kuhar, J.G. Humphreys, and J.A. Childs. 2003. Serologic evidence for a novel *Ehrlichia* species in woodchucks (*Marmota monax*) from Pennsylvania, USA. *Ann. New York Acad. Sci.* 990: 90-93.
- Palmer, D.B. and C.W. Wingo. 1972. Siphonaptera occurring on Missouri mammals. *Trans. Missouri Acad. Sci.* 6: 43-55.
- Parola, P., O.Y. Sanogo, K. Lerdtthusnee, Z. Zeaiter, G. Chauvancy, J.P. Gonzalez, R.S. Miller, S.R. Telford III, C. Wongsrichanalai, and D. Raoult. 2003. Identification of *Rickettsia* spp. and *Bartonella* spp. in fleas from the Thai-Myanmar border. *Ann. New York Acad. Sci.* 990: 173-181.
- Price, R. D., R. A. Hellenthal, R. L. Palma, K. P. Johnson, and D. H. Clayton. 2003. The chewing lice: World checklist and biological overview. Illinois Natural History Survey Special Publication 24. 501 pp.
- Roux, V., E. Rydkina, M. Ereemeeva, and D. Raoult. 1997. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. *Int. J. Syst. Evol. Microbio.* 47: 252-61.
- Sonenshine, D.E., F.M. Bozeman, M.S. Williams, S.A. Masiello, D.P. Chadwick, N.I. Stocks, D.M. Lauer, and B.L. Elisberg. 1978. Epizootiology of epidemic typhus (*Rickettsia prowazekii*) in flying squirrels. *Am. J. Trop. Med. Hyg.* 27: 339-349.
- Spach, D.H., A.S. Kanter, M.J. Dougherty, A.M. Larson, M.B. Coyle, D.J. Brenner, B. Swaminathan, G.M. Mater, D.F. Welch, R.K. Root, and W.E. Stamm. 1995. *Bartonella (Rochalimaea) quintana* bacteremia in inner-city patients with chronic alcoholism. *N. Engl. J. Med.* 332: 424-428.
- Traub, R., C.L. Wisseman, and A. Farhang-Azad. 1978. The ecology of murine typhus- a critical review. *Trop. Dis. Bull.* 75: 237-317.
- Webb, L., M. Carl, D.C. Malloy, G.A. Dasch, and A.F. Azad. 1990. Detection of murine typhus infection in fleas by using the polymerase chain reaction. *J. Clin. Microbiol.* 28: 530-534.
- Whitaker, J.O., Jr. and L.L. Schmeltz. 1973. External parasites of the woodchuck, *Marmota monax*, in Indiana. *Entomol. News* 84: 69-72.
- Williams, S.G., J.B. Sacci, Jr., M.E. Schrieffer, E.M. Andersen, K.K. Fujioka, F.J. Sorvillo, A.R. Barr, and A.F. Azad. 1992. Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. *J. Clin. Microbiol.* 30: 1758-1762.