CONCISE COMMUNICATIONS

Rats of the Genus *Rattus* are Reservoir Hosts for Pathogenic *Bartonella* Species: An Old World Origin for a New World Disease?

B. A. Ellis,[1] R. L. Regnery,[1] L. Beati,[1] F. Bacellar,[2] M. Rood,[3] G. G. Glass,[4] E. Marston,[1] T. G. Ksiazek,[1] D. Jones,[1] and J. E. Childs[1]

[1]Centers for Disease Control and Prevention, Atlanta, Georgia; [2]Centro de Estudos de Vectores e Doenças Infecciosas Instituto Nacional de Saúde, Águas de Moura, Portugal; [3]Los Angeles County Department of Health Services, Environmental Health—Vector Management Program, Monterey Park, California; [4]Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland

***Bartonella* species were isolated from the blood of 63 of 325 *Rattus norvegicus* and 11 of 92 *Rattus rattus* from 13 sites in the United States and Portugal. Infection in both *Rattus* species ranged from 0% (e.g., 0/87) to ~60% (e.g., 35/62). A 337-bp fragment of the citrate synthase (*glt*A) gene amplified by polymerase chain reaction was sequenced from all 74 isolates. Isolates from *R. norvegicus* were most similar to *Bartonella elizabethae,* isolated previously from a patient with endocarditis (93%–100% sequence similarity), followed by *Bartonella grahamii* and other *Bartonella* species isolated from Old World rodents (*Clethrionomys* species, *Mus musculus,* and *Rattus* species). These data suggest that *Rattus* species are a reservoir host for pathogenic *Bartonella* species and are consistent with a hypothesized Old World origin for *Bartonella* species recovered from *Rattus* species introduced into the Americas.**

The family Bartonellaceae has 12 named species that include 4 recognized human pathogens *(Bartonella bacilliformis, B. elizabethae, B. henselae,* and *B. quintana)* and 1 suspected pathogen *(Bartonella clarridgeiae).* Recent studies indicate that numerous *Bartonella* species circulate in wild mammals and that, in the United States, at least four unique *Bartonella* genogroups infect native rodents [1]. Recently, a new *Bartonella* species isolated from the blood of sylvatic *Rattus norvegicus* was described from isolation attempts from 4 animals [2].

In Baltimore, a high prevalence of antibodies to *B. elizabethae* (33%) was found among inner-city residents with a history of intravenous drug use [3]. This *Bartonella* species was isolated originally from a person with endocarditis in Brighton, Massachusetts [4]. To investigate the potential animal reservoir for an agent causing these infections, we examined rodents of the genus *Rattus.* These originally Old World species are among the most numerous commensal animals in urban environments, wherever they have been introduced. We also sampled *Rattus* species collected from the Old World to compare *Bartonella* isolates.

Materials and Methods

Specimen collection. Rats trapped in Los Angeles and Baltimore were collected specifically for this study. Specimens from 10 additional US sites were taken from frozen, stored specimens at the Centers for Disease Control and Prevention (table 1). In most cases, rats were collected from multiple sites at each locality. Specific information on habitat was not available, except for Los Angeles and Baltimore (inner-city). Whole blood specimens were also obtained from 5 *R. rattus* from Porto Santo Island, Madeira Archipelago, and 2 *R. norvegicus* from Aguas de Moura, Portugal.

Bacterial isolation and identification. Isolates were obtained by streaking 100 μL of whole blood onto heart-infusion agar supplemented with 5% rabbit blood (Becton Dickinson Microbiology Systems, Cockeysville, MD). Plates were incubated at 32°C in 5% CO_2 for up to 5 weeks.

DNA of putative *Bartonella* cultures was extracted (QIAamp Blood Kit; Qiagen, Chatsworth, CA). The polymerase chain reaction (PCR) master kit (Boehringer Mannheim, Indianapolis) was used with primers (*Bh*CS781.p and *Bh*CS1137.n), producing a 379-bp amplicon of the citrate synthase (*glt*A) gene in the PCR reaction [5]. Negative and positive controls (double-distilled H_20 and DNA from cultures of *B. henselae* [Houston-1] obtained from experimentally infected cats) were used in each PCR run. PCR product (12 μL) was electrophoresed in a 2% agarose gel. Products of the correct size were purified (Wizard PCR Prep; Promega, Madison, WI) and sequenced in both directions with the PRISM dye-terminator sequencing kit on an ABI Prism 377 (Applied Biosystems, Foster City, CA). Sequences of the novel isolates were submitted

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Reprints or correspondence; Dr. Barbara A. Ellis, Viral and Rickettsial Zoonoses Branch, Centers for Disease Control, MS G 13, 1600 Clifton Rd., N.E., Atlanta, GA 30333.

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Table 1. Survey of *Bartonella* species in *Rattus norvegicus* and *Rattus rattus* collected from United States and Portugal.

Locality	*R. norvegicus*	*R. rattus*	Total
United States			
California			
Los Angeles	19/42 (45.2)[a]		19/42 (45.2)
Palo Alto		0/11 (0.0)	0/11 (0.0)
Riverside		3/5 (60.0)[b]	3/5 (60.0)
Orange County		0/22 (0.0)	0/22 (0.0)
Miami, Florida	0/2 (0.0)	2/19 (10.5)	2/21 (9.5)
Atlanta, Georgia	0/13 (0.0)[c]	1/7 (14.3)	1/20 (5.0)
Spencer County, Indiana	0/16 (0.0)[c]		0/16 (0.0)
New Orleans, Louisiana	35/62 (56.4)[a]	2/12 (16.7)	37/74 (50.0)
Baltimore, Maryland	7/66 (10.6)[c]		7/66 (10.6)
Reno, Nevada	0/35 (0.0)[b]		0/35 (0.0)
New York, New York	0/87 (0.0)[a]		0/87 (0.0)
Kleberg, Texas		1/11 (9.1)	1/11 (9.1)
Portugal	2/2 (100)[c]	2/5 (40.0)	4/7 (57.1)
Totals	63/325 (19.4)	11/92 (12.0)	74/417 (17.7)

NOTE. Nos. of nucleotide sequence–confirmed *Bartonella* isolates of total no. tested by locality are given (%). Significant differences from expected overall prevalences as calculated by binomial distribution are given ([a]$P < .0001$, [b]$P < .01$, [c]$P < .05$).

to GenBank. Criteria used to define distinct genotypes included either genotypes consistently obtained from >1 isolate or, in the case of novel genotypes represented by a single isolate, a reproducible sequence.

The two strands were assembled with the GAP4 program of the Staden software package [6] and compared with sequences in GenBank using the BLAST program of GCG software (Wisconsin Sequence Analysis Package, Genetics Computer Group, version 8.1). Sequences were aligned using PILEUP (GCG); similarity values of sequences were calculated using length of the shorter sequences without gaps in the program OLDDISTANCES (GCG). We produced 100 bootstrap replicates of the alignment, using SEQBOOT [7]. A maximum parsimony analysis was performed with DNAPARS and a distance analysis (neighbor-joining) was done with DNADIST and NEIGHBOR to determine relationships with other known *Bartonella* species [7]; *B. bacilliformis* was the outgroup. CONSENSE [7] was used to construct a consensus tree, and trees were drawn using TreeView [8].

Statistical analysis of prevalence data. We examined focality of infection, using exact probabilities from the binomial distribution [9] computed by comparing numbers of infected animals at each site to an expected distribution based on the overall observed prevalence.

Results

Isolates identified as *Bartonella* species were obtained from 63 *R. norvegicus* (19%) and 11 *R. rattus* (12%; table 1). *Bartonella* infection in *R. norvegicus* was spatially focal. In 3/9 localities, a statistically significant higher-than-expected prevalence of *Bartonella* infection in *R. norvegicus* was observed (45% in California, 56% in Los Angeles, and 100% in Portugal), whereas prevalence of infection was less than expected from rats in 5 locales (Georgia, Indiana, Maryland, Nevada, and New York; table 1). *R. rattus* were infected with *Bartonella* species in 5/7 locations tested in the United States, with prevalences of 9%–60%. Except for Riverside, California (60%), prevalence of infection in *R. rattus* did not differ significantly from the overall prevalence. The overall prevalence of *Bartonella* bacteremia in both *Rattus* species was 18%.

Seven novel genotypes were among the 63 isolates of *Bartonella* obtained from *R. norvegicus* by the criteria used in this study. One isolate each from *R. norvegicus* collected in Louisiana and Maryland were identical to *B. elizabethae.* One of the frequently obtained genotypes (RN10149MD, 28/74) was 99% similar to *B. tribocorum* (1-bp difference; GenBank accession no. AJ005494). Most of the *R. norvegicus* isolates (87%, 55/63) were three genotypes: RN10149MD, RN10616LA, and RN10623LA (figure 1), and 1 of these (RN10149MD) was isolated from *R. norvegicus* captured in California, Louisiana, Maryland, and Portugal.

In general, the 11 isolates obtained from *R. rattus* captured in the United States were distinct from those from *R. norvegicus* (figure 1). The most frequent (6/11) genotypic variant was identical to that from an indigenous rodent, the cotton rat *(Sigmodon hispidus),* captured in Georgia (strain designation, SH8776GA). One variant matched another cotton rat genotype from Georgia, SH6397GA (see figure 1). Two of the *R. rattus* isolates from Louisiana were identical to one from an *R. norvegicus* captured in the same locality (RN10623LA; figure 1). The genotype obtained from 2 *R. rattus* captured in Portugal, RR13863PO, differed from an *R. norvegicus* isolate (RN10623LA) by 1 bp (99.7% similar).

The topology of the tree inferred by maximum parsimony analysis was characterized by modest to poorly supported branchings (bootstrap values <70%). Similar tree topologies were observed using the DISTANCE algorithm (data not shown). Nonetheless, sequences from 63 *R. norvegicus* isolates and 2 Portuguese *R. rattus* isolates formed a well-supported cluster (94% bootstrap support). These 7 novel genotypes clustered with *B. elizabethae,* a *Bartonella* isolate from a *Rattus* from Peru (C5RAT [rodent species not specified in the original paper], [10]), *B. grahamii* (from the bank vole, *Clethrionomys glareolus,* captured in the United Kingdom [11]), and an isolate from a house mouse *(Mus musculus)* captured in California (MM5136CA; figure 1). Genotypes from *Rattus* were most similar to *B. elizabethae* or *B. grahamii* (93%–100% similarity) and from 93% to 99.7% similar to each other, differing by 1–24 nucleotides.

The majority of the genotypes obtained from *R. rattus* (identical to strains SH8776GA and SH6397GA) were present in a well-supported clade containing isolates from native New World Sigmodontine rodents, including 1 from an *Oryzomys palustris* (rice rat) captured in Georgia, United States (OP6399GA), and 1 from a *Phyllotis* species captured in Peru (SRPHY1). The two genotypes most frequently obtained from *R. rattus* differed by 1 nucleotide (99.7% similarity). These genotypes were most similar to *Bartonella vinsonii* (93% similarity),

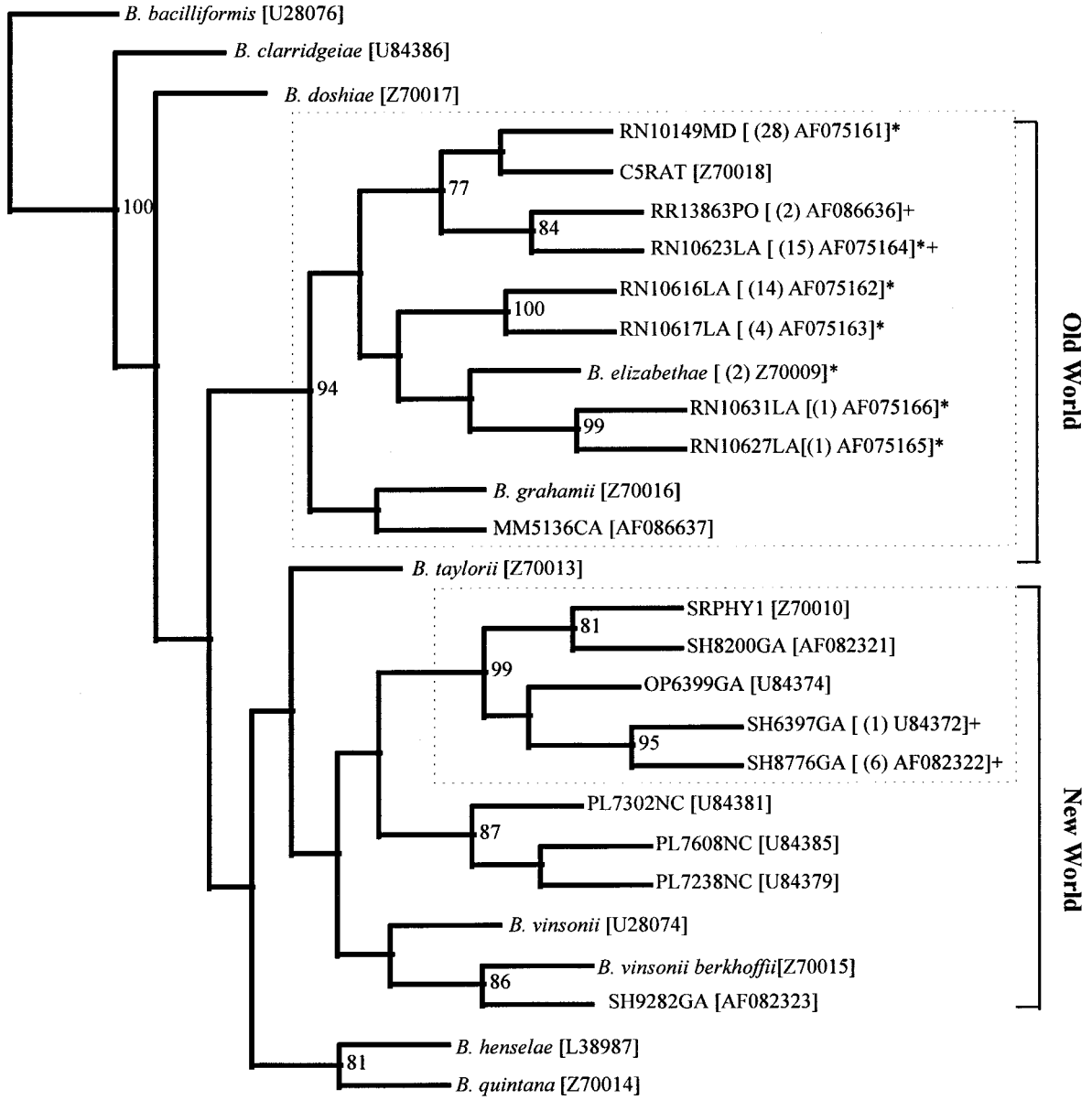

Figure 1. Maximum parsimony analysis of 337-bp sequence of *glt*A gene for *Rattus* isolates with representatives of other known *Bartonella* species. Strain designations are followed in brackets by frequency of genotype (in parentheses) and GenBank accession no. Only bootstrap replicates >70% are noted. *B. bacilliformis* is the out-group. Boxes delimit clades containing isolates obtained in this study from wild-captured *R. norvegicus* (*) or *R. rattus* (+) and hypothesized Old World or New World origin of Bartonellaceae.

isolated from a meadow vole *(Microtus pennsylvanicus)* from Canada [12].

A second well-supported clade included isolates from *Peromyscus leucopus* trapped in North Carolina (PL7302NC, PL7608NC, PL7238NC). Another recently characterized *P. leucopus* isolate [13] was within the same clade but was not included, because only 299 bp from the *glt*A gene were available from GenBank.

Discussion

The genus *Rattus* is commonly infected with *Bartonella* species, as are other rodents trapped in the United Kingdom [14] and in the United States [1]. Seven novel *Bartonella* genotypes were obtained from *R. norvegicus,* including 2 isolates identical by *glt*A partial gene sequence to *B. elizabethae.* The same genotypes were present in *R. norvegicus* captured from distant lo-

cations: *B. elizabethae* from Maryland and Louisiana (~1840 km distance); and RN10149MD and RN10623LA from California, Louisiana, and Maryland (~4430 km distance), as well as from Portugal. All 63 *Bartonella* isolates from *R. norvegicus* clustered into a well-defined clade.

Bartonella infection in *R. norvegicus* appeared to be spatially focal, ranging from 0% (0/87, New York) to 56% (35/62, Louisiana). In contrast, a significant deviation from overall prevalence was found only in 1 of 8 sites sampled for *R. rattus.* The underlying reasons for apparent focality may be related to the occurrence of disjunct populations of hosts that undergo local extinctions of bacteria, the transmission of *Bartonella* species from other mammal species, or unidentified vectors that may require different environmental conditions. The effects of specimen handling and temporal differences in prevalence among sites and small sample sizes at some sites also may have influenced these results.

Although the *glt*A segment used for this analysis was short, it is a reliable taxonomic tool for distinguishing differences among closely related organisms [10]. The phylogenetic relationships of *Bartonella* species isolated from *R. norvegicus* suggest that these rodents may have carried their infections from the Old World when introduced throughout the Americas. Data supporting the hypothesis of an Old World origin for *Bartonella* species obtained from *Rattus* include the widespread occurrence of genetically related isolates of *Bartonella* species in *R. norvegicus* from Portugal, the United States, and South America; the marked difference between the *Bartonella* isolates obtained from *Rattus* and those isolated and characterized from indigenous rodents of the New World (figure 1); and the presence of genetically related *Bartonella* species obtained from other Old World rodents from Europe (*B. grahamii* from a bank vole in the United Kingdom never exported to the United States [14]) or the United States (a house mouse, *M. musculus* [an Old World rodent introduced into the United States], captured in California [Regnery RL et al., unpublished data]).

The distance and time to effect a transoceanic passage of infected rats from Europe to the United States would not present significant obstacles to the dissemination of infected animals. *Bartonella* organisms can cause persistent bacteremia in the hosts, and this bacterium may be maintained within rodent populations by vertical transmission [15].

Although based on a relatively small number of isolates, the finding that most *Bartonella* genotypes in *R. rattus* were identical to those from New World Sigmodontine rodents is striking. *R. rattus* frequently occurs in rural environments, where it may come into contact with native New World species. Thus, the *Bartonella* species in *R. rattus* may be the result of a historical species transfer event from New World rodents, or it may represent ongoing "spillover" of infection from native species. The predominantly urban *R. norvegicus* would be much less likely to come into contact with native New World rodents. Infection of multiple rodent taxa with the same or similar *Bartonella* genotypes has been observed previously [1, 14], implying low host specificity. *Rattus* species may simply be infected with the bartonellae that are naturally hosted by the small mammals that are most common in their environment.

Few studies have examined diversity in the Bartonellaceae. Given the existence of *Bartonella* species in every mammal group examined to date, the diversity of the genus is probably much greater than has been observed among the relatively few strains examined to date. Thus, the Old and New World division of *Bartonella* seen in this study may prove to reflect gaps in our knowledge. Only a concerted effort to establish the evolutionary spectrum of the Bartonellaceae will affirm these apparent divisions within the family.

We report the finding of *Bartonella* species indistinguishable from the human pathogen, *B. elizabethae,* from 2 urban-captured *R. norvegicus* from Louisiana and Maryland. An isolate of *B. elizabethae* obtained from a *Rattus* species captured in Peru was previously reported by Birtles and Raoult [10]. These results suggest that *Rattus* species are a reservoir for this bacterium, and antibodies reactive to *B. elizabethae* among inner-city, intravenous drug users in Baltimore may indicate infection with this, or a related, *Bartonella* species [3].

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