DISSERTATION

DIVERSITY AND DYNAMICS OF RODENT-BORNE BARTONELLA BACTERIA ARE REGULATED BY RODENT COMMUNITY AND HOST POPULATION PARAMETERS

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY YING BAI ENTITLED "DIVERSITY AND DYNAMICS OF RODENT-BORNE BARTONELLA BACTERIA ARE REGULATED BY RODENT COMMUNITY AND HOST POPULATION PARAMETERS" BE ACCEPTED AS FULFULLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

DIVERSITY AND DYNAMICS OF RODENT-BORNE BARTONELLA BACTERIA ARE REGULATED BY RODENT COMMUNITY AND HOST POPULATION PARAMETERS

Bartonella infections are widely distributed in rodents of many species with high prevalences and high genetic diversity. Some rodent-associated bartonellae are also associated with human illnesses. After reviewing the current knowledge of bartonellae (Chapter 1), this dissertation focuses on analyses of associations of bartonellae with their rodent hosts in order to understand how dynamics and diversity of bartonellae are regulated by parameters of rodent communities and populations (Chapter 2).

Chapter 3 describes the relationship between bartonella prevalence and structure of rodent community and points out that bartonella prevalence is negatively correlated with diversity of rodent community, suggesting a dilution effect. Chapter 4 describes temporal dynamics of bartonella prevalence during rodent population cycles, successive bartonella infection in individual rodents, and the effects of some rodent population parameters, such as rodent age, on bartonella prevalence and level of bacteremia. Chapter 5 describes host-specificity of bartonellae in their rodent hosts and concludes that host-specificity occurs mostly at the level of the genus of the rodent host. Bartonellae occasionally can "jump" to non-specific host rodents. The northern grasshopper mouse, an exception, acquires non-specific bartonella strains commonly, and hosts multiple typical bartonella strains. Chapter 6 describes spatial distribution and deviation of genetic variants of

Bartonella species between colonial black-tailed prairie dogs, pointing out the effect of landscape features.

This research has provided data to help understand the ecological characteristics of bartonellae. Knowing information of rodent community and population, it is possible to predict bartonella dynamics and composition of bartonella strains in a particular rodent community and/or population. This is of epidemiological significance with regard to the public health concern of bartonellosis as an emerging disease.

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CHAPTER ONE

LITERATURE REVIEW

Bartonellae are mainly hemotropic, intracellular parasites associated with erythrocytes and endothelial cells of mammals (Anderson and Neuman, 1997; Schülein et al., 2001). In addition to knowledge that the agent of cat scratch disease is *Bartonella henselae*, but not *Afipia felis* (Chomel, 2000), bacteria of the genus *Bartonella* have been studied extensively. It has become increasingly obvious that *Bartonella* spp. organisms are highly adapted to intracellular persistence in a wide variety of vertebrates, principally mammals (Breitschwerdt and Kordick, 2000). Since the mid 1990s, there have been substantial advances in our understanding of the etiology, reservoir potential, vector transmission, and pathogenesis of bartonellosis in cats, dogs, and humans.

TAXONOMY

The genus *Bartonella* is named for Alberto L. Barton, a Peruvian physician who described the bacterium *B. bacilliformis* in 1909. Bartonellae belong to the α-2 subgroup of the class Proteobacteria and are all closely related, having > 98% homology in the sequences of their 16S rRNA genes. Evolutionarily, they are close to members of the genera *Brucella*, *Agrobacterium*, and *Rhizobium* (Breitschwerdt and Kordick, 2000). On the basis of 16S rRNA sequence data, DNA hybridization data, and the results of guanine-plus-cytosine content and phenotypic characterization studies, bacteria previously designated as *Rochalimaea* species and of the genus *Grahamella* were added

to the genus *Bartonella* (Brenner et al., 1993; Birtles et al., 1995). Historically, the genera *Rochalimaea*, *Grahamella* and *Bartonella* were all considered members of the order *Rickettsiales*. Now, however, the newly formed family Bartonellaceae has been removed from the order *Rickettsiales* (Brenner et al., 1993; Birtles et al., 1995).

PHENOTYPIC FEATURES

Bacteria of the genus *Bartonella* are generally short, pleomorphic, fastidious aerobic, gram-negative coccobacillary or bacillary (0.6 μm × 1.0 μm). In infected tissues, Warthin-Starry silver impregnation stain reveals small bacilli, which tend to appear as clumps of tightly compacted organisms. These organisms can be identified in red blood cells by May-Grünwald Giemsa staining. Some members of the genus, such as *Bartonella bacilliformis* and *B. clarridgeiae* have flagella and are motile. *B. henselae* and *B. quintana* do not have flagella, but typically display twitching motility in wet mounts due to the presence of pili. Polar structures resembling fimbriae have been observed on *B. tribocorum*. Bartonellae are catalase, oxidase, urease and nitrate reductase negative (Breitschwerdt and Kordick, 2000).

BARTONELLA SPECIES

Thirty species or subspecies have been described in the most recent list of members of the genus *Bartonella* (Table 1-1). A large number of strains isolated from a wide variety of mammalian hosts have been only partially characterized and further characterization is pending.

Table 1-1 Currently recognized *Bartonella* species

Species	Reservoir	Accidental host	Vector or potential vector
alsatica	Rabbit (*Oryctolagus cuniculus*)	Human	Fleas[b], ticks[b]
australis	Gray kangaroo (*Macropus giganteus*)	Unknown	Unknown
bacilliformis	Human	No	Sandflies (*Lutzomyia verrucarum*)[a]
birtlesii	Wood mouse (*Apodemus* spp.)	Unknown	Unknown
bovis (weissii)	Domestic cattle (*Bos taurus*)	Cat	Biting flies[b], ticks[b]
capreoli	Roe deer (*Capreolus capreolus*)	Unknown	Biting flies[b], ticks[b]
chomelii	Domestic cattle (*Bos taurus*)	Unknown	Biting flies[b], ticks[b]
clarridgeiae	Cat (*Felis catus*)	Human, dog	Fleas (*Ctenocephalides felis*)[a]
doshiae	Field vole (*Microtus agrestis*)	Unknown	Unknown
elizabethae	Rat (*Rattus norvegicus*)	Human, dog	Fleas[b]
grahamii	Bank vole (*Myodes glareolus*)	Human	Fleas[b]
henselae	Cat (*Felis catus*), rodent?	Human, dog	Fleas (*Ctenocephalides felis*), ticks[b]
koehlerae	Cat (*Felis catus*)	Human	Fleas (*Ctenocephalides felis*)[a]
peromysci	Field mouse (*Peromyscus* spp.)	Unknown	Unknown
phoceensis	Rat (*Rattus norvegicus*)	Unknown	Unknown
quintana	Human	Dog	Human body lice (*Pediculus humanis corporis*)[a]
rattimassiliensis	Rat (*Rattus norvegicus*)	Unknown	Unknown
rochalimae	Unknown	Human	Unknown
schoenbuchensis	Roe deer (*Capreolus capreolus*)	Unknown	Biting flies[b], ticks[b]
talpae	European mole (*Talpa europaea*)	No	Unknown
tamiae	Unknown	Human	Unknown
taylorii	Wood mouse (*Apodemus* spp.)	Unknown	Fleas[b]
tribocorum	Rat (*Rattus norvegicus*)	Unknown	Unknown
vinsonii subsp. *arupensis*	Deer mouse (*Peromyscus maniculatus*)	Human	Fleas[b], ticks[b]
vinsonii subsp. *berkhoffii*	Coyote (*Canis latrans*)	Human, dog	Ticks[b]
vinsonii subsp. *vinsonii*	Meadow vole (*Microtus pennsylvanicus*)	No	Ear mites (*Trombicula microti*)[b]
washoensis	California ground squirrel (*Spermophilus beecheyi*)	Human, dog	Fleas[b], ticks[b]
washoensis subsp. *cynomysii*	Black-tailed prairie dog (*Cynomys ludovicianus*)	Unknown	Fleas[b]

a: known vector; b: potential vector

Until 1993, the genus *Bartonella* contained only one species, *B. bacilliformis.* With the application of modern comparative phylogenetic methods, considerable diversity within the genus has been demonstrated. The unification of the *Rochalimaea* and *Grahamella* with the genus *Bartonella* resulted in the addition of nine *Bartonella* species: *B. quintana*, *B. vinsonii*, *B. henselae*, *B. elizabethae*, *B. talpae*, *B. peromysci*, *B. grahamii*, *B. taylorii*, and *B. doshiae* (Brenner et al., 1993; Birtles et al., 1995). The genus *Bartonella* continues to expand as new isolates are obtained from a variety of vertebrates. A subspecies of *B. vinsoni*, *B. vinsoni* subsp. *berkhoffii*, was isolated from dogs (Breitschwerdt et al., 1995); *B. washoensis* (GenBank accession number AF070463) was isolated from a human patient with cardiac disease; a second serogroup (Marseille) has been identified among *B. henselae* isolates (Drancourt et al., 1996); *B. clarridgeiae*, isolated from a cat, was characterized as a new species (Lawson and Collins, 1996); and *B. tribocorum* was isolated from bloods of Norway rats (Heller et al., 1998). During 1999, four species were added to the genus: *B. koehlerae*, *B. alsatica*, *B. vinsonii* subsp. *arupensis*, and *B. weissii* (GenBank accession number AF199502), isolated from bloods of domestic cats, wild rabbits, a cattle rancher, and a domestic cat, respectively (Droz et al., 1999; Heller et al., 1999; Welch et al., 1999). In 2000, a new species, *B. birtlesii*, isolated from *Apodemus* spp., was described (Bermond et al., 2000). Moreover, several *Bartonella* species have been isolated from wild roe deer (*Capreolus capreolus*) or cattle, including *B. schoenbuchensis* found in Germany (Dehio et al., 2001), *B. capreoli* and *B. bovis* (formerly described as *B. weissii*) found in France (Bermond et al., 2002). In addition, Maillard et al. (2004) described *B. chomelii* from French domestic cattle (*Bos taurus*). Two species, *B. rattimassiliensis* and *B. phoceensis*, were isolated from *Rattus*

norvegicus in France (Gundi et al., 2004). A strain isolated from febrile human patients in Thailand was described as *B. tamiae* (Kosoy et al., 2008). Very recently, a strain isolated from an American patient who traveled in Peru has been designated as *B. rochalimae* (Eremeeva et al., 2007). Finally, isolates obtained in kangaroos in Australia has been described as *B. australis* (Fournier et al., 2007).

BARTONELLAE AS FACULTATIVE INTRACELLULAR BACTERIA

In the natural cycle of bartonellae, these organisms are eventually seeded into the blood, where they encounter, associate with, and then invade erythrocytes. Within erythrocytes, bartonellae multiply and persist throughout the life of their host cells. Although occasionally found to be pathogens, bartonellae are more reasonably considered commensal with their hosts and have evolved to elegantly exploit their hosts.

Vascular endothelial cells serve as a primary niche for bartonellae prior to their entering the blood. Endothelial cells are invaded by two mechanisms: endocytosis of bacteria, similar to that which occurs with other intracellular bacteria; and engulfment of clustered bacteria by a unique host cell structure called the invasome (Dehio et al., 1997). Bartonellae infect erythrocytes and endothelial cells and cause prolonged intraerythrocytic bacteremia in their specific mammalian reservoir host (Dehio, 2004). The intraerythrocytic lifestyle appears to be the common and the most intriguing parasitic strategy of all bartonellae in their mammalian reservoirs. It appears that specific (polar flagella) and common (deformin, invasion-associated locus) mechanisms have been developed by various *Bartonella* spp. to adhere to and invade the erythrocytes. The low prevalence of clinical manifestations compared to the high prevalence of infection

underlines the elegance of these parasites, which exploit their hosts in a manner that optimizes transmission. Recent research efforts have begun to determine the strategies involved in this exploitation, and significant progress has been made in unraveling these unusually complex natural cycles.

BACTERIAL FACTORS INVOLVED IN PATHOGENESIS

Studies of bartonella pathogenesis have taken a large step forward in recent years. The combination of bacterial genetics and appropriate cell culture or animal models (Schülein et al., 2001) of bartonella infections have provided the first glimpses of their molecular mechanisms and improved our understanding of bartonella–host cell interactions. Some of the underlying virulence factors and pathogenic mechanisms may be common among all bartonellae, or specific to a particular *Bartonella* species and to its host cells.

Type IV secretion system and the *virB* operon -- transporting bacterial proteins

Type IV secretion systems (T4SS) consist of a multiprotein channel that transports DNA or protein from bacterium to host cell. These versatile transporters have evolved from bacterial conjugation systems and mediate translocation of bacterial effector molecules during interaction with host cells (Cascales and Christie, 2003). Bartonellae encode two distinct T4SS, *virB*-D4 and *trw*, both of which are essential for infection in an animal model. The *virB*-D4 T4SS is required at an early infection stage, before the onset of intraerythrocytic bacteremia (Schülein and Dehio, 2002). It may be involved in mediating endothelial cell interaction in both the mammalian reservoir and in the

incidental human host (Dehio, 2003). The primary role of the *trw* T4SS is to establish contact with erythrocytes by surface-expressed T4SS pili rather than to translocate effector proteins into host cells. Experimental animal models showed that mutants without either *virB*-D4 or *trw* were unable to cause intraerythrocytic bacteremia in rats (Schülein and Dehio, 2002; Seubert et al., 2003). To date these T4SS represent the only reliable pathogenesis factors of bartonellae and are major virulence determinants of these organisms for targeting multiple endothelial cell functions.

Angiogenic factor -- trigger of vasoproliferation

All three vasoproliferative *Bartonella* species, *B. bacilliformis*, *B. quintana*, and *B. henselae*, display this activity during infection of human umbilical vein endothelial cells (Garcia et al., 1990; Garcia et al., 1992; Conley et al., 1994; Maeno et al., 1999; Dehio, 2003). *groEL* was identified as a candidate cause of the observed mitogenic activity in *B. bacilliformis* (Minnick et al., 2003). Interestingly, a recent study on the zoonotic pathogen *Brucella abortus* suggested that *groEL* is actively secreted by its *virB* system (Watarai et al., 2003). It might be interesting to test whether secretion of *groEL* depends on a functional *virB*-D4 T4SS in bartonellae. Another question is whether *groEL* is directly involved in mediating endothelial proliferation, or whether it may act as the chaperone for the actual angiogenic factor. Additional experimental evidence is required to unequivocally demonstrate the role of *groEL* in vascular proliferation.

Compared with *B. bacilliformis*, much less is known regarding the angiogenic activity of other *Bartonella* species. Susceptibility to trypsin digestion suggests that the angiogenic activity of at least *B. henselae* is also of a proteinaceous nature (Conley et al.,

1994). To what extent the vasoproliferative factor(s) of *B. bacilliformis*, *B. henselae*, and *B. quintana* is related in structure and function, and whether they are benign endothelial mitogens or trigger proliferation indirectly via an autocrine loop, await further evidence.

Deformin -- deformation of erythrocyte membranes

B. bacilliformis produces a proteinaceous extracellular deformation factor that causes creasing and indentation of erythrocytes in the absence of intact bacteria (Mernaugh and Ihler, 1992). Deformin is a small hydrophobic molecule with a high affinity for albumin (Derrick and Ihler, 2001). Deformin can be extracted from albumin as a heat- and protease-resistant, water-soluble molecule with a molecular mass of about 1.4 kDa. A recent study indicates that a group of proteins of about 36 kDa present in the supernatant of *B. bacilliformis* are also required for high levels of deforming activity (Hendrix and Kiss, 2003). When these proteins are missing, as in the supernatant of a variant strain, or when they are removed by filtration or ammonium sulfate precipitation, the levels of deforming activity are abolished (Hendrix and Kiss, 2003). The 36-kDa proteins are considered either directly responsible for deformation of human erythrocytes -- they may be part of a deforming protein complex -- or may be necessary for secretion of the deforming activity (Hendrix and Kiss, 2003). It may be that the deformin-mediated invasion mechanism is shared among bartonellae of different species. Further work is required to reveal the precise molecular mechanisms of erythrocyte deformation by bartonellae.

Flagella -- strengthening erythrocyte interaction

B. bacilliformis is highly motile due to its possession of multiple unipolar flagella (Scherer et al., 1993). Flagella of *B. bacilliformis* facilitate erythrocyte invasion (Walker and Winkler, 1981). In vitro studies with human erythrocytes showed that bacterial motility correlates with bacterial adherence. Early work suggested that nonmotile variants bind poorly and do not invade erythrocytes (Benson et al., 1986; Mernaugh and Ihler, 1992). Consistently, antibodies against the flagellin subunit partially inhibited erythrocyte binding and almost completely abolished invasion (Scherer et al., 1993). A nonflagellated mutant obtained by site-directed mutagenesis of the flagellin gene displayed a 75% reduced binding capacity for human erythrocytes, and this phenotype could be partially rescued by complementation with the wild-type locus (Battisti and Minnick, 1999; Minnick and Anderson, 2000). It remains to be demonstrated whether flagella directly bind to erythrocytes or whether the energy-dependent process of motility simply enhances bacteria-erythrocyte collisions. The flagella of *B. bacilliformis* appear critical for the particularly high erythrocyte invasion rate observed in Oroya fever, a disease caused by these organisms (up to 80% infected erythrocytes) (Ihler, 1996). However, considering that several *Bartonella* spp. (e.g., *B. henselae*, *B. quintana*, and *B. tribocorum*) are nonflagellated but capable of invading erythrocytes (Batterman et al., 1995; Kordick and Breitschwerdt, 1995; Heller et al., 1998; Maeno et al., 1999; Rolain et al., 2001; Rolain et al., 2002), flagella cannot be essential for the process of erythrocyte invasion by these organisms.

Pili -- binding to nucleated cells

Pili appear to play an important role in mediating specific interaction with host endothelial cells and erythrocytes, leading to intracellular localization (Batterman et al., 1995; Dehio et al., 1997). Pili structures in *B. henselae*, *B. quintana*, *B. tribocorum*, and *B. alsatica* have been demonstrated by electron microscopy (Batterman et al., 1995; Heller et al., 1998; Heller et al., 1999; Maeno et al., 1999). These organisms may possess type IV pili owing to the presence of a number of properties typical for this type of pili, e.g., twitching motility and self-aggregation (Batterman et al., 1995).

Lipopolysaccharide -- unique structure with low endotoxicity

The remarkable interaction of *B. henselae* and *B. quintana* with endotoxin-sensitive endothelial cells, which eventually leads to vasoproliferation, and the apparent lack of septic shock as a result of bacteremia, suggest that the lipopolysaccharide (LPS) of these pathogens have reduced endotoxic activity (Matera et al., 2003). As measured by secretion of interleukin-8 in response to toll-like receptor 4 signaling, the endotoxic activity of highly purified LPS from *B. henselae* is 1,000 to 10,000 lower than that of enterobacterial LPS (Zähringer et al., 2004). The complete structural analysis of this *B. henselae* LPS revealed a short-chain structure with several unusual features, including a very short carbohydrate portion, an unusual core structure of lipid A, and penta-acetylation of lipid A. Some of the unusual structural features of *B. henselae* LPS, such as the long-chain fatty acid of lipid A, are shared by LPS of other intracellular bacteria that cause chronic infections (i.e., *Legionella* spp. and *Chlamydia* spp.). These features may

provide the structural basis for the low endotoxic potency of these LPS structures (Zähringer et al., 2004).

Invasion-associated locus (*ial*)

The genes of the invasion-associated locus (*ial*) identified in *B. bacilliformis* have been shown to confer an invasive phenotype to minimally invasive *Escherichia coli* strains (Mitchell and Minnick, 1995). The *ial* contains two genes, *ialA* and *ialB*. The *ial* locus is highly conserved in all bartonellae (Mitchell and Minnick, 1997). It encodes a nucleoside polyphosphate hydrolase of the *mutT* motif family (Cartwright et al., 1999; Conyers and Bessman, 1999). These enzymes are believed to eliminate toxic nucleotide derivatives from the cell and to regulate the levels of important signaling nucleotides and their metabolites (McLennan, 1999). The *ialB* gene encodes an 18-kDa protein that has been localized to the bacterial inner membrane (Coleman and Minnick, 2001). How *ialA* and/or *ialB* relate to the process of erythrocyte invasion is unknown, but these genes are thought to constitute a pathogenicity gene cluster. More importantly, several genes of this cluster have been sequenced in bartonellae of other species, and also in other facultatively intracellular bacteria, suggesting that some of the adhesion, invasion and persistence mechanisms used by bartonellae may be common to these species.

Bacteriophage

Phages may play an important role as vectors for the horizontal and vertical transfer of gene clusters or pathogenicity islands, which implicates a potential role for bacteriophages as mediators of the recombination events that take place in bartonellae.

Bacteriophage-like particles have been isolated from *B. henselae* (Anderson et al., 1994) and *B. bacilliformis* (Barbian and Minnick, 2000), and their presence has been inferred in *B. doshiae, B. grahamii*, and *B. quintana* by the detection of a 14-kbp extra chromosomal DNA element (Anderson et al., 1994; Anderson et al., 1997; Barbian and Minnick 2000; Zimmermann et al., 2003; Chenoweth et al., 2004). Recently, bacteriophage was also observed in *B. vinsonii* subsp. *berkhoffii* (Maggi and Breitschwerdt, 2005). Bacteriophages isolated from *B. henselae, B. quintana,* and *B. vinsonii* subsp. *berkhoffii* appear to share common structural and genetic features that are similar to those described for *B. bacilliformis* bacteriophages, such as size (40–50 nm in diameter), shape (icosahedral head) and packaging of the 14-kbp linear, double-stranded DNA molecule (Barbian and Minnick, 2000). In addition, several different phage-associated protein genes have been detected in the *B. henselae, B. quintana*, and *B. bacilliformis* genomes, including the *ialB* virulence gene in *B. bacilliformis* phage DNA (Barbian and Minnick, 2000). Only two bartonella phage-associated genes have been characterized in detail: *pap*31, which encodes a heme-binding protein in both *B. henselae* (Bowers et al., 1998; Zimmermann et al., 2003) and *B. quintana* (Carroll et al., 2000), and *papa*, which encodes a 36-kDa phage-capsid protein in *B. henselae* (Anderson et al., 1997).

Nevertheless, ultrastructural as well as preliminary genetic analyses indicate that bartonella bacteriophages differ in regard to selected characteristics. For example, host DNA packaging is near-random for *B. henselae* bacteriophages and loci specific for *B. bacilliformis* bacteriophages. Tail structures are 6–8 nm in length in *B. bacilliformis* bacteriophages, 60–80 nm in length in *B. vinsonii* subsp. *berkhoffii* bacteriophages, and appear to be absent in *B. henselae* and *B. quintana* bacteriophages.

Besides those described above, other factors, such as hemin-binding protein hbp/pap 31, hemolysin, inducible bartonella autotransporter, and outer membrane protein 43, are presumably involved in pathogenesis of bartonellae infections (Burgess et al., 2000; Carroll et al., 2000; Coleman and Minnick, 2001; Schülein et al., 2001; Seubert et al., 2002; Zimmermann et al., 2003). Quorum sensing in regulation of the intra-erythrocytic growth of bartonellae may also be possible.

KINETICS OF BACTEREMIA

The dynamics of erythrocyte exploitation by bartonellae has been monitored in several captive, naturally infected vertebrates and measured in experimentally infected vertebrates, including mice, rats, and cats (Abbott et al., 1997; Boulouis et al., 2001; Schülein et al., 2001), showing that bacteremia can be extremely chronic, lasting, for example, several weeks in rodents and several months in cats, although occasionally far more prolonged infections are encountered in naturally infected hosts. However, infections are not maintained at the same intensity throughout this period, but rather the concentration of bartonellae in the blood rises rapidly during the initial stages of the bacteremia and then gradually declines over the remainder of the infection period. For example, in the rat - *Bartonella tribocorum* model (Schülein et al., 2001), the onset of bacteremia occurs 5 – 6 days postinfection by a synchronous wave of bacterial adhesion and invasion into mature erythrocytes. The intracellular bacteria multiply until reaching a steady number, which is sustained for the remaining life of the infected erythrocytes (eventually exceeding several weeks). The initial wave of erythrocyte infection is followed by reinfection waves occurring at intervals of 3– 6 days. The phase of

intraerythrocytic bacteremia subsides spontaneously after a few months (typically 8–10 weeks; Kosoy et al., 1999). Prolonged periods of bacteremia also have been documented in naturally (sequentially sampled for at least 15 months) (Kordick et al., 1995) and experimentally (sampled for at least 454 days) (Kordick and Breitschwerdt, 1997; Kordick et al., 1999) infected specific-pathogen-free cats. Peak infection intensities appear to vary between hosts. However, given current difficulties in documenting low levels of bacteremia, the maximum period of persistence in animals of most species is undefined.

IMMUNE RESPONSE

Bartonella infections trigger an immune response which confers species-specific protective immunity, as challenging convalescent animals with the same bartonella strain does not result in reinfection (Regnery et al., 1996; Kosoy et al., 1999), whereas challenging with a different bartonella strain may cause bacteremia (Kosoy et al., 1999). A role for antigenic variation in the prolongation of bartonella infections has yet to be identified and, indeed, given that parasitism of erythrocytes affords bartonellae immunological privilege, the influence of host response on bartonella parasitism is unclear. Some data demonstrate an essential role for antibodies in abrogating the intraerythrocytic bacteremia of bartonellae. Using a mouse-*B. grahamii* model, Koesling et al. (2001) found that the bacteremia was transient and induced a strong humoral immune response in immunocompetent hosts. In contrast, bacteremia persists in immunocompromised B cell or T cell-deficient mice. Further, by immune serum transfer, the authors demonstrated that B cells or antibodies are required for immune control of

intraerythrocytic bartonella infection in mice. There appear to be multiple mechanisms by which antibody can influence the course of infections with intracellular pathogens (Casadevall, 1998). The plausible idea is that antibodies mediate killing of extracellular bartonellae or prevent their invasion into erythrocytes. It was shown that in rats, invasion of erythrocytes occurs in periodic waves every 3– 6 days, seeded from a yet uncharacterized primary niche (Schülein et al., 2001). If these reinfection waves are terminated by neutralizing antibodies, the intraerythrocytic bacteremia should remain until the infected, aging erythrocyte population is cleared.

Humoral response, however, varied by host species. Some reservoir hosts, such as cats, mount a significant humoral response, although bacteremia persists (Abbott et al., 1997). In other hosts, particularly in small mammals, despite the high prevalence of bacteremia, bartonella-specific antibodies are rarely detected, and the majority of those with antibodies had very low titers (Kosoy et al., 1997; Kosoy et al., 2004a). Thus the validity of extrapolation of humoral response in experimental infections to their naturally infected counterparts is debatable. Cellular immune responses have been considered to be a hallmark of immunity to intracellular bacterial pathogens (Schaible et al., 1999). For cellular immunity, the major histocompatibility complex (MHC) class I pathway plays an important role for antigen presentation to CD8+ T cells (Kaufmann, 1995; Pedersen et al., 1998). However, mammalian erythrocytes cannot present antigens to the immune system in an MHC-dependent way due to the lack of MHC on their surfaces. Intraerythrocytic bartonellae should thus be sequestered from a respective cellular immune response. Therefore, despite the presence of bartonella organisms inside the erythrocytes, the cellular immune response, especially the CD8+ pathway, may not be triggered, leading to

chronic bartonella infections (Dehio, 2001). However, recent studies have changed the view showing that *B. henselae*-infected BALB/c mice developed delayed-type hypersensitivity and secretion of interferon-γ (Karem et al., 1999); immunocompetent mice infected with a virulent *B. henselae* strain stimulated strong Th1 type immune responses (Arvand et al., 2001); and interleukin-4 (representative of activations of Th2) increased in cats infected with *B. henselae* (Kabeya et al., 2006). Although the mechanisms are unclear, it seems that cellular immunity plays a role in host defense against bartonella infections.

GENOME DIVERSITY AND LIFE-STYLE

The α-proteobacteria show extreme variation in genome size, accomplished by expansion and/or reduction (Casjens, 1998). The genome organization of bartonellae, studied for three species, revealed dramatic differences between *Bartonella* species. *B. quintana* (1.6 Mb) is a subset of *B. henselae* (1.9 Mb), which in turn is more or less a subset of *B. grahamii* (2.3 Mb). Alsmark and colleagues (2004) identified 301 genes unique to *B. henselae*, but only 26 unique to *B. quintana*. A majority, 62%, of the genes solely present in *B. henselae* are located on four main segments: a prophage region of 55 kb and three genomic islands (GIs) of 72, 34, and 9 kb. However, the *B. henselae* genome contains an unusually high fraction of repeated or partially repeated genes, pseudogenes and extensively degraded gene sequences. The *B. quintana* genome is derived from a larger *B. henselae*-like ancestral genome by reductive genome evolution, including loss of the prophage region and of the GIs in *B. henselae*. Preliminary studies of strain diversity in *B. henselae* reveal substantial variation in genome sizes and presence-absence

of GIs. However, none of the *B. henselae* strains studied so far are as highly reduced in genome size as is *B. quintana*, and some strains contain partial GIs, which supports the theory that the islands were present in a common ancestor of the two species and have been lost from *B. quintana* (Alsmark et al., 2004; Foucault et al., 2005; Lindroos et al., 2006).

The genomic heterogeneity within a bacterial species reflects lifestyle strategies and niche occupation in the natural growth environment. Evolutionary theory predicts that single-host pathogens (specialists) should be more successful than multihost pathogens (generalists), due to functional trade-offs that limit the fitness of generalists and because evolution is expected to proceed faster in narrower niches (Whitlock, 1996; Woolhouse et al., 2001). For vector-borne pathogens, the host-specificity and lifestyle of the vector is a major determinant of the potential for cross-species transmission. The initial status as a specialist or a generalist may reflect the host range size of the vector and the expression of virulence traits that enhance the survival potential in novel hosts. With time, however, narrow niches will make specialists less able to cope with environmental changes because of extensive gene loss and limited opportunities for novel gene acquisitions. Unless stabilized by host-selected functions, species specialists may in the long term be out-competed by invading generalists, with improved phenotypes acquired during selective sweeps in their broader host–vector systems. *B. quintana* infection in humans may reflect recent cross-species transmission events from other, related host – vector systems. The inactivation and loss of ancestral genes that are retained in their contemporary closest relatives with a different host range size, would explain the unique lifestyle and growth niche of *B. quintana*, a louse-borne human specialist. In contrast to the narrow host range

of the human body louse (Page et al., 1998), fleas can feed on a broader range of mammals (Rust and Dryden, 1997), and thus a larger genome is necessary for the generalist *B. henselae* to be able to accommodate change and to invade novel environmental niches.

DETECTION OF BARTONELLAE

Serologic testing

In humans, serologic testing (mainly indirect immunofluorescence assay [IFA]) is the most commonly used reference test for diagnosis of cat scratch disease (Fournier et al., 2002; Jacomo and Kelly, 2002). An IgG anti-*B. henselae* antibody titer ≥ 64 is considered positive when patients are tested at least two weeks after a suspected infection. Bartonella-associated endocarditis in humans and animals is usually associated with much higher IFA IgG antibody titers (> 800) (Fournier et al., 2002; Jacomo and Kelly, 2002). Zangwill et al. (1993) estimated the sensitivity and specificity of a *B. henselae*-based IFA to be 84% and 96%, respectively. In cats, serologic testing of single serum is of limited diagnostic value, as many cats, especially stray cats are likely to be seropositive against *B. henselae* (Chomel et al., 1995). Testing is indicated in kittens or recently adopted cats, because seronegative cats are more likely not to be bacteremic. Similarly, persons who may have immunocompromising conditions should require that an IFA test detecting antibodies against *B. henselae* be performed on cats prior to adoption. However, bacteremia in seronegative cats has been reported in a few instances and the antibodies usually cross-react with several bartonella antigens (Chomel et al., 2004). In dogs, as for humans, diagnosis of bartonella infection is largely based on the

presence of antibodies to specific antigens. Although serologic testing is widely used, identification of species-specific seroreactivity was not possible. Because of these limitations for serologic testing, bacterial isolation or PCR assay are necessary to identify the infecting species.

Bacterial isolation

A positive culture of blood or other tissue is the most reliable test for definitive diagnosis of active bartonella infection (Guptill, 2003). Usually, Bartonellae are cultured from blood using direct plating or lysis-centrifugation and freeze–thaw techniques followed by inoculation of a blood or chocolate agar plate (Regnery et al., 1992a; Welch et al., 1992). Anticoagulated blood is plated (usually after freezing to induce RBC lysis) onto fresh rabbit blood agar and incubated for at least four weeks at 35°C (except for *B. bacilliformis*, which grows best at 28 °C) with an atmosphere containing 5% carbon dioxide. Bartonellae grow best on blood (rabbit, sheep or horse)–enriched agar as they are highly hemin-dependent (Chomel and Kasten, 2004). Growth of organisms of any bartonella on blood agar typically is slow and requires an extended incubation period. It takes from 5 to 15 days, and up to 45 days on primary culture, to form visible colonies (Breitschwerdt and Kordick, 2000; Chomel, 2000). In subcultures, colonies usually appear after 3 to 5 days.

However, the traditional method of culturing bartonellae is rarely successful for humans with CSD or dogs with bartonella infections. It was reported that isolation of bartonellae from immunocompromised reservoir hosts, such as patients with bacillary angiomatosis, is much easier than is isolation of those organisms from an

immunocompetent individual, or from a non-reservoir host, perhaps due to an extremely low number of circulating organisms (Rolain et al., 2001; Rolain et al., 2002; Gouriet et al., 2005). Recently, cell culture systems have been developed for the cultivation of bartonellae. To date, alternative methods of isolation have proven to be of limited diagnostic utility. Recently, a novel liquid culture medium (*Bartonella*/alpha-*Proteobacteria* growth media, BAPGM) has been developed (Maggi et al., 2005a; Duncan et al., 2007). This is a chemically modified, insect growth based medium that is enriched with amino acids, yeast extract and other nutrients. The medium supported the growth of organisms of at least seven *Bartonella* species and co-cultures consisting of different *Bartonella* sp. bacteria, and facilitated isolation of *B. henselae* from bloods and other body fluids of naturally infected cats (Maggi et al., 2005a).

A combined approach using liquid pre-enrichment to increase bacterial numbers coupled with highly sensitive, molecular-based diagnostic detection has been used for detection of bartonellae. This method clearly facilitated the detection and subsequent isolation of single and co-infections with *B. henselae* and *B. vinsonii* subsp. *berkhoffii* in bloods of naturally infected dogs (Duncan et al., 2007). This combined approach can provide a substantial improvement in microbiological documentation of bartonella infection when compared to traditional inoculation of blood agar plates. The use of a combinational approach of pre-enrichment culture and polymerase chain reaction (PCR) likely will assist in the diagnostic confirmation of bartonellosis in animals and humans.

Molecular methods

Extraction of DNA from tissue samples and polymerase chain reaction assay have been more successful than has culturing for detection of bartonellae in humans and dogs (Regnery et al., 1992b; MacDonald et al., 2004). Frozen tissue samples or fresh biopsy specimens can be easily tested.

Molecular genetic methods, such as restriction fragment length polymorphisms (RFLP) of genes coding for the citrate synthase (*gltA*) and 16S rRNA and, more recently, analysis based on PCR of random, repetitive extragenic palindromic sequences have been used to identify and distinguish *Bartonella* strains and species. Using DNA fingerprinting, *B. henselae* isolates can be differentiated into two types (type I and type II), based upon differences in the 16S rRNA gene sequence (Bergmans et al., 1996), or four variants (variants I to IV) on the basis of several alternative DNA fingerprinting methods (Sander et al., 1997; Sander et al., 1998b). Although the same *B. henselae* subtype can be identified in CSD patients and in the bloods of their cats, definitive correlations between subtypes and specific disease manifestations have not yet been reported (Sander et al., 1998a).

Very recently, a new technique, DNA sequencing after PCR amplification both directly from specimens or pure cultures, has been widely adapted for detecting and characterizing bartonellae. In the case of uncultivated bacteria, sequencing approaches are all that is available for identifying the diverse species. This technique is extremely useful for *Bartonella* species identification, as bartonellae are such fastidious organisms. DNA sequence variation and sequence-derived phylogenies have allowed systematists to characterize the species diversity and may provide a universal criterion for classifying

these bacteria. The existence of multiple species within a group can now be identified by sequencing data. Stackebrandt et al. (2002) proposed to sequence at least five house-keeping genes for species differentiation. Besides 16S rRNA and *gltA*, the 16S-23S rRNA intergenic spacer region (ITS) (Roux and Raoult, 1995), the heat shock protein (*groEL*), the riboflavin synthase gene (*ribC*), the RNA polymerase beta subunit (*rpoB*), a cell division protein (*ftsZ*) and a 17 kDa antigen (La Scola et al., 2003) have been variously used for characterization of bartonellae.

OCCURRENCE OF BARTONELLAE IN ANIMALS

Bartonella infections have been encountered in vertebrates of virtually all species surveyed, which to date have extended to members of at least eight different orders of mammals, including Artiodactyla, Cetacea, Carnivora, Chiroptera, Insectivora, Lagomorpha, Primates, and Rodentia (Boulouis et al., 2005; Concannon et al., 2005; Maggi et al., 2005b). Results have demonstrated that the prevalence of bacteremia can range from 0 to almost 100% in vertebrate populations. Persistent infections in domestic and wild animals result in a substantial reservoir of bartonellae in nature and that these can serve as sources of human infections.

Rodents

Rats and mice of numerous species are known to have intraerythrocytic bartonella bacteremias. Many *Bartonella* spp. and subspecies have been identified in rodents, with some of them having been associated with human infections.

B. talpae was first observed in 1905 in the erythrocytes of moles (*Talpa europaea*) in the United Kingdom (described as *Grahamella talpae* at that time but later reclassified as a *Bartonella*) (Birtles et al., 1995). *B. vinsonii*, isolated from meadow voles (*Microtus pennsylvanicus*), was another early described species (designated as *Rochalimaea* at the time). Three new *Bartonella* species, *B. grahamii*, *B. taylori*, and *B. doshiae* were isolated from bloods of small woodland mammals, including wood mice (*Apodemus sylvaticus*), yellow-necked mice (*A. flavicollis*), bank voles (*Myodes glareolus*), field voles (*Microtus agrestis*) and water shrews (*Neomys fodiens*) in the United Kingdom (Birtles et al., 1995). *B. birtlesii* was isolated from *Apodemus* spp. in the United Kingdom and France. *B. elizabethae*, a human pathogen, was isolated from *Rattus norvegicus* (Ellis et al., 1999a). *B. tribocorum*, a species genetically close to but distinguishable from *B. elizabethae* also has been isolated from *Rattus* spp. rats living in a rural environment in France (Heller et al., 1998). *B. vinsonii* subsp. *arupensis*, another human pathogen, was isolated from white-footed mice (*Peromyscus leucopus*) (Wong et al., 1995; Hofmeister et al., 1998; Welch et al., 1999). *B. washoensis* was isolated from California ground squirrels (*Spermophilus beecheyi*) (Kosoy et al., 2003). Bartonellae, only a very small portion of strains isolated from rodents, have been described at the species level, and many of them remain uncharacterized or only partially characterized. For example, a previously unknown *Bartonella* sp. has been identified in the western United States in prairie dogs (*Cynomys ludovicianus*) and in the fleas that they carried (Stevenson et al., 2003).

Pathogenic effects are not commonly observed in rodents, although bacteremia can persist, and antibodies may be detected (Kosoy et al., 1999). In an experimental study, rodent reproductive disorders were observed (Boulouis et al., 2001).

Cats and wild felids

Four *Bartonella* spp., *henselae*, *clarridgeiae, koehlerae* and *weissii* have been isolated from the bloods of cats from the United States (Koehler et al., 1994; Demers et al., 1995; Keret et al., 1998), Japan (Maruyama et al., 1996), Australia (Flexman et al., 1995; Branely et al., 1996), New Zealand (Joseph et al., 1997), The Netherlands (Bergmans et al., 1997), France (Heller et al., 1997), Indonesia (Marston et al, 1999) and many other countries.

B. henselae commonly infects feral and domestic cats (*Felis catus*) (Childs et al., 1995; Jameson et al., 1995; Norman et al., 1995), with infection occurring in the erythrocytes of bacteremic cats. Seroepidemiologic studies have revealed worldwide distribution of *B. henselae* infection in domestic cats with different prevalence by regions. The seroprevalence of *B. henselae* in cats was 6.7% in Portugal, 11.9% in Egypt, 15.1% in Japan, 28.2% in the United States, and 32% in Jordan (Childs et al., 1995; Ueno et al., 1995; Al-Majali, 2004). Two main genotypes/serotypes of *B. henselae* have been identified in humans and cats on the basis of partial sequencing of the 16S rRNA gene and are presently designated as either genotype I and genotype II or genotype Houston I and genotype Marseille (Bergmans et al., 1996; Drancourt et al., 1996; La Scola et al., 2002). Major regional variations in the prevalence of *B. henselae* type I (Houston I) and type II (Marseille) have been reported in domestic cat populations. For example, in a limited study performed in the United States, the prevalence of *B. henselae* type I in 20 cats from northern California was only 18%, whereas this bacterium represented 21 (52%) of 40 isolates from cats in the southeastern United States. Similarly, in 271 young pet cats, prevalence of *B. henselae*-associated bacteremia ranged from 33% in Florida and 28% in

southern California to 12% in Washington, DC, and only 6% in Chicago (Guptill et al., 2004). In that study, most of the isolates (81%) in southern California were *B. henselae* type II, whereas both *B. henselae* types were uniformly distributed in Florida. In most areas of Europe, *B. henselae* type II is the dominant type (Chomel et al., 2002), whereas type I represents 70% to 80% of *B. henselae* isolates detected in eastern Asia (Chomel et al., 1999; Maruyama et al., 2000; Maruyama et al., 2001). Co-infection of cats with these two genotypes also has been reported (Bergmans et al., 1997; Gurfield et al., 1997). Prevalence of bacteremia in cats ranges from 15% to 55% in Australia, the Americas, Europe, Asia, and Africa. Although bacteremia in cats can last for several weeks to a few years (Kordick et al., 1995; Abbott et al., 1997), they are typically asymptomatic and appear to be healthy carriers of *B. henselae*, as limited pathology has been associated with natural infection (Koehler et al., 1994). The prevalence of bacteremia in young cats (< 1 year old) is usually higher than it is in adult cats (Chomel et al., 1995).

Due to the high prevalence of infection in cats, it has been difficult to attribute specific clinical signs to cats naturally infected with *B. henselae* (Chomel et al., 1995; Chomel, 2000). Naturally infected cats were more likely to have lymphadenitis and gingivitis (Ueno et al., 1996). Such an association also was demonstrated between *B. henselae* seropositivity and presence of stomatitis or urological disease (Glaus et al., 1997). *B. henselae* has been implicated as a cause of anterior uveitis (Lappin et al., 2000) and endocarditis in cats (Chomel et al., 2003c). Under experimental conditions, cats infected with *B. henselae* (mainly type II, feline isolates) have developed various clinical signs (Guptill et al., 1997; Kordick et al., 1999; O'Reilly et al., 1999). The most commonly reported sign is fever but local inflammation (erythema, edema) at the site of

inoculation, localized or generalized lymphadenopathy, lethargy and anorexia also have been observed. Some cats also developed mild neurological signs, such as nystagmus, tremors, focal motor seizures, and behavioral changes (Guptill, 2003). Reproductive disorders (stillbirths, pregnancy failure or pregnancy only after repeated breedings) were reported in experimentally infected female cats (Guptill et al., 1998). Variation in pathogenicity of strains has been suggested for differences in clinical signs observed under experimental conditions (O'Reilly et al., 1999).

B. clarridgeiae is another common *Bartonella* species found in cats. Feline infection with *B. clarridgeiae* has been reported in many parts of the world, including France, The Netherlands (Bergmans et al., 1997; Gurfield et al., 2001), Indonesia, Thailand, Japan, Philippine (Chomel et al., 1999; Marston et al., 1999; Maruyama et al., 2000; Maruyama et al., 2001), and the southeastern United States (Kordick et al., 1997). Coinfection with *B. clarridgeiae* and *B. henselae* has been reported in cats (Gurfield et al., 1997; Kordick et al., 1999; Gurfield et al., 2001; Maruyama et al., 2001). Another species, *B. koehlerae*, has been isolated from domestic cats in northern California, in cat fleas and from a pet cat in France (Droz et al., 1999; Breitschwerdt and Kordick, 2000; Rolain et al., 2003a; Rolain et al., 2003b), and from a bacteremic stray cat in Israel (Avidor et al., 2004). Finally, a fourth species, *B. weissii* (now combined with *B. bovis*) (Bermond et al., 2002), was isolated from each of four domestic cats from Utah and Illinois.

Besides domestic cats, bartonella infections have also been reported in wild felids: *B. henselae* has been isolated from a cheetah (*Acinonyx jubatus*) (Kelly et al., 1998); antibodies to *B. henselae* have been found in 53% of bobcats (*Felis rufus*), 35% of

mountain lions (*Felis concolor*) (Yamamoto et al., 1997), and 18% of free-ranging Florida panthers (*Puma concolor coryi*) (Rotstein et al., 2000). Feral cats are more likely to be infected than are domestic cats from the same region and this has been correlated with flea infestation (Chomel et el., 1995).

Dogs and wild canids

The first isolation of bartonella from a dog was from a case of endocarditis in 1993 (Breitschwerdt et al., 1995), an isolate later described as *B. vinsonii* subsp. *berkhoffii* (Kordick et al., 1996). Since then, this bacterium has been identified as an important cause of canine endocarditis (Breitschwerdt et al., 1999). However, *B. vinsonii* subsp. *berkhoffii* has also been isolated from clinically healthy dogs, with one having had a persistent infection for 16 months, suggesting that dogs may be long-term carriers of the bacterium (Kordick and Breitschwerdt, 1998). In addition to the United States, *B. vinsonii* subsp. *berkhoffii* has been found in Israel and in China (Baneth et al., 1998; Li et al., 2006). Antibodies against *B. vinsonii* subsp. *berkhoffii* were detected in dogs in North Africa (Henn et al., 2006), Israel (Baneth et al., 1998) and Thailand (Chomel et al., 2004). Overall prevalence of antibodies to this subspecies in pet dogs is quite low (<5%) (Pappalardo et al., 1997; Henn et al., 2005), but can be higher in specific populations (Baneth et al., 1998; Honadel et al., 2001). The recognized clinical spectrum of infection with *B. vinsonii* subsp. *berkhoffii* in dogs is expanding, as it now has been associated not only with endocarditis, but with cardiac arrhythmias, myocarditis, granulomatous lymphadenitis and granulomatous rhinitis, peliosis hepatitis, syncope, and sudden death (Breitschwerdt et al., 1999; Pappalardo et al., 2000). Based upon serological evidence,

infection with *B. vinsonii* subsp. *berkhoffii* may also cause immune-mediated hemolytic anemia, neutrophilic or granulomatous meningo-encephalitis, neutrophilic polyarthritis, cutaneous vasculitis, and uveitis in dogs (Breitschwerdt et al., 2004).

In addition to *B. vinsonii* subsp *berkhoffii*, several other *Bartonella* spp. have been identified in dogs (Kitchell et al., 2000; Mexas et al., 2002; Gillespie et al., 2003), including *B. clarridgeiae*, *B. washoensis*, *B. elizabethae*, *B. henselae*, and *B. quintana. B. clarridgeiae* and *B. washoensis* were isolated from bloods of dogs with endocarditis (Chomel et al., 2001; Chomel et al., 2003b). *B. clarridgeiae* DNA was detected by PCR assay in a dog with hepatic disease (Gillespie et al., 2003). The DNA of *B. elizabethae* has been detected in dogs with various clinical conditions (Mexas et al., 2002). *B. henselae* DNA was detected in dogs with peliosis hepatitis (Kitchell et al., 2000), granulomatous hepatopathy (Gillespie et al., 2003), and other clinical entities (Mexas et al., 2002), and the first isolation was made from a dog from Gabon in Central Africa (Gundi et al., 2004). Antibodies against *B. henselae* were reported in dogs from different areas with a variety of prevalences: 7.7% in Japan (Tsukahara et al., 1998); 14% in Zimbabwe (Kelly et al., 2004);10% in the eastern United States (Solano-Gallego et al., 2004); and a much higher prevalence (27%) in sick dogs from the eastern United States (Solano-Gallego et al., 2004). In addition, *B. quintana* was identified in the blood of a dog from the United States with aortic valve endocarditis and probably also from the mitral valve of a dog with endocarditis in New Zealand (Kelly et al., 2006).

Recent studies have demonstrated bartonella infections in wild canids. Coyotes (*Canis latrans*) appear to be a major wildlife reservoir of *B. vinsonii* subsp. *berkhoffii* in the western United States, with 76% of them seropositive and bacteremia being present in

28% of animals (Chang et al., 2000). Coyotes with antibodies against *B. vinsonii* subsp *berkhoffii* were more likely to have been living in coastal counties than inland counties (Chang et al., 1999).

Ruminants

Bartonellae have been isolated from several ruminant species (mule deer [*Odocoileus hemionus*], elk [*Cervus elaphus*], and dairy and beef cattle) in the United States, Europe and, very recently, in Africa. Breitschwerdt et al. (2001) found 16S rRNA gene of bartonellae similar or closely related to *B. weissii* (*B. bovis*) in North Carolina beef cattle. In the western United States, the prevalences of bartonella bacteremias have been determined to be 15% in elk, 90% in mule deer, 0% in 84 bighorn sheep (*Ovis canadensis*), and 49% in domestic cattle (*Bos taurus*) (Chang et al., 2001). The same group found that isolates from all cattle, deer, and elk are of the same species, indicating the potential for inter- and intraspecies transmission among ruminants. In Europe, several species have been isolated from wild roe deer (*Capreolus capreolus*), including *B. schoenbuchensis* in Germany (Dehio et al., 2001), *B. capreoli* and *B. bovis* in France. *B. schoenbuchensis* also was found in French cattle (Rolain et al., 2003c). *B. bovis* originated from a cow from a French herd (Bermond et al., 2002), and it was found that *B. bovis* caused endocarditis in cattle (Maillard et al., 2007). In Africa, *B. bovis* also was found in cattle (Raoult et al., 2005). Maillard et al. (2004) described *B. chomelii* isolated from French domestic cattle. Recently, bartonellae were isolated from sheep blood (Bemis and Kania, 2007).

Other vertebrates

Besides from animals mentioned above, bartonellae have been isolated from wild rabbits (*Oryctolagus cuniculus*) in eastern France (Heller et al., 1999), from gray foxes (*Urocyon cinereoargenteus*) and bobcats (*Lynx rufus*) in California (Riley et al., 2004), from porpoise (*Phocoena phocoena*) in North Carolina (Maggi et al., 2005b), from cynomolgus monkey (*Macaca fascicularis*) in Vietnam (O'Rourke et al., 2005), and recently bartonella DNA was detected from bats (*Microchiroptera*) in the UK (Concannon et al., 2005)

VECTOR TRANSMISSION

In general, knowledge related to transmission of bartonellae is incomplete. However, the chronic and long-lasting bacteremia (several weeks in rodents and several months in cats and dogs) clearly facilitates uptake of bartonellae by blood-feeding arthropods, which supports studies indicating that bartonellae are transmitted between mammalian hosts by hematophagous arthropods. Sandflies (Garcia-Caceres and Garcia, 1991), the human body louse (Roux and Raoult, 1999), the cat flea (Chomel et al., 1995; Chomel et al., 1996; Higgins et al., 1996), the vole ear mite (Baker, 1946), and ticks (Pappalardo et al., 1997; Welch et al., 1999), have been implicated in bartonella transmission. The vector varies with the organism involved.

Sandflies of the genus *Lutzomyia* have been demonstrated to transmit *B. bacilliformis* (Garcia-Caceresand Garcia, 1991). The human body louse (*Pediculus humanis corporis*) is a well-known vector of *B. quintana* (Byam et al., 1919; Drancourt et al., 1995; Spach et al., 1995; Roux and Raoult, 1999). Cat fleas (*Ctenocephalides felis*)

have been proposed as potential vectors transmitting *B. henselae*, *B. clarridgeiae* and *B. koehlerae* between cats. This was supported by the concordant distribution of feline infections with *B. henselae* and the flea (Jameson et al., 1995). In regions where fleas are endemic, bartonella bacteremias have been found in up to 50% of domestic and feral cats (Heller et al., 1997). In addition, warm and humid climates are strongly associated with the presence of antibodies against *B. henselae* and ectoparasite infestation of cats, further supporting arthropod vector involvement in bartonella transmission (Jameson et al., 1995). Identification of *B. clarridgeiae* and *B. koehlerae* DNA in cat fleas in France strongly supported flea-associated transmission of these bacteria (Rolain et al., 2003b). Fleas also have been implicated as potential vectors transmitting bartonellae among rodents. *Oropsylla* fleas (13.1%) collected from abandoned prairie dog burrows in Colorado were positive for bartonella (Stevenson et al., 2003). Another rodent flea *Ctenophthalmus nobilis* was shown to be a competent vector of at least two rodent-associated *Bartonella* species, *B. grahamii* and *B. taylorii* (Kerkhoff et al., 1999; Serratrice et al., 2003; Bown et al., 2004). *Ctenophthalmus lushuiensis* collected from the nests of voles were infected with the vole-associated bartonella and *B. clarridgeiae* in China (Li et al., 2007). *Xenopsylla cheopis* collected from rats were commonly infected (61%) with bartonellae, including *B. elizabethae*. *X. cheopis* may transmit bartonellae from rats to humans (Breitschwerdt and Kordick, 2000). As the vectors of many zoonotic pathogens, ticks also have been proposed to transmit bartonellae among animals, especially among ruminants, such as deer or cattle, as fleas infrequently parasitize these species. *Ixodes scapularis* ticks in the United States and *Ixodes ricinus* ticks in The Netherlands frequently appear to be infected with bartonellae (Hofmeister et al, 1998;

Schouls et al., 1999). Ticks have been implicated as potential vectors of *B. vinsonii* subsp. *berkhoffii* in the US (Pappalardo et al., 1997; Chang et al., 2001). A seroepidemiological study has suggested tick exposure as a potential risk factor for the presence of antibodies to *B. vinsonii* in dogs (Pappalardo et al., 1997). *B. henselae* DNA was detected in a biting fly, raising questions about the role of these insects as vectors or intermediate hosts of bartonellae (Chung et al., 2004).

Although arthropods appear to play an essential role in the natural cycle of all *Bartonella* species, serving as vectors and possibly as reservoir hosts, very little is known about the nature of arthropod exploitation or the underlying molecular mechanisms that may occur. An experimental model of *B. quintana* infection in its natural vector, the human body louse, has been described and has been used to demonstrate that bartonellae maintain a lifelong extracellular infection of the louse midgut (Fournier et al., 2001). There was no evidence that bartonellae invaded midgut epithelial cells or other parts of the louse body. Infection did not appear to have an effect on the lice, with the survival rate of infected lice being no different from that of uninfected lice. In another experiment, *B. henselae* was transmitted by transferring *Ctenocephalides felis* fleas from bacteremic cats to specific-pathogen-free cats (Chomel et al., 1996). In that study, it was not established whether the fleas served as mechanical or biologic vectors, or both; however, cat fleas can become infected and support the multiplication of *B. henselae* following ingestion of a blood meal from an infected cat (Higgins et al., 1996). Bartonellae are visible in dissected flea guts and can be cultured from their feces up to 9 days after fleas were fed infected blood (Higgins et al., 1996). More recently, cats have been experimentally infected with *B. henselae* by intradermal inoculation of feces from

infected fleas (Foil et al., 1998). In the same study, oral transmission, using fleas or flea feces, was not accomplished. Such results suggest that, just as are their mammalian hosts, bartonellae are able to establish prolonged infections in their arthropod vectors. Furthermore, given that infections appear to be limited to the midgut, transmission of bartonellae from arthropods to mammals is not a direct process, but rather is mediated by infected fecal material. Such a strategy seems somewhat inefficient; however, given that the arthropod vectors exploited by bartonellae share an obligate and intimate relationship with their hosts, the extremely frequent contacts between them provide ample opportunities for deposition of infected feces onto the host skin. Moreover, arthropod bites generally induce irritation of the skin, provoking the scratching that may result in the introduction of infected fecal material into the host body. Furthermore, bartonellae have not been detected in either eggs or larvae produced by infected lice, indicating that vertical transmission in them probably did not occur.

Besides arthropod transmission, there may be other transmission mechanisms that occur. It was found that embryos and neonates from naturally infected rodents were infected by bartonellae, suggesting that vertical transmission in hosts is possible (Kosoy et al., 1998). Most importantly, direct transmission between individuals in a vector-free environment has not been detected (Abbott et al., 1997; Guptill et al., 1998).

BARTONELLA AS HUMAN PATHOGENS

Bartonellae were virtually unrecognized as pathogens of humans until the 1990s. Identifications of bartonellae as agents of cat-scratch disease, bacillary angiomatosis, urban trench fever, and recent outbreaks of Carrión's disease have left little doubt about

the emerging medical importance of these bacteria. Bartonellae have attained their current level of notoriety following the identification of new species within the genus and the implication of these, and the previously established species, in a wide range of syndromes. Based on either isolation of the bacterium or PCR testing, at least 13 *Bartonella* species or subspecies have been recognized as emerging human pathogens or zoonotic agents. These include *B. bacilliformis*, *B. quintana*, *B. henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. vinsonii* subsp. *berkhoffii*, *B. elizabethae*, *B. grahamii*, *B. vinsonii* subsp. *arupensis*, *B. washoensis*, *B. alsatica*, *B. tamiae*, and very recently, *B. rochalimae*.

Bartonella bacilliformis and Carrión's disease

B. bacilliformis was the first member of the genus *Bartonella* to be formally described as a species. The bacterium is limited to the Andes mountain region of South America (Peru, Ecuador, and Colombia) and cause Carrión's disease among the local inhabitants (Bass et al., 1997). Carrión's disease was named after Daniel Alcides Carrión, a medical student who died from Oroya fever in 1885 after deliberate inoculation with blood from a verruga peruana patient. During the late 19th and early 20th centuries, Carrión's disease was a major impediment to European settlement of western South America. In fact, there is credible speculation that bartonellosis was well recognized among pre-Columbian Native Americans (Alexander, 1995). The disease is biphasic and the classic forms consist of Oroya fever and verruga peruana. Oroya fever is a life-threatening febrile anemia resulting from the massive invasion of erythrocytes by bartonella organisms. Patients surviving this acute intraerythrocytic phase may develop verruga peruana as the chronic secondary tissue phase: bacteria colonize the vascular

endothelium and cause vasoproliferative eruptions of the skin, which develop within 1 to 2 months and persist for months to years (Strong et al., 1915; Loutit, 1997).

To date, humans are the only known reservoir for *B. bacilliformis*. As for a vector of *B. bacilliformis*, it has been suspected that the limited geographic distribution of the sandfly *Lutzomyia verrucarum* was responsible for the apparent limited distribution of the human disease within a certain altitude stratum of the Andean foothills, referred to as the 'verruga zone'.

Recently, Carrión's disease appears to be more prevalent and more widespread across western South America than previously recognized, occurring outside the recognized verruga zone in areas where *L. verrucarum* is not the predominant sandfly (Ellis et al., 1999b). In addition, chronic, asymptomatic individuals have been identified in proximity to patients with active disease in regions where the disease is endemic (Ellis et al., 1999b; Chamberlin et al., 2002; Chomel et al., 2003a). Moreover, there is an absence of the verruga lesion-producing stage in recent human disease episodes outside the verruga zone. These observations raise intriguing questions. For example, can humans with asymptomatic bacteremia serve as adequate reservoirs for transmission to other humans in the absence of a nonhuman vertebrate reservoir? Do these new isolates represent possible emergent strains of *B. bacilliformis,* strains which may be able to exploit additional vector species and thus vastly increase the geographic distribution of human disease, perhaps one with an altered clinical presentation?

Bartonella quintana and trench fever

B. quintana was the agent of trench fever among troops during World War I and World War II. Transmitted through human body lice, trench fever is characterized by an intraerythrocytic bacteremia (Rolain et al., 2001) with recurrent, five-day cycling fever, headache, and leg pain (Bass et al., 1997). During the World War I era it was demonstrated that body lice could serve to transmit the organism of trench fever from persons infected many months previously. Like *B. bacilliformis*, humans serve as a reservoir for *B. quintana.* This apparent close association with long-term human transmissibility and the absence of any as yet recognized nonhuman vertebrate reservoir suggests that *B. quintana*, perhaps like *B. bacilliformis*, can exist without an intervening nonhuman vertebrate reservoir. Unlike sandflies that are distributed in limited regions, the body louse is a widely distributed arthropod and *B. quintana* is distributed worldwide. Under conditions in which personal hygiene is difficult to achieve, trench fever can attain epidemic proportions.

Although almost forgotten to medical science since the end of World War II, *B. quintana* reemerged as an important agent of disease toward the end of the twentieth century as an agent of disease among the homeless, drug addicts, and members of other disadvantaged social groups who are at least occasionally infested by body lice (Raoult et al., 1996; Spach and Koehler, 1998) and their illnesses have been referred to as "urban trench fever" (Spach and Koehler, 1998). Perhaps as expected, lousiness is a significant risk factor (Koehler et al., 1997). More recently, *B. quintana* also has been associated with endocarditis and recurrent protracted bacteremia and fever, primarily among those living under poor socioeconomic conditions, including the inner-city homeless of

industrialized countries and refugees in central Africa (Drancourt et al., 1995; Spach et al., 1995). *B. quintana* also has been shown to be a modern era agent of disease among the immune-impaired, in whom the disease can manifest as bacillary angiomatosis, a syndrome characterized by vasoproliferative lesions, first described in 1983 (Stoler et al., 1983; Relman et al., 1990; Koehler et al., 1992; Koehler et al., 1997) and which is an AIDS-related disease. Moreover, asymptomatic carriage of the bacterium by humans is becoming increasingly recognized (Raoult et al., 2001; Brouqui et al., 2005).

Bartonella henselae **and cat scratch disease (CSD)**

Although French physician Debré (1954) described CSD in patients suffering from suppurative lymphadenitis following cat scratches in France more than 50 years ago, it is only in the last decade that *B. henselae* was recognized as the etiological agent of this disease (Regnery et al., 1992c; Dolan et al., 1993; Zangwill et al., 1993). *B. henselae* was first isolated from bacteremic, immune-impaired persons, some of whom presented with recurrent fever (Regnery et al., 1992a; Welch et al., 1992). Based on the antigen derived from one of these isolates, serologic testing to determine the risk for *B. henselae* infections among immune-impaired persons led to the first experimental data linking *B. henselae* to CSD (Regnery et al., 1992c). *B. henselae* was first identified in 1990 by PCR amplification of the gene for bacterial 16S rRNA (Relman et al., 1990) and characterized as a new species in 1992 (Regnery et al., 1992a).

Unlike *B. bacilliformis* and *B. quintana*, each of which requires a human reservoir, *B. henselae* infections are zoonoses. Cats represent the main reservoir of this bacterium. *B. henselae* transmission from cats to humans occurs directly by scratch and possibly via

bite (Carithers, 1985) or by the bite of an infected flea or tick (Lucey et al., 1992; Zangwill et al., 1993; Rolain et al., 2003b). However, it is now suggested that flea bites are an unlikely mode of infection and that flea feces may be the only infecting material that could be inoculated by a cat scratch (Foil et al., 1998; Finkelstein et al., 2002).

CSD human cases have been reported from North America, Europe, and Australia, and from most countries where investigators have looked for such infections (Breitschwerdt and Kordick, 2000; Chomel et al., 2004). Several thousand cases of CSD may occur every year in many countries (Jackson et al., 1993; Bergmans, 1997). In the United States, an estimated 24,000 cases of CSD occur each year, with 10% of cases requiring hospitalization (Jackson et al., 1993). Seroprevalence varies among different groups, 28.9% in cat owners, 7.1% in veterinary professionals, and 5.9% in healthy people (Noah et al., 1997; Blanco et al., 1999). CSD occurs in all age groups but is more likely to occur in children and young adults (Carithers, 1985). A study from the UK reported an overall prevalence of 16.3% but 19.4% and 20.7% in 0-9 years and 10-19 years in patients whose specimens were submitted for estimation of bartonella antibodies (Harrison and Doshi, 1999). The same study also reported that cases were more frequent among males than females (18.5% vs. 13.9%).

B. henselae can cause a variety of clinical manifestations in humans. In immunocompetent hosts, the pathologic response is granulomatous, suppurative, extracellular and intracellular, and generally is self-limited (Bass et al., 1997). Classical CSD is mainly characterized by a benign regional lymphadenopathy (Carithers, 1985; Margileth, 1993; Chomel, 2000). *B. henselae* has been identified as a frequent cause of prolonged fever in children (Jacobs and Schutze, 1998; Tsukahara et al., 2000; Tsujino et

al., 2004). Low-grade fever, malaise and aching are often reported; and in some instances, headache, anorexia and splenomegaly occur. As is *B. quintana*, *B. henselae* is a major cause of endocarditis (Hadfield et al., 1993; Heller et al., 1998). It is estimated that bartonellae account for 3-4% of all human endocarditis cases (Chomel et al., 2003a; Lamas and Eykyn, 2003). Ocular infections, peliosis hepatitis (Perkocha et al., 1990) which is associated with proliferating endothelial cells (Manders, 1996), relapsing bacteremia with fever, and an increasingly recognized number of other manifestations (Chomel et al., 2004) have been reported as due to *B. henselae* infection. Like *B. quintana, B. henselae* is also another important cause of bacillary angiomatosis in immunocompromised individuals (Relman et al., 1991; Koehler et al., 1992). *B. henselae* also can cause a related vasoproliferative disease in inner organs. Along with the finding that a number of clinical syndromes in immunocompetent and immunocompromised patients are related to infection with *B. henselae*, and knowledge of its global distribution, this bacterium has become the most medically important member of the genus.

Newly recognized pathogens

Within the last 15 years, new bacteria of the genus of *Bartonella* were isolated from several mammalian reservoirs, including rodents, cats, dogs, and rabbits, and recognized as emerging zoonotic agents. Most of these zoonotic pathogens have a rodent reservoir. The epidemiological importance of rodent-borne bartonellae as causes of disease in animals and humans is emerging. It is likely that new rodent-borne bartonellae will be identified in the near future, some of which likely will be zoonotic.

B. elizabethae was first isolated from a patient with severe endocarditis in Saint Elizabeth's Hospital, Brighton, Massachusetts (Daly et al., 1993). Comparative serologic results indicated that *B. elizabethae* has also been a cause of Lever's neuroretinitis (O'Halloran et al., 1998). *B. elizabethae* infection seems to be prevalent (>33%) in inner-city residents with a history of intravenous drug abuse and in homeless people in various parts of the USA and Sweden (Comer et al., 1996; Comer et al., 2001; McGill et al., 2003), based on serological studies. A natural reservoir for *B. elizabethae* was not found until 1996 when Birtles and Raoult identified a strain of *Bartonella* obtained in Peru from an unspecified rat, presumably a species of *Rattus*, that had citrate synthase and 16SrRNA gene sequences that matched the sequences for the respective genes of *B. elizabethae* (Birtles and Raoult, 1996). Since then, this *Bartonella* species has been isolated from urban rats from various parts of the world, including the USA (Louisiana, Maryland), Portugal and Peru (Birtles and Raoult, 1996; Easterbrook et al., 2007). In Yunnan, southern China, *Rattus* spp. rats are infected with a large number of *Bartonella* strains that are genetically related to *B. elizabethae* (Ying et al., 2002). More recently, *B. elizabethae*-like bacteria also were found in rodents from Thailand (Castle et al., 2004). A hypothesis of Old World origin of these bacteria has been proposed based on the finding of *B. elizabethae*-like strains among *Rattus* species (Childs et al., 1999; Ellis et al., 1999a). The potential public health significance of these newly described organisms is undefined but of international interest because their rat reservoir has been introduced throughout the world.

B. grahamii has been associated with human ocular infection, such as neuroretinitis and retinal artery occlusion (Kerkhoff et al., 1999; Serratrice et al., 2003). The bacterium

has been isolated mainly from bank voles (*Myodes glareolus*) in the UK (Birtles et al., 1994) and Poland (Bajer et al., 2001), from yellow-necked mice (*Apodemus flavicollis*) in Sweden and Russia (Holmberg et al., 2003; Markov et al., 2006), from red-backed voles (*Myodes gapperi*) in Canada (Jardine et al., 2005), but also has been isolated from rats in the USA and from a house mouse (*Mus musculus*) captured in California (Ellis et al., 1999a). *B. vinsonii* subspecies *arupensis* was originally isolated from white-footed mice (*Peromyscus leucopus*) and later was found in many deer mice (*P. maniculatus*) from western US. It has been associated with a human case with fever, bacteremia and neurological symptoms (Welch et al., 1999). California ground squirrels (*Spermophilus beecheyi*) are the main reservoir of *B. washoensis* that was identified in a human case of myocarditis (Kosoy et al., 2003). Recently, *B. henselae* type Houston I was isolated from three long-tailed field mice (*Apodemus sylvaticus*) in Denmark (Engbaek and Lawson, 2004). This raises the question about the possibility of natural infection of wild rodents with *B. henselae*. Further studies will be required to determine whether rodents could be a natural reservoir of this bacterium, in addition to its feline reservoir. Finally, in the rural southwestern USA, serological investigation suggested rodent-associated bartonellae as etiologic agent of febrile illness, because in some patients, high antibody titers were found against a strain of *Bartonella* isolated from a white-throated wood rat (*Neotoma albigula*) (Iralu et al., 2006).

Besides rodent-associated bartonellae, other animal-associated bartonellae have been identified as human pathogens. *B. vinsonii* subspecies *berkhoffii*, a common dog pathogen, was found causally associated with human endocarditis (Roux et al., 2000). Because of the close association of dogs with humans, and the similar disease spectrum in

dogs, dogs may serve as good sentinels for human exposure to bartonellae; two cat-associated species, *B. clarridgeiae* and *B. koehlerae*, have been related to human diseases. Serological investigations indicated that *B. clarridgeiae* may be a minor causative agent of CSD, with detection of antibodies against *B. clarridgeiae* in a suspected case of CSD and in a patient with a chest-wall abscess (Kordick et al., 1997; Margileth and Baehren, 1998; Sander et al., 2000). Furthermore, anti-flagella specific antibodies against *B. clarridgeiae* were detected in 3.9% of 724 patients with lymphadenopathy (Sander et al., 2000). However, significant cross-reactivity between *B. henselae* and *B. clarridgeiae* was noted in a recent study from Japan (Tsuneoka et al., 2004). The first human case of endocarditis associated with *B. koehlerae* was reported from Israel (Avidor et al., 2004); *B. alsatica*, a rabbit-associated strain, was identified by serology and culturing and by PCR assay of an aortic valve specimen (Raoult et al., 2006).

Very recently, two novel *Bartonella* species, *B. tamiae*, isolated from febrile Thai patients (Kosoy et al., 2008), and *B. rochalimae*, isolated from an American patient who traveled in Peru and developed fever and splenomegaly after return (Eremeeva et al., 2007). However, the reservoir remains unknown as do the mode of transmission, pathogenesis, and many other characteristics of these organisms.

ECOLOGY OF BARTONELLAE IN RODENTS

World distribution and prevalence of bartonella infection in rodents

Bartonellae have been found to be widely distributed in rodents throughout the world, including all continents except Australia. In Europe, rodent-associated bartonellae were reported from the United Kingdom (Birtles et al.,1994; Bown et al., 2002;

Concannon et al., 2005), Finland (Laakkonen et al., 1998), France (Heller et al., 1998; Bermond et al., 2000; Gundi et al., 2004), Portugal (Ellis et al., 1999a), Poland (Bajer et al., 2001; Pawelczyk et al., 2004), Sweden (Holmberg et al., 2003), Greece (Tea et al., 2004), Ireland (Telfer et al., 2005), Denmark (Engbaek and Lawson, 2004), Russia (Markov et al., 2006) and Slovenia (Knap et al., 2007). In the Americas, bartonella infections in rodents have been reported from Canada (Jardine et al., 2005), USA (Kosoy et al., 1997; Hofmeister et al., 1998; Ellis et al., 1999a), and Peru (Birtles et al., 1999). In Asia, bartonellae were found in rodents in China (Ying et al., 2002), Thailand (Castle et al., 2004), Japan (Kabeya et al., unpub.), Korea (Kim et al., 2005), Indonesia (Winoto et al., 2005), Russia-Far East (Mediannikov et al., 2005), and Bangladesh (Bai et al., 2007b). In Africa, bartonellae in rodents were reported from Tunisia (Fichet-Calvet et al., 2000) and South Africa (Pretorius et al., 2004).

Rodents of many species have been found infected with bartonellae. The prevalence of bacterimia in rodents is generally high but varies by location and rodent species. Ellis et al. (1999a) analyzed whole blood specimens from 325 *R. norvegicus* rats and 92 *R. rattus* rats trapped in Portugal or at 13 different sites in the United States. Bartonellae were isolated from bloods of 63 *R. norvegicus* rats and 11 *R. rattus* rats. The overall prevalence of bacteremias in both species was 18%, with the prevalence in *R. norvegicus* rats being significantly higher in Los Angeles, California (45%); New Orleans, Louisiana (56%); and Portugal (100%). The prevalence in *R. rattus* rats did not differ significantly from the overall prevalence except in Riverside, California, where the prevalence was 60%. In the United Kingdom, it was found that 23 of 37 (62.2%) blood samples of small woodland mammals were bacteremic (Birtles et al., 1994). In the southeastern United

States, bartonellae were isolated from119 of 279 (42.6%) rodents (Kosoy et al., 1997). In Canada, 57% of rodents were infected with bartonellae, but ranged from 49% for Richardson's ground squirrel (*Spermophilus richardsonii*) to 90% for Franklin's ground squirrels (*S. franklinii*) (Jardine et al., 2005). In the study in southern China, the overall prevalence of bartonellae in rodents was 43.5%, with 19% in oriental voles (*Eothenomys miletus*), and 41.4% in *Rattus* spp. (Ying et al., 2002). In Finland, bartonellae were visualized in 10% of Cinereus shrew (*Sorex cinereus*), 20% of smoky shrew (*Sorex fumens*), and 14% of Northern short-tailed shrew (*Blarina brevicauda*) (Laakkonen et al., 1998). Reasons for differences of prevalence of bartonellae have been discussed in the literature. Parameters of rodent hosts, such as age and sex have been found to contribute to the prevalence of bartonellae in some rodent populations as well. In a study of bartonellae in cotton rats, prevalence of bartonella infection increased to > 90% among juvenile and sub-adult rats before declining to < 40% among the largest, oldest individuals (Kosoy et al., 2004a). The same author also reported that the level of bacteremia (measured by colony forming unit) is significantly higher in male than in female cotton rats. In Canada, Jardine et al. found that juvenile Richardson's ground squirrels were significantly more likely to be infected with bartonellae than were adults, and juvenile animals also were significantly more likely to have high levels of bacteremia compared to adult animals (Jardine et al., 2006b). In Tunisia, Fichet-Calvet et al. (2000) found that bartonella prevalence within a population of fat sand rats (*Psammomys obesus*) decreased as the rats aged. These patterns can be explained by bacterial clearance through acquired immunity (Kosoy et al., 2004a). They also found that transmission of

bartonellae occurred mostly during the summer and fall, suggesting seasonal dynamics of bartonellae in their natural hosts (Fichet-Calvet et al., 2000).

Host-specific relationships between bartonellae and rodents

Because bartonellae are blood-borne intracellular organisms, preferential infectivity in specific hosts appears to play a major role in determining which will become infected with a particular *Bartonella* species. Adaptation by bartonellae to exploit a diversity of mammalian hosts has generally resulted in some degree of host-specificity. The 30 or so well-characterized *Bartonella* species described to date are capable of parasitizing mammals of only a limited number of species (Boulouis et al., 2005) and all appear to be associated mainly with a single or principle mammalian host. There is, apparently, a species or subspecies-specific association between bartonellae and their hosts or vectors (Ellis et al., 1999a; Kosoy et al., 2000; Houpikian and Raoult, 2001).

Although the overall picture of bartonellae and their hosts suggests host-specificity at the taxon level, the situation of bartonellae with natural rodent hosts is controversial. Evidence varies among studies around the world. Bartonellae obtained from *Rattus* rats in southern China were specific for the host species (Ying et al., 2002). In Canada, bartonellae isolated from Richardson's ground squirrels and rodents of other species also showed very strong host-specificity association (Jardine et al., 2005). In the southeastern United States, bartonellae isolates from mice of three *Peromyscus* species were all in the same phylogenetic cluster (Kosoy et al., 1997). Furthermore, *B. vinsonii* seems to have no specific hosts, as several subspecies have been identified in very distant hosts, i.e., dogs are the host reservoir of *B. vinsonii* subsp. *berkohoffii* but *B. vinsonii* subsp. *arenpensis*

was isolated from white-footed mice, whereas *B. vinsonii* subsp. *visonii* was isolated from meadow voles. In Europe, there is no evidence to support host-specificity of bartonellae in rodents. In the United Kingdom, the study of bartonellae in small woodland mammals revealed three distinct *Bartonella* species, with one of them frequently infecting five local rodent species (*Apodemus sylvaticus, A. flavicollis, Myomys glareolus, Microtus agrestis* and *Neomys fodiens*) (Birtles et al., 1994). Holmberg and colleagues (2003) studied bartonella infection in sylvatic small mammls of central Sweden and found results similar to those obtained in the UK. A single species, *B. grahamii*, frequently infects voles (*M. glareolus*), Apodemus mice (*A. flavicollis, A. sylvaticus*) and house mice (*Mus musculus*), although *A. flavicollis* appeared to be the most common host.

Most assumptions about reservoir hosts are based on isolation rather than experimental infections, and therefore we cannot be sure that all relationships represent real reservoir-bacteria associations. As bartonellae have the potential to exploit alternative hosts, incidental and/or permanent cross-transmissions are possible, resulting in non-specific host-parasite relationships. For example, *B. elizabethae*, *B. washoensis* and *B. grahamii* generally parasitize rodents but also have been isolated from humans. Phylogenetic inferences of the evolutionary relationships among *Bartonella* species have indicated that organisms associated with a particular mammalian host are not specifically related. Furthermore, there is no apparent congruence between the phylogeny of bartonellae and that of the specific hosts they parasitize (Pretorius et al., 2004). These observations suggest that the adaptation of bartonellae to specific hosts was not an ancient occurrence, but rather members of different lineages adapted to their current hosts

more recently, a hypothesis that also may indicate that the basis for host-specificity (if it occurs) is not particularly complex.

Co-occurrence of *Bartonella* spp.

Within a rodent-bartonella community, a combination of bacteria that belong to different *Bartonella* species or strains can co-occur sympatrically (Kosoy et al., 1999; Kosoy et al., 2004b). Theoretically, several genetically different bartonellae populations can be associated with one rodent population. Some hosts are more likely to be infected by different *Bartonella* species. In the study of bartonellae in cotton rats in the southwestern US, coexistence of bartonella isolates that belong to three phylogenetic groups in the same population of cotton rats was detected (Kosoy et al., 1997). Birtles and colleagues (1994) found three *Bartonella* species in *Microtus agrestis* in their study, although there was no evidence of concurrent infections by more than one of these bartonellae. In the study by Holmberg et al., they also found that bartonellae of multiple species could infect hosts of the same species, i.e., *B. grahamii* and *B. birtlesii* were both isolated from *A. sylvaticus* and *B. grahamii, B. taylorii* and another novel genotype (most similar to *B. clarridgeae*) were isolated from *A. flavicollis* (Holmberg et al., 2003).

CONCLUSION

Available data on bartonella have expanded rapidly in recent years as this group of organisms has been found to be responsible for a growing spectrum of emerging and reemerging diseases. We now have new insights into the natural histories of a variety of *Bartonella* species and understand that these bacteria have adapted to their mammalian

reservoir hosts in unique ways. Sometimes they can cause chronic intraerythrocytic infections, with up to half of the reservoir host populations being bacteremic at any one time. Bartonellae bacteremias, however, cause few or no clinical signs in their specific reservoir hosts. Considering the extensive animal reservoirs and the large number of insects that have been implicated in transmission of *Bartonella* spp., both animal and human exposure to these organisms may be more common than is currently believed.

Recent advances in our understanding of parasitism by bartonellae have been greatly facilitated by the development of useful infection models, the introduction of effective molecular tools, and the availability of complete genome sequence data for *B. henselae* and *B. quintana* (Alsmark et al., 2004). Despite substantial and rapid progress during the past several years, *Bartonella* spp. still have many characteristics that can only be understood by investigators in the future.

CHAPTER TWO

OBJECTIVES OF THE STUDY

Interest in rodents as natural reservoir hosts of bartonellae has led to numerous studies. Results of investigations of the natural history of bartonellae challenge many concepts of bacterial ecology and evolution. Organisms of various *Bartonella* species have been isolated from rodent worldwide, with an extremely wide range of prevalence and a high level of genetic diversity in rodents of a wide variety of species (Birtles et al., 1994; Kosoy et al., 1997; Ying et al., 2002; Jardine et al., 2005). Although much has been accomplished, it is still poorly understood how dynamics and diversity of bartonellae populations are regulated under natural conditions. None of the studies reported were intended to address even a portion of this question by studying bartonellae in a variety of rodent communities.

I hypothesize that dynamics and diversity of rodent-borne bartonellae populations under natural conditions are regulated by the rodent community structure and the density of the host rodent population. To prove the hypothesis, the study focused on four specific aims: 1) To determine whether bartonella prevalence associates with community diversity; 2) To determine whether changes in bartonella prevalence parallel population cycles of their rodent hosts and to verify the effects of rodent host parameters on bartonella prevalence; 3) To determine whether bartonellae exhibit host-specificity; and 4) To determine whether spatially structured population dynamics of *Bartonella* spp. bacteria depends on landscape ecology.

CHAPTER THREE

ASSOCIATION OF BARTONELLA PREVALENCE WITH DIVERSITY OF RODENT COMMUNITIES

The importance of diversity within an ecological community in the performance of ecosystem functions has been widely discussed, and a potential connection between species diversity and disease transmission has been recognized. Medical entomologists suggested a connection between species diversity and transmission of vector-borne diseases in human a long time ago, when they applied the concepts to malaria and malarial transmission dynamics and argued that malaria transmission might be reduced if alternative hosts for mosquito vectors (e.g. livestock) were placed at areas around human habitation, an approach termed "zooprophylaxis" (Garrett-Jones, 1964). Later, a connection between diversity and disease was recognized by Elton (1958) who proposed that plant diseases could be ameliorated in 'complex' ecosystems if this complexity reduces density of the host plant of the disease; an insight that was subsequently supported both by empirical research on plant diseases (Burdon and Chilvers, 1982; Boudreau and Mundt, 1997) and by epidemiological models demonstrating the sensitivity of disease transmission to host density (Anderson and May, 1981).

This concept became more popular after its application to Lyme disease, the most common vector-borne disease in the United States (Lane et al., 1991; Barbour and Fish, 1993). Because the natural history of the pathogen (a spirochete, *Borrelia burgdorferi*), the primary tick vector (the black-legged tick, *Ixodes scapularis*), and its vertebrate host

(white-footed mouse, *Peromyscus leucopus*) are relatively well understood (Lane et al., 1991; Piesman and Gray, 1994; Ostfeld, 1997), Lyme disease has been used as the classical model to study the effect of biodiversity on disease risk. Within a community of high species diversity, the most common non-mouse hosts are relatively poor reservoirs for the Lyme spirochete, as they are fed on but rarely become infected via ticks. By diluting the effects of the white-footed mouse, high species diversity in the community of tick hosts reduces infection prevalence and reduces the prevalence of the disease (LoGiudice, 2003).

Several recent theoretical studies have attempted to delineate under what general conditions host diversity should increase or decrease disease prevalence (Dobson, 2004; Holt et al., 2003; Rudolf and Antonovics, 2005). Based on Lyme disease, Ostfeld and others (Ostfeld and Keesing, 2000a; Ostfeld and Keesing, 2000b; Schmidt and Ostfeld, 2001) have proposed a general theoretical model for vector-borne diseases, describing a mechanism by which increasing diversity of potential vertebrate host species would result in a lower prevalence of the infection in the vector and lower risk of the infection of humans, referred to as the 'dilution effect'. According to the dilution-effect model, adding species to the host community will either diminish the population density of the primary reservoir host, e.g., via predation or resources competition, or reduce the absolute vector burden on the reservoir host, e.g., by diverting vector meals from the reservoir host to incompetent reservoirs (Schmidt and Ostfeld, 2001). Moreover, the presence of alternative host species with low reservoir competence may reduce the effective reservoir competence of hosts of other species by reducing encounter rates between infected vectors and susceptible hosts (Schauber and Ostfeld, 2002). Together,

these would result in lower infection prevalence in the vector population, which will in turn decrease disease risk to human (Ostfeld and Keesing 2000b; Schmidt and Ostfeld, 2001).

Although the dilution-effect model was developed for Lyme disease, it has been applied to many other zoonoses, including both vector-borne and non-vector-borne diseases. During investigation of an outbreak of Hantavirus pulmonary syndrome, a significant difference in small mammal community structure was found between case sites and a negative control site (Ruedas et al., 2004). Similar effects also were observed in rodent-borne illness caused by hantaviruses and arenaviruses (Mills, 2006). Interestingly, in some disease systems, it was found that community diversity not only could decrease, but also, increase the disease risk. For example, the infection rate of mosquito-borne West Nile virus in wild birds, the primary reservoir hosts, was found to be significantly lower in avian communities consisting of higher diversities of non-passerine birds, whereas was not associated with passerine species richness (Ezenwa et al., 2006). Another example is that the transmission of Louping ill virus, a tick-borne virus, among grouses was diluted in a community containing a high population density of deer, but increased in a community with a high population density of hare. Ostfeld explained this as a result of "rescue effect" (Ostfeld and Keesing, 2000b). If added species function as alternative sources of infection, or if host communities contain many species of incompetent reservoirs that could increase the density of vectors by providing the vector population with more feeding opportunities than they would have in species-poor communities, and increased diversity could thereby increase transmission efficiency and disease risk (Dobson, 2004; Gilbert et al., 2001; Holt and Pickering, 1985; Norman et

al., 1999; Ostfeld and Keesing, 2000a; Saul, 2003; Schmidt and Ostfeld, 2001). In the system of West Nile virus, passerine birds are competent hosts for the virus, whereas non-passerines are poor hosts. The non-passerines dilute the infection by acting as alternative blood meal sources for mosquitoes but do not amplify the virus (Ezenwa et al., 2006). In the system of Louping ill virus, deer are hosts that maintain ticks but do not transmit the virus (diluting the infection and causing it to die out) whereas hares can amplify both the ticks and virus and allow the virus to persist (Gilbert et al., 2001). More likely, various mechanisms work in concert and, as a result, net effects of community diversity on disease risk become less predictable.

The genus *Bartonella* includes a variety of species that are widely distributed among many rodent species and many other mammalian hosts, including humans (Birtles et al., 1994; Chang et al., 2000; Jardine et al., 2005; Kosoy et al., 1997; Kosoy et al., 2003; Welch et al., 1999; Ying et al., 2002). Infection rate varies among different rodent species. In North America, studies showed that a specific bartonella commonly infects rodents of one species or their close relatives (Bai et al., 2007b; Jardine et al., 2006a; Kosoy et al., 1997) indicating a co-speciation of bartonella with their natural hosts. Although transmission mechanisms by which individual rodents acquire bartonella infections are not fully understood, arthropods, such as fleas, have been implicated as potential vectors (Breitschwerdt and Kordick, 2000; Li et al., 2007; Stevenson et al., 2003) and experimental studies have demonstrated that fleas can transmit bartonellae between rodents (Bown et al., 2004). Other routes, such as vertical transmission (Kosoy et al., 1998), are possible.

Bartonella infection has been increasingly associated with human illnesses. It has raised public health concern and drawn the attention of scientists studying these organisms. Rodents of some species have been found as reservoir hosts of some bartonellae that are human pathogens (Birtles et al., 1995; Ellis et al., 1999a; Iralu et al., 2006; Kosoy et al., 2003; Welch et al., 1999). Previous studies indicated that community structure could affect bartonella prevalence (Kosoy et al., 1997). Recently, Telfer and his coworkers reported that introduction of bank voles (*Myodes glareolus*) into woodland rodent community significantly reduced bartonella prevalence in the local rodent population (Telfer et al., 2005). Understanding the mechanisms of the impact of diversity on bartonella infections is critical for evaluating the generality of patterns, predicting net effects, and preventing occurrence of human disease.

Community diversity contains two components: species richness (the number of species) and evenness (equality in the abundances of each species). Diversity indices, taking both species richness and relative abundance of different species into account, can provide more information about community composition than can either one of these parameters, and are usually employed to measure community diversity (Magurran, 1988). Many different indices have been developed, such as the Shannon index, Simpson index, Brillouin index, and Berger-Parker index (Krebs, 1999). One index may work better than another in a particular study but the Shannon index is the one that has been the most widely used in ecological studies.

Conducted in a large number of rodent communities, the work reported here was designed to determine the effects of diversity of rodent community (measured by three components: richness, proportion, and Shannon index at both species and genus level) on

bartonella prevalence and to address the hypothesis that the presence of a diverse assemblage of rodents can dilute bartonella prevalence in a community. I measured prevalence of bartonella infections by percentage of culture-positive rodents in samples collected in 24 rodent communities. Bartonella prevalence was measured at different levels: overall bartonella prevalence in the entire rodent community; bartonella prevalence at the level of rodent host genus; and bartonella prevalence at the host species level. Diversity of the rodent community was estimated by three criteria: number of rodent genera or species in a community, Shannon index at genus or species level, and proportion of a rodent genus or a rodent species in a community. Analyzing the proportion, I concentrated on analysis of bartonella prevalence in two genera and one species: the genera *Peromyscus* and *Onychomys*, and the species *P. maniculatus*. Other rodent genera or species were not analyzed, as the sample sizes were not large enough. I tested the association of overall bartonella prevalence at each site with community genus/species richness, the association of overall bartonella prevalence at each site with the Shannon index at both genus and species level, and the association of bartonella prevalence in a rodent genus/species with the proportion of the genus/species in the community.

MATERIALS AND METHODS

Study sites

Twenty-four sites that represent diverse rodent communities within various habitats, including short grassland, bush shrubland, sagebrush shrubland, montane shrubland, conifer forest, and broadleaf woodland were selected for this study. Except for one site

(Janos) located in northern Mexico, the other 23 sites are located in nine of the western United States, including four sites in Arizona (Apache, Fort Huachuca, Phoenix, and Yuma), one in California (Orange), six in Colorado (Boulder, Comanche, Fort Collins, Fort Lewis, Loveland, and Red Feather Lakes), one in Kansas (Cimarron), two in Nevada (Clark and Vya), four in New Mexico (Placitas, Rio Rancho, Sevilleta, and Socorro), two in South Dakota (Badlands and Wind Cave), two in Utah (Mojave and Pinto), and one in Wyoming (Thunder Basin) (Table 3-1).

Trapping, animal processing and blood collecting were done by the author and/or by collaborators from the Centers for Disease Control and Prevention, Fort Collins, University of Colorado, Colorado State University, Kansas State University, University of New Mexico, and US Army during 1995-2006. All rodents were trapped using Sherman live traps (8 cm x 9 cm x 23 cm; H.B. Sherman Traps, Inc., Tallahassee, FL). Blood were collected from the retro-orbital plexus. Animals were then released at the capture site.

Bartonella culturing

Isolation of bartonellae followed methods published early (Kosoy et al., 1997), with some modifications. Frozen whole blood was thawed and diluted 1:4 in brain heart infusion (BHI) medium (BBL, Becton Dickinson Microbiology System, Sparks, MD), supplemented with 5% fungizone (Amphotericin B). Fungizone has no effect on bartonella growth but reduce the likelihood that fungal contaminants would overgrow the fastidious and slow-growing bartonella colonies.

Table 3-1 Twenty-four sites chosen for the study, of which 23 are located in western US, one in northern Mexico

Site	State	County	GPS coordinates	Habitat	Trapping date (month/year)
Apache	Arizona	Apache	35°25′56″N, 109°26′33″W	Sagebrush shrubland	5-6/2000
Badlands	South Dakota	Pennington	43°45′0″N, 102°30′0″W	Short grassland	5, 7/2002
Boulder	Colorado	Boulder	40°01′03″N, 105°16′47″W	Short grassland	6-8/2003
Cimarron	Kansas	Gray	37°7′27″N, 101°47′24″W	Short grassland	6-8/2003
Clark	Nevada	Clark	36°12′0″N, 1115°01′12″W	Bush shrubland	4/2006
Comanche	Colorado	Otero	37°20′12″N, 103°4′07″W	Short grassland	6-7/2003
Fort Collins	Colorado	Larimer	40°33′33″N, 105°4′41″W	Forest-plain interface	8-10/1999
Fort Huachuca	Arizona	Cochise	N/A	Bush shrubland	3-4/1997
Fort Lewis	Colorado	La Plata	37°13′31″N, 108°10′51″W	Montane shrubland	7/1999
Janos	Chihuahua, Mexico		28°16′12″N, 106°52′12″W	Short grassland	4, 5, 8, 9, 10/2001-2003
Loveland	Colorado	Larimer	40°24′17″N, 105°5′9″W	Conifer forest	6, 8/1999
Mojave	Utah	Washington	37°00′N, 113°15′W	Bush shrubland	6/2000
Orange	California	Orange	33°47′16" N, 117°51′00" W	Broadleaf woodland	4-9/2000
Phoenix	Arizona	Maricopa	33°26′54" N, 112°4′26" W	Bush shrubland	9/2001
Pinto	Utah	Washington	37°32′20″N, 113°32′16″W	Sagebrush shrubland	6/2000
Placitas	New Mexico	Sandoval	35°19′3″N, 106°27′7″W	Conifer forest	10/2000
Red Feather	Colorado	Larimer	40°48′28″N, 105°34′43″W	Conifer forest	5/1999
Rio Rahcho	New Mexico	Sandoval	35°17′10" N, 106°40′14" W	Sagebrush shrubland	2/2000
Sevilleta	New Mexico	Socorro	34°21′10" N, 106°52′55" W	Sagebrush shrubland	12/1995; 3, 4/1997
Socorro	New Mexico	Socorro	34°3′42″N, 106°53′58″W	Sagebrush shrubland	11/1997
Thunder Basin	Wyoming	Weston	43°41′48″N, 104°59′39″W	Short grassland	6, 8/2003
Vya	Nevada	Washoe	41°35′32″N, 119°51′38″W	Conifer forest	8/2000
Wind Cave	South Dakota	Custer	43°34′0″N, 103°29′0″W	Short grassland	5, 7/2003
Yuma	Arizona	Yuma	32°41′32″N, 114°36′55″W	Bush shrubland	2, 3/2001

Diluted blood (0.1 mL) was pipetted onto heart infusion agar plates containing 5% rabbit blood. Agar plates were incubated aerobically at 35°C in 5% carbon dioxide for as long as four weeks. Plates were monitored for bacterial growth at least once per week after initial plating. Bartonellae were tentatively identified based on bacterial colony morphology and microscopic examination of Gram-stained bacteria after subculturing to determine purity. Morphologically distinct colony types from each sample were identified individually. Single colonies were harvested from initial plates and from subsequent passages; subculturing was continued until a pure culture, free from contamination, was obtained. Colonies were harvested by adding 5 ml of BHI plus 10% glycerol to each plate, gently scraping the layer of bacteria from the surface of the agar plate, and storing the material in individual polypropylene vials at -20°C until further testing.

Verification of bartonellae by PCR

Bartonella isolates were verified by polymerase chain reaction (PCR) amplification of a region in the citrate synthase gene (*gltA*) that is specific for bartonella (Norman et al., 1995). Genomic DNA was extracted from bacterial cultures using a Qiagen DNA extraction kit (Qiagen, Chatsworth, CA). PCR amplifications were performed in a 50-µL reaction mixture containing 5µl 10X PCR buffer, 5 pmol of each primer, 200 µM each dNTP (Invitrogen, Cergy-Pontoise, France), 2.5 U Taq DNA polymerase (EuroblueTaq, Eurobio, Les Ulis, France), and 2.5 µL DNA. Two oligonucleotides, BhCS781.p (5'-GGGGACCAGCTCATGGTGG-3') and BhCS1137.n (5'-AATGCAAAAAGAACAGTAAACA-3'), were used as PCR primers to generate a 379-

bp amplicon of the *gltA* gene (Norman et al., 1995). Positive and negative controls were included in each PCR run to evaluate the presence of appropriately sized amplicons and contaminants, respectively. Each PCR was carried out in a PTC 200 Peltier thermal cycler (MJ Research, Inc., Waltham, MA) using the following program parameters: an initial 5-min denaturation at 95°C followed by 35 cycles of 1-min denaturation at 95°C, 1-min annealing at 56°C, and 1-min elongation at 72°C. Amplification was completed by holding the reaction mixture at 72°C for 10 min. PCR products were analyzed for the presence of amplicons of the correct size by electrophoresis in 1.5% agarose gel with ethidium bromide staining.

Shannon index (H) calculation

The Shannon index was measured at genus (H1) and species (H2) level, respectively, and was calculated from the proportional abundances (P*i*) of each genus or species in the entire community as

$H = -\sum Pi \ln Pi$ ($i = 1$ to s), where s is the total number of genus/species in the community, and P*i* is the proportion of s comprising the ith genus/species.

Statistical analysis

All statistical analyses were performed using specific programs within the SAS package (Statistical Analysis System, version 9.1, SAS Institute Inc., Cary, North Carolina). Statistical tests for significance of difference or correlation between compared groups were performed at the 0.05 probability level.

i. Goodness-of-Fit test

All datasets, including genus/species richness, Shannon index (H1 and H2), overall bartonella prevalence in each community, genus, and species, as well as proportion of rodents of a particular genus and species in a rodent community were first tested for the normal distribution using the Wilks-Shapiro test within goodness-of-fit test before further analyses.

ii. Chi-square analysis and Fisher's exact test

Bartonella prevalence was compared among geographic locations, genera, and species. Generally, chi-square analyses were done to determine whether prevalence differed significantly. Fisher's exact test was used if the sample size of any compared group was equal to or smaller than 60.

iii. Simple linear regression and correlation

The associations between bartonella prevalence and genus/species richness, Shannon's index, and proportion of a rodent genus or species in a rodent community were determined by performing simple regression and correlation analyses. The correlation coefficient (r) then was computed and the statistical significance was determined.

RESULTS

Small mammals

A total of 2,159 small mammals belonging to 54 species of 19 genera within 5 families of Rodentia were captured from the 24 study sites (Table 3-2). *Peromyscus* mice were the most common animals and accounted for 53.9% (n = 1163); *Onychomys* mice

Table 3-2 Rodent species tested for presence of bartonella and bartonella prevalence by species

Species	Common name	NC	PP (%)	NT	NP	PV (%)
Ammospermophilus leucurus	White-tailed antelope squirrel	5	0.2	5	2	40.0
Baiomys taylori	Northern pygmy mouse	13	0.6	13	3	23.1
Chaetodipus californicus	California pocket mouse	7	0.3	7	0	0.0
Chaetodipus fallax	San Diego pocket mouse	1	0.1	1	0	0.0
Chaetodipus hispidus	Hispid pocket mouse	61	2.8	58	1	1.7
Chaetodipus intermedius	Rock pocket mouse	46	2.1	42	0	0.0
Chaetodipus penicillatus	Desert pocket mouse	4	0.2	3	1	33.3
Chaetodipus sp.		5	0.2	1	0	0.0
Subtotal		*124*	*5.7*	*112*	*2*	*1.8*
Dipodomys merriami	Merriam's kangaroo rat	13	0.6	9	2	22.2
Dipodomys ordii	Ord's kangaroo rat	42	2.0	37	18	48.7
Dipodomys spectabilis	Banner-tailed kangaroo rat	14	0.7	4	0	0.0
Subtotal		*69*	*3.2*	*50*	*20*	*40.0*
Lemmiscus curtatus	Sagebrush vole	1	0.1	1	0	0.0
Microdipodops pallidus	Pale kangaroo mouse	1	0.1	0	0	
Microtus montanus	Montane vole	2	0.1	2	0	0.0
Microtus ochrogaster	Prairie vole	24	1.1	21	6	28.6
Microtus pennsylvanicus	Meadow vole	5	0.2	5	1	20.0
Microtus spp. (unidentified)		6	0.3	5	2	40.0
Subtotal		*37*	*1.7*	*33*	*9*	*27.3*
Mus musculus	House mouse	18	0.8	18	1	5.6
Myodes gapperi	Southern red-backed vole	2	0.1	2	1	50.0

Table 3-2 continued

Neotoma albigula	White-throated woodrat	78	3.6	49	18	36.7
Neotoma fuscipes	Dusky-footed woodrat	8	0.4	7	3	42.9
Neotoma lepida	Desert woodrat	51	2.4	36	15	41.7
Neotoma mexicana	Mexican woodrat	35	1.6	35	17	48.6
Neotoma micropus	Southern plains woodrat	3	0.1	2	1	50.0
Subtotal		*175*	*8.1*	*129*	*54*	*41.9*
Onychomys leucogaster	Northern grasshopper mouse	217	10.1	209	148	70.8
Onychomys torridus	Southern grasshopper mouse	22	1.0	19	11	57.9
Subtotal		*239*	*11.1*	*228*	*159*	*69.7*
Perognathus flavus	Silky pocket mouse	11	0.5	7	0	0.0
Perognathus formosus	Long-tailed pocket mouse	19	0.9	18	6	33.3
Subtotal		*30*	*1.4*	*25*	*6*	*24.0*
Peromyscus boylii	Brush mouse	77	3.6	58	34	58.6
Peromyscus californicus	California mouse	30	1.4	29	18	62.1
Peromyscus crinitus	Canyon mouse	1	0.1	1	0	0.0
Peromyscus difficilis	Rock mouse	3	0.1	3	2	66.7
Peromyscus eremicus	Cactus mouse	119	5.5	101	16	15.8
Peromyscus leucopus	White-footed mouse	58	2.7	56	13	23.2
Peromyscus maniculatus	Deer mouse	805	37.3	754	358	47.5
Peromyscus truei	Pinyon mouse	70	3.2	68	32	47.1
Subtotal		*1163*	*53.9*	*1070*	*473*	*44.2*
Rattus rattus	Black rat	5	0.2	5	0	0.0
Reithrodontomys fulvescens	Fulvous harvest mouse	17	0.8	17	0	0.0
Reithrodontomys megalotis	Western harvest mouse	62	2.9	58	3	5.2
Reithrodontomys montanus	Plains harvest mouse	5	0.2	4	0	0.0
Subtotal		*84*	*3.9*	*79*	*3*	*3.8*

Table 3-2 continued

Sigmodon arizonae	Arizona cotton rat	13	0.6	13	4	30.8
Sigmodon hispidus	Hispid cotton rat	4	0.2	3	0	0.0
Subtotal		*17*	*0.8*	*16*	*4*	*25.0*
Spermophilus beecheyi	California ground squirrel	20	0.9	17	2	11.8
Spermophilus lateralis	Golden-mantled ground squirrel	14	0.7	14	7	50.0
Spermophilus spilosoma	Spotted ground squirrel	3	0.1	3	0	0.0
Spermophilus tridecemlineatus	Thirteen-lined ground squirrel	65	3.0	60	25	41.7
Spermophilus variegatus	Rock squirrel	6	0.3	6	0	0.0
Subtotal		*108*	*5.0*	*100*	*34*	*34.0*
Tamias dorsalis	Cliff chipmunk	10	0.5	10	7	70.0
Tamias minimus	Least chipmunk	33	1.5	33	20	60.6
Tamias quadrivittatus	Colorado chipmunk	5	0.2	3	2	66.7
Tamias umbrinus	Uinta chipmunk	18	0.8	18	12	66.7
Subtotal		*66*	*3.1*	*64*	*41*	*64.1*
Thomomys bottae	Botta's pocket gopher	1	0.1	1	1	100.0
Thomomys talpoides	Northern rocket gopher	1	0.1	1	0	0.0
Subtotal		*2*	*0.1*	*2*	*1*	*50.0*
Total		2159	100.0	1952	813	41.6

NC=Number of rodents captured; PP=Proportion of the species in all captures; NT=Number of rodents tested; NP=Number of rodents positive for bartonella; PV=Prevalence of bartonella in the species.

Table 3-3 Community structure and bartonella prevalence by study sites

Site	Species	NC	PP (%)	NT	NP	PV (%)
	Neotoma albigula	2	6.5	2	1	50.0
	Peromyscus maniculatus	5	16.1	5	3	60.0
Apache	*Peromyscus truei*	20	64.5	20	13	65.0
	Tamias dorsalis	4	12.9	4	2	50.0
	Total	*31*	*100.0*	*31*	*19*	*61.3*
	Chaetodipus hispidus	2	1.5	2	0	0.0
	Microtus ochrogaster	9	6.5	9	5	55.6
	Onychomys leucogaster	22	15.9	18	4	22.2
Badlands	*Peromyscus maniculatis*	97	70.3	86	42	48.8
	Spermophilus tridecemlineatus	8	5.8	8	3	37.5
	Total	*138*	*100.0*	*123*	*54*	*43.9*
	Chaetodipus hispidus	36	21.3	36	1	2.8
	Microtus montanus	2	1.2	2	0	0.0
	Microtus ochrogaster	1	0.6	1	0	0.0
	Microtus pennsylvanicus	5	3.0	5	1	20.0
	Microtus spp. (unidentified)	4	2.4	4	2	50.0
Boulder	*Mus musculus*	2	1.2	2	0	0.0
	Neotoma mexicana	3	1.8	3	0	0.0
	Peromyscus maniculatus	98	58.0	98	56	57.1
	Reithrodontomys megalotis	18	10.7	18	1	5.6
	Total	*169*	*100.0*	*169*	*61*	*36.1*
	Chaetodipus hispidus	14	6.9	12	0	0.0
	Dipodomys ordii	21	10.4	18	8	44.4
	Microtus ochrogaster	1	0.5	0	0	
	Neotoma micropus	3	1.5	2	1	50.0
	Onychomys leucogaster	96	47.5	96	86	89.6
Cimarron	*Perognathus flavus*	2	1.0	2	0	0.0
	Peromyscus leucopus	7	3.5	7	4	57.1
	Peromyscus maniculatus	43	21.3	39	13	33.3
	Reithrodontomys montanus	4	2.0	3	0	0.0
	Spermophilus tridecemlineatus	11	5.5	11	5	45.5
	Total	*202*	*100.0*	*190*	*117*	*61.6*

Table 3-3 continued

	Ammospermophilus leucurus	1	0.9	1	0	0.0
	Chaetodipus penicillatus	1	0.9	1	1	100.0
	Dipodomys merriami	3	2.7	1	0	0.0
	Microdipodops pallidus	1	0.9	0	0	
	Neotoma albigula	9	8.1	9	0	0.0
	Neotoma lepida	4	3.6	4	0	0.0
Clark	*Perognathus formosus*	16	14.4	15	6	40.0
	Peromyscus eremicus	8	7.2	8	2	25.0
	Peromyscus maniculatis	32	28.8	32	8	25.0
	Peromyscus truei	25	22.5	25	10	40.0
	Reithrodontomys megalotis	11	9.9	9	2	22.2
	Total	*111*	*100.0*	*105*	*29*	*27.6*
	Chaetodipus hispidus	8	7.1	7	0	0.0
	Dipodomys ordii	3	2.7	2	1	50.0
	Microtus ochrogaster	1	0.9	1	0	0.0
	Mus musculus	1	0.9	1	0	0.0
	Onychomys leucogaster	46	41.1	44	36	81.8
Comanche	*Peromyscus maniculatus*	31	27.7	30	0	0.0
	Reithrodontomys megalotis	1	0.9	1	0	0.0
	Reithrodontomys montanus	1	0.9	1	0	0.0
	Spermophilus tridecemlineatus	20	17.9	15	3	20.0
	Total	*112*	*100.0*	*102*	*40*	*39.2*
	Microtus ochrogaster	9	16.4	9	0	0.0
	Mus musculus	8	14.6	8	1	12.5
	Neotoma mexicana	17	30.9	17	11	64.7
Fort Collins	*Peromyscus difficilis*	2	3.6	2	2	100.0
	Peromyscus maniculatus	19	34.6	19	13	68.4
	Total	*55*	*100.0*	*55*	*27*	*49.1*
	Baiomys taylori	13	10.2	13	3	23.1
	Chaetodipus sp.	5	3.9	1	0	0.0
	Mus musculus	3	2.4	3	0	0.0
	Neotoma albigula	7	5.5	0	0	
	Onychomys torridus	1	0.8	1	1	100.0
	Perognathus flavus	2	1.6	1	0	0.0
	Peromyscus boylii	19	15.0	10	7	70.0
Fort Huachuca	*Peromyscus eremicus*	4	3.2	4	0	0.0
	Peromyscus leucopus	16	12.6	14	2	14.3
	Peromyscus maniculatus	27	21.3	26	5	19.2
	Reithrodontomys fulvescens	17	13.4	17	0	0.0
	Reithrodontomys megalotis	12	9.5	11	0	0.0
	Sigmodon arizonae	1	0.8	1	1	100.0
	Total	*127*	*100.0*	*102*	*19*	*18.6*

Table 3-3 continued

	Peromyscus maniculatus	28	54.9	28	26	92.9
Fort Lewis	*Tamias minimus*	23	45.1	23	16	69.6
	Total	*51*	*100.0*	*51*	*42*	*82.4*
	Chaetodipus penicilatus	1	1.0	1	0	0.0
	Dipodomys merriami	7	7.1	5	0	0.0
	Dipodomys ordii	1	1.0	1	0	0.0
	Dipodomys spectabilis	14	14.1	4	0	0.0
	Mus musculus	1	1.0	1	0	0.0
	Neotoma albigula	14	14.1	12	1	8.3
Janos	*Onychomys leucogaster*	5	5.1	5	4	80.0
	Onychomys torridus	21	21.2	18	10	55.6
	Perognathus flavus	6	6.1	3	0	0.0
	Peromyscus leucopus	6	6.1	6	1	16.7
	Peromyscus maniculatus	20	20.2	19	4	21.1
	Spermophilus spilosoma	3	3.0	3	0	0.0
	Total	*99*	*100.0*	*78*	*20*	*25.6*
	Neotoma mexicana	15	48.4	15	6	40.0
	Peromyscus maniculatus	12	38.7	12	4	33.3
Loveland	*Tamias umbrinus*	4	12.9	4	2	50.0
	Total	*31*	*100.0*	*31*	*12*	*38.7*
	Ammospermophilus leucurus	2	6.9	2	1	50.0
	Dipodomys merriami	1	3.5	1	1	100.0
	Neotoma albigula	1	3.5	1	0	0.0
	Neotoma lepida	6	20.7	6	5	83.3
	Perognathus formosus	3	10.3	3	0	0.0
Mojave	*Peromyscus boylii*	9	31.0	9	6	66.7
	Peromyscus crinitus	1	3.5	1	0	0.0
	Peromyscus eremicus	2	6.9	2	0	0.0
	Peromyscus truei	2	6.9	2	2	100.0
	Tamias dorsalis	2	6.9	2	2	100.0
	Total	*29*	*100.0*	*29*	*17*	*58.6*
	Chaetodipus californicus	7	4.0	7	0	0.0
	Chaetodipus fallax	1	0.6	1	0	0.0
	Neotoma fuscipes	8	4.6	7	3	42.9
	Neotoma lepida	41	23.3	26	10	38.5
	Peromyscus boylii	14	8.0	14	8	57.1
	Peromyscus californicus	30	17.1	29	18	62.1
Orange	*Peromyscus eremicus*	31	17.6	21	8	38.1
	Peromyscus maniculatus	11	6.3	9	5	55.6
	Rattus rattus	5	2.8	5	0	0.0
	Reithrodontomys megalotis	8	4.6	8	0	0.0
	Spermophilus beecheyi	20	11.4	17	2	11.8
	Total	*176*	*100.0*	*144*	*54*	*37.5*

Table 3-3 continued

	Chaetodipus intermedius	44	51.2	40	0	0.0
	Neotoma albigula	9	10.5	0	0	
	Peromyscus eremicus	18	20.9	17	0	0.0
Phoenix	*Reithrodontomys megalotis*	1	1.2	0	0	
	Sigmodon arizonae	12	14.0	12	3	25.0
	Spermophilus variegatus	2	2.3	2	0	0.0
	Total	*86*	*100.0*	*71*	*3*	*4.2*
	Peromyscus maniculatus	45	76.3	45	25	55.6
	Peromyscus truei	5	8.5	5	2	40.0
	Spermophilus variegatus	4	6.8	4	0	0.0
Pinto	*Tamias dorsalis*	4	6.8	4	3	75.0
	Thomomys bottae	1	1.7	1	1	100.0
	Total	*59*	*100.0*	*59*	*31*	*52.5*
	Peromyscus boylii	33	67.4	23	12	52.2
	Peromyscus truei	11	22.5	9	4	44.4
Placitas	*Tamias quadrivittatus*	5	10.2	3	2	66.7
	Total	*49*	*100.0*	*35*	*18*	*51.4*
	Myodes gapperi	2	3.6	2	1	50.0
	Peromyscus difficilis	1	1.8	1	0	0.0
	Peromyscus maniculatus	24	42.9	24	8	33.3
Red Feather	*Spermophilus lateralis*	14	25.0	14	7	50.0
	Tamias minimus	1	1.8	1	0	0.0
	Tamias umbrinus	14	25.0	14	10	71.4
	Total	*56*	*100.0*	*56*	*26*	*46.4*
	Ammospermophilus leucurus	2	4.8	2	1	50.0
	Dipodomys ordii	10	23.8	10	5	50.0
	Onychomys leucogaster	4	9.5	4	3	75.0
	Peromyscus leucopus	17	40.5	17	3	17.7
Rio Rancho	*Peromyscus maniculatus*	4	9.5	4	2	50.0
	Peromyscus truei	2	4.8	2	1	50.0
	Reithrodontomys megalotis	3	7.1	3	0	0.0
	Total	*42*	*100.0*	*42*	*15*	*35.7*
	Neotoma albigula	24	53.3	24	16	66.7
	Onychomys leucogaster	4	8.9	4	1	25.0
	Peromyscus eremicus	4	8.9	4	0	0.0
	Peromyscus leucopus	2	4.4	2	2	100.0
Sevilleta	*Peromyscus maniculatus*	4	8.9	4	2	50.0
	Peromyscus truei	5	11.1	5	0	0.0
	Reithrodontomys megalotis	2	4.4	2	0	0.0
	Total	*45*	*100.0*	*45*	*21*	*46.7*

Table 3-3 continued

	Chaetodipus intermedius	2	3.6	2	0	0.0
	Dipodomys merriami	1	1.8	1	1	100.0
	Dipodomys ordii	1	1.8	1	0	0.0
	Neotoma albigula	12	21.4	1	0	0.0
	Onychomys leucogaster	1	1.8	1	1	100.0
	Perognathus flavus	1	1.8	1	0	0.0
Socorro	*Peromyscus boylii*	2	3.6	2	1	50.0
	Peromyscus eremicus	1	1.8	1	0	0.0
	Peromyscus leucopus	10	17.9	10	1	10.0
	Peromyscus maniculatus	17	30.4	16	1	6.3
	Reithrodontomys megalotis	6	10.7	6	0	0.0
	Sigmodon hispidus	2	3.6	2	0	0.0
	Total	*56*	*100.0*	*44*	*5*	*11.4*
	Dipodomys ordii	6	2.5	5	4	80.0
	Lemmiscus curtatus	1	0.4	1	0	0.0
	Microtus spp. (unidentified)	2	0.9	1	0	0.0
	Onychomys leucogaster	39	16.5	37	13	35.1
Thunder Basin	*Peromyscus maniculatus*	164	69.5	142	83	58.5
	Spermophilus tridecemlineatus	23	9.8	23	14	60.9
	Thomomys talpoides	1	0.4	1	0	0.0
	Total	*236*	*100.0*	*210*	*114*	*54.3*
	Peromyscus maniculatus	26	74.3	26	16	61.5
Vya	*Tamias minimus*	9	25.7	9	4	44.4
	Total	*35*	*100.0*	*35*	*20*	*57.1*
	Chaetodipus hispidus	1	1.0	1	0	0.0
	Microtus ochrogaster	3	2.9	1	1	100.0
Wind Cave	*Peromyscus maniculatis*	96	93.2	88	42	47.7
	Spermophilus tridecemlineatus	3	2.9	3	0	0.0
	Total	*103*	*100.0*	*93*	*43*	*46.2*
	Chaetodipus penicillatus	2	3.3	1	0	0.0
	Dipodomys merriami	1	1.6	1	0	0.0
	Mus musculus	3	4.9	3	0	0.0
Yuma	*Peromyscus eremicus*	51	83.6	44	6	13.6
	Peromyscus maniculatus	2	3.3	2	0	0.0
	Sigmodon hispidus	2	3.3	1	0	0.0
	Total	*61*	*100.0*	*52*	*6*	*11.5*
Grand total		*2159*		*1952*	*813*	*41.7*

NC=Number of animals captured; PP=Proportion of the species in the community; NT=number of animals tested; NP=Number of animals positive for bartonella; PV=Prevalence of bartonella of the species in the community.

accounted for 11.1% (n = 239); *Neotoma*, *Chaetodipus*, and *Spermophilus* accounted for 8.1% (n = 175), 5.7% (n = 123), and 5.0% (n = 108), respectively. Other 14 mammalian genera accounted for a very small portion ranging from <0.1% to 3.9%. At species level, deer mice (*P. maniculatus*) were the most common species, accounting for 37.23% of the total captures; the northern grasshopper mice (*Onychomys leucogaster*) comprised the second largest population, accounting for 10.1%. Other 52 species accounted together for 52.6% of the population and ranged from 0.04% to 5.5%.

Community diversity

i. Species/genus richness

Community diversity varied by location. Among sites, the genus richness (number of rodent genera) ranged from two (Fort Lewis, Placitas, Vya) to nine (Fort Huachuca, Cimarron) and species richness (number of rodent species) ranged from two (Fort Lewis, Vya) to13 (Fort Huachuca) (Table 3-4). The distribution of both genus richness and species richness was normal (Wilks-Shapiro test, W=0.95 and 0.95, p=0.21 and 0.23).

ii. Shannon index

The Shannon index ranged from 0.32 to 1.68 at the genus level (H1) and from 0.32 to 2.23 at the species level (H2) among study sites (Table 3-4). The community at Wind Cave had the lowest diversity at both genus and species levels (H1=H2=0.32), with four genera and four species captured at the site; the community at Janos had the highest diversity at the genus level (H1=1.68), with rodents of eight genera captured at the site; and the community at Fort Huachuca had the highest diversity at the species level (H2=2.23), with rodents of 13 species captured at the site. The Shannon indices of

Table 3-4 Richness, Shannon index, and bartonella prevalence within study sites

Site	NC	NG	NS	H1	H2	NT	NP	PR (%)
Apache	31	3	4	0.61	1.02	31	19	61.3
Badlands	138	5	5	0.95	0.95	123	54	43.9
Boulder	169	6	9	1.2	1.28	169	61	36.1
Cimarron	202	9	10	1.49	1.59	190	117	61.6
Clark	111	7	11	1.26	1.94	105	29	27.6
Comanche	112	8	9	1.41	1.48	102	40	39.2
Fort Collins	55	4	5	1.38	1.43	55	27	49.1
Fort Huachuca	127	9	13	1.43	2.23	102	19	18.6
Fort Lewis	51	2	2	0.69	0.69	51	42	82.4
Janos	99	8	12	1.68	2.12	78	20	25.6
Loveland	31	3	3	0.98	0.98	31	12	38.7
Mojave	29	6	10	1.41	2.01	29	17	58.6
Orange	176	6	11	1.34	2.11	144	54	37.5
Phoenix	86	6	6	1.32	1.32	71	3	4.2
Pinto	59	4	5	0.58	0.85	59	31	52.5
Placitas	49	2	3	0.83	0.83	35	18	51.4
Red Feather	56	4	6	1.18	1.32	56	26	46.4
Rio Rancho	42	5	7	1.23	1.63	42	15	35.7
Sevilleta	45	4	7	1.05	1.5	45	21	46.7
Socorro	56	8	12	1.4	1.96	44	5	11.4
Thunder Basin	236	7	7	0.96	0.96	210	114	54.3
Vya	35	2	2	0.57	0.57	35	20	57.1
Wind Cave	103	4	4	0.32	0.32	93	43	46.2
Yuma	61	5	6	0.56	0.7	52	6	11.5

NC=Number of rodents captured; NG=Number of rodent genus in a community; NS=Number of rodent species in a community; NT=Number of rodents tested for bartonella; NP=Number of rodents positive for bartonella; H1=Shannon index at genus level; H2=Shannon index at species level; PR=bartonella prevalence in the entire community.

communities from other sites varied between the two indices at either genus or species level. For example, H1=H2=1.32 with six genera or six species was in Phoenix and H1=0.56, H2=0.7 with five genera or six species was in Yuma. The Shannon indices at both genus and species levels were normally distributed (W=0.94 and 0.96, p=0.14 and 0.42).

Proportion of *Peromyscus* mice, *Onychomys* mice and others in the communities

Peromyscus mice were present at all 24 sites with their proportions ranging from 20.9 to 93.2% per community. The number of species of this genus varied within a site from one to four (Table 3-5). The proportion of *Peromyscus* mice at each site was normally distributed (W=0.96, p=0.41). Deer mice were absent in Phoenix, Placitas, and Mojave, and distributed at the other 21 sites with as 3.3% to 93.2% of the population and was the dominant species at 12 of the study sites. The proportion of the deer mice at each site also fits the normal distribution (W=0.93, p=0.16). The pinyon mouse (*P. truei*), found at seven sites, was the dominant species at Apache (64.5%). The brush mouse (*P. boylii*) was found at five sites and was the dominant species in Mojave (31.0%) and Placitas (67.4%). Cactus mice (*P. eremicus*) and white-footed mice (*P. leucopus*) were found at seven and six sites, respectively, and were the respective dominant species in Yuma (83.6%) and Rio Rancho (40.5%). Mice of another three species *Peromyscus*, the California mouse (*P. californicus*), the canyon mouse (*P. crinitus*) and the rock mouse (*P. difficilis*), were found only at one or two sites (Table 3-5).

Table 3-5 Distribution and proportion of *Peromyscus* mice in rodent community and bartonella prevalence in *Peromyscus* mice and deer mice

Site	PEBO	PECA	PECR	PEDI	PEER	PELE	PEMA	PETR	NS	TPP	PVPE	PVPM
Apache							16.1	64.5	2	80.7	64.0	60.0
Badlands							70.3		1	70.3	48.8	48.8
Boulder							58.0		1	58.0	57.1	57.1
Cimarron						3.5	21.3		2	24.8	37.0	33.3
Clark					7.2		28.8	22.5	3	58.6	30.8	25.0
Comanche							27.7		1	27.7	0.0	0.0
Fort Collins				3.6			34.6		2	38.2	71.4	68.4
Fort Huachuca	15.0				3.2	12.6	21.3		4	52.0	25.9	19.2
Fort Lewis							54.9		1	54.9	92.9	92.9
Janos						6.1	20.2		2	26.3	20.0	21.1
Loveland							38.7		1	38.7	33.3	33.3
Mojave	31.0		3.5		6.9			6.9	4	48.3	57.1	
Orange	8.0	17.1			17.6		6.3		4	48.9	53.4	55.6
Phoenix					20.9				1	20.9	0.0	
Pinto							76.3	8.5	2	84.8	54.0	55.6
Placitas	67.4							22.5	2	89.9	50.0	
Red Feather				1.8			42.9		2	44.7	33.3	33.3
Rio Rancho						40.5	9.5	4.8	3	54.8	26.1	50.0
Sevilleta					8.9	4.4	8.9	11.1	4	33.3	26.7	50.0
Socorro	3.6				1.8	17.9	30.4		4	53.6	10.3	6.3
Thunder Basin							69.5		1	69.5	58.5	58.5
Vya							74.3		1	74.3	61.5	61.5
Wind Cave							93.2		1	93.2	47.7	47.7
Yuma					83.6		3.3		2	86.9	13.0	0.0

PEBO=*Peromyscus boylii*; PECA=*P. californicus*; PECR=*P. crinitus*; PEDI=*P. difficilis*; PEER=*P. eremicus*; PELE=*P. leucopus*; PEMA=*P.maniculatis*; PETR=*P. truei*; NS=Number of rodent species in a community; TPP= Total proportion of *Peromyscus* mouse in a rodent commnity; PVPE=Bartonella prevalence in *Peromyscus* mouse; PVPM=Bartonella prevalence in *P. maniculatus.*

Table 3-6 Distribution and proportion of *Onychomys* mice in rodent community and bartonella prevalence in the mouse

Site	ONLE	ONTO	NS	TPP	Prevalence (%)
Badlands	15.9		1	15.9	22.2
Cimarron	47.5		1	47.5	89.6
Comanche	41.1		1	41.1	81.8
Janos	5.1	21.2	2	26.3	60.9
Thunder Basin	16.5		1	16.5	35.1

ONLE=*Onychomys leucogaster*; ONTO=*O. torridus*; NS=Number of rodent species in a community; TPP=Total proportion of *Onychomys* mouse in rodent community.

Onychomys mice were captured at nine sites with population proportion ranging from 0.8% to 47.5% per community. The northern grasshopper mouse was captured in eight sites and was the dominant species in Commanche (41.1%) and Cimarron (47.5%); the southern grasshopper mouse (*O. torridus*) was captured at two site and was the dominant species at Janos (21.2%) (Table 3-6).

Rats of three species of *Neotoma*, white-throated woodrat (*N. albigula*), desert woodrat (*N. lepida*), and Mexican woodrat (*N. mexicana*) were the dominant species at Sevilleta, Orange, and Loveland, respectively. The rock pocket mouse (*Chaetodipus intermedius*) was the dominant species at the Phoenix site (Table 3-3).

Prevalence of bartonella infection by rodent genus and species

As shown in Table 3-2, bartonellae were detected in 813 of 1,952 (41.6%) rodents from all study sites. The positive rodents belonged to 36 species of 16 genera and prevalence varied by genus and species. Among these rodents, *Onychomys* mice were the most likely to have bartonellae, with 69.7% (159/228) of individuals infected. Both species within the genus had bartonella infection, with prevalence 70.8% (148/209) in northern grasshopper mice and 57.9% (11/19) in southern grasshopper mice. Fisher's exact test showed the prevalence did not differ significantly between the two species ($p= 0.2967$).

Prevalence of bartonella also was high in the genus *Tamias* (64.1%, 41/64). Rodents of the four species tested had a high prevalence of bartonella infection: 60.6% (20/33) in the least chipmunk (*T. minimus*), 66.7% (12/18) in the Uinta chipmunk (*T. umbrinus*), 66.7% (2/3) in the Colorado chipmunk (*T. quadrivittatus*), and 70% (7/10) in the cliff

chipmunk (*T. dorsalis*) (Table 3-2). There was no significant difference in bartonella prevalence among the four species (Fisher's exact test, p=0.9176).

The average prevalence of bartonella in mice of the genus *Peromyscus* was 44.2% (473/1070). Bartonellae were found in mice of seven over eight *Peromyscus* species tested. Bartonella prevalence was 0% (0/1) in the canyon mouse, 15.8% (16/101) in the cactus mouse, 23.2% (13/56) in the white-footed mouse, 47.1% (32/68) in the pinyon mouse, 47.5% (358/754) in the deer mouse, 58.6% (34/58) in the brush mouse, 62.1% (18/29) in the California mouse, and 66.7% (2/3) in the rock mouse (Table 3-2). Compared to the mean prevalence, the prevalence in the brush mouse and the California mouse was significantly higher (Fisher's exact test, $0.0258 < p < 0.0473$), whereas that in the cactus mouse and the white-footed mouse was significantly lower (Fisher's exact test, $p < 0.001$). The prevalence in mice of the other four species did not differ significantly from the mean (Fisher's exact test, $0.1239 < p < 0.5542$).

The prevalence of bartonella in the rats of genus *Neotoma* was 41.9% (54/129), with 36.7% (18/49), 41.7% (15/36), 42.9% (3/7), 48.6% (18/37) and 50% (1/2) in the white-throated woodrat, the desert woodrat, the dusky-footed woodrat (*N. fuscipes*), the Mexican woodrat, and the southern plains woodrat (*N. micropus*), respectively (Table 3-2). Fisher's exact test showed the prevalence variation among these four species was not significant (p=0.7208).

Twenty of 50 *Dipodomys* rats that of three species had bartonella infections, with 22.2% (2/9) in Merriam's kangaroo rats (*D. merriami*) and 48.7% (18/37) in Ord's kangaroo rats (*D. ordii*), and 0% (0/4) in banner-tailed kangaroo rats (*D. spectabilis*). Only 2 (1.8%) of 112 *Chaetodipus* mice were infected by bartonella. Mice of six species

of *Chaetodipus* were tested but all except two (one hispid pocket mouse [*Chaetodipus hispidus*] and one desert pocket mouse [*Chaetodipus penicillatus*]) were free of bartonella. The prevalence also was very low in *Reithrodontomys* (3.8%, 3/79) and *Mus* mice (5.6%, 1/18). The other genera had moderate prevalence: 25% (4/16) in *Sigmodon* rats, 26.9% (7/26) in *Perognathus* mice, 27.3% (9/33) in *Microtus* voles, and 34% (34/100) in *Spermophilus* ground squirrels (Table 3-2).

Prevalence of bartonella infection by rodent community

The bartonella prevalence at the study sites ranged from 4.2% to 82.4%. The lowest prevalence was at Phoenix, and the highest prevalence was at Fort Lewis (Table 3-4). The distribution of prevalence at all study sites was normal (W=0.97, p=0.67).

Bartonella prevalence within the same genus or species has also varied by study site. The prevalence in *Peromyscus* mouse from the 24 sites varied from 0% to 92.9% and fitted a normal distribution (W=0.97, p=0.72). The same variation in bartonella prevalence was observed in deer mice from 21 sites, and also fitted a normal distribution (W=0.95, p=0.38) (Table 3-5). In *Onychomys* mouse, prevalence was 22.2% at Badlands, 35.1% at Thunder Basin, 60.9% in Janos, 81.8% in Comanche, and 89.6% in Cimarron (Table 3-6).

Associations between bartonella prevalence and community diversity

The Goodness-of-Fit test indicated all datasets fitted the normal distribution (all p>0.05), assured the validity of the following analyses.

i. Bartonella prevalence and genus/species richness

Using data from all 24 sites, simple linear regression and correlation analysis demonstrated that bartonella prevalence was significantly and negatively correlated with both the genus richness of the community (r=-0.49; p=0.0141) and the species richness of the community (r= -0.50, p=0.0132). The correlation became more apparent after two sites, Phoenix and Yuma, were removed from the analysis because of biases that will be discussed below (r=-0.58, p=0.0049 at the genus level; r=-0.67, p=0.0006 at the species level) (Figures 3-1, 3-2).

ii. Bartonella prevalence and Shannon index

Using data from all 24 study sites, simple linear regression and correlation analysis indicated that bartonella prevalence was not significantly associated with the Shannon index at either the genus level (r=-0.36, p=0.0821) or the species level (r=-0.40, p=0.0552), although a trend was observed. However, a significant correlation was observed between the two variables after two sites, Phoenix and Yuma, were removed from the analysis (r=-0.52, p=0.0121 at the genus level; r=-0.61, p=0.0026 at the species level) (Figures 3-3, 3-4).

Associations between Bartonella prevalence and proportion of rodents in community

Analyses were conducted for two genera (*Peromyscus* and *Onychomys*) and one species (*P. maniculatus*) because of the limited number of representatives of other genera and species.

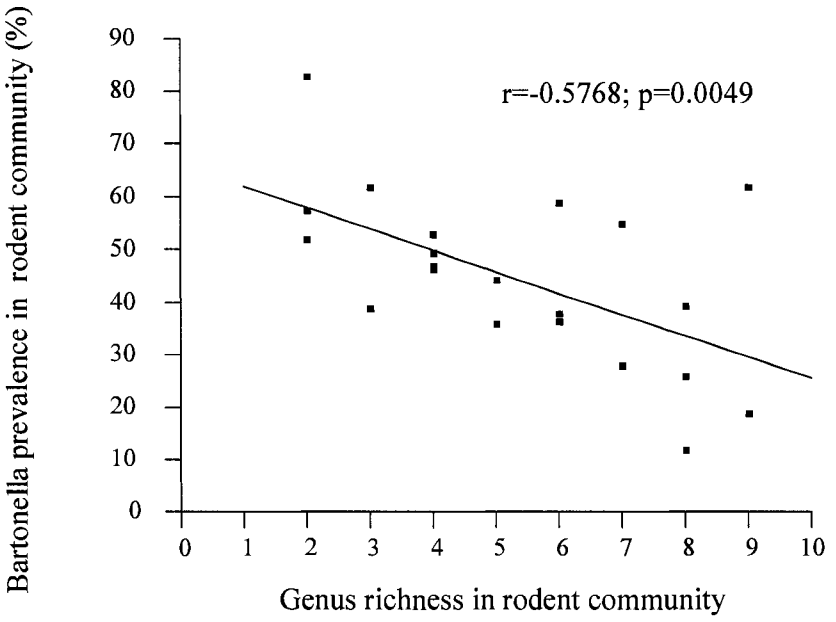

Figure 3-1 Association of bartonella prevalence with genus richness in rodent communities. Bartonella prevalence was measured by percentage of culture-positive rodents of all tested individuals in the rodent community. Genus richness represents the number of genera of rodents in a community. Simple linear regression was performed after removal of two sites (Phoenix and Yuma), where members of dominant species were non- or low- susceptible to bartonella. Bartonella prevalence declines when the number of genus of hosts increases in a community. A significant negative correlation was demonstrated.

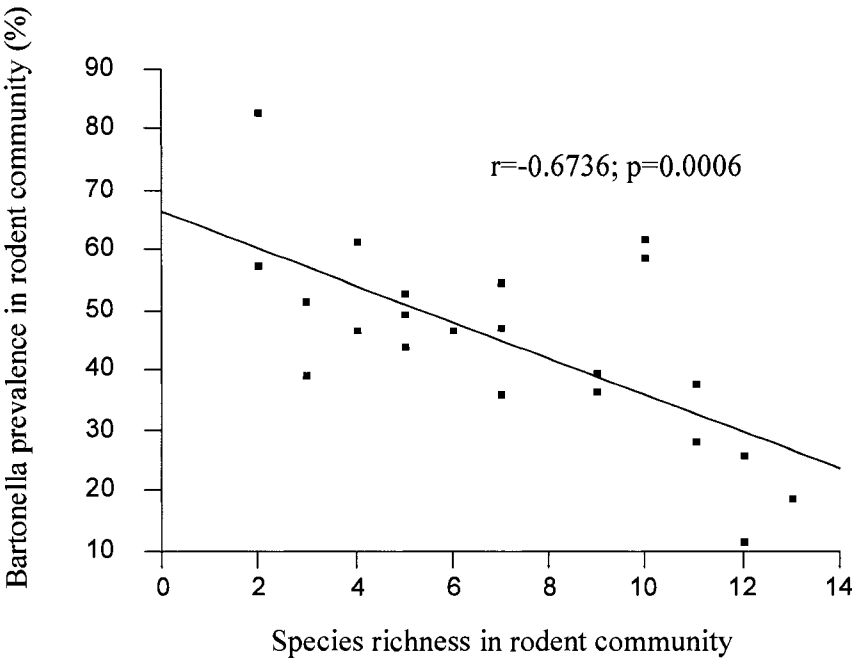

Figure 3-2 Association of bartonella prevalence with species richness in rodent communities. Bartonella prevalence was measured by percentage of culture-positive rodents of all tested individuals in the rodent community. Species richness represents the number of species of rodents in a community. Simple linear regression was performed after removal of two sites (Phoenix and Yuma), where members of dominant species were non- or low- susceptible to bartonella. Bartonella prevalence declines when the number of species of hosts increases in a community. A significant negative correlation was demonstrated.

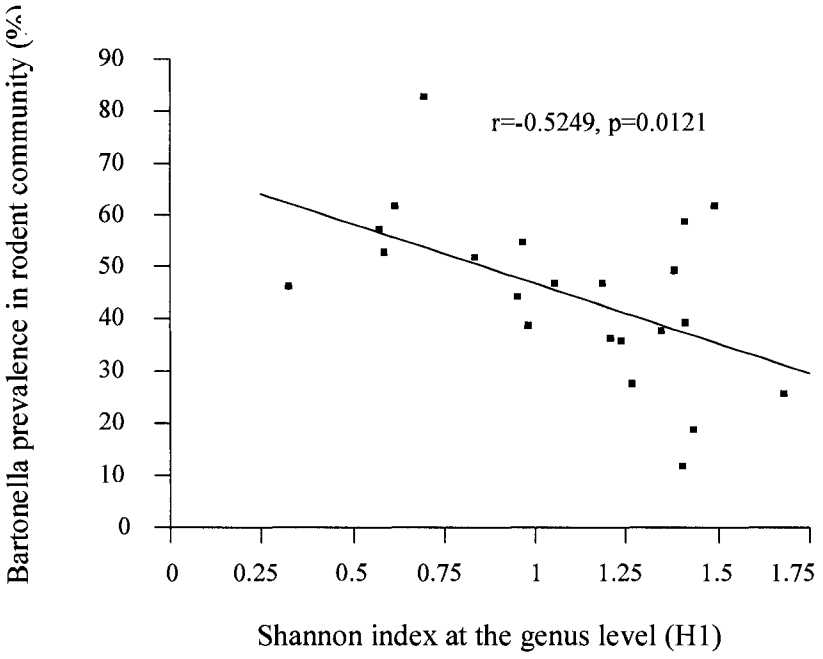

Figure 3-3 Association of bartonella prevalence with Shannon index at genus level (H1) in rodent communities. Bartonella prevalence was measured by percentage of culture-positive rodents of all tested individuals in a rodent community. Shannon index was calculated from proportional abundances of each genus of rodent in a rodent community. Simple linear regression was performed after removal of two sites (Phoenix and Yuma), where members of dominant species were non- or low- susceptible to bartonella. Bartonella prevalence declines while Shannon index at genus level increases in a rodent community. A significant negative correlation was demonstrated.

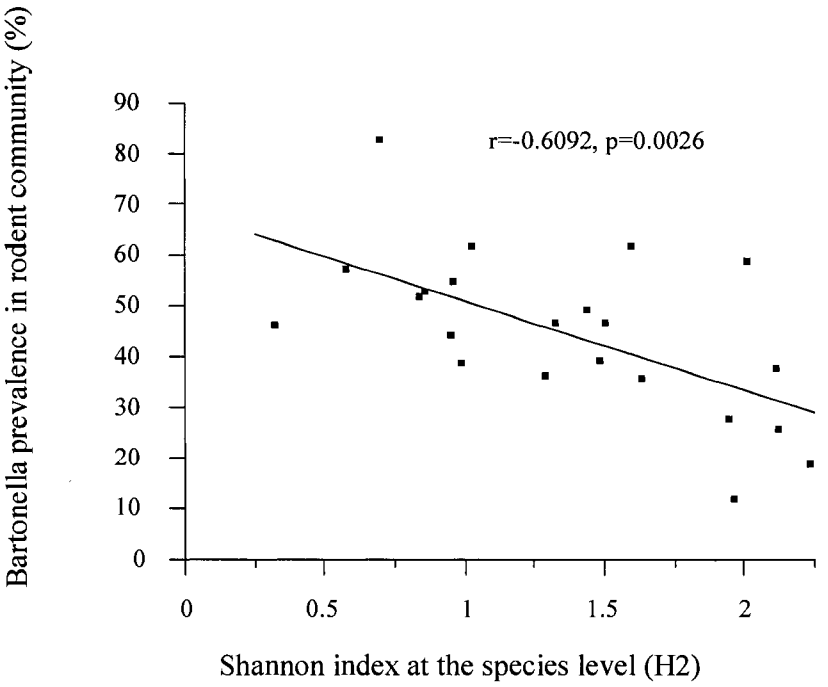

Figure 3-4 Association of bartonella prevalence with Shannon index at species level (H2) in a rodent community. Bartonella prevalence is the percentage of positive rodents of all tested rodents in a rodent community. Shannon index was calculated from proportional abundances of each rodent species in a rodent community. Simple linear regression was performed after removal of two sites (Phoenix and Yuma), where members of dominant species were non- or low- susceptible to bartonella. Bartonella prevalence declines while Shannon index at species level increases in a rodent community. A significant negative correlation was demonstrated.

Regression and correlation analyses showed that bartonella prevalence in both all *Peromyscus* mice and deer mice alone were positively associated with the proportion of the mice in the community at either the genus or the species level ($r=0.40$, $p=0.0556$ at the genus level; $r=0.42$, $p=0.0613$ at the species level) with marginal significance (Figure 3-5a; Figure 3-5b).

Although *Onychomys* mice were captured at nine sites, data from only five of these sites (Badlands, Cimarron, Comanche, Janos, Thunder Basin) were used to analyze the relationship between the bartonella prevalence and the proportion of the mice in the community. Data from the other four sites (Fort Huachuca, Rio Rancho, Sevilleta, Socorro) were excluded due to the very small sample size ($n = 1\sim4$). Simple linear regression and correlation analysis detected a highly significant and positive correlation of bartonella prevalence in *Onychomys* mice with the proportion of mice of the genus in the community ($r=0.9716$, $p=0.0057$; Figure 3-5c).

DISCUSSION

Transmission of agents of infectious diseases is an inherently ecological process involving interactions among hosts, pathogens and, when they are involved, vectors. Not surprisingly, the species diversity of ecological communities can potentially affect the disease dynamics, either increasing or decreasing the risk (Ostfeld and Keesing, 2000a; Ezenwa et al., 2006). The present study showed that bartonella prevalence varied in rodent communities with different compositions (Table 3-3), indicating that community diversity impacted the prevalence or rate of bartonella infection. The results in general suggested a negative correlation between bartonella prevalence and community diversity.

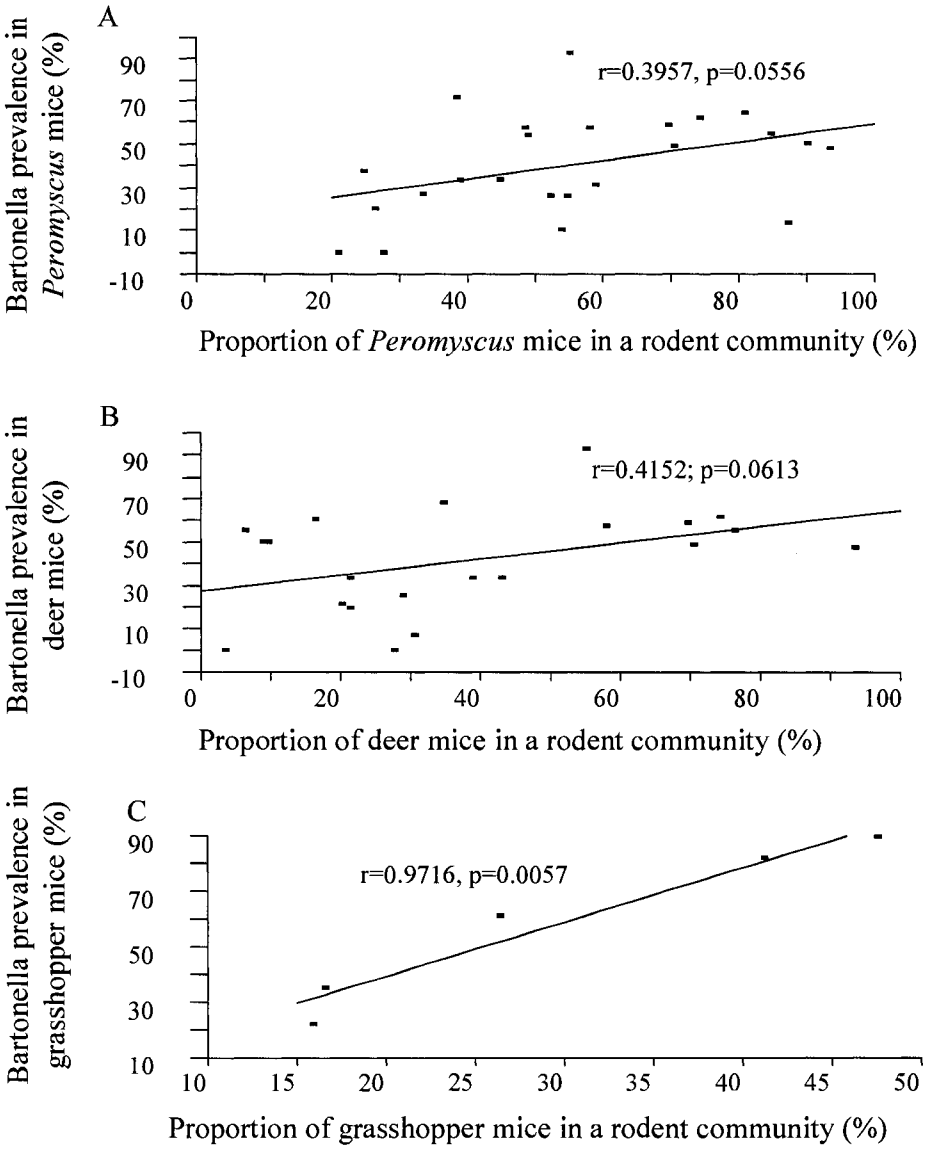

Figure 3-5 Association of bartonella prevalence with proportion of all *Peromyscus* mice (A), deer mice, (B), and *grasshopper* mice (C) in a rodent community. Bartonella prevalence is the percentage of positive rodents of each category of all tested rodents of the category. Proportion of each rodent category is the percentage of the number of captures of the category of all captures in a rodent community. Simple linear regression was performed using data from all 24 sites for *Peromyscus* mice, 21 sites for deer mice, and 5 sites for *Onychomys* mouse. Bartonella prevalence was highly positively related to the proportion of *Onychomys* mice but only marginally positively related to the proportion of *Peromyscus* mice and deer mice.

That is, within a rodent community with higher diversity, bartonella prevalence is lower, and *vice versa.*

As relatively newly discovered organisms, we know little about bartonellae. The genus *Bartonella* includes many species (multiple pathogens) that can infect a variety of rodent species (multiple hosts), and likely are transmitted by fleas and other possible vectors (multiple vectors). These and other factors make the system of bartonella infection very complex. Community diversity may affect the rate of infection through many different ways within such a system. Discussed below are three models to explain the observed effects of diversity of rodent community on bartonella prevalence.

Within-genus /species transmission model (encounter reduction)

As its co-speciation implying, the same bartonella strain rarely or does not infect rodents of species that are taxonomically distant. This suggests that the bartonella transmission rate must be higher within-species than between-species. In a multi-host system, if transmission rates are higher within-species than between-species, then host diversity should decrease infection rates, and *vice versa* (Begon et al., 1992; Begon and Bowers, 1994; Bowers and Begon, 1991; Dobson 2004; Holt and Pickering, 1985; Rudolf and Antonovics, 2005). This is because adding non-host species to the population would reduce the probability of encounters between hosts, and/or limit or regulate host numbers through resource competition. As a result, increasing rodent community diversity would decrease bartonella prevalence. Nevertheless, rodents of different species but the same genus, for example, *Peromyscus*, may share the same *Bartonella* species (Kosoy et al., 1997), suggesting that transmission of bartonella occurs both within- and between-

species. Rodents of closely related species could function as alternative sources of infection in the same genus. Adding a *Peromyscus* species to a community that already has one *Peromyscus* species would increase the total number of susceptible hosts and the rate of encounter between the hosts, therefore increase the infection rate. Furthermore, rodents of related species not only share the same bartonella species, but also share fleas of the same species. Adding rodents of related species would provide the vector with more feeding opportunities and would increase the density of the vector population. Vector regulation likely occurs for bartonella transmission among individuals of related species of the same genus. The prevalence of bartonella in a host community comprising several *Peromyscus* species may not be lower than in a community containing only one *Peromyscus* species, but may remain same or increase. In the study reported here, the prevalence of bartonella is marginally associated with both the proportion of deer mice and the total population of all *Peromyscus* mice, further supporting the observation that within- and between-species transmission have occurred in the same system. Similar results were obtained with *Onychomys* mice. Transmission between rodents of related species of different genera may apply to the entire rodent-bartonella system.

Frequency-dependent model (transmission reduction)

A key factor for defining the effects of community diversity on risk of infection in a multi-host system appears to be whether transmission of a pathogen is a function of the absolute density of infected hosts (density-dependent) or whether it is a function of the proportion of the total population that is infected with the pathogen (frequency-dependent). Vector-borne diseases frequently are considered to conform broadly to

frequency-dependent models of transmission (Thrall et al., 1993). In the present study, the finding of association of the prevalence of bartonella with the proportion of hosts in the entire rodent community of a particular habitat further suggests that the frequency-dependent model pertains to natural systems of bartonella infection. In a frequency-dependent manner, adding hosts will decrease infection risk whether or not the added hosts reduce the abundance of the principal hosts. This is because added hosts decrease the proportion of infected individuals in the community, resulting in a reduction of contact between susceptible and infected individuals, and leading to a lower rate of transmission, again assuming that transmission between individuals of different species is lower than transmission within individuals of a single species.

Dominant species effect (susceptible host regulation)

Bartonella prevalence in rodents at Phoenix site was surprisingly low, as compared with that in other rodent communities with a similar or higher diversity. A similar situation also was observed at Yuma site. Although bartonellae are distributed throughout a wide range of rodent hosts with overall high prevalence (Birtles et al., 1994; Kosoy et al., 1997; Ying et al., 2002; Holmberg et al., 2003), they rarely have been found to infect rodents of certain genera or species, such as *Chaetodipus* mice, *Reithrodontomys* mice, and cactus mice. Rodents that are not susceptible or that are of low susceptibility could play a crucial role in determining the mean infection rate of arthropod vectors. If the dominant species in a community is not susceptible to bartonella infection, the overall prevalence in the community will be low because the dominant species would constrain the abundance of susceptible hosts through competition of limited resources, a process

called "susceptible host regulation" (Keesing et al., 2006). The rock pocket mouse and cactus mouse were the dominant species in Phoenix (51.2%) and Yuma (83.6%), respectively (Table 3-3). However, the rock pocket mouse has never been found culture positive for bartonella, and the level of infectivity of bartonella among cactus mice was evidently lower compared to other species of *Peromyscus* mice. The dominance of these rodents in both communities can explain the overall low prevalence of bartonella in two mentioned sites regardless of the community diversity. Although the cactus mouse was also the dominant species in Rio Rancho, the prevalence of bartonella was higher there than in Yuma, though the diversity was higher in Rio Rancho (Table 3-3, 3-4). This can be explained by the lower proportion of cactus mice in Rio Rancho compared to Yuma (40.5% versus 83.6%), which would have fewer effects on rodents of other host species. The dominant rodents at other 21 sites, including pinyon mice, deer mice, brush mice, white-footed mice, desert woodrats, white-throated woodrats, Mexican woodrat , northern grasshopper mice, and southern grasshopper mice were all susceptible to bartonellae based on this and other studies (Bai et al., 2007b; Kosoy et al., 1997; Morway, personal communication). As mentioned, the correlation of bartonella prevalence with community diversity became more apparent after exclusion of the Phoenix and Yuma sites from the analyses, suggesting that dilution effects were more evident when susceptible reservoirs were dominants in rodent communities (Schmidt and Ostfeld, 2001). Those results indicated a potential bias associated with the level of host susceptibility when analyzing relationships between diversity and infection risk.

Potential bias affecting the current analysis

Although it was observed during these studies that community diversity generally dilutes bartonella infection, the effects differ among diversity components. It appears that species richness can better explain variations in bartonella prevalence in these communities than a proportion of rodents of certain species in a community does. The Shannon index, incorporating both richness and evenness (another way to show proportion), seems to support such a statement by showing the correlation with bartonella prevalence. Bartonella prevalence is always lower in a community consisting of more diverse rodent species or genus, but is not always lower when the proportion of rodents of a species in a community is smaller. In other words, genus/species richness has consistent effects on bartonella prevalence, whereas the proportion of rodents in the communities may vary by rodent genus or species. Particularly, bartonella prevalence in *Onychomys* mice was highly related to the proportion of these mice in the rodent communities at the study sites, with 94% variation of the prevalence being explained by the proportion of *Onychomys* mice in the communities (Figure 3-5c); whereas bartonella prevalence was only marginally associated with the proportion of *Peromyscus* mice, with only ~16% variation of the bartonella prevalence explained by the proportion of the mice (Figure 3-5a). This may indicate that it is not appropriate to determine the effects of proportion of rodents on bartonella prevalence using a combined proportion of all relative species within a genus if bartonella prevalence in each species varies between species. Mice of the eight *Peromyscus* species tested in the study were different with respect to their susceptibilities to bartonella infection, as shown by low prevalence in cactus mice and white-footed mice, but high prevalence in brush mice, deer mice, and pinyon mice (Table

3-5). If an added species is a more susceptible one, it will increase the prevalence; if the added species is less susceptible to bartonella infection, a decreased prevalence would be likely. Two *Onychomys* spp. mice, however, did not show differences in their susceptibilities to bartonella infection; thus, the combined proportion would be of value analyses. However, as a relationship of bartonella prevalence also was not observed with proportions of deer mice in the communities, it is suggested that other factors might exist. Differing contributions of rodent proportions on bartonella prevalence may be related to a potential bias caused by trapping design, trapping season or other factors. For example, the trapping in five sites used for analysis of *Onychomys* mice was done within the same season by the same team using the same design, making the data more appropriate for analysis. Samples for analyses of *Peromyscus* mice were collected during different seasons and were less comparable.

A particular situation was observed in Commanche, where bartonella was not found in any of 30 tested deer mice and this result was not related to community diversity. At least two hypotheses might explain this phenomenon: (1) the current deer mouse population was newly established after a recent crash of the population and this population remained free of bartonella not having sufficient time to re-establish bartonella infection in this rodent population; and (2) the newly established population of deer mice at Comanche represented only a small fraction of the genetic variation in deer mice (founder effect), and mice of the new population were not susceptible to bartonella infection.

Vector regulation likely does not occur

Dilution effect based on Lyme disease model requires vectors be generalist species and acquire pathogens orally (as opposed to exclusively transovarial transmission). A vertebrate community with high species diversity can deflect vector meals away from the most competent reservoirs, thereby reducing infection prevalence. The above-mentioned requirements do not apply to bartonella infections. First, mechanisms of transmission of bartonella between rodents remain unclear. Although the role of fleas or other potential vectors is generally accepted as a mechanism for bartonella transmission (Bown et al., 2004; Breitschwerdt and Kordick, 2000; Stevenson et al., 2003), there is evidence showing that transovarial transmission of bartonella occurs in rodents, suggesting that transmission by arthropod vectors is not only mean by which bartonellae are transmitted between rodents. More importantly, rodent fleas are usually specialists feeding on specific rodent hosts, at least at the genus level, although some rodent flea species, such as *Aetheca wagneri, Orchopeas leucopus*, and *Pleochaetis exilis*, can share hosts of different genera, such as grasshopper mice and deer mice (Thomas, 1988). However, as specialists, most rodent fleas will not be drawn away from their hosts by adding non-host species to the community. Therefore, the density of the flea population will not increase, but remain at the same or a similar level. The investigation of bartonella infection within vole populations in Ireland by Telfer et al. (2005) demonstrated that flea prevalence did not increase with overall rodent density increases, suggesting that vector involvement did not increase. I argue that the requirements to apply a dilution effect model should be reconsidered in regard to generalist vectors. In infection systems with specialist vectors

(bartonella infection) or non-vector systems (hantavirus), dilution effects also occur within a diverse community.

CONCLUSION

Community diversity has significant influence on the interaction between agents and their vertebrate hosts (Ostfeld and Keesing, 2000b). The potential effects of diversity on risk of infection with bartonellae have drawn the attention of ecologists, conservation planners, and physicians, who work together to understand the factors responsible for disease risk and for maintenance of biological diversity. The present study highlights the critical role of rodent community ecology in risk of bartonella infection. These results provided empirical support for theoretical models applicable in ecological epidemiology. In addition, they demonstrate that the presence of a diverse assemblage of rodents can bring about a reduction in bartonella prevalence in a rodent community through differing mechanisms.

The occurrence and peculiarities of bartonella infection are far more complex than what we recognize. For example, multiple *Bartonella* species can coexist in the same individual or different individuals of same host species, individuals of different host species can share the same bartonella, and multiple vectors may transmit the organisms among multiple hosts. Complex interaction of organism, hosts, and vectors, as well as many other factors make the net effects of rodent community diversity on bartonella prevalence unpredictable. Additional studies are warranted to more fully understand the relationship between rodent community structure and bartonella prevalence.

CHAPTER FOUR

DYNAMICS OF BARTONELLA INFECTIONS IN RODENTS AND EFFECTS OF HOST POPULATION PARAMETERS

Close contact between rodents and humans throughout the world makes the study of rodent-borne bartonellae essential in order to determine the extent to which rodents may serve as sources of human infections. Information on the ecology of bartonellae and their potential reservoir hosts is of public health interest. Numerous surveys have been conducted in a variety of rodent community at many locations and demonstrated that bartonellae are highly prevalent, with or without co-speciation in their rodent hosts (Birtles et al. 1994; Kosoy et al. 1997; Ying et al., 2002; Jardine et al., 2005; Bai et al., 2007b).

Although a great deal of information has been collected, most previous surveys to detect and characterize bartonellae in small mammals have been cross-sectional. Host-parasite systems are subject to high variation in the short term because of extrinsic factors. This has made our understanding of the epidemiology of bartonellae in their rodent hosts very limited, and many questions remain unanswered. For example, what host factors regulate bartonella prevalence and bacteremias at the individual and population levels? How do populations of rodents maintain high prevalence of bartonella infections? Is high prevalence an indication that infection is chronic or is there a rapid turnover of multiple *Bartonella* species by recurring acute infections? How are the dynamics of bartonella infections in rodents linked to the population dynamics of their hosts? What are the

dynamics of bartonella infection within repeatedly sampled individuals? Does infection with bartonella of one species provide protection against subsequent infection with bartonella of other species or with other strains of the same species?

To address some of these issues, detailed ecological studies of the demography of bartonellae in their rodent hosts are needed. By monitoring population dynamics and temporal patterns, longitudinal studies done by mark-release-recapture can elucidate the natural history of associations between parasites and their hosts (Douglass et al., 1996; Mills et al., 1999a). This approach has been applied to the study of the prevalence of antibody to hantaviruses in rodents, to the prevalence of West Nile virus in wild birds, and to many other zoonotic agents during the population cycles of their hosts (Banet-Noach et al., 2004; Calisher et al., 2007; Mills et al., 2007; Yates et al., 2002). The results describe the relations between these agents and their hosts, insights that are crucial to our understanding of the ecology of these parasites and their hosts and crucial in developing preventive measures and predictive models (e.g. Mills et al., 1999a, b).

Several longitudinal studies have partially explored the temporal dynamics of bartonella infections in wild rodents and have provided valuable information regarding these dynamics (Birtles et al., 2001; Fichet-Calvert et al., 2000; Kosoy et al., 2004a; Jardine et al., 2006b; Telfer et al., 2007a, b). In the UK, Birtles et al. (2001) studied the dynamics of bartonella in a woodland rodent community over a 12-month period. They concluded that, although bartonella often persist in most animals, there was a continuous turnover of *Bartonella* genotypes in these animals. In Tunisia, Fichet-Calvet et al. (2000) found that transmission of bartonella within populations of fat sand rats occurred mostly in the summer – fall period. In the USA, Kosoy et al. (2004a) reported that bartonella

infection was common in hispid cotton rats, with seasonal variations of the prevalence. Multiple *Bartonella* species were detected in cotton rats, and cotton rats could be infected with one of them or in mixed infection during the course of their bacteremias (Kosoy et al., 2004b). In Canada, a seasonal pattern of bartonella infections in Richardson's ground squirrels (*Spermophilus richardsonii*) was reported, the prevalence of infection being lowest in May and highest in late summer to early autumn (Jardine et al., 2006a, b).

Parameters of rodent hosts, such as age, have been found to contribute to the prevalence of bartonella in rodents of some populations. An inverse association between mass (as an indicator of age) and bartonella prevalence has been demonstrated. Fichet-Calvet et al. (2000) reported that bartonella prevalence decreased as the fat sand rats aged; Kosoy et al. (2004a) reported negative correlations of age and bartonella prevalence with level of bacteremia in cotton rats; and Jardine et al. (2006a) found that juvenile Richardson's ground squirrels were more likely to be infected and to have high levels of bacteremia than were adult animals.

Describing and understanding the ecology of zoonotic pathogens in their natural hosts is of epidemiologic importance, and warrants further investigations with the aim of developing preventive measures and predictive models (e.g. Mills et al., 1999a, b). Although much is known, it is still poorly understood how the diversity and dynamics of bartonella populations are regulated under natural conditions. In this chapter, I reported the evaluation of studies of the effects of host parameters, including host age (mass-based) and sex, on bartonella infections and bacteremia level; explored temporal dynamics of bartonella infections during population cycles of rodent hosts; examined the correlation of bartonella prevalence with relative abundance of host population; and estimated

successive bartonella infections in individual rodents. Data generated from three mark-release-recapture studies that were conducted in different areas were used to fulfill the goals. The prevalence of bartonella infection was measured by the percentage of culture-positive rodents in the rodents. Relative abundance of hosts was calculated as the number of individual animals captured per 100 trap-nights (Wilson et al., 1996).

MATERIAL AND METHODS

Study sites and trapping

Boulder

Boulder, northern Colorado (39°83′9″N, 109°31′1″W), is in the midst of an urban corridor on the east side of the Rocky Mountains. The area is characterized by patches of short- and mixed-grass prairie interspersed with urban development (Johnson and Collinge, 2004). Black-tailed prairie dogs (*Cynomys ludovicianus*), a recognized keystone species in western prairie ecosystems (Kotliar et al. 1999), deer mice (*Peromyscus maniculatus*), and rodents of many other species are widely distributed in the area. The present study focuses on analyses of prairie dogs and deer mice.

Prairie dogs, deer mice and other rodents were trapped in twenty selected prairie dog colonies (1A-20A). Prairie dogs were trapped in each colony once during each of the summers of 2003-2006, using a 5 x 10, 6 x 8 or 7 x 7 trapping grid, depending on colony shape. Tomahawk live traps (Tomahawk Live Trap, Inc., Tomahawk, WI) were set at each station at either 20 m (2003) or 25 m (2005) intervals for four consecutive days and were checked within three hours of being set each day. For technical reasons, only samples collected in summers of 2003 and 2005 were analyzed for the present study.

Deer mice and other small rodents were trapped in each colony in two sessions during 2003-2006, the first session in June, and the second in August-September, over four consecutive days each. Sherman live traps (8 cm x 9 cm x 23 cm; H.B. Sherman Traps, Inc., Tallahassee, FL) were placed 20 m apart and arranged in a 7 x 7 grid. The traps were checked each morning. Deer mouse samples from 2003 were analyzed in the present study.

Fort Lewis

Fort Lewis is located in southwestern Colorado (37°13′31″N, 109°10′51″W). The vegetation at this site is dominated by montane shrubland superimposed on intrusive igneous rocks forming laccoliths (Baars, 1995; Fitzgerald et al., 1994).

A mark-release-recapture study of hantavirus infection in deer mice performed by Calisher et al. from CSU was conducted at two trapping webs from June 1994 to September 2006, with trapping sessions each six weeks, as weather and logistics permitted. Trapping was not conducted during the winter and early spring months (December–April). Small-mammals were trapped for three consecutive nights on each trapping occasion. Each web comprised 145 Sherman live traps arranged along 12 lines radiating from a center trap, the first four traps in each line being placed 5 m apart, the next eight placed 10 m apart; lines were 30 degrees from each other. Traps were checked each morning of the trapping period. Samples collected during October 2003 to June 2006 were provided by Calisher for the present study. The rodent community mainly consisted of deer mouse. Other species accounted for a very small portion. The present study was restricted to analysis of deer mice data.

Placitas

Placitas is located in northern New Mexico (35°19′3″N, 106°27′7″W). A canyon on the west side of Sandia Mountain was selected as the trapping site. The trapping area extended upslope of 2.8 km from an elevation of 1820 m to 2165 m. Habitat varied from juniper woodland to juniper-scrub oak and into a mix of pinyon, ponderosa pine, Douglas fir, and Gambel's oak, then abruptly returns to pinyon-juniper, with very little scrub oak as the canyon ends.

A total of 14 trap lines, each consisting of 10 Sherman live traps, and oriented perpendicularly to the canyon walls, were set each 200 m along the length of the canyon. Trap lines were set for three nights each month between June 2000 and July 2002, weather permitting, yielding a total of 420 trap-nights per month. Traps were checked each morning of the trapping period. The local rodent community consisted of several species, dominated by peromyscines, among which brush mouse (*Peromyscus boylii*) constituted the largest portion. Analyses conducted in the present study were restricted to brush mice alone and combined *Peromyscus* species (including brush mice).

Animal processing

Captured rodents from all sites were processed following safety procedures published by the US Centers for Disease Control and Prevention (Mills et al., 1995a, b). Captured rodents were anesthetized with a mixture of isofluorane and oxygen, species recorded, sex determined, reproductive status accessed, and weight and other standard measurements taken. Capture status (first capture, recapture [different trapping session], or repeater [within same trapping session]) was noted. Bloods were collected from the

retro-orbital plexus of the rodents. New captures were marked with uniquely numbered ear tags. When they had recovered from anesthetization, rodents were released at the capture site. Repeaters were not re-processed.

Bartonella culturing

Culturing procedures were the same as those described in Chapter 3. Additionally, the level of bacteremia was estimated by counting the number of colonies on the original plates after incubation and re- calculated as colony forming units (CFU) per ml of blood.

Statistical analysis

Statistical analyses were performed using specific programs within the SAS package (Statistical Analysis System, version 9.1, SAS Institute Inc., Cary, North Carolina). Relative abundance and proportion of rodents within certain classes were first tested for normal distribution using a goodness-of-fit test before further analyses. Chi-square analyses were done to determine whether bartonella prevalence differed significantly between years, seasons, or sex. Fisher's exact test was used if sample size was ≤ 60. Simple linear regression and correlation was performed to determine the associations of bartonella prevalence and rodent mass, relative abundance or logarithm (base e) transformed relative abundance, and proportion of a specific group of rodents in a population. The correlation coefficient (r) was computed and the statistical significance was tested. In addition, 95% confidence intervals (CI) were calculated to compare the mean mass of rodents; median mass was determined; an odds ratio was computed to compare prevalence of bartonella infection in different mass classes. Statistical tests for

significance of difference or correlation between any groups were performed at the 0.05 probability level.

RESULTS

Trapping summary and population structures

Boulder

i. Prairie dogs

A total of 849 individual prairie dogs were captured during the study period, with 325 and 524 captured in 2003 and 2005, respectively. The numbers of female and male prairie dogs were 163 and 161 in 2003 (the sex was not determined for one animal), 289 and 235 in 2005, respectively. Twenty-nine prairie dogs that had been captured in 2003 were recaptured in 2005, for a total of 58 captures.

The mass of prairie dogs varied from 185 g to 1410 g in 2003 with median mass as 860 g and mean mass as 826.7 g (95% CI = {800.3, 853.0}). The population was highly skewed to large mass (W=0.98, $p<<0.01$), with 72.0% of individuals weighing ≥700 g. In 2005, the mass of prairie dogs varied from 220 g to 1470 g with median mass of 780 g and mean mass of 746.7 g (95% CI = {726.5, 766.8}). The proportion of individuals in mass class of <700 g, 41.4%, was significantly higher in 2005 than that in 2003 (28.0%) ($\chi^2=10.0$, $p<<0.01$) (Figure 4-1).

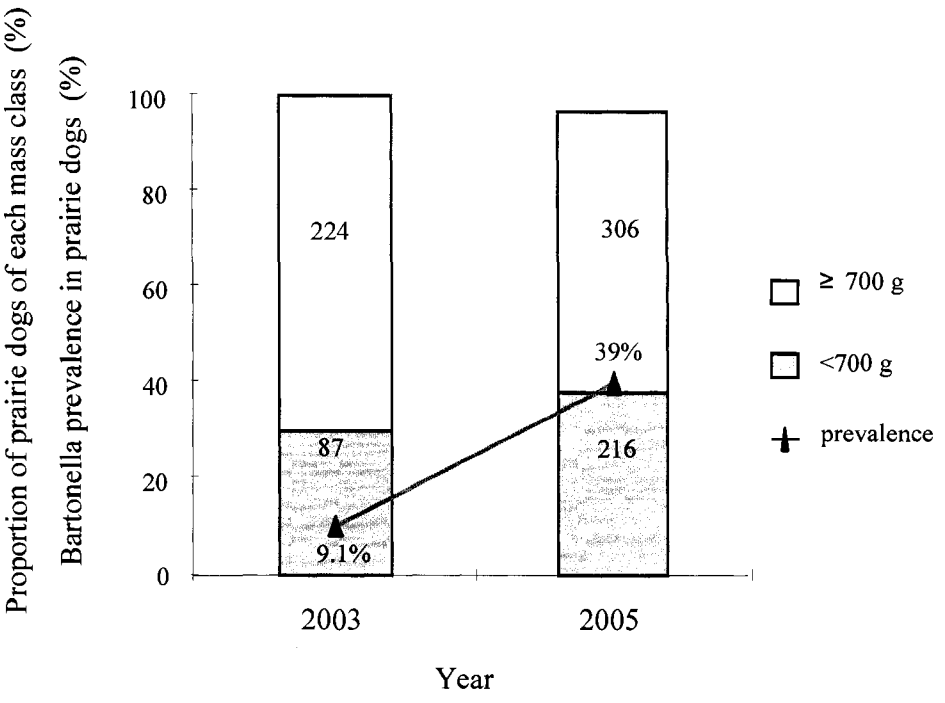

Figure 4-1 Bartonella prevalence in prairie dogs versus proportion of prairie dogs within each mass class, Boulder, Colorado, 2003 and 2005. The numbers shown in bars represented the number of prairie dogs in mass class of <700 g or ≥ 700 g. The percentages were overall bartonella prevalence in prairie dogs in each year. Increased bartonella prevalence was coincided with the increased proportion of prairie dogs within mass class <700 g in 2005.

ii. Deer mice

A total of 471 individual deer mice were captured within the trapping colonies, among which 139 were captured in the summer of 2003, and the other 332 in the fall of 2003. The numbers of female and male deer mice were 208 and 254, respectively (the sex was not determined for 9 mice).

The masses varied from 6 g to 34 g with median 19 g and mean 18.7 g (95% CI ={18.3, 19.1}). In summer, the masses varied from 8 g to 30 g among 134 mice (median = 20 g, mean = 19.7 g, 95% CI = {19.0, 20.3}) (the mass was not determined for 5 mice). Mice within mass class of <19 g (49) and of ≤14 g (9) accounted for 36.6% and 6.7% of the population, respectively. In the fall, the masses of 312 mice (the masses were not determined for 20 mice) exhibited broader variation, from 6 g to 34 g (median = 19 g; mean = 18.3 g, 95% CI = {17.8, 18.8}). The proportion of 146 individuals with mass <19 g was 46.8% and did not significantly differ from that in summer (χ^2=2.43, p=0.12), but the proportion of 65 individuals with mass ≤ 14 g increased to 20.8%, significantly higher than that in summer (6.7%) (Fisher's exact test, p<<0.01) (Figure 4-2).

Fort Lewis

Rodent samples were collected in 10 trapping sessions during the study period (2003-2006). In all, 1,171 individuals were recorded, consisting of 1,079 (92.1%) of deer mouse and 92 (7.9%) of other rodent. Of the 1079 deer mice, 737 were randomly selected for the present study. Female and male deer mice were evenly distributed, 365 and 372 of the captures, respectively.

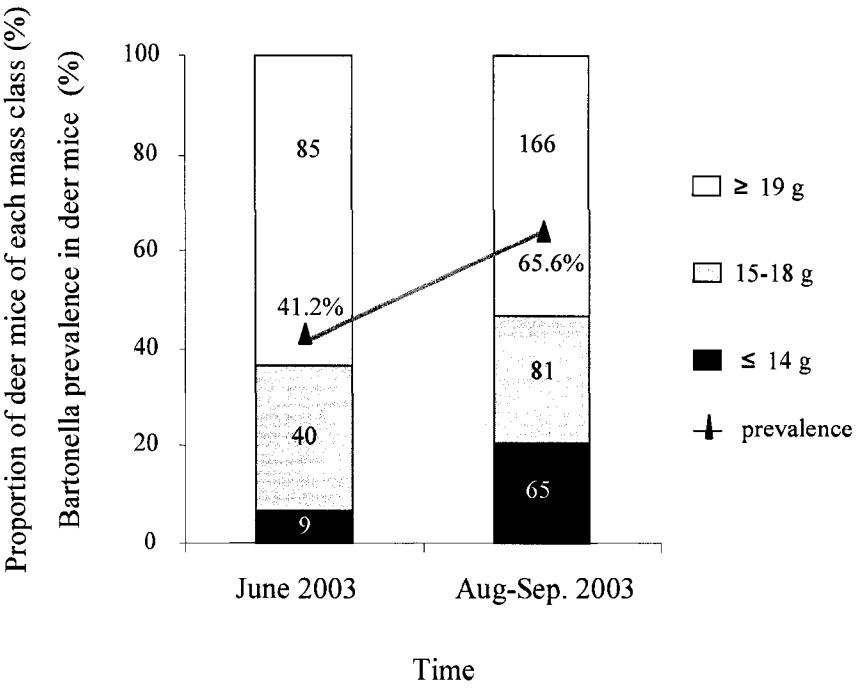

Figure 4-2 Bartonella prevalence in deer mice versus proportion of deer mice within each mass class, Boulder, Colorado, June and August-September of 2003. The numbers shown in bars represented the number of deer mice in mass class ≤ 14 g, 15-18 g, and ≥ 19 g accordingly. The percentages were bartonella prevalence in deer mice in each trapping session. Increased bartonella prevalence coincided with an increased proportion of deer mice within mass class ≤ 14 g in fall.

The deer mouse population fluctuated during the trapping period. The relative abundance ranged from 4.8% to 24.5% and fits a normal distribution (W=0.90, p=0.23). Specifically, the deer mouse population began high in October 2003, declined in May, June and July 2004, then increased the next month, peaked in September, remained high in October, then declined again (Table 4-1; Figure 4-3A).

The mass of deer mice ranged from 6 g to 34 g, with median mass of 18 g and mean mass of 17.5 g (95% CI = {17.2, 17.9}). The average proportion of mice within mass class of <19 g was 57.5% (ranging from 16.7% to 100%) during the trapping period, and fit a normal distribution (W=0.94, p=0.62), and was in concordance with the fluctuation of the entire deer mouse population (r^2=0.53, p=0.04; Table 4-1; Figure 4-3A).

Of 133 individual deer mice captured more than once, 75 were captured twice, and 36, 16, 4, and 2 were captured 3, 4, 5 and 6 times, respectively, for a total of 354 captures. The time between two captures ranged 6-54 weeks (Tables 4-2, 4-3, 4-4).

Placitas

Seventeen trapping sessions occurred during the study period, resulting in 398 captures of rodents. Of these, 359 (90.2%) were of the genus *Peromyscus*. The remaining 39 (9.8%) belonged to seven species of six genera. The 359 captures of peromyscines consisted of 237 (66%) brush mice, 92 (25.6%) pinyon mice (*P. truei*), 29 (8.1%) white-footed mice (*P. leucopus*), and 1 (0.3%) deer mouse. Male/female ratio was 135/100 for brush mice, and 207/149 for all peromyscines (including brush mice).

The size of peromyscine populations changed during the trapping period (2000-2002) with relative abundance varying from 2.1% to 10.5%, which fits a normal distribution after logarithmic transformation. The populations exhibited a general pattern

Table 4-1 Seasonal dynamics of population structure and bartonella prevalence in deer mice (*Peromyscus maniculatus*) in Fort Lewis, October 2003 - June 2006

Trapping date yr.mo.date-date	No. captured	No. tested	No. positives	Proportion with mass ≤18 g (%)	Relative abundance (%)	Bartonella prevalence (%)
2003.10.21-23	134	133	118	87.2	15.4	88.7
2004.5.18-20	84	84	70	27.4	9.7	83.3
2004.6.29-7.1	81	81	63	43.2	9.3	77.3
2004.8.10-12	121	120	98	55.8	13.9	81.7
2004.9.21-23	213	189	173	68.8	24.5	91.5
2004.10.26-28	179	41	33	56.1	20.6	80.5
2005.6.7-9	42	24	18	16.7	4.8	75
2005.7.19-21	69	23	16	17.4	7.9	69.6
2006.4.25-27	69	3	1	100.0	7.9	33.3
2006.6.6-8	87	39	17	48.7	10	43.6
Total	1079	737	607	57.5	12.4	82.4

Table 4-2 Bartonella infection in deer mice captured twice during different periods of time, Fort Lewis, October 2003 - June 2006, considering time point of first capture is week 0

No. weeks after 1st capture	No. mice infected at both captures	No. mice infected at one capture	No. mice not infected at either capture	Total
6	34	10	2	46
12	5	3	0	8
18	3	2	0	5
30	9	0	1	10
36	2	0	1	3
42	0	0	1	1
48	2	0	0	2
Total	55	15	5	75

Table 4-3 Patterns of bartonella infection in 36 deer mice captured three times during different period of time, Fort Lewis, October 2003 - June 2006, considering time point of first capture is week 0

Week 0	Week 6	Week 12	Week 18	Week 30	Week 36	Week 42	Week 48	Week 54	Total number of individuals exhibiting the pattern
+	+	+							15
+	-	+							3
+	+	-							1
-	+	+							1
+	-	-							1
-	-	+							2
+	+		+						2
+				+	+				4
+	+					+			2
+					+	+			1
+				+			+		1
+				-	-				1
+							-	-	1
-	+						+		1

" + " = positive for bartonella infection; "-"= negative for bartonella infection

Table 4-4 Status of bartonella infection in multiply captured individuals of deer mice, Fort Lewis, October 2003 - July 2005

Mouse ID	No. captures	Oct. 2003	May 2004	Jun. 2004	Aug. 2004	Sep. 2004	Oct. 2004	Jun. 2005	Jul. 2005
1	4	+	+	-	+				
2	4	+	-	+	+				
3	4	+	+	NC	+	+			
4	4	+	+	+	+				
5	4	+	+	+	NC	+			
6	4		+	+	+	+			
7	4		+	NC	+	+	+		
8	4		+	-	+	+			
9	4		-	-	+	+			
10	4		+	+	-	+			
11	4		+	NC	+	+	+		
12	4			+	+	+	NC	+	
13	4			+	+	+	+		
14	4				+	+	+	+	
15	4					+	+	+	+
16	4		+	NC	+	+	-		
17	5	+	-	-	-	+			
18	5	+	+	+	+	+			
19	5		+	+	+	+	+		
20	5			-	+	+	NC	-	-
21	6	+	+	+	+	-	+		
22	6	+	+	-	-	+	+		

" + " = positive for bartonella infection; "-"= negative for bartonella infection; NC = no capture at this time.

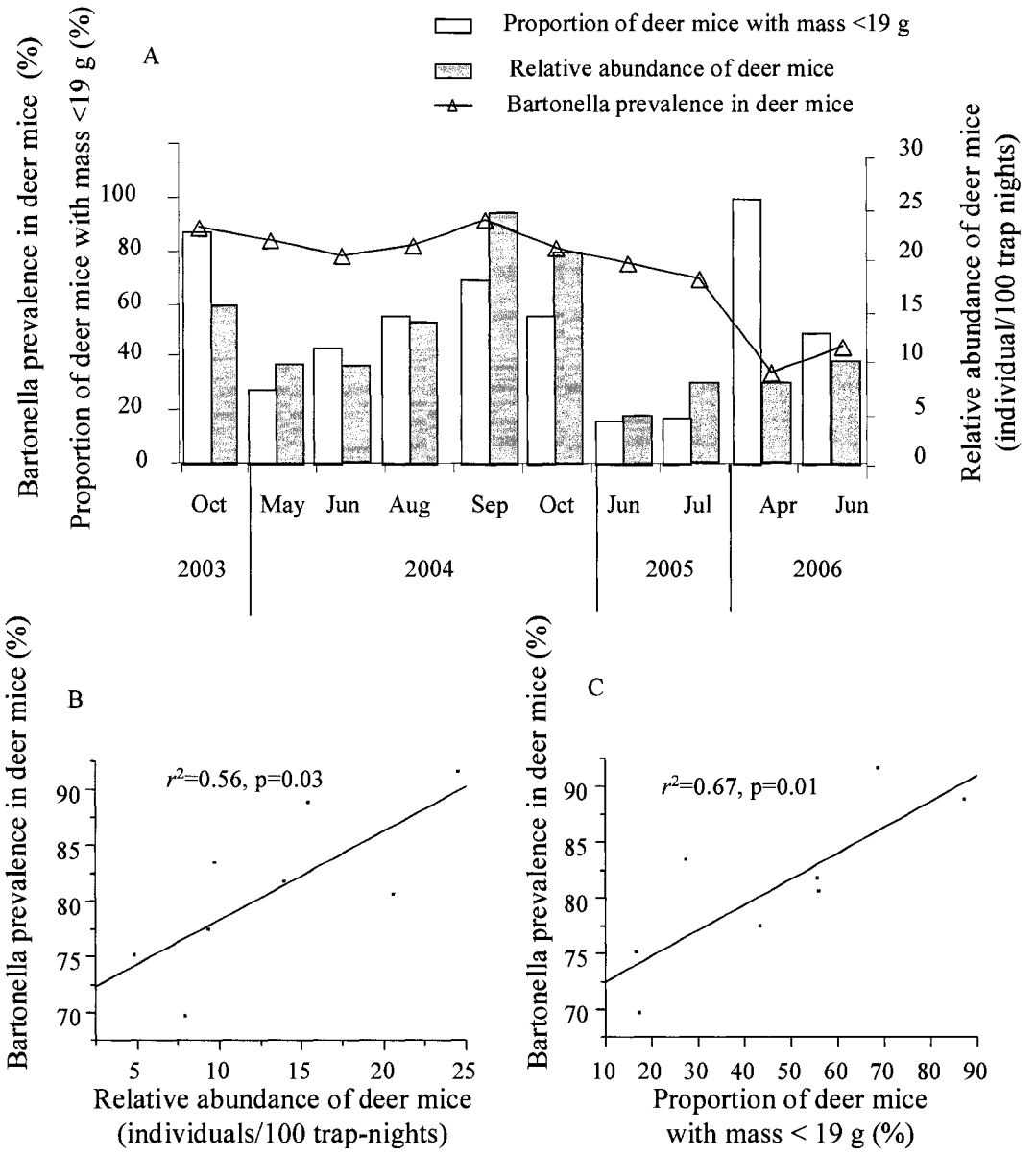

Figure 4-3 Dynamics of the relative abundance of deer mice, proportion of deer mice with mass <19 g, and bartonella prevalence in the mice during October 2003 - June 2006 at Fort Lewis, Colorado (A), correlation of bartonella prevalence with relative abundance of deer mice (B), and correlation of bartonella prevalence with proportion of deer mice with mass <19 g (C). Bartonella prevalence was significantly correlated with the relative abundance of host population and proportion of deer mice within mass class <19 g.

of increasing in summer and fall and decreasing in winter and spring. The number of peromyscines steadily declined over the winter of 2000-2001 from 10.5% in October 2000 to 2.4% in March 2001. The peromyscine population decreased considerably thereafter and did not recover until fall 2001, after which it maintained a similar population size (6.4%-7.6%) until the end of that trapping season (July 2002) (Table 4-5; Figure 4-4). The pattern of brush mouse population paralleled to that of the entire peromyscine populations, with the variation of relative abundance from 1% to 7.9%. This also fits a normal distribution after logarithm transformed (Table 4-5; Figure 4-4).

Among 233 brush mice, mass ranged 10 g - 39 g, with the median mass 21 g and the mean mass 20.8 g (95% CI = {20.2, 21.3}). The average proportion of mice within mass class of <19 g was 28.4% and varied 0 - 44.8% during the trapping period (Table 4-5). The proportion of mice within the mass class of <19 g fits a normal distribution but did not correspond to the change of population size during the trapping period.

Fifty-three brush mice were captured multiple times. The time between two captures ranged 1-13 months. Of these repeatedly captured brush mice, 34 were captured twice, 11 captured 3 times, 7 captured 4 times, and 1 captured 8 times (Table 4-6).

Overall bartonella infection in rodents

At the Boulder site, of 838 prairie dogs, 232 were bartonella culture-positive, giving an overall prevalence of 27.7%; and 245 of 416 deer mice were positive, giving an overall prevalence of 58.9%. At Fort Lewis, 607 of 737 deer mice were positive, giving a prevalence of 82.4%. At Placitas, the overall prevalence of bartonella was 32.4% (91/281) in all peromyscines and 39.4% (76/193) in brush mice alone.

Table 4-5 Seasonal dynamics of bartonella infection in all peromyscines or brush mice alone, Placitas, June 2000 - July 2002

Trapping date	All peromyscines					Brush mice					
(yr.mo.date-date)	No. captured	No. tested	No. positives	RA (%)	Prevalence (%)	No. captured	No. tested	No. positives	RA (%)	Proportion with mass ≤18 g (%)	Prevalence (%)
2000.6.27-29	10	10	8	2.4	80	6	6	6	1.4	0.0	100
2000.7.25-27	18	13	6	4.3	46	9	7	4	2.1	11.1	57
2000.8.15-17	18	18	12	4.3	67	9	9	9	2.1	0.0	100
2000.9.26-28	39	37	24	9.3	65	29	29	24	6.9	44.8	83
2000.10.26-28	44	33	16	11	49	33	24	12	7.9	30.3	50
2000.11.28-30	29	25	8	6.9	32	19	16	7	4.5	44.4	44
2001.2.20-22	15	15	7	3.6	47	8	8	4	1.9	25.0	50
2001.3.27-29	10	10	4	2.4	40	8	8	4	1.9	0.0	50
2001.4.24-26	15	13	1	3.6	7.7	7	6	1	1.7	42.9	17
2001.5.22-24	9	8	2	2.1	25	4	4	2	1	0.0	50
2001.6.27-29	9	9	0	2.1	0	6	6	0	1.4	16.7	0
2001.8.15-17	13	11	0	3.1	0	11	10	0	2.6	9.1	0
2001.9.26-28	11	7	0	2.6	0	10	6	0	2.4	40.0	0
2001.11.7-9	27	14	2	6.4	14	20	13	2	4.8	30.0	15
2002.3.13-15	28	11	0	6.7	0	20	10	0	4.8	30.0	0
2002.5.8-10	32	18	0	7.6	0	15	11	0	3.6	13.3	0
2002.7.10-12	32	29	1	7.6	3.4	23	20	1	5.5	43.5	5
Total	359	281	91	5	32	237	193	76	3.3	28.4	39

RA = Relative abundance

Table 4-6 Pattern of bartonella infection in 53 brush mice captured multiple times during June 2000 - July 2002 in Placitas, considering time point of first capture is month 0 (M0)

M0	M1	M2	M3	M4	M5	M6	M7	M8	M10	M11	M13	TNIETP
+	+											7
+	-											1
-	+											2
-	-											8
-		-										9
+			-									1
-			-									1
-				-								3
-					-							1
-										-		1
+	-	-										1
+	+	-										1
-	+	+										1
-	-	-										2
+	+	+										1
-	+	-										1
-		-		-								1
-		+				-						1
-		-				-						1
-				-		-						1
+	+	+	+									1
+	-	-				-						1
-	-			+	+							1
-	-			-	-							1
-	+			+	+							1
-	-		+				-					1
-	+						+	-				1
-	+			+	+	+	-		-		-	1

" + " = positive for bartonella infection; "-"= negative for bartonella infection; M=Month; TNIETP=Total number of individuals exhibiting the pattern

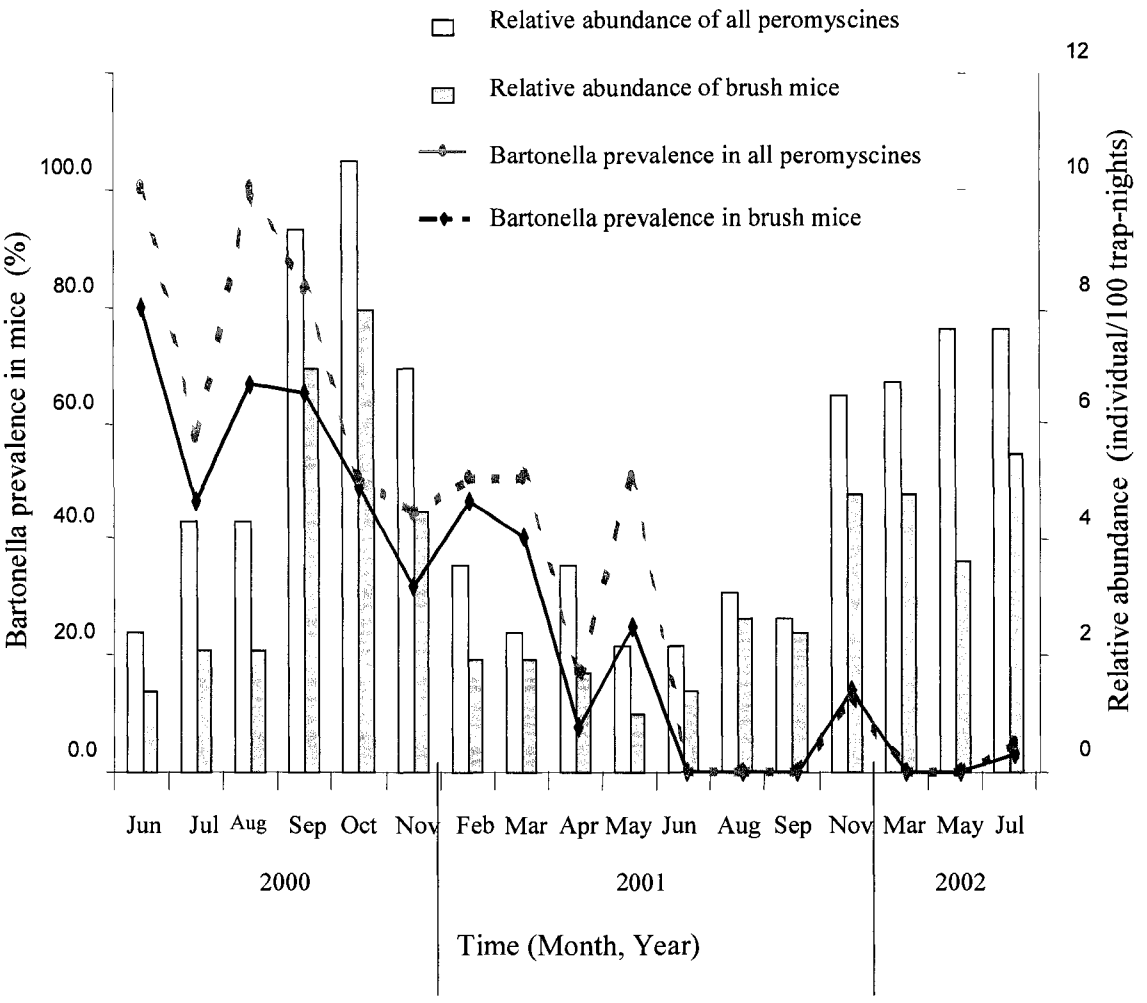

Figure 4-4 Dynamics of the relative abundance of all peromyscines and brush mice alone, and bartonella prevalence in these mice during June 2000 - July 2002 at Placitas, New Mexico. Bartonella prevalence declined through the whole study period and did not correspond to the change of the mice.

Host sex and bartonella prevalence

i. Prairie dogs

Prairie dogs at Boulder comprised 449 (53.6%) females and 389 (46.4%) males. Although prevalence in both sexes varied by year (9.9% and 37.2% for females, 8.3% and 41.4% for males, in 2003 and 2005, respectively), there was no significant difference in bartonella prevalence between sexes ($\chi^2 \leq 0.96$, $p \geq 0.33$).

ii. Deer mice

Similar to that in prairie dogs, bartonella prevalence in deer mice did not significantly differ between females and males at any study site: at Boulder, bartonella prevalence was 60.4% (113/187) in females and 60.0% (131/226) in males ($\chi^2=0.26$, $p=0.61$); at Fort Lewis, bartonella prevalence was 84.1% (307/365) in females and 80.6% (300/372) in males ($\chi^2=1.52$, $p=0.22$).

iii. Brush mice

With regard to sex, bartonella prevalence in brush mice from Placitas exhibited the same pattern as was observed in rodents of other species. The prevalence was 40.5% (32/79) in females and 38.6% (44/114) in males ($\chi^2=0.07$, $p=0.79$).

Host age (mass-based) and bartonella prevalence

To analyze the association between age and bartonella prevalence, body mass was used as a surrogate for age because age-dependance of animal weight is widely used as aparameter in ecologic studies.

i. Prairie dogs

Prairie dogs (n=822) captured in Boulder were classified into 9 mass classes, from <300 g to ≥ 1000 g by 100-g increments. Bartonella prevalence varied highly among mass classes, with the highest in class of 300-399 g (71.4%) and the lowest in class of 900-999 g (7.1%) and exhibited a sigmoidal response to body mass. Prevalence in the smallest juveniles (class <300 g; 42.9%) was relatively low, but increased quickly (class 300-399 g; 71.4%), then declined in parallel with increase of body mass, but did not decrease below 44% until the mass reached 700 g, after which prevalence declined dramatically (≤16.1; Figure 4-5). The odds of bartonella infection were more than four times as high for prairie dogs weighing < 700 g as for those weighing ≥ 700 g (OR=4.1, χ^2=16.78, p<<0.01). Statistical analysis demonstrated a highly significant negative correlation between bartonella prevalence and host mass for prairie dogs weighing > 300 g (all sampled classes; r^2=0.90, p<<0.01).

ii. Deer mice

The correlation between bartonella prevalence and body mass of deer mice was analyzed by site. Deer mice were divided into five mass classes at 4-g increments: ≤14 g, 15 -18 g, 19 -22 g, 23 -26 g, and ≥27 g.

In Boulder, analysis based on a total of 400 individuals showed that bartonella prevalence decreased with increasing mass, from 71.4% in the smallest/youngest mice to 45% in the largest/oldest mice (Fisher's exact test, p=0.03; Figure 4-6A). The prevalence was significantly different between mass classes of ≤14 g and 19 -22 g (Fisher's exact test, p=0.01), but did not differ between classes with mass ≤14 g and 15 -18 g, or the classes with mass 19 -22 g, 23 -26 g, and ≥27 g. A significantly higher prevalence was

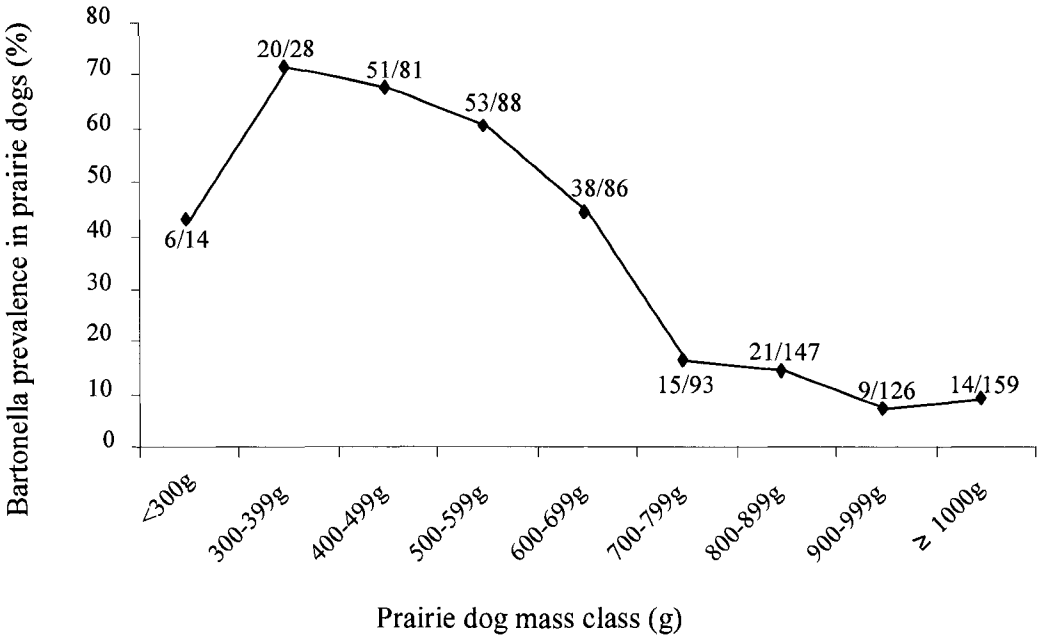

Figure 4-5 The pattern of bartonella prevalence in prairie dogs versus prairie dog mass. The fraction at each point represents the number of infected prairie dogs over the number of tested in each mass class. Bartonella exhibited a sigmoidal response to prairie dog mass. A significant negative correlation was observed between bartonella prevalence and host mass for prairie dogs weighing >300 g (r^2=0.90, p<<0.01). Bartonella prevalence significantly dropped after prairie dog mass past 700 g (OR=4.1, p<<0.01).

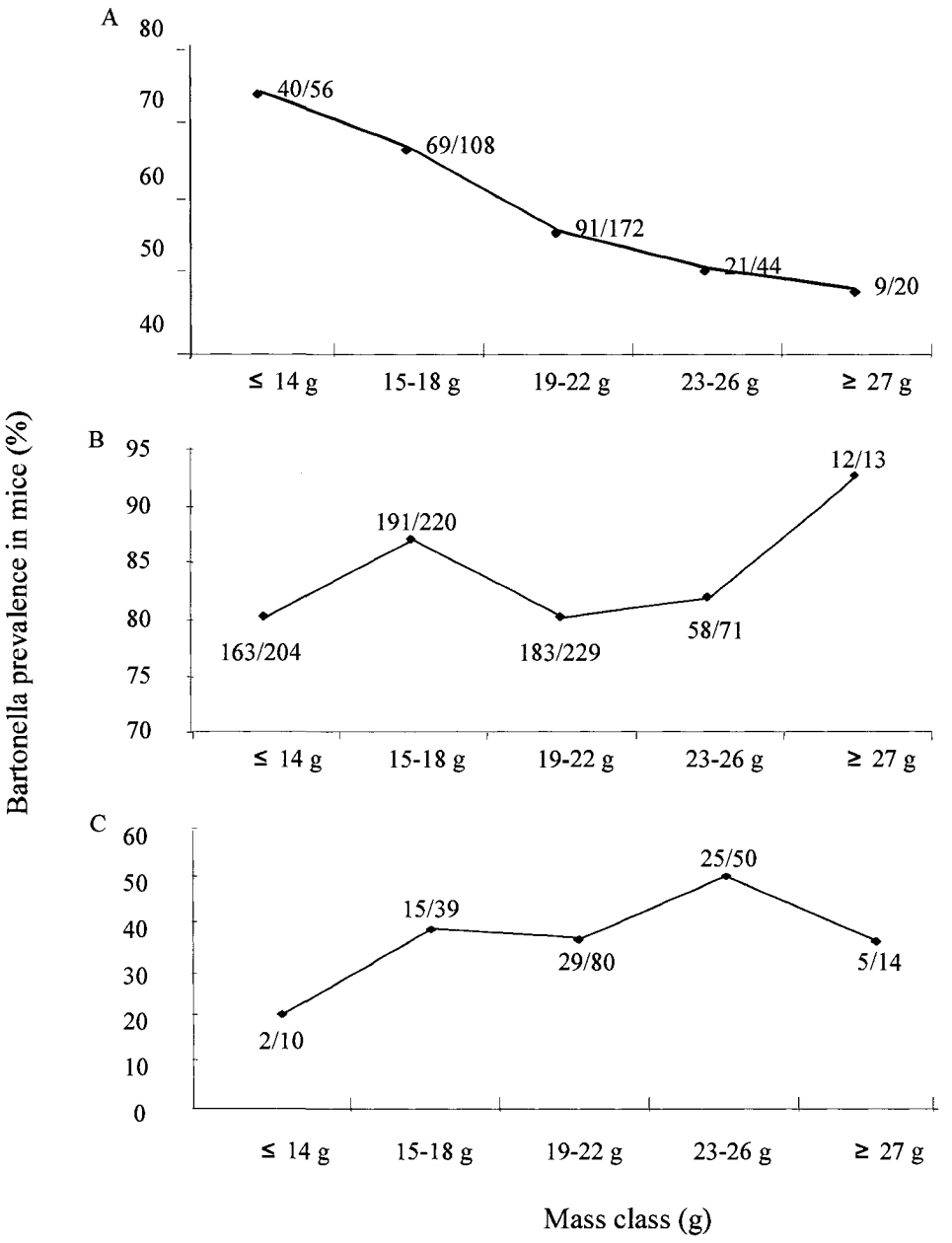

Figure 4-6 The patterns of bartonella prevalence in mice versus mouse mass. The fraction at each point represented the number of infected mice over the number of tested in each mass class. A highly significant negative correlation between bartonella prevalence and host mass was observed in deer mice in Boulder (r^2=0.94, p<<0.01). Bartonella prevalence is significantly higher before reaching mass 19 g (OR=1.9, p<<0.01) (A). No correlations were detected between bartonella prevalence and mice mass at both Fort Lewis (B) and Placitas (C).

demonstrated between deer mice weighing <19 g and those weighing ≥19 g (OR=1.9, χ^2=9.1, p<<0.01). Statistical analysis demonstrated a significant negative correlation (r^2=0.94, p<<0.01) between bartonella prevalence and deer mouse mass.

At Fort Lewis, bartonella prevalence was 79.9%, 86.8%, 79.9%, 81.7%, and 92.3% for the five mass classes mentioned above, respectively (Figure 4-6B). There was no apparent difference in bartonella prevalence between classes (χ^2=5.7, p=0.22) and no correlation between bartonella prevalence and mouse mass.

iii. Brush mice

At Placitas, 193 brush mice were classified into the same five mass classes, as were deer mice at other sites. The prevalence of bartonella in brush mice was 20%, 38.5%, 36.3%, 50%, and 35.7%, respectively (Figure 4-6C), with no significant difference between classes (Fisher's exact test, p=0.39). No correlation was observed between bartonella prevalence and mouse mass.

Temporal patterns of bacteremia level

i. Prairie dogs

The level of bartonella bacteremia in prairie dogs (n=232) varied from 40 to 12,000 CFU per ml of blood. The distribution of bacteremia was highly skewed to a low CFU, with the median being 280 CFU and only 18.3% samples with >1000 CFU. Bacteremia levels did not differ significantly between males and females. Prairie dogs with larger mass were less likely to have high loads of bacteria. Forty (23.1%) of 173 individuals weighing <700 g had bacteremias at >1000 CFU, whereas only 7 (11.9%) of 59 weighing ≥700 g had the same level (Fisher's exact test, p<<0.01) (Figure 4-7).

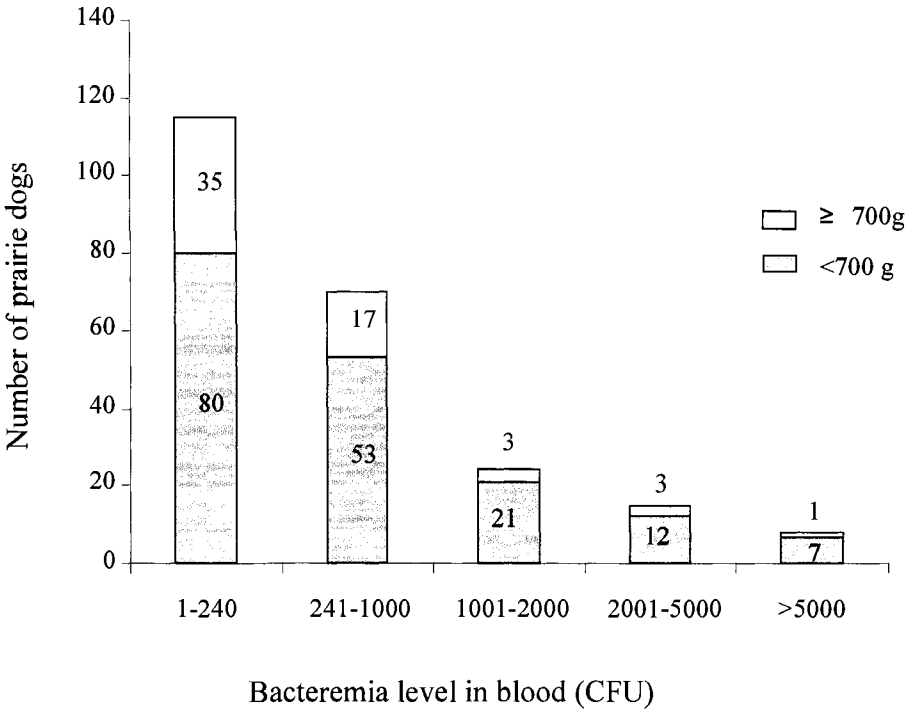

Figure 4-7 The distribution pattern of bartonella bacteremia levels (measured as number of colony forming units or CFU) in prairie dogs. The numbers shown in or above bars represented the number of bartonella-infected prairie dogs in mass class of <700 g or ≥ 700 g.

ii. Deer mice

Bartonella bacteremia in deer mice varied by site. At Boulder, the bacteremia in deer mice (n=230) ranged from 80 to 40000 CFU per ml of blood, with a median of 12,000 and did not differ between males and females. Bacteremia level was related to host mass, with a significant reverse relationship: a high proportion of high bacteremia (>20,001 CFU) in young hosts, a high proportion of low bacteremia (<4,000 CFU) in old hosts (Fisher's exact test, $p<<0.01$). The proportion of moderate level bacteremia (between 4,001 and 20,000 CFU) did not significantly differ between mass classes (Figure 4-8A).

At Fort Lewis, bacteremia in deer mice (n=602) ranged from 40 to 400,000 CFU per ml of blood. No significant difference was observed between females and males (median CFU = 8,000). The pattern of bacteremia in responding to host mass at Fort Lewis was similar to that at Boulder: a higher proportion of higher bacteremia in younger mice and a higher proportion of lower bacteremia in older mice (Fisher's exact test, $p<<0.01$). The proportion of moderate level of bacteremia (between 4,001 and 20,000 CFU also did not significantly differ among mass classes (Figure 4-8B).

iii. Brush mice

At Placitas, bacteremia in brush mice (n=72) ranged from 80 to 400,000 CFU per ml of blood with a median of 20,000. Bacteremias did not differ significantly between males and females. As in deer mice, bacteremia showed some association with body mass of brush mice. Due to the relatively small sample size of bartonella-infected brush mice, the samples were classified into 2 groups: ≤ 21 g and ≥ 22 g. The proportion of high

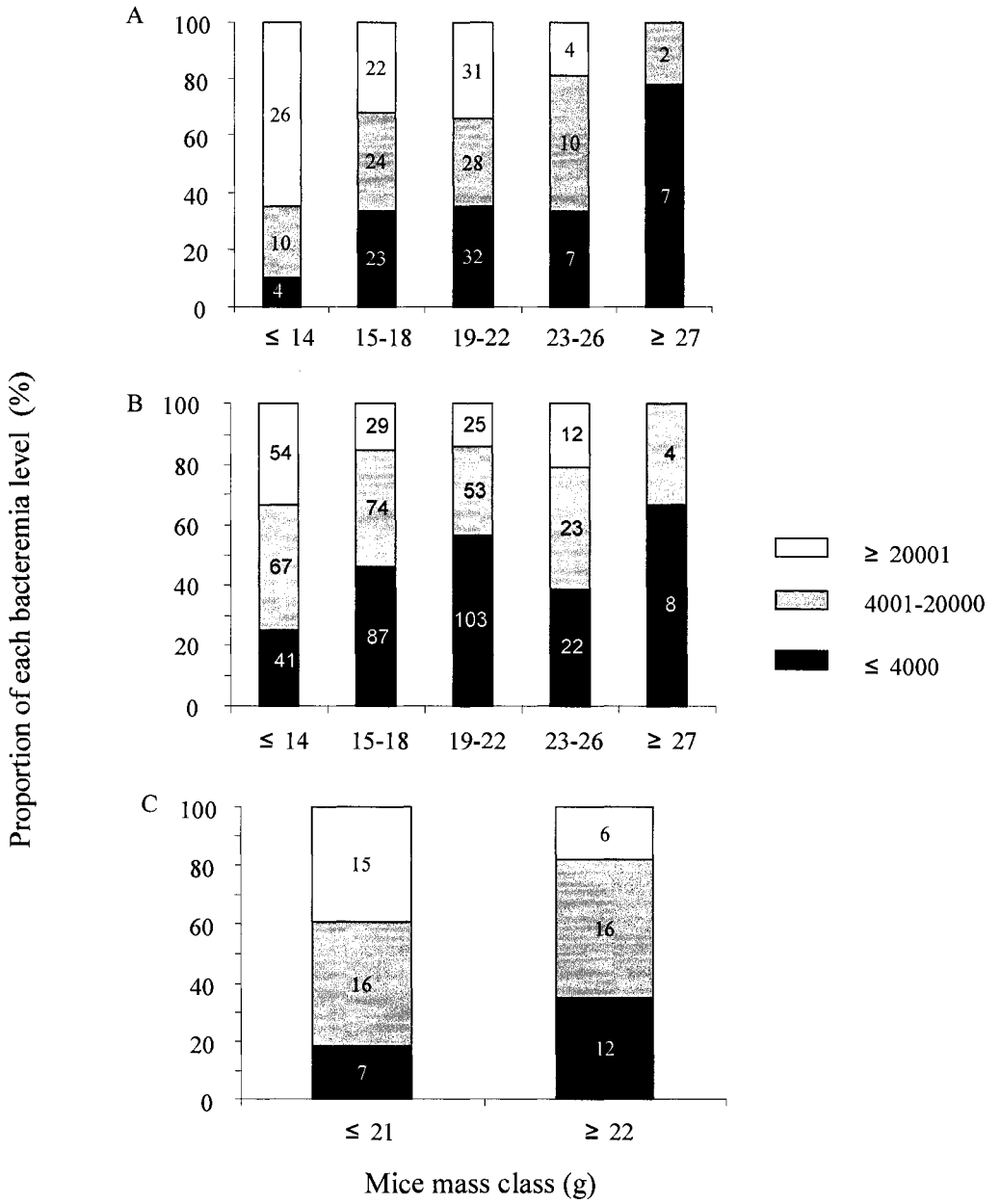

Figure 4-8 The distribution patterns of bartonella bacteremia versus mouse mass. The numbers shown in bars represented the number of mice observed in each bacteremia level. A similar reverse trend was observed between bartonella bacteremia level and mouse mass at Boulder (A), Fort Lewis (B), and Placitas (C), showing higher portion of higher bacteremia in younger hosts, and higher portion of lower bacteremia level in older host ($p<0.01$).

bacteremia (>20,000 CFU) was higher in younger brush mice than it was in older brush mice (Fisher's exact test, p=0.03).

Successive infection with bartonellae in recaptured rodents

i. Prairie dogs (Boulder)

Bartonellae were detected in three of 29 twice-captured prairie dogs (58 captures), once in each. Among all 58 captures, 6 (10.3%) weighed <700 g, the other 52 (89.7%) weighed from 725 g to 1180 g.

ii. Deer mice (Fort Lewis)

The overall prevalence of bartonella infection in repeatedly captured deer mice at Fort Lewis was 83.9% (297/354). Among the 75 mice captured twice, 55 (73.3%) were positive for bartonella at both captures, 15 (20%) were positive at one capture, and 5 (6.7%) were negative at both captures (Table 4-2). Of 58 mice captured 3-6 times and positive for bartonella at least once, 5 were positive at one capture, 53 were positive at 2-5 captures. Persistency of bartonella in hosts varied from six weeks to a year, or the infection was cleared, but recurred at a later time. Details are shown in Tables 4-3 and 4-4.

iii. Brush mice (Placitas)

The overall prevalence of bartonella infection in repeatedly captured brush mice at Placitas was 32.8% (45/137). Bartonella infection was not detected in 29 individuals captured 2-4 times within 1 - 11 months. Nine individuals captured 2 - 4 times within 1 - 7 months were positive for bartonella once. The other 15 mice were positive for bartonella 2-4 times within 1-13 months (Table 4-6).

Temporal dynamics of bartonella prevalence and association with host population structures

Boulder

i. Prairie dogs

In 2003, bartonella infection was detected in 29 of 318 (9.1%) prairie dogs. In 2005, bartonellae were detected in 203 of 520 (39.0%) prairie dogs. The prevalence was significantly higher than that in 2003 (χ^2=88.2, p<<0.01). The increased prevalence in prairie dogs in 2005 corresponded with an increased proportion of those within mass class <700 g (Figure 4-1).

ii. Deer mice

The prevalence of bartonella infection in deer mice at Boulder was 41.2% (47/114) in June 2003 and significantly increased to 65.6% (198/302) in August-September (χ^2=20.24, p<<0.01). This pattern also was concordant with the increased proportion of deer mice within mass class ≤14 g in the fall (Figure 4-2).

Fort Lewis

Bartonella prevalence varied from 33.3% to 91.5% during the trapping period at Fort Lewis (Figure 4-3). Corresponding to the change of relative abundance of deer mice, bartonella prevalence was high (118/133, 88.7%) in fall 2003 (October 2003, the first trapping session), but declined (133/165, 80.6%) in summer 2004 (May, June, and July), increased (98/120, 81.7%) in August 2004, reached a peak (173/189, 91.5%) in September 2004, began to decline (33/41, 80.5%) in October 2004, then maintained a relatively low prevalence (<75%) (Figure 4-3A). Due to the availability of very few samples from the last two trapping sessions, prevalence data from these two sessions

were excluded from analyses. Statistical analysis indicated significant correlations of bartonella prevalence with relative abundance of deer mice (r^2=0.56, p=0.03, Figure 4-3B), and with the proportion of deer mice with mass <19 g (r^2=0.67, p=0.01, Figure 4-3C).

Placitas

The prevalence of bartonella in all peromyscines at Placitas underwent a considerable decline during the study period. Starting with a high (8/10, 80%) prevalence of infection in June 2000 (the first trapping session), the prevalence declined (6/13, 46%) during that summer and the fall. The decline became more apparent in November (8/25, 32%), when the host population size also declined. Bartonella finally was no longer detected in the host population in June 2001, one month after the relative abundance of peromyscines decreased to their lowest level (2.1%) in May 2001. No bartonella was detected during summer 2001, when a decrease in the peromyscine populations was observed. Two mice were found bartonella-positive in November 2001, but the bartonella population was not re-established. This situation was maintained until July 2002, when trapping was ended at Placitas (Table 4-5; Figure 4-4). The same pattern of bartonella prevalence was observed in brush mice, with variation between trapping sessions, ranging from 0 to 100% (Table 4-5; Figure 4-4). No correlation was observed between bartonella prevalence in peromyscines and relative abundance of the peromyscines; and no correlation was observed between bartonella prevalence in brush mice and proportion of brush mice with mass <19 g in the population ($F<0.43$, $p>0.52$).

DISCUSSION

Although bartonellae are widely distributed in wild rodents (Birtles et al., 1994; Castle et al., 2004; Jardine et al., 2005; Kosoy et al., 1997; Ying et al., 2002), the prevalence and bacteremia level can vary by rodent species, as demonstrated by the present study. Furthermore, the present study provided new information in regard to bartonella infection and host parameters, and to dynamics of bartonella infection during the host population cycle.

The age of rodents in a population has been found to be a very important factor in the prevalence of bartonella (Fichet-Calvet et al., 2000; Kosoy et al., 2004a; Jardine et al., 2006b). In the present study, bartonella exhibited prevalence patterns in response to the age (mass-based) of rodents of various species, or of the same species but of different geographic distributions.

A significant negative correlation was observed between bartonella prevalence and prairie dog mass >300 g. Although there may be extrinsic factors, the higher prevalence of bartonella in 2005 than in 2003 was more likely explained by the different age structures of prairie dog population in these two years (Figure 4-1). The negative correlation between bartonella prevalence and host age may be explained by bacterial clearance through acquired immunity (Kosoy et al., 2004a). Cellular immunity associated with an increase in CD4 Th1 cells (Arvand et al., 2001) might be important because bartonella-specific antibodies were found in only a small proportion of animals (Kosoy et al., 2004a). Humoral immunity might not be a major feature of bartonella infections in rodents, but may play a role in feline infections (Childs et al., 1995). Alternatively, older, infected adult rodents might be more vulnerable to predation or starvation than might

their uninfected counterparts. Mortality or loss of fitness induced by the parasite, either alone or in synergy with other factors, is known to regulate host populations (Holmes, 1995; Scott and Dobson, 1989).

A very interesting finding in bartonellae - prairie dogs system is the difference in host susceptibility to infection before and after reaching a mass of 700 g ($\lambda \geq 4.1$). This suggested two reasonable hypotheses: a. Prairie dogs may experience a drastic alteration in immune responses when they reach this size. Thus, 700 g might be used as a threshold to evaluate the likelihood of bartonella infection in prairie dogs. b. Alternatively, the observed "threshold effect" may reflect prairie dog demography. The majority of those weighing < 700 g are born within the year of capture, while the majority of those weighing > 700 g were born in the previous year, or earlier. The utility of 700 g as a predictive threshold may depend on the timing of the study. It may be useful for studies conducted in the summer, when most adults weigh > 700 g, but less useful in the fall, when first-year animals exceed 700 g while still in the early stages of infection.

Similarly, a negative linear relationship was observed between bartonella prevalence and deer mouse mass at Boulder (Figure 4-6a), which might also provide explanations for the variation of bartonella prevalence in deer mice between two trapping seasons (Figure 4-2). However, mass-dependant prevalence was not observed at Fort Lewis or Placitas. Telfer et al. (2007b) proposed an impact of population age structure on zoonotic risk. In the present study, as the likelihood of bartonella infection in deer mice of mass <19 g is almost twice as high as in those of mass ≥19 g, members of the deer mouse population (mean mass =17.5 g) at Fort Lewis were susceptible to bartonella infection, as they exhibited an extremely high prevalence. Nevertheless, the prevalence

would not correlate with host mass, as was observed in Boulder, because there was no significant prevalence difference in mice with mass <19 g, regardless of the mass variation among the individuals. On the contrary, the relatively old population (mean mass = 20.8 g) at Placitas, which may have included mostly less susceptible individuals, may explain the low prevalence there. This population also did not exhibit mass-dependant prevalence, because the prevalence did not differ in older mice. Only the deer mice in Boulder, of moderate age (mean mass = 18.7 g, 95% CI = {18.3, 19.1}), exhibited a mass - prevalence correlation. Alternatively, all deer mice from Boulder were trapped under similar conditions as they were trapped at approximately the same season, whereas deer mice form Fort Lewis and brush mice from Placitas were trapped over several years. Changes in environmental conditions, as well as population structures influenced by these changes in environmental conditions likely affect the prevalence of infection. For example, fluctuations of flea loads were observed along with changes in size of the host population (Telfer et al., 2007b). Such variables may have major effects in determining whether an individual mouse would become infected. In addition, susceptibility to the infection of individual mice may differ at different time points, even if the mice are of comparable age. In this complex situation, the contribution of body mass to bartonella prevalence is difficult or impossible to determine and the prevalence is not predictable based on body mass. Habitat type, ectoparasite and endoparasite burden, meteorological conditions and events, availability of food, predators, and other ecological factors must be measured for a better understanding of the pattern of bartonella infection in hosts.

Although in this study bartonella prevalence was not always associated with age, younger mice were more likely to have higher levels of bacteremia, even though bacteremia levels varied between species. This pattern, observed in all species and all locations in the present study (Figure 4-8), suggests increasing immune competence of rodents as they age.

It has been reported that there is no association between bartonella prevalence and sex of rodent hosts (Kosoy et al., 2004a; Jardine et al., 2006b). In the present study, bartonella prevalence also was found to be essentially the same between male and female prairie dogs, deer mice, and brush mice.

Seasonal dynamics of bartonella infections in rodent hosts has been reported (Fichet-Calvet et al., 2000; Kosoy et al., 2004a; Jardine et al., 2006b; Telfer et al., 2007a). At Fort Lewis, bartonella prevalence generally increased in fall but decreased in winter (Figure 4-3A). This pattern coincided with the change of relative abundance of deer mouse (r^2=0.56, p=0.03; Figure 4-3B). By detecting the significant correlation between bartonella prevalence and the proportion of deer mice with mass <19 (r^2=0.67, p=0.01; Figure 4-3C), the present study demonstrated that the observed dynamics of bartonella prevalence in deer mice was largely determined by age structure of the host population (Fichet-Calvet et al., 2000; Kosoy et al., 2004a; Telfer et al., 2007b).

Not associated with the fluctuation of host population size, the presence of bartonella in brush mice steadily decreased during the study period at Placitas until they disappeared from the host population in June 2001 (Figure 4-4). It was observed that the host population had the lowest relative abundance (2.1%) a month earlier (May 2001). This may suggest delayed relative abundance-dependence, a pattern observed in studies

of prevalence of Sin Nombre virus infection in deer mice (Madhav et al., 2007). The bartonella population was not re-established at Placitas, whereas the relative abundance of brush mice remained low. This observation suggests that decrease of the host population impacts bartonella prevalence, which supports the hypothesis proposed in Chapter 3 regarding the observation of bartonella-free deer mice at Comanche, Colorado.

It is not known why bartonella prevalence seemed to correlate differently with relative abundance of host populations at Fort Lewis and Placitas. In addition to aging, habitat heterogeneity at Placitas might have had an effect on rodent activity, which in turn affected bartonella multiplication. Conditions such as flea activity, food resources, presence of other infections, and climatic conditions would be "averaged" if the host population were continuously distributed (such as at Fort Lewis) but could vary if the host population were discretely distributed (such as at Placitas).

The pattern of successive bartonella infections in the present study varied by rodent species. On many occasions, bartonellae were multiply detected in repeatedly captured deer mice, continuously, or intermittently for as long as one year. As a result, all mice became infected during their lifetimes. Kosoy et al. (2004a) reported a high prevalence of bartonella in cotton rats and explained it being the result of re-infection with bartonellae of different species, a result of low cross-protection. However, replacement of one bartonella by another does not explain the pattern observed in deer mice, because deer mice carry bartonella only of a single species (Kosoy et al., 1997). A plausible explanation for this finding is that the deer mice were not free of infection, but that their bacteremia levels were too low to be detected. An alternative explanation is that these bacteria persist in a "hidden niche" in the latent form, and can be re-activated from

sequestration only under specific conditions. It is likely that many mice have chronic, persistent infections, in which bartonellae never completely disappear. Another, albeit unlikely, explanation is that antibodies produced by deer mice persist only for a short period of time after the bacteremia is cleared. In such a case, mice would be unprotected from the same strain and could, therefore, become re-infected.

Brush mice exhibited some differences from deer mice in regard to patterns of successive infection by bartonella. Only about half of the brush mice were found infected and these infections lasted for a shorter time than did those in deer mice. Interestingly, intermittent infection was not observed in brush mice. Once the infection was cleared, they became free of infection and did not become re-infected.

Patterns of successive bartonella infection in prairie dogs were distinct. Infection lasted for a short period of time and was detected only once. This likely is related to the increased immunity acquired during the lifetime of prairie dogs. The majority of recaptured prairie dogs (89.7%) in the present study was relatively old ($\geq$700 g) and had already passed through the active bacteremic phase of their infection, in accordance with the observation of low odds of infection in this group. This observed lack of persistent bacteremia could explain the relatively low prevalence of bartonellae in prairie dogs.

CONCLUSIONS

The present study provided information regarding temporal variations in prevalence of bartonella infections in their natural rodent hosts during host population cycles. Also, the data demonstrate that rodent sex does not affect bartonella prevalence and bacteremia level, but that rodent age does. The relationship between bartonella prevalence and

relative host abundance suggested a role of age in determining seasonal variation of bartonella prevalence.

The observations of the present study emphasize the importance of long-term studies, of continuity, and of replication in studying the dynamics of bartonella infections in their hosts and within their host populations. However, extrapolation of the patterns observed in the present study to those in other geographic areas, habitats, or climatic conditions may not be appropriate. To better understand the regulation of bartonellae in their rodent hosts, further analyses, modeling, and longer-term studies are required to discern any effects or to identify trends or cycles that have a multiyear periodicity. Such studies should be as complete as possible.

CHAPTER FIVE

HOST-SPECIFIC RELATIONS BETWEEN BARTONELLAE AND THEIR RODENT HOSTS

Investigations of bartonellae in rodents in Europe, North America, and Asia have indicated differences in the rodent host-specificity of bartonellae, and the degree of host-specificity. The pioneering work on bartonellae in rodents from the United Kingdom, by Birtles and his colleagues (1994), questioned host-specificity of bartonellae by finding that multiple bartonella species (*B. grahamii, B taylorii,* and *B. doshiae*) were circulating among woodland mammals of all dominant rodent species (*Apodemus sylvaticus, A. flavicollis, Myomys glareolus, Microtus agrestis* and *Neomys fodiens*). Subsequent investigations of bartonellae in rodent communities in Sweden found that *B. grahamii* frequently infected *Microtus* voles (*M. glareolus*), *Apodemus* mice (*A. flavicollis*, *A. sylvaticus*) and house mice (*Mus musculus*) (Holmberg et al., 2003). Recent longitudinal studies of the dynamics of bartonella infections in rodents from the UK demonstrated that a slightly noticeable level of bacterium – host selection among *Bartonella* species might occur (Telfer et al., 2007a).

At the same time, investigations of rodent-borne bartonella infections in North America and some Asian countries suggested completely different characters of bartonella – rodent relationships from those found in Europe, indicating definite host-specificity of bartonella strains and rodent species (Kosoy et al., 1997; Ying et al., 2002; Stevenson et al., 2003; Castle et al., 2004; Jardine et al., 2006a; Bai et al., 2007b).

Experimental evidence also supported those field observations. Hispid cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) inoculated with bartonellae became bacteremic only when the bartonellae were originally isolated from rodents of the same species or from rodents of a taxonomically close species (Kosoy et al., 2000).

Dramatic differences in observations of host-specificity between bartonellae and rodents from different geographic areas have presented an interesting model for understanding evolutionary trends in bacterium – rodent co-adaptation, considering a high level of natural bartonella infection among diverse rodent species and wide genetic diversity of the strains (Birtles et al., 1994; Kosoy et al., 1997; Ying et al., 2002). Taking into account that more and more species of rodent-borne bartonellae have been associated with human illness (Daly et al., 1993; Kerkhoff et al., 1999; Iralu et al., 2006), it is important to know the relationships between bartonellae and their rodent hosts. Matching isolates from human patients and rodents in the same area can provide useful information for identifying the source of the human infection.

It would be difficult, if not impossible, to study the phenomenon of host-specificity of phylogenetic group (species) without a clear definition of the status of the group. Poor definitions of bacterial species are common in bacteriology, but become especially clear in regard to bartonellae because of high genetic diversity and ecological plasticity of these bacteria. The former definitions of bacterial species that included determination of phenotypic characteristics and DNA-DNA homology are no longer popular. These approaches are especially inconvenient for the rapid characterization of fastidious bacteria, such as bartonellae.

Instead, gene-sequence-based criteria have been proposed to replace DNA-DNA hybridization, with a comparison of at least five housekeeping genes (Stackebrandt et al., 2002). La Scola et al. (2003) indicated two genes, RNA polymerase beta-subunit gene (*rpoB*) and citrate synthase gene (*gltA*), were the most potent in defining *Bartonella* species. The *gltA* became especially wide-used by scientists from all over the world and many sequences of this gene have been deposited in the GenBank database for public release. Other genes, such as cell division protein gene (*ftsZ*), riboflavin synthase gene (*ribC*), and 16S ribosomal RNA gene (16S rRNA) are also commonly used to characterize of *Bartonella*. In North America, in spite of the large number of strains from rodents of many species, only a limited number of rodent-associated *Bartonella* species have been described. Discrete clusters of related strains have been identified, usually using one genetic marker, commonly the *gltA*, but most clades have never been described at species or subspecies level.

In this chapter, I further summarize the level of host-specificity between bartonellae and their rodent hosts using large-scale sampling of rodents of multiple species from a variety of sites in the United States. I followed the most accepted approach to estimate the extent to which a parasite (bartonella) taxon is restricted in the number of host (rodent) species analyzed. For measuring the level of specificity to the rodent host, I used three categories, as follows: 1) known *Bartonella* species characterized by genetic and phenotypic approaches; 2) proposed *Bartonella* subspecies or genogroups, combining a cluster of closely related genetic variants by *gltA* sequences within a defined or proposed species; and 3) enumerated unique *gltA* variants distinguished by one or few nucleotides between them.

The objectives of this chapter are to compare bartonella strains isolated from rodents of a taxonomic group based on the similarities of partial sequences of *gltA*; to estimate host-specificity of bartonella strains having a wide range of taxonomic groups of the rodent hosts at levels of genus, species, or higher taxonomic categories; and to verify reliability of the *gltA* fragment as a marker for measuring host-specific relationships by additional genetic characterizations using four housekeeping genes.

MATERIALS AND METHODS

Bartonella isolates and host rodents

Bartonella cultures available for analysis were obtained from rodents trapped in 11 states of the U. S. (Arizona, California, Colorado, Georgia, Kansas, Nevada, New Mexico, North Carolina, South Dakota, Utah, and Wyoming). A total of 1,452 bartonella isolates were included for the analyses (Table 5-1).

The rodent hosts from which the bartonella isolates used in the present study were obtained were of two families (Cricetidae and Sciuridae); six genera (*Cynomys*, *Neotoma*, *Onychomys*, *Peromyscus*, *Spermophilus*, and *Tamias*); and 17 species (*Cynomys ludovicianus* [black-tailed prairie dog], *Neotoma albigula* [white-throated woodrat], *N. lepida* [desert woodrat], *N. mexicana* [Mexican woodrat], *N. micropus* [southern plains woodrat], *Onychomys leucogaster* [northern grass hopper mouse], *Peromyscus boylii* [brush mouse], *P. leucopus* [white-footed mouse], *P. maniculatus* [deer mouse], *P. truei* [pinyon mouse], *Spermophilus beecheyi* [California ground squirrel], *S. lateralis* [golden-mantled ground squirrel], *S. tridecemlineatus* [thirteen-lined ground squirrel],

Table 5-1 The number of bartonella isolates from each rodent species used for the phylogenetic study and the geographic origins of the rodents

Rodent species	Common name	Geographic origin and number of isolates	Total No. bartonella isolates	No. variants
Cynomys ludovicianus	Black-tailed prairie dog	CO (400)	400	9
Neotoma albigula	White-throated woodrat	AZ (1), CO (3), NM (21)	25	10
Neotoma lepida	Desert woodrat	CA (5), UT (6)	11	4
Neotoma mexicana	Mexican woodrat	AZ (2), CO (10)	12	8
Neotoma micropus	Southern plains woodrat	KS (1), NM (34)	35	5
Onychomys leucogaster	Northern grass hopper mouse	CO (20), KS (54), SD (5), WY (11)	90	10
Peromyscus boylii	Brush mouse	AZ (5), CA (6), CO (5), NM (49), UT (6)	71	16
Peromyscus leucopus	White-footed mouse	AZ (1), GA (2), KS (4), NC (22), NM (6)	35	6
Peromyscus maniculatus	Deer mouse	AZ (7), CA (2), CO (507), GA (4), KS (9), NC (13), NM (12), NV (8), SD (41), UT (2), WY (35)	640	26
Peromyscus truei	Pinyon mouse	AZ (12), NM (11), UT (5), NV (1)	29	14
Spermophilus beecheyi	California ground squirrel	NV (6), NM (1)	7	4
Spermophilus lateralis	Golden-mantled ground squirrel	CO (6) NV(4)	10	3
Spermophilus tridecemlineatus	Thirteen-lined ground squirrel	CO (4), KS (4), SD (1), WY (16)	25	2
Spermophilus variegatus	Rock squirrel	NM (15)	15	7
Tamias dorsalis	Cliff chipmunk	AZ (9), UT (4), NM (1)	14	5
Tamias minimus	Least chipmunk	CO (6), NM (1), NV (13)	20	10
Tamias umbrinus	Uinta chipmunk	CO (13)	13	5

AZ: Arizona; CA: California; CO: Colorado; GA: Geogia; KS: Kansas; NC: North Corolina; NM: New Mexico; NV: Nevada; SD: South Dakota; UT: Utah; WY: Wyoming.

S. variegatus [rock squirrel], *Tamias dorsalis* [cliff chipmunk], *T. minimus* [least chipmunk], and *T. umbrinus* [Uinta chipmunk]). The taxonomic relationships of these rodents are shown in Figure 5-1.

PCR amplification

Genomic DNA was extracted from bacterial cultures using a Qiagen DNA extraction kit (Qiagen, Chatsworth, CA). A 379-bp fragment of the *gltA* gene was amplified using specific primers (Table 5-2). PCR procedures for the *gltA* were the same as those described in Chapter 3. The *gltA* fragment was amplified for all bartonella cultures used in the present study.

Four additional genes, 16S rRNA, *rpoB*, *ftsZ*, and *ribC* were selected for characterizing bartonellae associating with black-tailed prairie dogs. Five bartonella isolates obtained from black-tailed prairie dogs that each represents a known *gltA* variant (see "RESULTS") were used to amplify the four target genes. The primers used for amplification of each gene have been described elsewhere (Marchesi et al., 1998; Schwieger and Tebbe, 1998; Renesto et al., 2001; Schmalenberger et al., 2001; Zeaiter et al., 2002; Johnson et al., 2003) and are presented in Table 5-2. For each gene, PCR amplifications were performed in a 50-μL reaction mixture containing 5μl 10X PCR buffer, 5 pmol of each appropriate primer, 200 μM of each dNTP (Invitrogen, Cergy-Pontoise, France), 2.5 U Taq DNA polymerase (EuroblueTaq, Eurobio, Les Ulis, France), and 2.5 μL DNA. Positive and negative controls were included in each PCR run to evaluate the presence of appropriately sized amplicons and contaminants, respectively. All PCRs were carried out in a PTC 200 Peltier thermal cycler (MJ Research, Inc.,

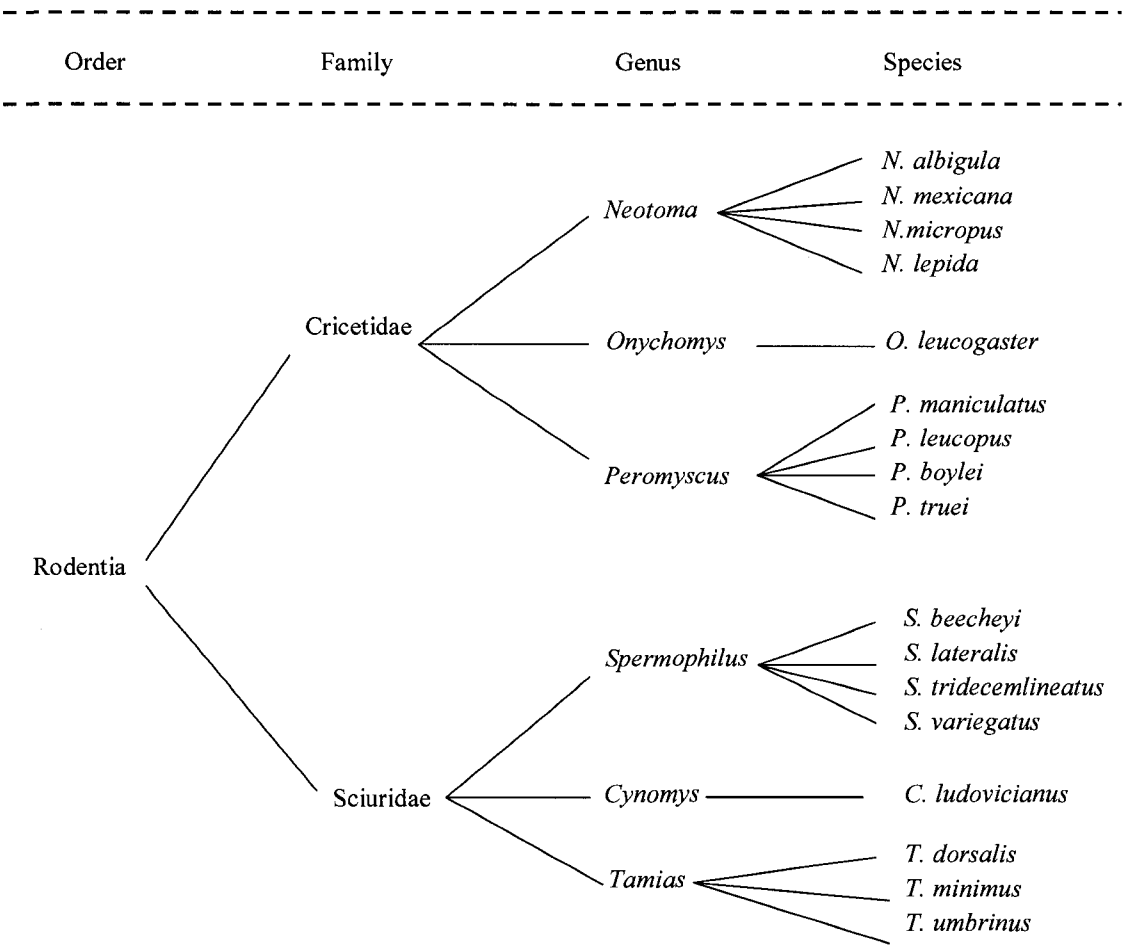

Figure 5-1 Taxonomic relationships of the related rodents in the present study

Table 5-2 Primers used for Polymerase Chain Reaction (PCR) amplification and/or sequencing of five genes of bartonellae associating with black-tailed prairie dogs

gene	Primer *	Primer sequence
gltA	BhCS781p (ap, sp)	5'-GGG GAC CAG CTC ATG GTG G-3'
	BhCS1137n (ap, sp)	5'-AAT GCA AAA AGA ACA GTA AAC A-3'
16S rRNA	63f (ap, sp)	5'-CAG GCC TAA CAC ATG CAA GTC-3'
	101f (sp)	5'-ACT GGC GGA CGG GTG AGT AA-3'
	519f (sp)	5'-CAG CAG CCG CGG TAA TAC-3'
	537r (sp)	5'-CGT ATT ACC GCG GCT GCT GG-3'
	926r (sp)	5'-CCG TCA ATT CCT TTG AGT TT-3'
	1387r (ap, sp)	5'-GGG CGG WGT GTA CAA GGC-3'
rpoB	1400f (ap, sp)	5'-CGC ATT GGC TTA CTT CGT ATG-3'
	1596f (sp)	5'-GGA CAA ATA CGA CCA TAA TGC G-3'
	1873r (sp)	5'-TCY TCC ATM GCW GAM AGA TAA A-3'
	2300r (ap, sp)	5'-GTA GAC TGA TTA GAA CGC TG-3'
ftsZ	Bfp1 (ap, sp)	5'-ATT AAT CTG CAY CGG CCA GA-3'
	Bfp2 (ap, sp)	5'-ACV GAD ACA CGA ATA ACA CC-3'
	Bfp3 (sp)	5'-TTA CAA AAA TCY GTT GAT AC-3'
	Bfp4 (sp)	5'-GTA TCA ACR GAT TTT TGT AA-3'
ribC	BARTON-1 (ap, sp)	5'-TAA CCG ATA TTG GTT GTG TTG AAG-3'
	BARTON-2 (ap, sp)	5'-TAA AGC TAG AAA GTC TGG CAA CAT AAC G-3'

* Abbreviations: ap, amplification primer; sp, sequencing primer.

Waltham, MA), and all amplicons were resolved by 1.5% agarose gel electrophoresis and visualized by staining with ethidium bromide. The program parameters for each gene are provided as below:

16S rRNA: PCR amplification included 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min followed by a final extension step of 5 min at 72°C.

rpoB: following a first denaturation step (94°C for 2 min), a three-step cycle of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min was repeated 35 times. The PCR program was ended by a single 2-min extension step at 72°C.

ftsZ: a 4-min denaturation at 94°C was followed by 44 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 60 s. Amplification was completed by maintaining the reaction mixture at 68°C for 10 min.

ribC: an initial denaturation at 95°C for 10 min was followed by 37 cycles of 95°C for 1 min, 51°C for 1 min, and 72°C for 1 min. Amplification was completed by a final incubation at 72°C for 3 min.

Sequencing

The PCR products from *gltA* and the other four genes were purified using the QIAquick PCR Purification Kit (Qiagen, Germantown, Maryland) following the instructions of the manufacturer, and were sequenced in both directions directly, using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). Sequencing reactions were carried out in a PTC 200 Peltier Thermal cycler. The primers used for each gene are presented in Table 5-2, at a concentration of 1.6 μM. Cycle parameters for the sequencing reactions were 45 cycles at

96°C for 20 seconds, 50°C for 20 seconds, and 60°C for 4 minutes. The reaction products then were purified using a DyeEx 2.0 Spin Kit (Qiagen). Removal of the primer sequences from the full-length amplicons resulted in fragments of 338bp, 1289bp, 828bp, 910bp, and 587bp for *gltA*, 16S rRNA, *rpoB*, *ftsZ*, and *ribC*, respectively.

Phylogenetic analysis

All sequences were analyzed using Lasergene sequence analysis software (DNASTAR Inc, Madison, WI) to determine consensus sequences for the amplified region of each gene. The Clustal V program within Megalign (DNASTAR) was used to align and compare homologous bartonella sequences from the present study and from the GenBank database. Identifications of unique variants were based on the comparison of homogeneity for each gene.

Using the PAUP software program (Center for Biodiversity, Illinois Natural History Survey, Champaign, IL), the sequences of the five genes of bartonellae in black-tailed prairie dogs were concatenated and aligned with the homologous genes for other bartonellae obtained from GenBank, using a partition homogeneity test. Phylogenetic relationships among various species of *Bartonella* and black-tailed prairie dog-associated bartonellae were estimated using maximum likelihood. Support for nodes in the tree was assessed using bootstrapping with 1000 replicates, assuming three different objective criteria: parsimony, distance, and maximum likelihood. For the parsimony bootstrap analysis, all changes were unordered and assigned the same weight. For the distance analysis, the neighbor-joining clustering algorithm was used. For maximum likelihood, the heuristic search algorithm was used. In addition, it was asked whether each gene

showed the same support as the analysis based on all genes combined, using maximum likelihood. All tests were performed under an assumption of the HKY model.

RESULTS

Bartonella variants in ground squirrels of the genus *Spermophilus*

The genus *Spermophilus* is the largest genus of ground squirrels and the one that contains the species that are most common in North America. A total of 57 bartonella *gltA* sequences from ground squirrels of four species (*S. beecheyi, S. lateralis, S. tridecemlineatus,* and *S. variegatus*) were analyzed. Details are presented by host species, as below:

S. beecheyi: seven sequences obtained from California ground squirrels were analyzed. The squirrels were captured in Nevada (6) and New Mexico (1) (Table 5-1). The seven sequences, identical or similar to each other, were assigned to four variants. One variant with two sequences is identical to *B. washoensis* that was originally obtained from a human patient from Nevada (Kosoy et al., 2003) and is the prototype to compare sciurid-associated bartonellae in this study. The other three variants contained 3, 1, and 1 sequence, respectively, with divergence ranging from 1.8% to 3.3%, and are clustered with *B. washoensis*.

S. variegatus: 15 sequences were obtained from rock squirrels captured in New Mexico, and were assigned to seven variants. Two variants, with 5 and 1 sequence for each, are identical to two variants detected in *S. beechyi*, including the one identical to *B. washoensis*. The other five variants contain 1, 2, 1, 3, and 2 sequences, respectively.

Genetic divergences between the variants range from 0.3% to 4.2%, and these variants also are clustered with *B. washoensis*.

S. lateralis: 10 sequences were obtained from golden-mantled ground squirrels captured in Colorado (6) and Nevada (4). These sequences were assigned to three unique *gltA* variants with 5, 4, and 1 sequence for each. These variants, with divergences of 1.5% to 2.7% between them, did not match bartonellae from other *Spermophilus* squirrels or from rodents of other species, but also are clustered together in close proximity to *B. washoensis*.

S. tridecemlineatus: 25 sequences were obtained from thirteen-lined ground squirrels captured from Colorado (4), Kansas (4), South Dakota (1), and Wyoming (16). These sequences, which are almost identical, were assigned to two variants with 17 and 8 sequences for each. The two variants, distinguished by only one nucleotide difference (0.3% divergence), also are close to *B. washoensis*. These sequences had not been detected in ground squirrels of other species; but one variant is identical to a common variant found in black-tailed prairie dogs (see below).

Overall, phylogenetic analyses of 57 bartonella *gltA* sequences from ground squirrels of the genus *Spermophilus* demonstrated 14 unique *Bartonella gltA* variants. Two variants, one of which is identical to the human isolate of *B. washoensis*, were detected in both California ground squirrels and rock squirrels. The other 12 variants each were related to a specific *Spermophilus* species. These variants, with divergence of 0.3% - 4.2%, are identical or close to the prototype strain of *B. washoensis* (Figure 5-2).

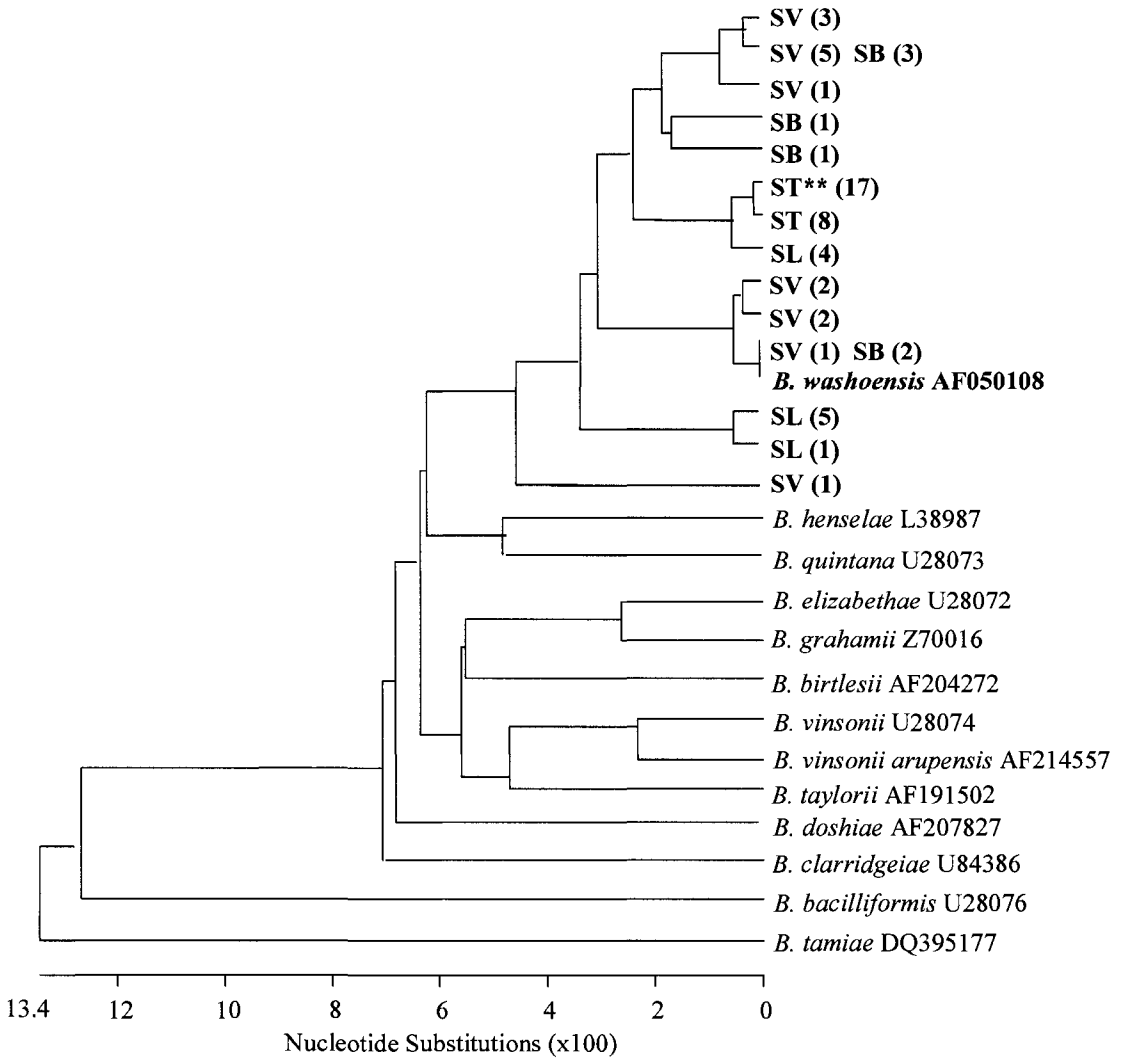

Figure 5-2 Phylogenetic relationships between *Spermophilus*-associated *Bartonella* variants and reference *Bartonella* species based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*). Numbers in parentheses indicate frequency of bartonella sequences belonging to the same variant obtained from a certain *Spermophilus* species. ** the variant identical to a variant detected in black-tailed prairie dogs. SB: *Spermophilus beecheyi*; ST: *S.tridecemlineatus*; SL: *S. lateralis*; SV: *S. variegatus*.

Bartonella variants in prairie dogs of genus *Cynomys*

The intensive long-term study conducted by the University of Colorado to study disease dynamics in black-tailed prairie dogs from different colonies has provided a large collection of blood samples of these rodents from Boulder County, Colorado.

A total of 400 bartonella *gltA* sequences were obtained from black-tailed prairie dogs, a species belonging to the family Sciuridae and evolutionarily close to ground squirrels. Phylogenetic analyses revealed nine unique variants among these sequences, with number of sequences as 169, 75, 61, 26, 23, 17, 17, 7, and 5 within each. All variants, with divergence ranging from 0.3% to 2.4%, form a separate group within *B. washoensis* clade. One of these variants is identical to a variant detected in thirteen-lined ground squirrels; beyond that, none of the variants identified in other species of *Spermophilus* or in other rodents belong to this cluster formed by variants of bartonellae from black-tailed prairie dogs. This group with divergence of 3.6% - 8.8% to *B. washoensis* presumably represents a subspecies of *B. washoensis* (Figure 5-3). I conducted additional characterizations of this group of bartonellae, with the results being provided below.

Characterization of black-tailed prairie dog-associated bartonellae

To verify the prediction that bartonellae associating with black-tailed prairie dogs represents a separate subspecies, I conducted a multi-gene examination of five isolates representing five identified *gltA* variants, targeting 16S rRNA, *rpoB*, *ftsZ*, and *ribC*.

Comparison of sequences obtained from these five isolates demonstrated 2, 3, 5, and 4 unique sequences for 16SrRNA, *rpoB*, *ftsZ*, and *ribC*, respectively.

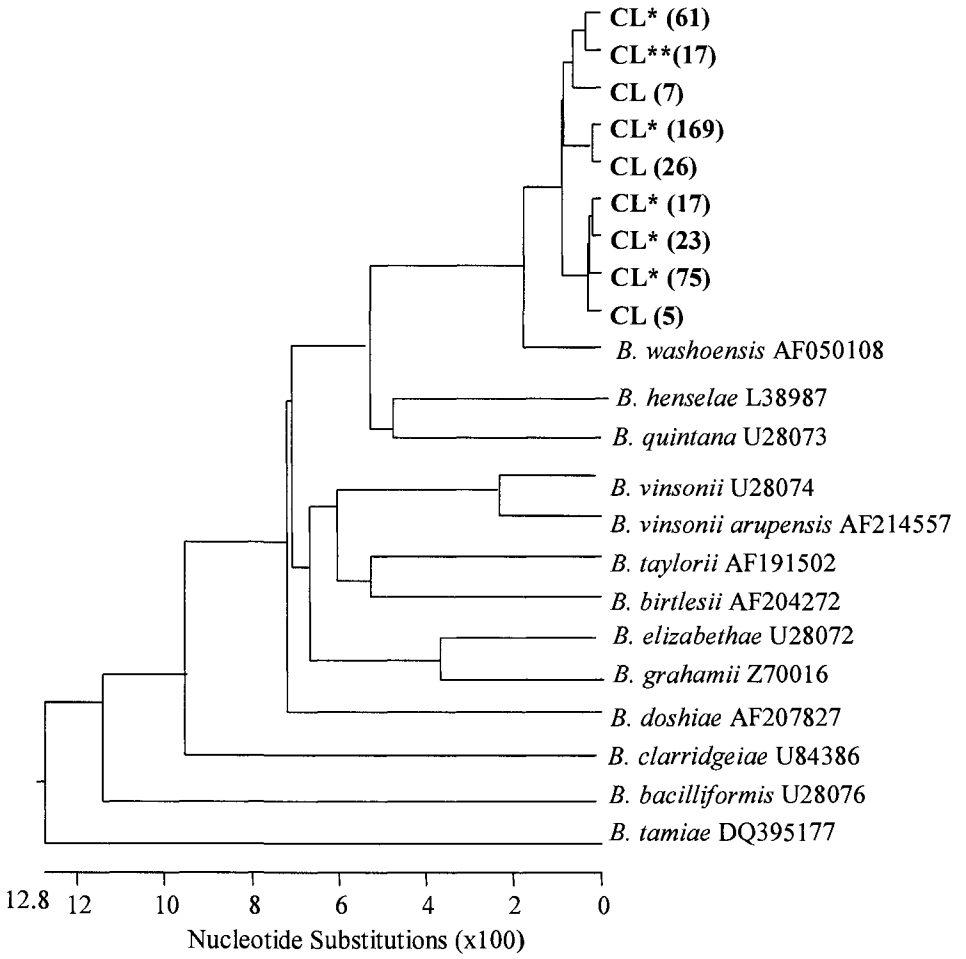

Figure 5-3 Phylogenetic relationships between *Cynomys*-associated *Bartonella* variants and reference *Bartonella* species based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*). Numbers in parentheses indicate frequency of bartonella sequences belonging to the same variant obtained from black-tailed prairie dogs. * variants used for characterization. ** the variant also detected in thirteen-lined ground squirrels. CL: *Cynomys ludovicianus.*

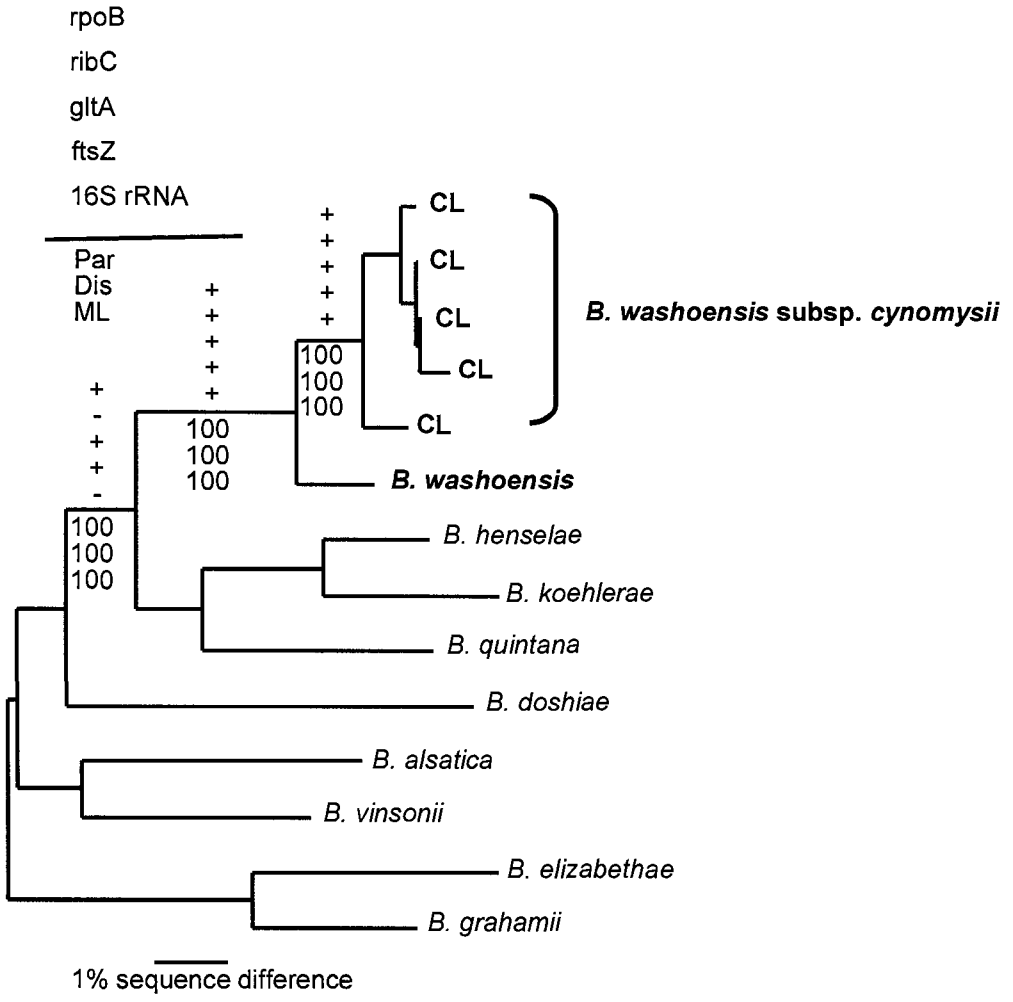

Figure 5-4 Phylogenetic relationships between the newly recognized subspecies associated with black-tailed prairie dogs (*B. w. cynomysii*) and reference *Bartonella* species inferred from the concatenated sequences for five genes (*rpoB*, *ribC*, *gltA*, *ftsZ*, and 16S rRNA) using maximum likelihood assuming a HKY model of evolution. Numbers below key branches defining relationships of the new subspecies are bootstrap values for analyses using parsimony, distance and maximum likelihood methods. A plus or a minus above the branches indicates whether particular genes analyzed separately using maximum likelihood match the topology generated from all genes combined, where a plus indicates that the clade was present and a minus indicates the particular clade was not present when only the specific gene was analyzed.

Phylogenetic analyses of the five genes for the five variants defined by the *gltA* sequences provided strong support for a distinct monophyletic clade most closely related to the *B. washoensis* (Figure 5-4). The new strains grouped together in 100% of the bootstrapped trees for all three phylogenetic methods employed. All five genes supported the close association of the strains. Moreover, all five genes grouped the new strains with *B. washoensis*. This group of bartonellae has been named Candidatus *Bartonella washoensis* subsp. *cynomysii*, with the type strain CL8606co^{T} being deposited in the American Type Culture Collection, USA (ATCC BAA-1342 ^{T}) and in the Culture Collection of the University of Göteborg, Sweden (CCUG 53213 ^{T}) (Bai et al., 2008).

Bartonella* variants in chipmunks of genus *Tamias

Chipmunks are small squirrel-like rodents. A total of 47 bartonella *gltA* sequences from chipmunks of three species (*T. dorsalis*, *T. minimus*, and *T. umbrinus*) were analyzed. Details are presented below, by host species.

T. dorsalis: 14 sequences were obtained from cliff chipmunks collected in Arizona (9), New Mexico (1), and Utah (4) (Table 5-1). Phylogenetic analyses revealed five variants among these sequences. Of these, four variants, with number of sequences as 6, 5, 1, and 1 for each, were found only in cliff chipmunks. With divergences of 0.9% - 1.8% between them, the four variants are clustered together with *B. washoensis* (Figure 5-5), but form a group separated from bartonellae from ground squirrels or prairie dogs.

The last variant, with a single sequence, is >9% divergent from other variants, but is identical to a variant typical of deer mice in its *gltA* sequence (Figure 5-5). Likely, the bartonella was accidentally jumped onto the chipmunk (Woolhouse et al., 2005).

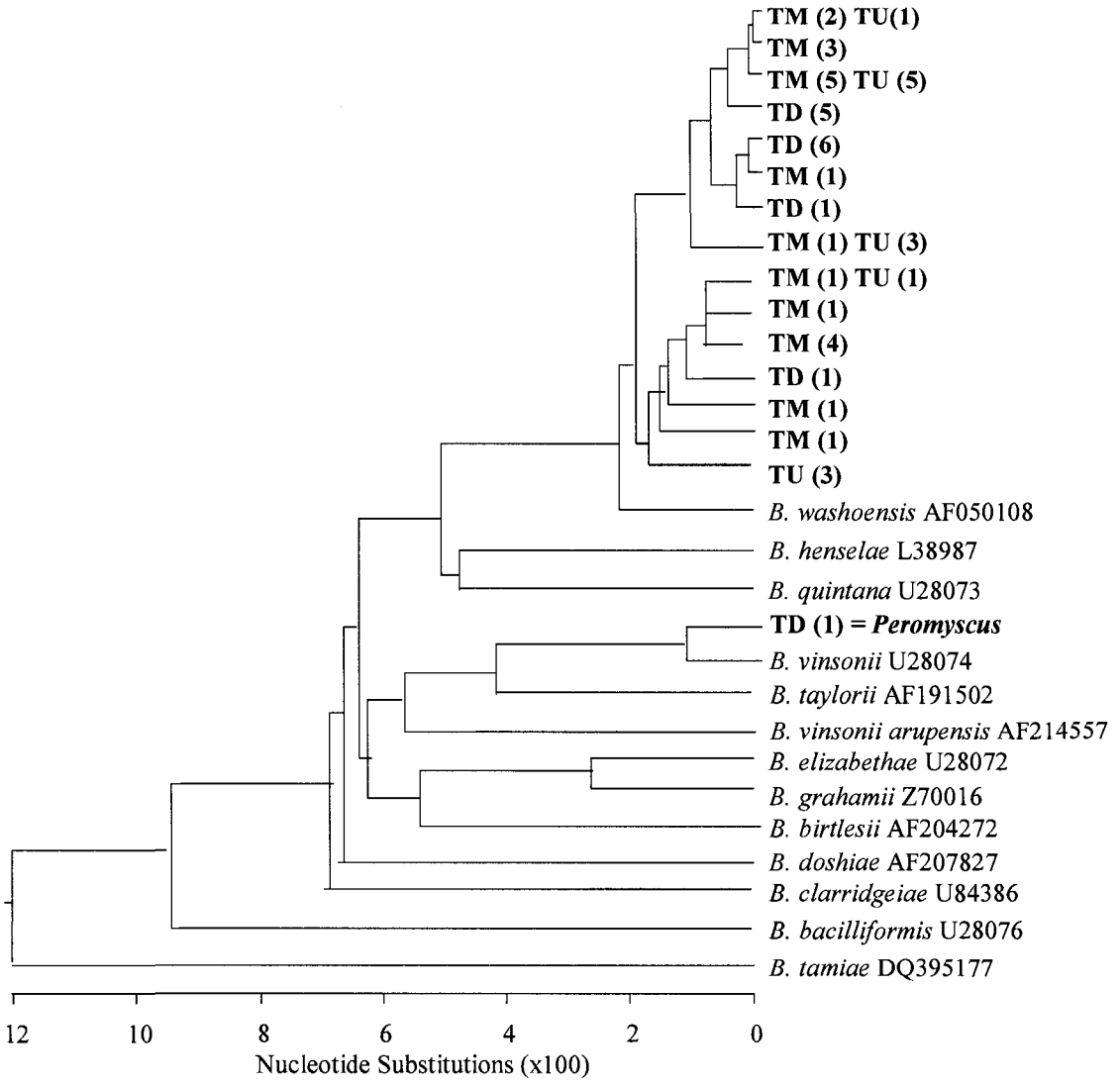

Figure 5-5 Phylogenetic relationships between *Tamias*-associated *Bartonella* variants and reference *Bartonella* species based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*), showing the group that is specific to *Tamias*, and one non-specific variant. Numbers in parentheses indicate frequency of bartonella sequences belonging to the same variant obtained from a certain *Tamias* species. TD: *Tamias dorsalis*; TM: *T. minimus*; TU: *T. mbrinus*.

T. minimus: 20 sequences were obtained from least chipmunks captured in Colorado (6), New Mexico (1), and Nevada (13) (Table 5-1). Phylogenetic analyses revealed 10 *gltA* unique variants among these sequences, with number of sequences ranging from 1 to 5 for each. These variants, with divergences of 0.3% - 2.4%, are all clustered together with bartonella from the cliff chipmunk next to *B. washoensis*, and are separated from bartonellae recovered from ground squirrels or prairie dogs.

T. umbrinus: The Uinta chipmunk, collected in Colorado, was represented with 13 bartonella *gltA* sequences. The sequences were assigned to five *gltA* variants, with the number of sequences ranging 1-5 per variant. These variants, with divergences of 0.6% - 1.2%, are clustered near *B. washoensis*; four of them are identical to those identified in least chipmunks.

Overall, phylogenetic analyses of 47 bartonella *gltA* sequences from chipmunks of the genus *Tamias* revealed 16 unique variants. Of these, 15 variants (46 sequences), with divergences of 0.3% - 2.4% among them, form a separate group within *B. washoensis* clade (Figure 5-5). This group contains no variants from rodents of other genera, and presumably represents a subspecies of *B. washoensis* with divergences of 2.1% - 3.4% from *B. washoensis*. Four variants were shared between least chipmunks and Uinta chipmunks. The other 11 variants each were related to a specific *Tamias* species. The remaining variant likely was the result of a bartonella jumping from its specific host to a non-specific host.

Bartonella variants in peromyscines of genus *Peromyscus*

The genus *Peromyscus* consists of large number of species, with *P. maniculatus* and *P. leucopus* as the most common in the continental United States. *P. maniculatus* also is the most populous mammalian species in the USA, which can explain why a large number of bartonella isolates were obtained from them. A total of 775 sequences from peromyscines of four species (*P. boylii*, *P. leucopus*, *P. maniculatus*, and *P. truei*) were analyzed. Details are presented below by species.

P. maniculatus: 640 bartonella *gltA* sequences were obtained from deer mice from Arizona (7), California (2), Colorado (507), Georgia (4), Kansas (9), Nevada (8), New Mexico (12), North Carolina (13), South Dakota (41), Utah (2), and Wyoming (35). Phylogenetic analyses demonstrated 26 variants among them. One variant, containing 460 sequences, apparently is the most common. This variant, together with 19 other variants of 172 sequences (1-30 sequences per variant), and with divergence of 0.3% - 2.4% between them, form a group within the clade of *B. vinsonii* (prototype used to compare cricetid-associated bartonellae in this study) and belongs to *B. vinsonii* subsp. *arupensis*, a strain that originally had been isolated from a patient from Wyoming (Welch et al., 1999). In fact, one variant with 26 sequences is identical to the human isolate by *gltA* sequence (Figure 5-6). That 632 of 640 sequences of bartonella from deer mice belong to the same group suggests that this is a genotype typical of deer mice.

Four of the 26 variants, with divergence of 0.3% - 2.4% and containing 6 sequences, fall into another group, namely, "boylii-truei", within *B. vinsonii* clade. In fact, this group is typical of brush mice and pinyon mice (see below). The divergence was 3.0% - 4.2% between variants of this group and those of "arupensis" group.

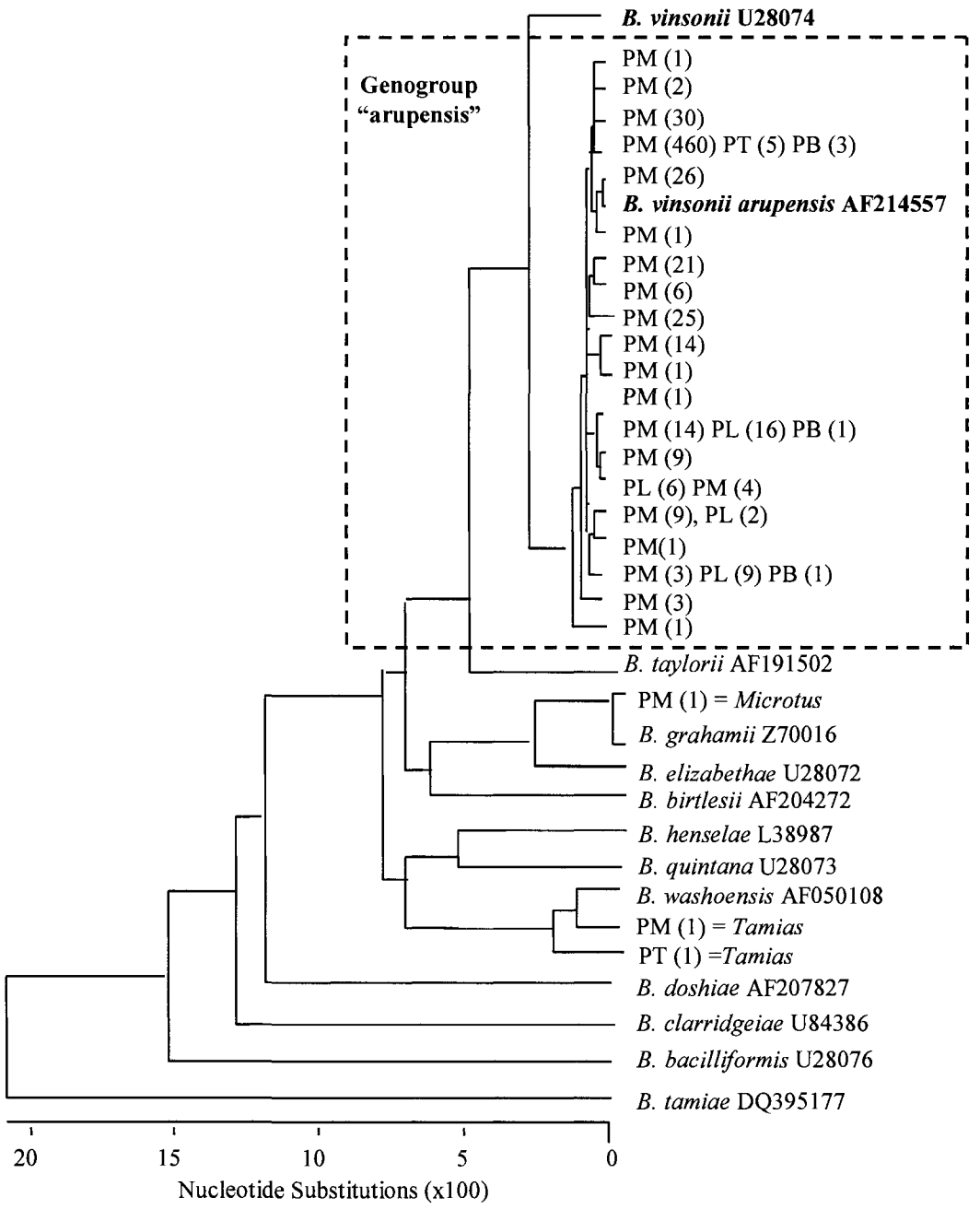

Figure 5-6 Phylogenetic relationships between *Peromyscus*-associated *Bartonella* variants and reference *Bartonella* species based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*). The variants form the group "arupensis" that is typical of PM and PL. Numbers in parentheses indicate frequency of bartonella sequences belonging to the same variant obtained from a certain *Peromyscus* species. PB: *Peromyscus boylii*; PL: *P. leucopus*; PM: *P. maniculatus*; PT: *P. truei.*

The last two variants, each contains a single sequence, with divergence >8.4%, and did not belong to either group described above. Instead, the two variants each are identical or close to bartonellae specific for other rodent species: one variant identified in a deer mouse from Colorado is identical to a variant of chipmunks; the second variant of deer mouse from South Dakota is identical to a previously described variant of microtine voles (the genus *Microtus*). These observations suggest that bartonellae may have jumped to deer mice from rodents of other species, although rarely.

P. leucopus: Phylogenetically, white-footed mice are very close to deer mice. Of 35 sequences obtained from white-footed mice collected in Arizona (1), Georgia (2), Kansas (4), New Mexico (6), and North Carolina (22), 33 are very close to each other and are assigned to 4 variants with divergence of 0.3% - 0.9%. These variants are identical to those described in deer mice, and belong to the "arupensis" group (Figure 5-6). The other two sequences, with divergence of 1.8%, each represent a distinct variant and belong to the "boylii-truei" group.

P. boylii: 71 bartonella *gltA* sequences were obtained from brush mice collected in Arizona (5), California (6), Colorado (5), New Mexico (49), and Utah (6). These sequences were assigned to 16 variants, with divergences ranging from 0.3% to 4%. One variant, containing 25 sequences, is the most common. This variant, together with 12 other variants of 41 sequences (1-6 sequences per variant), and with divergences of 0.3% - 2.7%, form the "boylii-truei" group that is typical of brush mice and pinyon mice, as mentioned above (Figure 5-7). The other three variants, containing 5 sequences, with divergence of 0.3% - 0.5%, are identical to variants from deer mice and belong to the "arupensis" group.

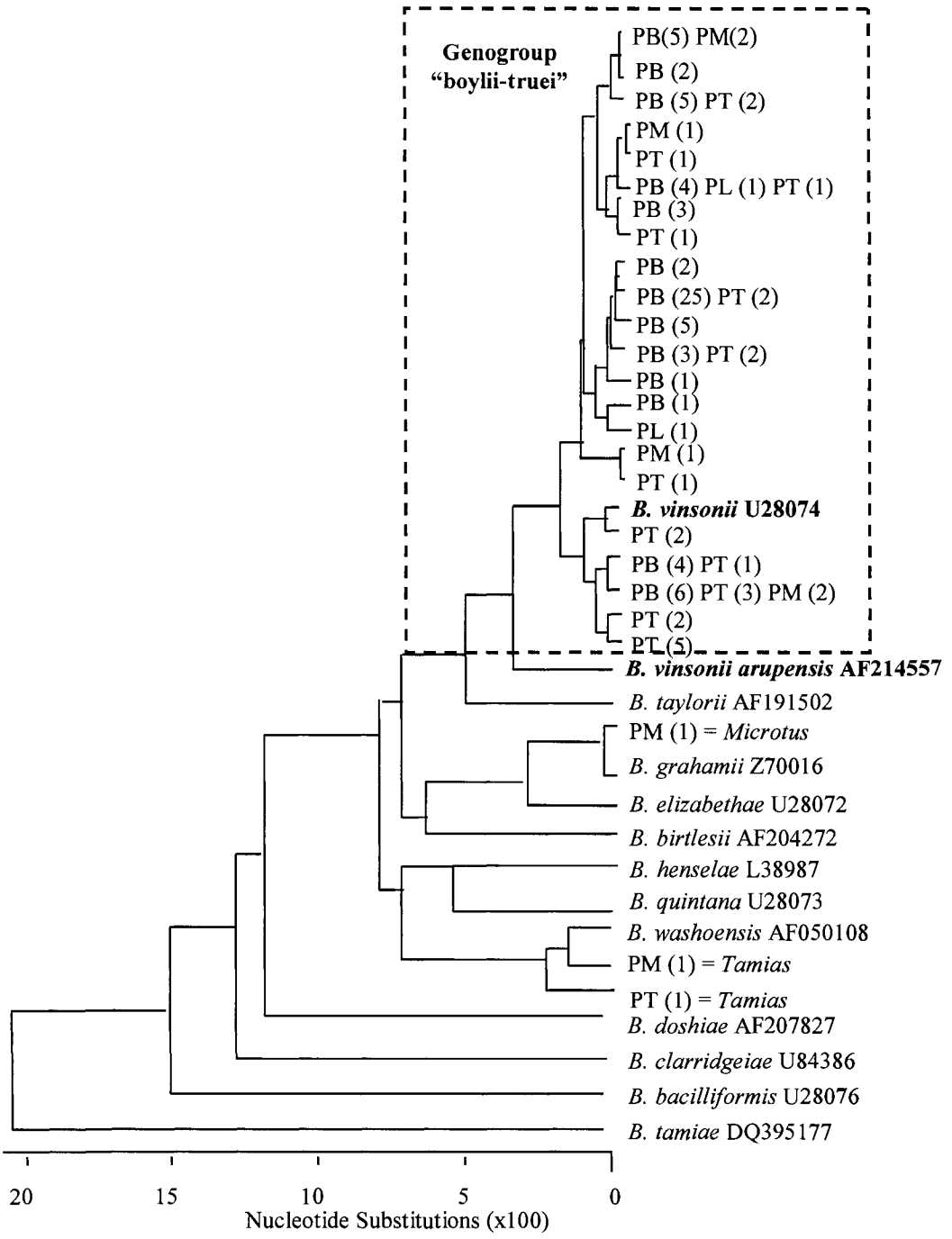

Figure 5-7 Phylogenetic relationships between *Peromyscus*-associated *Bartonella* variants and reference *Bartonella* species based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*). The variants form the group "boylii-truei" that is typical of PB and PT. Numbers in parentheses indicate frequency of bartonella sequences belonging to the same variant obtained from a certain *Peromyscus* species. PB: *Peromyscus boylii*; PL: *P. leucopus*; PM: *P. maniculatus*; PT: *P. truei.*

P. truei: 29 sequences were obtained from pinyon mice collected in Arizona (12), Nevada (1), New Mexico (11), and Utah (5), and were assigned to 14 variants. Of these, 12 variants of 23 sequences, with divergences ranging from 0.3% to 3%, belong to the "boylii-truei" group (Figure 5-7); one variant with 5 sequences was identical to a variant identified in deer mice and belongs to the "arupensis" group; the last variant with a single sequence from a pinyon mouse trapped in Arizona is identical to a variant of bartonella from cliff chipmunks, showing a bartonella jump from chipmunks to brush mice.

Overall, phylogenetic analyses showed that peromyscine-associated bartonellae are in the *B. vinsonii* clade, but represent two genogroups of the species. Although the variants of any of the four species studied can cross the genogroups, one genogroup contains bartonellae principally obtained from deer mice and white-footed mice, and another genogroup contains bartonellae mainly from brush mice and pinyon mice (Figure 5-8). Sometimes, peromyscine mice can acquire bartonellae that are not specific to them, but such events appear to be rare (3 of 775 as observed).

Bartonella variants in woodrats of the genus *Neotoma*

A total of 84 *gltA* sequences obtained from woodrats of four species, including *N. albigula*, *N. lepida*, *N. mexicana*, and *N. micropus* were analyzed. Bartonellae associating with these rodents exhibited a large heterogeneity in *gltA* sequences.

N. albigula: 25 sequences were obtained from white-throated woodrats captured in Arizona (1), Colorado (3), and New Mexico (21). Phylogenetic analyses revealed 10 *gltA* variants, with the number of sequences ranging 1-12 for each. Of the variants, nine are within *B. vinsonii* clade, with variant divergences of 0.3% - 5.3%. The last variant with

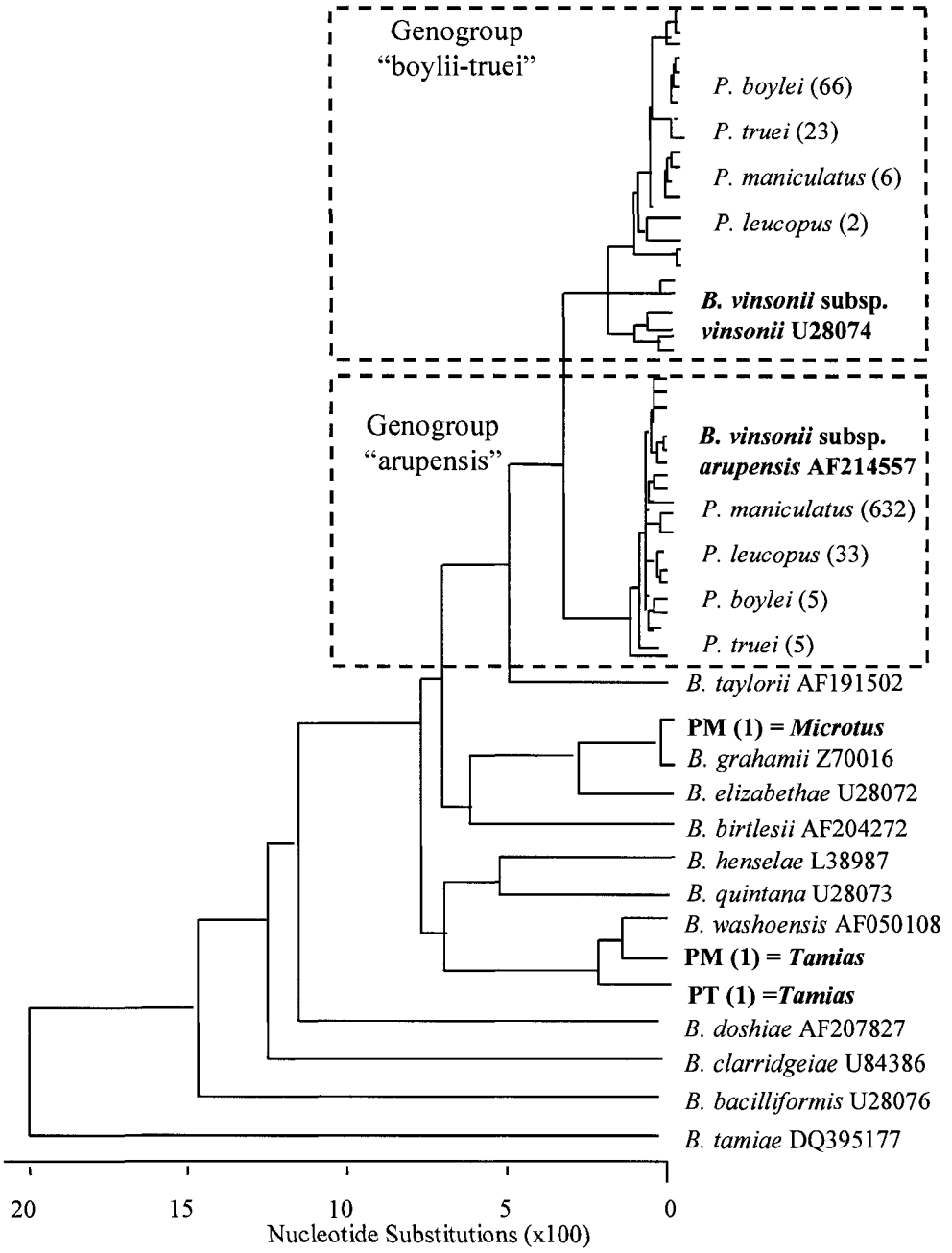

Figure 5-8 Phylogenetic relationships between *Peromyscus*-associated *Bartonella* variants and reference *Bartonella* species based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*), showing two genogroups identified among all variants, and non-specific bartonellae acquired by *Peromyscus* mice. Numbers in parentheses indicate frequency of bartonella sequences obtained from a certain *Peromyscus* species. PB: *Peromyscus boylii*; PL: *P. leucopus*; PM: *P. maniculatus*; PT: *P. truei.*

a single sequence is distant from all other variants in white-throated woodrats, with divergence of 9.3% - 11.1%, but identical to a variant typical of microtine voles (Figure 5-9).

N. lepida: 11 sequences were obtained from desert woodrats captured in California (5) and Utah (6). Four variants were identified among the sequences, one of which also was detected in white-throated woodrats. All variants are within the *B. vinsonii* clade, with divergence ranging from 0.9% to 6.9%.

N. mexicana: 12 sequences were obtained from Mexican woodrats captured in Arizona (2) and Colorado (10), and were assigned to eight variants. Seven of these are within the *B. vinsonii* clade, with divergence ranging 1.2% - 6.9%. The last variant is distant from all others, with divergence of 10.4% -12.1%, and is identical to a variant found in chipmunks (Figure 5-9).

N. micropus: 35 sequences were obtained from southern plains woodrats captured in Kansas (1) and New Mexico (34). Phylogenetic analyses revealed five *gltA* variants. One variant, containing 26 sequences, including the one from Kansas, appears to be the most common in southern plains woodrats. Moreover, this variant was not detected in woodrats of other species. This variant, and three (6 sequences) other that were also detected in woodrats of other species (2 variants in Mexican woodrats, 1 in white-throated woodrats), are clustered together, with divergence ranging from 0.9% to 2.7%, and are within the *B. vinsonii* clade. The last variant was ≥9.6% divergent from all others, and is actually close to a variant found in microtine voles (Figure 5-9).

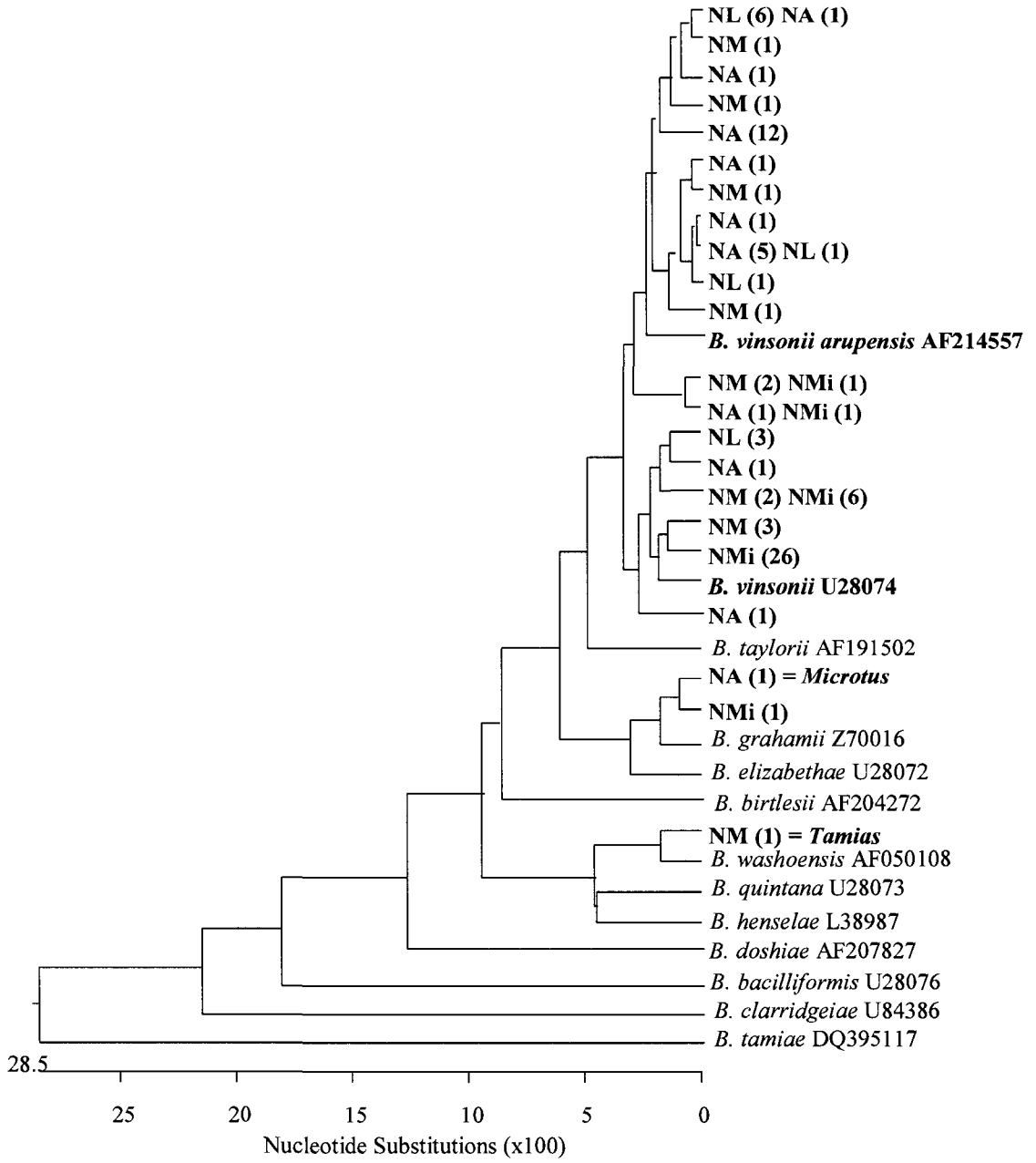

Figure 5-9 Phylogenetic relationships between *Neotoma*-associated *Bartonella* variants and reference *Bartonella* species based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*), showing the genogorup specific to *Neotoma*, and non-specific bartonellae acquired by *Neotoma* woodrats. Numbers in parentheses indicate frequency of bartonella sequences belonging to the same variant obtained from a certain *Neotoma* species. NA: *Neotoma albigula*; NM: *N. mexicana*; NMi: *N. micropus*; NL: *N. lepida*.

Overall, 22 bartonella *gltA* variants were identified among 84 sequences in woodrats, with five variants shared by rats of different *Neotoma* species, including two in white-throated woodrats and desert woodrats, two in Mexican woodrats and southern plains woodrats, and one in white-throated woodrats and southern plains woodrats; and the other variants each are related to a particular host species. Of all variants, 19 (81 sequences) are within *B. vinsonii* clade; 11 (34 sequences) of the 19 variants, with divergence of 0.3% - 2.7%, are clustered more closely with each other and form a separate group. This group, with divergence of 2.1% - 4.6% from *B. vinsonii* subsp. *arupensis*, presumably represents another subspecies of *B. vinsonii*, and is typical of woodrats of the genus *Neotoma* (Figure 5-9). Acquisition of non-specific bartonella strain also was observed rarely among woodrats.

***Bartonella* variants in grasshopper mice (genus *Onychomys*)**

The genus *Onychomys* is a member of Cricetidae family. A total of 90 sequences from the northern grasshopper mouse were analyzed. The host mice were from Colorado (20), Kansas (54), South Dakota (5), and Wyoming (11).

The bartonella *gltA* sequences from these mice exhibited considerable heterogeneity. Phylogenetic analyses revealed 11 unique genetic variants and they are clustered into four phylogenetic groups (Figure 5-10) with divergence from 3.3% to 11.6%. Of the four genogroups, two contain bartonellae only from grasshopper mice, and are different from all previously described bartonella variants found in any other rodents, thus are specific to grasshopper mice; the other two genogroups are identical to bartonella found in rodents of other species (see below).

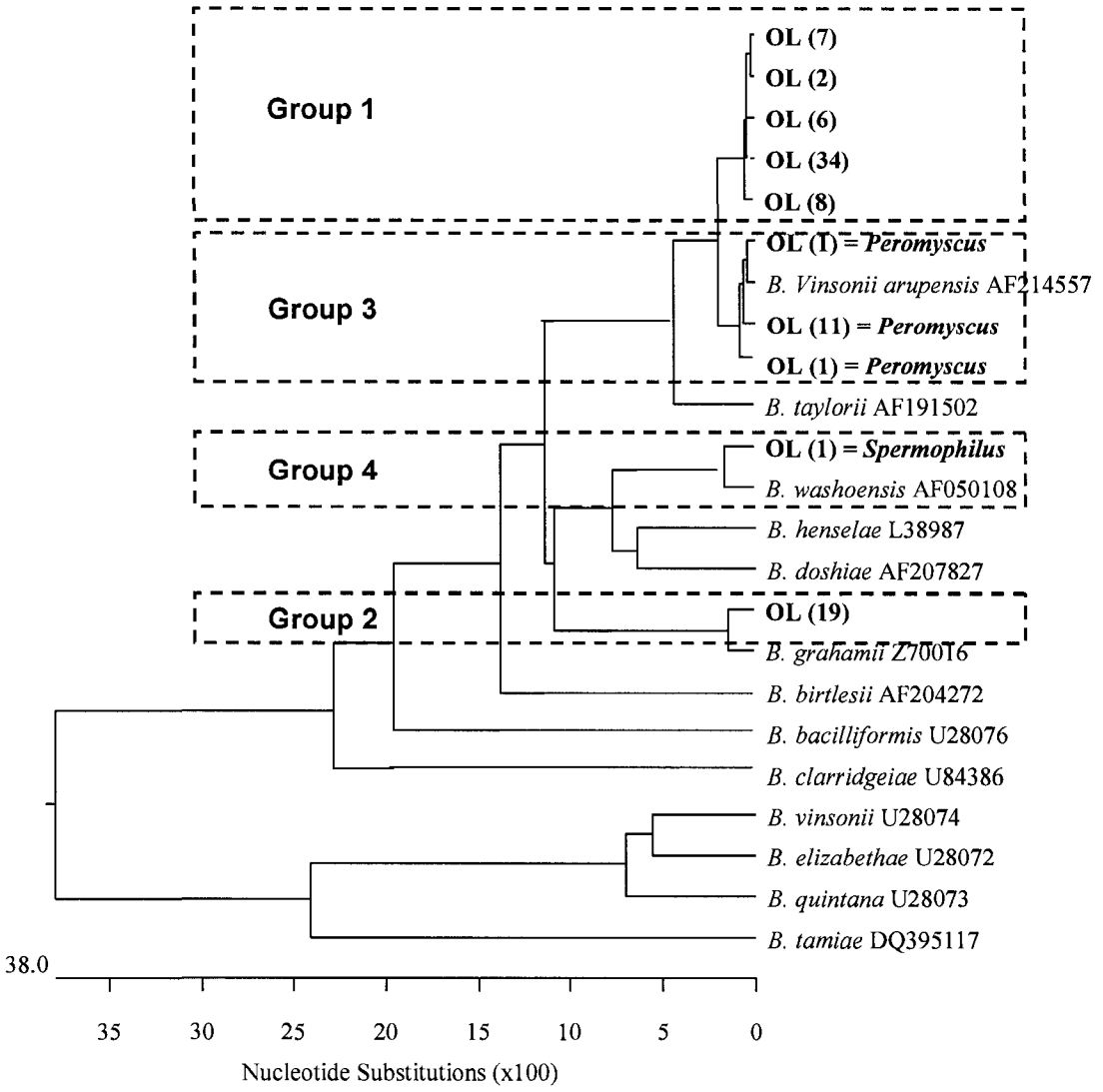

Figure 5-10 Phylogenetic relationships between *Bartonella* variants obtained from the northern grasshopper mouse based on the analysis of a 338-bp sequence in the citrate synthase gene (*gltA*), showing two genogroups that are typical to the genus of *Onychomus*, and non-specific bartonellae acquired by the northern grasshopper mouse. Numbers in parentheses indicate frequency of bartonella sequences belonging to the same variant obtained from the northern grasshopper mouse. OL: *Onychomys leucogaster*.

The first group, the largest of the four, contains 5 variants of 57 sequences from northern grasshopper mice from Kansas (39), Colorado (17), and Wyoming (1). These variants, with divergences ranging from 0.3% to 1.1%, form a group that is within the *B. vinsonii* clade. This group, with divergence of 4.3% - 4.6% to *B. vinsonii* subsp. *arupensis*, is typical of grasshopper mice of the genus *Onychomys* and presumably represents another subspecies of *B. vinsonii* (Figure 5-10). The second group, with divergence of 9.5% - 10.5% from the first group, contains only one variant with 19 identical sequences from northern grasshopper mice trapped in Kansas (15), Colorado (3), and Wyoming (1). This variant also is different from other described bartonella variants and appears to be specific to grasshopper mice (Figure 5-10). The third group contains 3 variants of 13 sequences from northern grasshopper mice obtained in South Dakota (5) and Wyoming (8), with similarities ranging from 0.3% to 1.1%. These sequences were identical to *gltA* sequences from deer mice, and the bartonellae belong to *B. vinsonii* subsp. *arupensis*. The last sequence was obtained from a northern grasshopper mouse captured in Wyoming. This sequence is identical to the sequence from thirteen-lined ground squirrels and belongs to *B. washoensis*, indicating that bartonella has jumped from other rodent species to grasshopper mice.

DISCUSSION

The relationship of host-specificity of bartonellae and their rodent hosts has been controversial. By comparing bartonella strains from rodents belonging to different taxonomic groups and covering broad geographic areas, the present study demonstrates the host-specific associations of bartonellae and their rodent hosts in the United States.

Nevertheless, the specificity was largely complex. Discussed below are three types of relationships observed in this study.

One phylogenetic group of bartonellae – one taxonomic group of rodents

This relationship, demonstrating host-specificity of bartonellae and their natural rodent hosts, as reported in some previous studies around the world (Ying et al., 2002; Kosoy et al., 2003; Castle et al., 2004; Jardine et al., 2006a), is the most common and has been observed in most instances in this study. However, this specificity is very complex in regard to the level at which it occurs. A phylogenetic group of bartonellae can be a species, a subspecies, or a genogroup, and taxonomic groups of rodents can be a family, a genus, or a species.

Broadly, the present study demonstrated that bartonellae associating with rodents from the same family are of the same species: sciurid-associated bartonellae are of *B. washoensis*; and cricetid-associated bartonellae are of *B. vinsonii*. Nevertheless, bartonellae of the same species can occur as different genogroups, and some of them might represent different subspecies, with each genogroup being specific to rodents of a certain genus. For example, *Cynomys*- and *Tamias*-associated bartonellae each form a distinct group within *B. washoensis*; *Peromyscus*- and *Neotoma*-associated bartonellae also are grouped separately within *B. visonii*, showing specificity at the genus level.

Moreover, some of my observations suggest that host-specificity can also be expressed at the rodent species level. Comparison of *gltA* sequences from peromyscine mice of four *Peromyscus* species demonstrated that peromyscine-associated bartonellae can be further divided into two genogroups, with one genogroup "arupensis" being

typical of deer mice and white-footed mice, and the other one "boylii-truei" being typical of brush mice and pinyon mice, although bartonellae from these species sometimes can have "jumped" the genogroups. These results verified the finding of Kosoy et al. (1997) that bartonella isolates obtained from deer mice, white-footed mice, and cotton mice (*P. gossypinus*) form one phylogenetic group.

The present study demonstrates the potential of *gltA* in defining *Bartonella* species (La Scola et al., 2003). Bartonellae can be easily classified using a combination of *gltA* sequence data and host information. However, definition of a taxonomic group is not sufficiently stringent when it is based on application of a single parameter. The robust multi-gene phylogenetic analysis (following Stackebrandt 2002) used here showed the occurrence of novel *Bartonella* subspecies comprised of strains recovered from black-tailed prairie dogs. All strains, for which sequences of *gltA*, 16S rRNA, *rpoB*, *ftsZ*, and *ribC* genes were determined, formed a unique cluster within *B. washoensis* but different from strains recovered from other sciurids. Although one of these *gltA* variants was also detected in thirteen-lined ground squirrels, considering that this variant is within the specific cluster formed by variants found in black-tailed prairie dog, and low variability of the sequences from thirteen-lined ground squirrels (only one nucleotide difference for the two variants detected in thirteen-lined ground squirrels in this study, which more likely represents a nucleotide mutation rather than a stable genetic difference), this bartonella clone likely is specific to the prairie dogs but adapted to thirteen-lined ground-squirrels after occasional interspecies transfer and was successively distributed in populations of thirteen-lined ground squirrels. To indicate the host, this bartonella lineage, has been proposed Candidatus *Bartonella washoensis* subsp. *cynomysii* (Bai et al., 2008).

Multiple species of bartonellae – one taxonomic group of rodents

Within a rodent-bartonella community, an assemblage of bartonellae belonging to numerous species or strains can occur sympatrically, and several genetically different bartonella populations can be associating with one rodent population (Kosoy et al., 1999; Kosoy et al., 2004b). In a study of bartonellae in cotton rats in the southwestern US, Kosoy et al. (1997) found bartonellae of three phylogenetic groups in the same population of cotton rats (Kosoy et al., 1997).

Supporting such a relationship, two phylogenetic distant species of bartonellae, group 1 and 2, were found typical of grasshopper mice in the present study (Figure 5-10). Group 1, locating within *B. vinsonii* clade that is typical of cricetid rodents, and containing the larger number of bartonella sequences than group 2 (57 versus 19), may be more common to grasshopper mice. My results may indicate that the more common bartonella may have had longer relationship with grasshopper mice in terms of their co-speciation. I hypothesize that one of the multiple *Bartonella* species adapted to a specific rodent host can better fit the model of bartonella co-speciation reflecting rodent taxonomy.

Acquisition of non-specific bartonellae

As bartonellae have the potential to exploit alternative hosts, a bartonella strain that is specific to hosts of a particular species can be incidentally transmitted to and within a non-specific host species. Such events were observed albeit rarely in early studies: a single Norway rat (*Rattus norvegicus*) acquired *Bartonella* that is typical of field mice (the genus *Apodemus*) in China (Ying et al., 2002); a single black rat (*R. rattus*) acquired

bartonella that is typical of greater bandicoot rat (*Bandicota indica*) in Thailand (Castle et al., 2004). Acquisition of non-specific bartonella strains also was observed in the present study, mostly occurring at random bases. For example, only 3 of 775 bartonella-infected peromyscines acquired bartonellae typical of rodents of other genera; 3 of 84 bartonella-infected woodrats acquired bartonellae typical of rodents of other genera. Compared to peromyscine mice and woodrats, grasshopper mice can more readily acquire non-specific bartonellae from rodents of other genera, with 14 transfers identified within 90 isolates.

Acquisition of non-specific bartonellae may relate to the ecological and biological conditions of the host. Special behaviors of individual rodents might increase the chance of acquisition of infections from other rodents. Two hypotheses may explain the apparently common bartonella acquisition by northern grasshopper mice. First, usurping the burrows of other small mammals could allow the grasshopper mice to acquire fleas that usually parasitize rodents of other species. Fleas of some certain species, such as *Aetheca wagneri, Orchopeas leucopus*, and *Pleochaetis exilis* are commonly found on northern grasshopper mice, deer mice and thirteen-lined ground squirrels. Given that bartonellae can be transmitted by fleas, this might help in explaining why bartonella strains were shared between the grasshopper mouse and other rodents. As small mice of other species are prey for grasshopper mice, it is not difficult to speculate that bartonella strains are transferred from rodents of other species to grasshopper mice while grasshopper mice consume bartonella-infected rodents; this hypotheis requires experimental testing. Furthermore, it was found that the relative abundance of potential hosts within rodent communities plays a key role in this process of bartonella transfer,

and bartonella prevalence in the rodents also plays a role in such an outcome (Bai et al., 2007a).

CONCLUSIONS

The genetic analyses of bartonellae cultured from rodents of numerous species in the United States provided data to answer a controversial issue regarding relationships between *Bartonella* species and their natural hosts. In general, the results reported here suggest that the bartonella – rodent relationship is specific. However, the relationship is not that simple and straightforward. There are many more complicated characters of associations between specific variants of these bacteria and the genetic parallels with the taxonomic status of their hosts. Indeed, the phylogenetic study of bartonellae challenges some long used aspects and definitions of parasite-host specificity and suggests that additional information on ecology and evolution of both parasites and animal hosts be incorporated into such definitions in the future. One interesting conclusion is that the level of host-specificity can vary substantially between rodents of different taxa or between bartonella of various species. These observations are quite different from those observed in rodents from Europe, and probably reflect the evolutionary history of a particular bartonella species more than they reflect environmental differences. If rodents migrated from one continent (America) to another (Europe), bartonellae carried by them would exploit opportunities for host switching, and colonizing new rodent hosts.

Apparent congruence between the phylogeny of bartonellae and their specific hosts in America and absence of similar congruence in Europe suggests that bartonellae have long adapted to American rodent species, but European bartonellae have not come into

equilibrium with their rodent hosts. Alternatively, the preference of fleas and other vectors may facilitate co-speciation. If rodents of different species in Europe are sharing the same flea species that associate with specific bartonella species, rodent-barotnella relationship will be different from that observed in America where fleas do not share their rodent hosts. These hypotheses require testing. Discovery of transfer of some bartonella variants from specific hosts to occasional hosts suggests that there are even more complexities to this system than we recognize, but also suggest that this system would be used as a good model for reconstructing recent evolutionary changes in bartonella populations. In spite all this complexity, the data included here can be used in epidemiological practice, when information on host specificity can help to establish a link between homologous genetic variants isolated from humans and those found in a wide range of potential rodent reservoirs.

CHAPTER SIX

SPATIAL-TEMPORAL DISTRIBUTION OF GENETIC VARIANTS OF BARTONELLAE ASSOCIATED WITH BLACK-TAILED PRAIRIE DOGS

Interest in rodents as reservoir hosts of bartonellae led to numerous studies that have found not only high prevalence but also high genetic diversity in rodents of a variety of species world-wide (Birtles et al., 1994; Kosoy et al., 1997; Fichet-Calvet et al., 2000; Ying et al., 2002; Jardine et al., 2005; Bai et al., 2007b). Frequent or not, concurrent infections with multiple genotypes in a single animal have been reported in some studies (Kosoy et al. 2004b; Jardine et al., 2006a). Arthropod vectors, such as fleas, have been implicated as potential vectors (Breitschwerdt and Kordick, 2000; Stevenson et al., 2003) and experimental studies have demonstrated that fleas can transmit bartonellae to rodents (Bown et al., 2004).

The potential role of parasites in affecting the population dynamics of their hosts is now becoming more widely recognized (Grenfell and Dobson, 1995). Studying zoonotic pathogens in their natural hosts could address questions in numerous disciplines, ranging from epidemiology to conservation. Bartonellae, due to their frequently high prevalence and high genetic diversity and without obvious effects on rodent mortality, can serve as an excellent ecological marker to study the population structure of their natural hosts. From an ecological standpoint, the study of bartonella infections in rodents can potentially provide much-needed data to test certain theoretical models of infectious disease dynamics within naturally susceptible populations (Grenfell and Dobson, 1995).

Black-tailed prairie dog (BTPD), *Cynomys ludovicianus*, the most widespread of five prairie dog species, has been recognized as a keystone species in western prairie ecosystems (Kotliar et al., 1999). As a keystone species, BTPD not only influence biological diversity and ecosystem functions, but may also aid conservation efforts that help protect other species (Kotliar et al, 1999). However, the BTPD population has drastically declined throughout its range during the last two centuries due to habitat alteration, agricultural control, "recreational" shooting and, most recently, introduction of sylvatic plague, caused by the bacterial pathogen, *Yersinia pestis* and which causes nearly 100% mortality in BTPD (Biggins and Kosoy, 2001). As a consequence of habitat fragmentation, BTPDs currently exist in spatially isolated colonies or discrete patches (Johnson and Collinge, 2004) connected to different degrees by dispersal (Hoogland, 1995) brought about by relatively frequent movement of individuals between colonies (Roach et al., 2001). Physical and ecological variability in the landscapes inhabited by prairie dogs likely influence dispersal and gene flow among colonies (Hanski, 1999; Wiens, 1996). Topographic variation, tall vegetation, and areas of urban or agricultural development are important barriers to dispersal and to expansion of colonies (Koford, 1958), but other landscape features, such as roads and trails, may facilitate movement (Knowles, 1986; Garrett and Franklin, 1988). Recently, ecologists began studying the genetic structure of prairie dogs to better understand the processes of dispersal. Population genetic studies have demonstrated moderate levels of subdivision between colonies (Roach et al., 2001).

As a strictly colonial species, BTPDs contract infections more often than do rodents of solitary species. Infected BTPDs potentially spread infections within and between

colonies. In Chapter 4, bartonella is reported as being widely distributed in BTPDs and the fragmented distribution of prairie dogs considered affecting dynamics and diversity of BTPD-associated bartonellae. As the prevalence of bartonella infection in juvenile BTPDs is much higher than in adult BTPDs (Chapter 4), dispersal of juvenile BTPDs is an especially important factor in studying bartonella in them. Dynamics of bartonellae in spatially structured population of BTPDs might reflect fragmentation effects, which can provide very useful model for understanding and delineating their dispersal.

In Boulder County, Colorado, humans and BTPDs have partitioned the mostly flat prairie landscape into a checkerboard pattern of different habitat types. Some colonies are completely bounded by urban or agricultural land use, while others exist within more natural "open space" habitats (Johnson and Collinge, 2004). Prairie dog population differentiation is expected among BTPD colonies, and so are bartonellae. The research goals of the present study were to investigate the genetic structure of BTPD-associated bartonellae and to understand the influences of ecological factors on bartonella transmission in human-altered landscapes. BTPDs were sampled in 20 prairie dog colonies in Boulder County, Colorado from 2003 to 2005. These samples were subjected to test for the presence of bartonella and, further, classification of genetic variants. The objectives of the study were to characterize bartonellae obtained from BTPDs using genetic analyses; describe spatial distribution of bartonella among prairie dog colonies; illustrate the population genetic structure of these parasites, and their relation to landscape features; and estimate potential dispersal of BTPDs from place to place using BTPD-associated *Bartonella* genetic variants as ecological markers. In this study, I

define bartonellae from all 20 colonies as a population, and bartonellae within a single colony as a subpopulation.

MATERIALS AND METHODS

Study sites and trapping

The 20 BTPD colonies (1A-20A) described in Chapter 4 were used in this study. These colonies are distributed within a approximately 900 km^2 area (about 45 kilometers from north to south and 20 kilometers from west to east) in Boulder County, Colorado, in the midst of an urban corridor on the east side of the Rocky Mountains. Distribution of colonies was restricted by topography and other barriers, such as urbanization, streams, and lakes. Two major roads (State highways 119 and 36) bisect the study area (Figure 6-1). For spatial analysis, the study areas were divided into three areas separated by the two highways: seven colonies (1A, 2A, 4A, 5A, 8A, 17A, 19A) comprise the "northern" study area, and are separated from other colonies by highway 119; six colonies (6A, 7A, 11A, 12A, 14A, 20A) comprise the "southern" study area, separated from other colonies by highway 36; and seven colonies (3A, 9A, 10A, 13A, 15A, 16A, 18A) located between the two highways comprise the "central" study area. Prospective sampling using mark-release-recapture was conducted from June to August in 2003-2005. Trapping procedures and animal processing are presented in detail in Chapter 4.

Bartonella detection

Bartonella was detected by either culturing (2003 and 2005 samples) or nested PCR

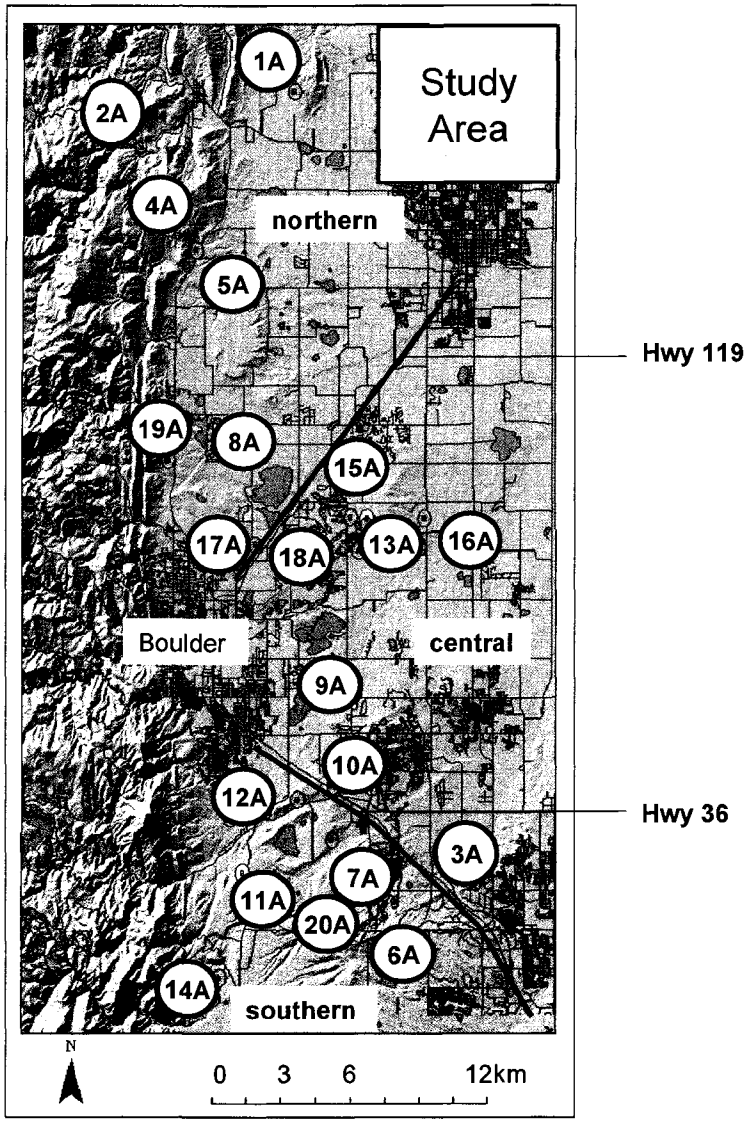

Figure 6-1 Study area, showing 20 black-tailed prairie dog colonies (1A-20A) in urban contexts and short-grass prairie in Boulder County, Colorado. For spatial analyses, the study area was divided into northern, central, and southern areas, with reference to two major highways crossing the study area.

(2004 samples). Culturing procedures and PCR confirmation of cultures as bartonella were the same as those described in Chapter 3.

The presence of bartonella in samples from 2004 was detected using nested PCR due to a freezer failure that killed most bartonella in bloods, making culturing impossible. Nested PCR was applied directly to DNA extracted from blood samples for detection of bartonella. DNA was extracted from animal blood using a QIAamp Kit (Qiagen, Chatsworth, CA), following procedures recommended by the manufacturer. For comparison with culturing results, a group of surviving samples (n=64) was tested using both methods. Eighty-six percent (55/64) of tested samples had concordant results by the two methods (34 samples were negative and 21 samples were positive by both culturing and nested PCR); two samples were positive by culturing but negative by nested PCR; seven samples were negative by culturing but positive by nested PCR. Chi-square test indicated an agreement between culturing and nested PCR results (χ^2=0.81, p=0.37). The nested PCR used two pairs of primers designed at the Gamaleya Institute of Epidemiology and Microbiology, Moscow, Russia (Kirillov et al., 2007). The first step of amplification used the pair of outer primers G003o_F 5'-TATTACAGATCCGCAACAGAGAATGA-3' and G003o_R 5'-ACTCGATGACCAAAACCCATAA-3' to generate a 427-bp amplicon of the bartonella *gltA* gene using the following program parameters: 5-min denaturation at 95°C, followed by 4 cycles of 1-min denaturation at 95°C, 1-min annealing at 56°C, 1-min elongation at 72°C, 35 cycles of 55-sec denaturation at 95°C, 55-sec annealing at 56.3°C, 55-sec elongation at 72°C, 1-min final denaturation at 95°C, and 1-min annealing at 56.3°C. Amplification was completed by holding the reaction mixture at 72°C for 10 min. The

PCR products were subjected to the second step of amplification using the pair of inner primers G004i_F: TTCACAGGTCCCAACTCTTGCCGC TAT and G004i_R: TGCCAGACGTACAGTGGATGTAGAAGC to generate a 324-bp amplicon of the bartonella *gltA* gene following the procedure outlined for single PCR (Chapter 3).

Sequencing and analysis of DNA

Sequencing procedures and phylogenetic analyses were the same as those described in Chapter 5. Novel sequences from the current study were submitted to GenBank and assigned unique accession numbers.

Statistical analyses

Chi-square analysis ($n>60$) or Fisher's exact test ($n\leq 60$) was performed to determine whether bartonella prevalence differed significantly among colonies or areas, and to compare the change of proportion of variants over the years observed. All analyses were performed using related programs within the SAS package (Statistical Analysis System, version 9.1, SAS Institute Inc., Cary, North Carolina). Statistical tests for significance of difference between compared groups were determined at the 0.05 probability level.

RESULTS

Prairie dog captures and bartonella infection in the animals

A total of 1,362 BTPDs were captured in the 20 colonies during the study period, with 325, 513, and 524 individuals captured in 2003, 2004, and 2005, respectively. Of

these, bloods collected from 1,302 individuals were tested for bartonella, with 318, 464, and 520 samples obtained from each year accordingly. Bartonella was detected in 301 individuals, including 29 and 203 culture positive for 2003 and 2005 samples, and 69 nested-PCR positive for 2004 samples (Table 6-1).

Spatial dynamics of bartonella infection

Over the study period, bartonellae were detected in BTPDs distributed in all 20 colonies, but with a high variation in prevalence between colonies: from 4.8% (3/62, 8A) to 41.7% (40/96, 16A) (Table 6-1). Average prevalence among colonies was significantly different over three years (χ^2=85.69, p<<0.01).

In 2003, bartonella-infected BTPDs were found only in 10 of the 20 colonies (Table 6-1; Figure 6-2A). The overall prevalence was 9.1% (29/318) but varied from 0 to 33.3% (8/24; 19A) per colony. The majority of infected BTPDs (75.9%, 22/29) were distributed in six colonies located in the northern study area, with the highest number of bartonella-positive animals detected in colonies located nearest to the foothills (8 in 19A and 7 in 2A). Colony 17A, adjacent to highway 119, was the only colony in which bartonella infection was not detected in northern study area. In the other part of the study area, bartonella was detected only in one southern colony and three central colonies (2 in 6A and 5 total in 3A, 9A and 15A).

In 2004, bartonella spread to most of the study area (Table 6-1; Figure 6-2B). The infection was detected in 69 BTPDs that were distributed in 18 colonies. The overall prevalence was 14.9% (69/464) but varied from 0 to 36.4% (8/22; 5A) per colony. Although the colony with the highest prevalence of bartonella was located in the northern

Table 6-1 Prevalence of bartonella infection in black-tailed prairie dogs of 20 colonies, Boulder, Colorado, 2003-2005. Infection detected by culturing (*), by nested PCR (¶), or by either method (§)

colony	2003*			2004¶			2005*			total§		
	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%
1A	16	3	18.8	38	2	5.3	25	3	12	79	8	10
2A	35	7	20	41	1	2.4	25	4	16	101	12	12
4A	5	1	20	9	0	0	6	2	33.3	20	3	15
5A	20	2	10	22	8	36.4	26	12	46.2	68	22	32
8A	16	1	6.3	19	0	0	27	2	7.4	62	3	4.8
17A	2	0	0	10	1	10	28	16	57.1	40	17	43
19A	24	8	33.3	49	11	22.5	35	11	31.4	108	30	28
3A	17	2	11.8	30	1	3.3	36	14	38.9	83	17	21
9A	27	1	3.7	39	8	20.5	27	5	18.5	93	14	15
10A	12	0	0	13	1	7.7	13	1	7.7	38	2	5.3
13A	8	0	0	42	7	16.7	40	24	60	90	31	34
15A	24	2	8.3	14	3	21.4	28	19	67.9	66	24	36
16A	16	0	0	34	5	14.7	46	35	76.1	96	40	42
18A	8	0	0	22	2	9.1	29	9	31	59	11	19
6A	16	2	12.5	8	1	12.5	6	3	50	30	6	20
7A	30	0	0	16	3	18.8	29	15	51.7	75	18	24
11A	11	0	0	7	2	28.6	15	6	40	33	8	24
12A	10	0	0	13	4	30.8	22	8	36.4	45	12	27
14A	6	0	0	16	5	31.3	29	8	27.6	51	13	26
20A	15	0	0	22	4	18.2	28	6	21.4	65	10	15
total	318	29	9.1	464	69	14.9	520	203	39	1302	301	23

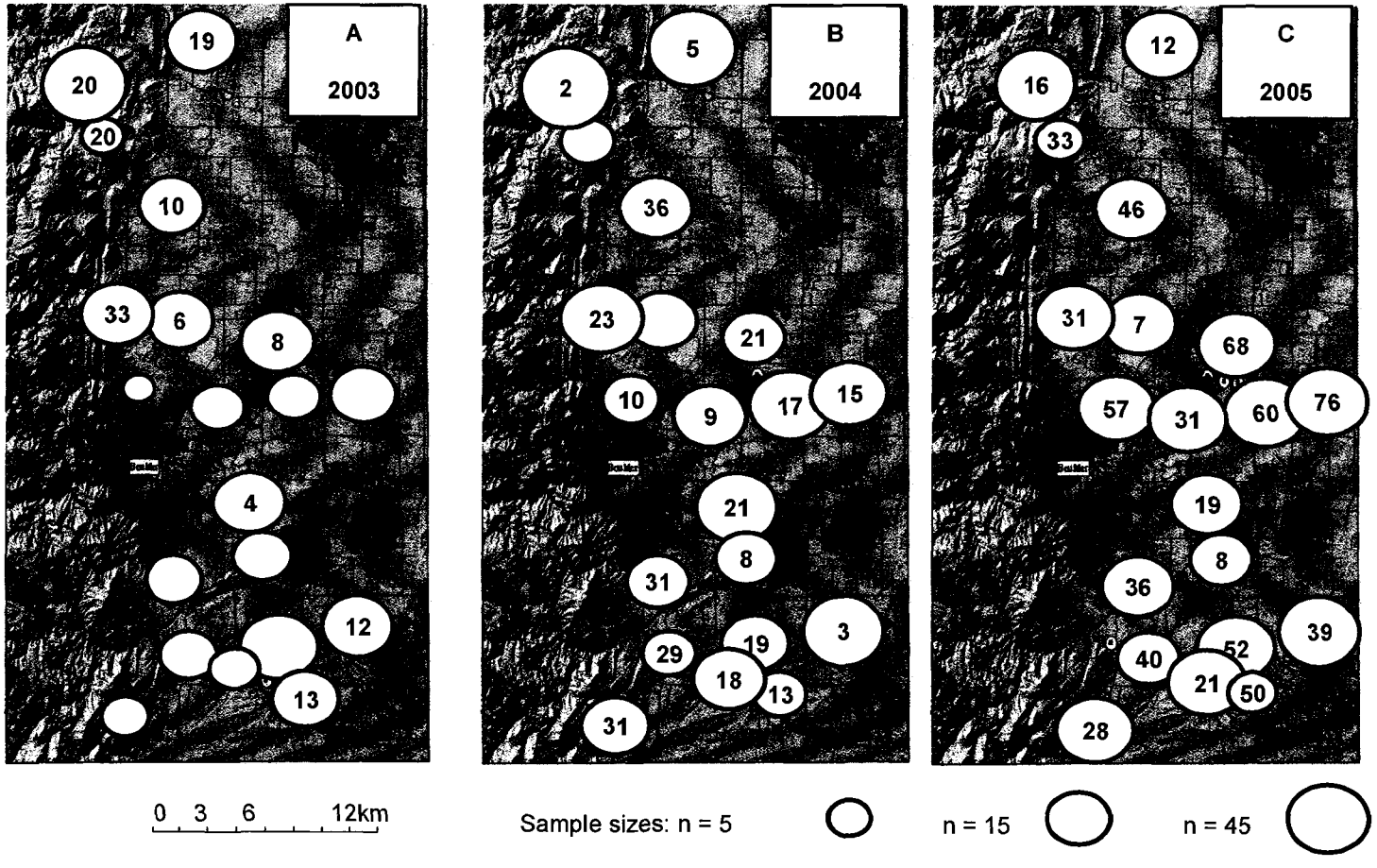

Figure 6-2 Spatial distribution of bartonella-infected black-tailed prairie dogs at 20 study colonies in 2003 (A), 2004 (B), and 2005 (C), indicating spread from northern to central and southern colonies. The number in each circle represents the percentage of infected BTPDs detected within the colony, while the size of each circle represents the sample size of BTPDs tested for bartonella (see Table 6-1 for details). No bartonellae were detected black-tailed prairie dogs in colonies represented by blank circles.

area, the largest number of infected BTPDs was detected in the central study area. Compared to the previous year, it was evident that bartonella spread towards colonies located in the central and southern areas. In contrast, bartonella disappeared from two colonies (4A, 8A) in the northern area, where the infection had been detected in 2003.

In 2005, bartonella-positive BTPDs were found in all colonies (Table 6-1; Figure 6-2C). Infection was detected in 39.0% (203/520) tested BTPDs. Average prevalence increased in 2005, varying from 7.4% (2/27; 8A) to 76.1% (35/46; 16A) per colony. The majority, 52.7% (107/203) of infected animals were found within colonies in the central area. Colonies with the highest number of infected BTPDs with high prevalence were 13A (60%, 24/40), 15A (67.9%, 19/28), and 16A (76.1%, 35/46), all in the central area, plus 17A (57.1%, 16/28), the most southern colony in the northern area.

Genotypic variants of BTPD-associated bartonellae and sequence accession numbers

A total of 284 sequences were obtained from 242 isolates amplified from 232 BTPDs (years 2003 and 2005) and from 42 bartonella-positive DNAs (in 2004). Analysis of the sequences demonstrated nine genetic variants, named in order of letters A to I. Each variant was assigned a unique GenBank accession number as follows: DQ897367, AF440273, AF440275, AY589564, AY589569, AF440274, AF440731, AF440732, and AY584570. Phylogenetic analysis showed that the nine variants clustered into a single clade, with the sequence divergence ranging from 0.3% to 2.4%. These variants are closely related (96.9% similarity) to *Bartonella washoensis*, the species considered to be a source of human cardiac illness and that was found in California ground squirrels

(*Spermophilus beecheyi*) and rock squirrels (*S. variegatus*) (Kosoy et al., 2003). BTPD-associated bartonellae has been characterized and proposed as Candidatus *Bartonella washoensis* subsp. *cynomysii* (Bai et al., 2008).

Temporal dynamics of BTPD-associated *Bartonella* variants (Figure 6-3)

Variant A was the dominant genotype of the nine identified variants: of the 284 sequences, 119 (41.9%) were of variant A; variants B and C each accounted for 18% (51 sequences for each); variant G accounted for 7% (21 sequences); variants F, D, I, E, and H accounted for 5% (13), 4%(12), 3% (9), 2% (5), and 1% (3), respectively (Figure 6-3A).

In 2003, only four variants, A, B, C, and D were detected among 29 bartonella-positive BTPDs, with the majority BTPDs (18, 62.1%) having variant A. Variant B, C, D were found in 4 (13.8%), 4 (13.8%), and 3 (10.3%) BTPDs, respectively (Figure 6-3B).

In 2004, six variants were determined in 42 bartonella-positive BTPDs. In addition to variants A, B, and C, identified in 2003, three new variants (E, G, H) were detected. Variant D, identified in 2003, was not detected in 2004. The frequency of each variant varied from 1 to 22. Found in 22 (52.4%) BTPDs, variant A still was the most prevalent, although the proportion decreased compared to that in 2003 (Fisher's test, p=0.29); variant B was found in 5 BTPDs and remained in a proportion (11.9%) similar to that in 2003; variant C was found in 8 BTPDs, with an increase in proportion (19.0%) but not significant compared to 2003 (Fisher's exact test, p=0.40). The three new variants, G, E and H, were found in 4 (9.5%), 2 (4.8%), and 1 (2.4%) BTPD, respectively (Figure 6-3C).

In 2005, in addition to the variants identified previously, two more new variants (F

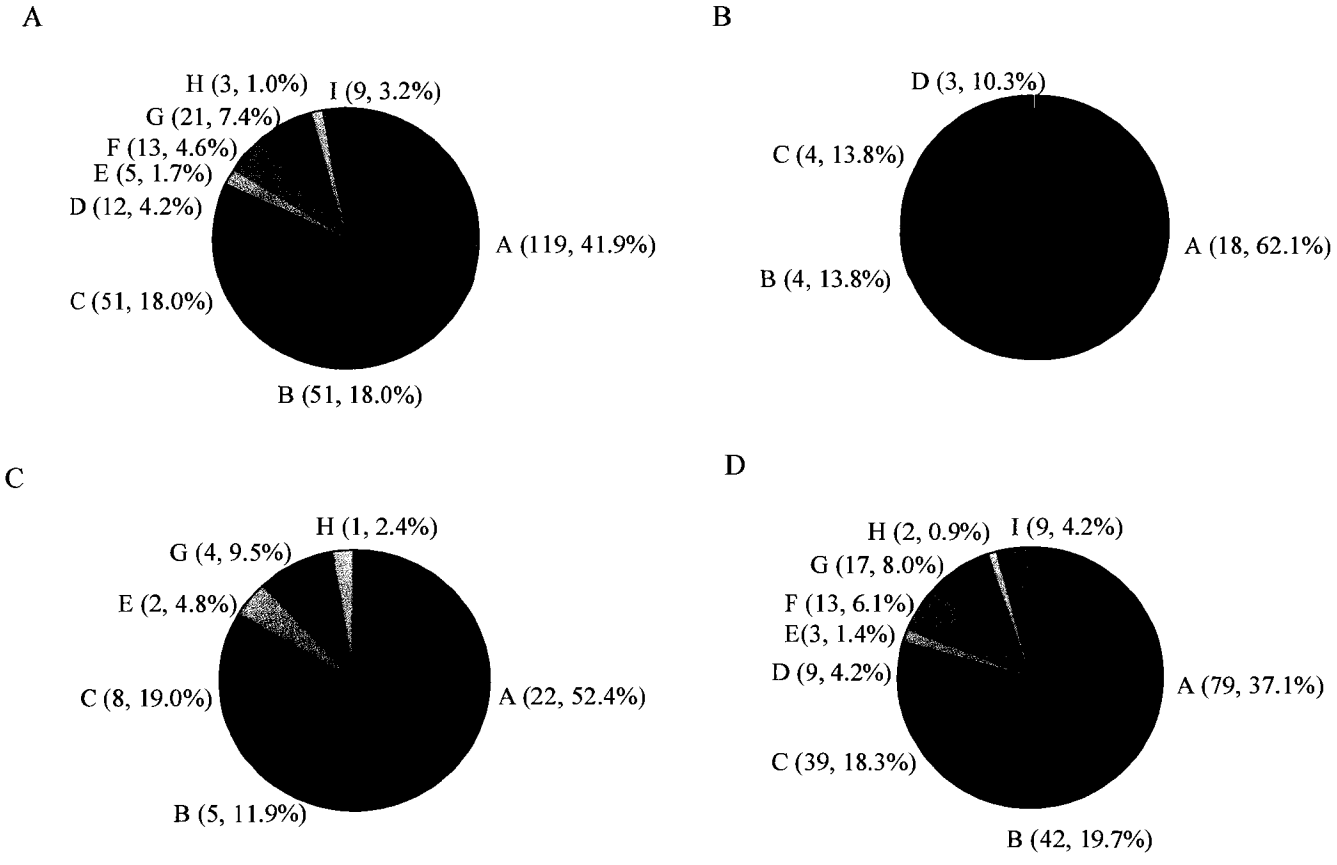

Figure 6-3 Composition of genetic variants of black-tailed prairie dog-associated bartonellae based on the *gltA* gene, showing frequency and percentage of each variant in all years combined (A), year 2003 (B), 2004 (C), and 2005 (D). Each variant is marked by a particular color.

and I) were detected, resulting in a total of 9 variants being detected in 203 bartonella-positive BTPDs. Detected in 79 BTPDs, the proportion of variant A (37.1%) was continuously and significantly decreased in 2005 compare to that of the previous years (Fisher's exact test, $p \leq 0.048$); variant B was detected in 42 BTPDs, with a non-significant increase in proportion (20.7%) compared to that of the previous years (Fisher's exact test, $p > 0.16$); variant C was detected in 39 BTPDs, and also showed an insignificant increase in proportion (19.2%) of the variant in all positive BTPDs (Fisher's exact test, $p > 0.38$); variant F and G were detected in 13 (6.4%) and 17 BTPDs (8.4%), respectively. The other four variants were detected in 2-9 BTPDs and accounted for 0.9%-4.2% (Figure 6-3D) of the entire bartonella population.

Temporal variations of *Bartonella* genetic diversity within selected BTPD colonies (Figure 6-4)

Bartonella subpopulations within six colonies (5A, 7A, 14A, 15A, 16A, and 19A) were selected to compare fluctuations in *Bartonella* variants and frequency within each BTPD colony through the 3-year period. Colonies were selected on the basis of having available adequate data for analysis. Increasing diversity was exhibited within each subpopulation during this period, from 0-2 variants in 2003 to 4-7 variants in 2005. For example, at colony 19A, only one variant was detected in 2003, but three were detected in 2004 and five were detected in 2005. At the same time, the proportion of predominant variant A among all detected variants has decreased to 50% in 2004 and 45.5% in 2005. At colony 5A, two, three, and six variants were detected in 2003, 2004, and 2005, respectively. Within colonies 7A, 14A, and 16A, bartonella was not detected in 2003, but

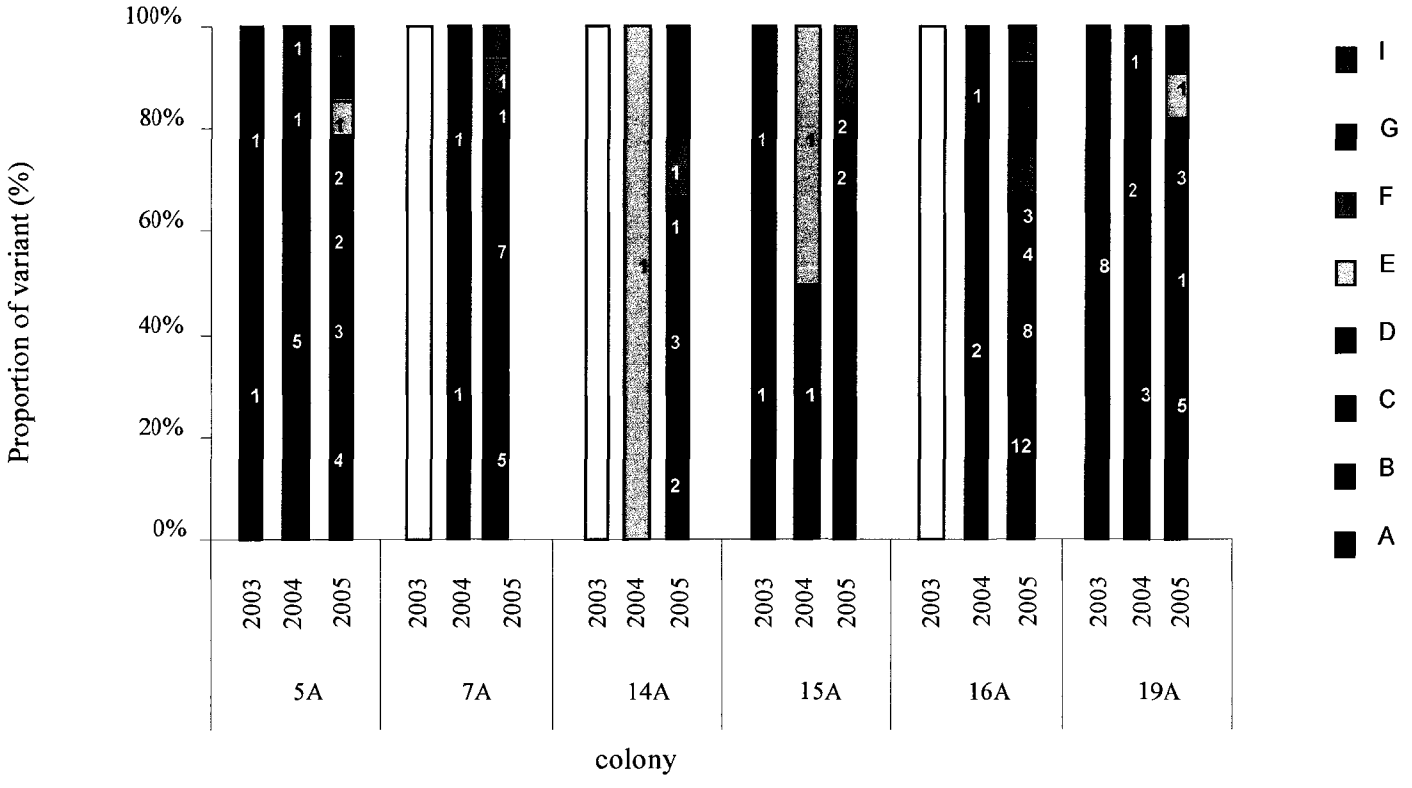

Figure 6-4 Genetic variation within bartonella subpopulations at BTPD colonies, 2003-2005, showing the increasing diversity and change of proportion of a particular variant. The bars represent *Bartonella* variants and are distinguished by color. The numbers shown in bars represent the frequency of a particular variant detected at given colony. A blank bar indicates no bartonella was detected in that year.

1-2 variants began to circulate in 2004, and these subpopulations became well established, with 5-7 variants circulating in 2005. Within colony 15A, bartonella diversity was less variable during the three years, with two variants detected in 2003 and 2004, and four variants in 2005.

Spatial dynamics of *Bartonella* variants in prairie dog colonies (Table 6-2, Figures 6-5, 6-6, 6-7)

The frequency of each variant differed at each colony. Combining data from the three years, the number of variants per colony was 1 (10A) to 7 (16A), and the frequency of all variants per colony was 2 (10A) to 43 (16A). Variant A was found in BTPDs of all 20 colonies, with frequencies per colony ranging from 1 (8A) to 16 (19A). Variant B was found in BTPDs of 17 colonies, with frequencies ranging from 1 to 8. Variant C was found in BTPDs of 16 colonies, with frequencies ranging from 1 to 11. Other variants were detected among some few colonies with low frequencies. For details see Table 6-2 and Figure 6-5.

Distribution of Bartonella variants in 2003

In 2003, variant A (62.1%, 18/29) was found in BTPDs of seven colonies in the northern and central areas, with frequencies from 1 to 8; variant B (4) was found in BTPDs of four colonies in the northern and southern ares, with one infected BTPDs in each colony; variant C (4) was found in BTPD of four colonies in the northern and central areas, with one infected BTPD at each colony; variant D was found in 3 BTPDs from three colonies, with one of each located at northern, central, and southern areas. In the northern area, variant A accounted for 72.7% (16/22) of all sequences obtained from

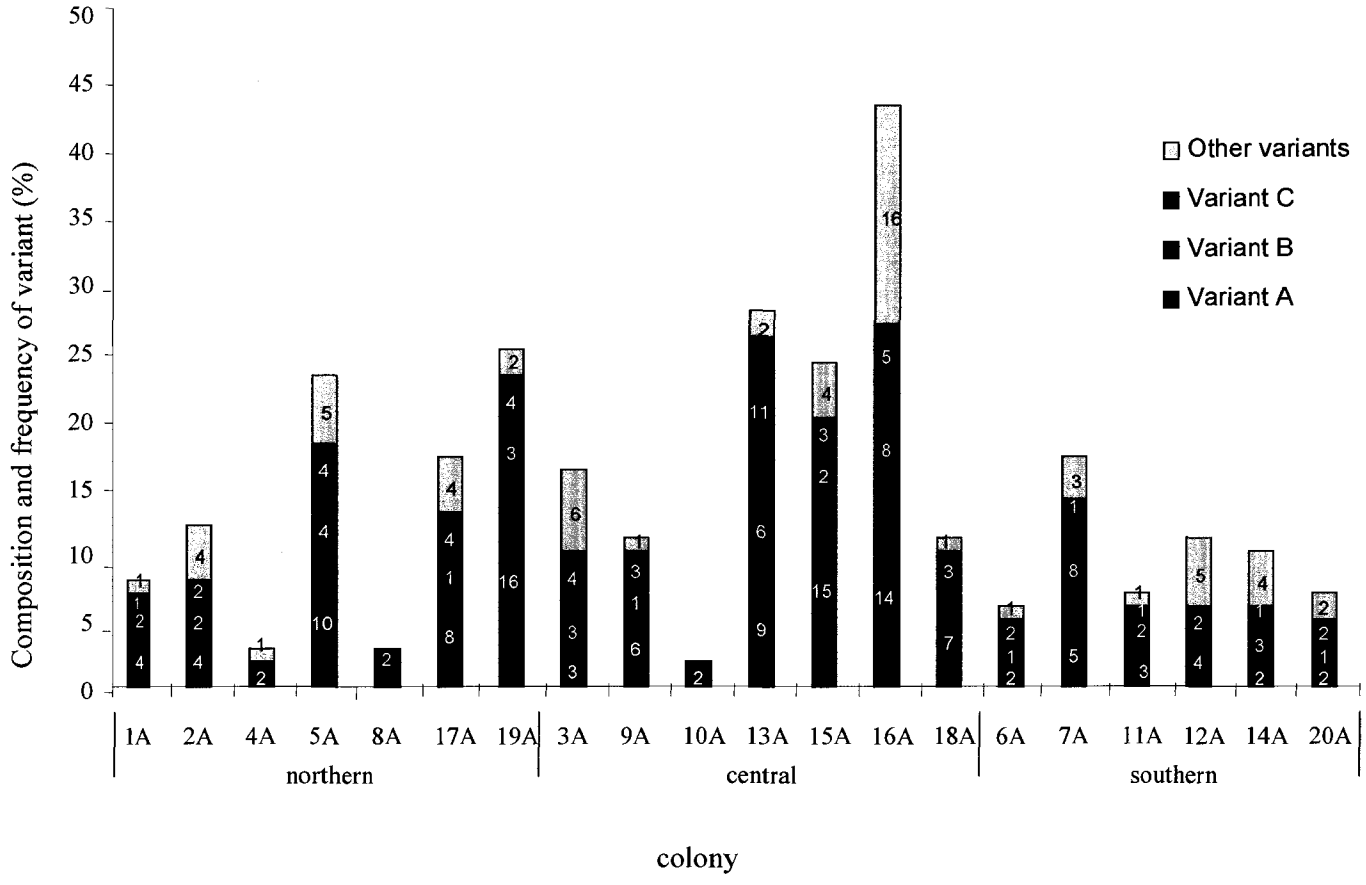

Figure 6-5 Overall distribution (all years combined) of *Bartonella* variants among 20 black-tailed prairie dog colonies, showing the structure of bartonella subpopulations at each colony. The bars represent *Bartonella* variants and are distinguished by color. The numbers shown in bars represent the frequency of each particular variant detected at that colony.

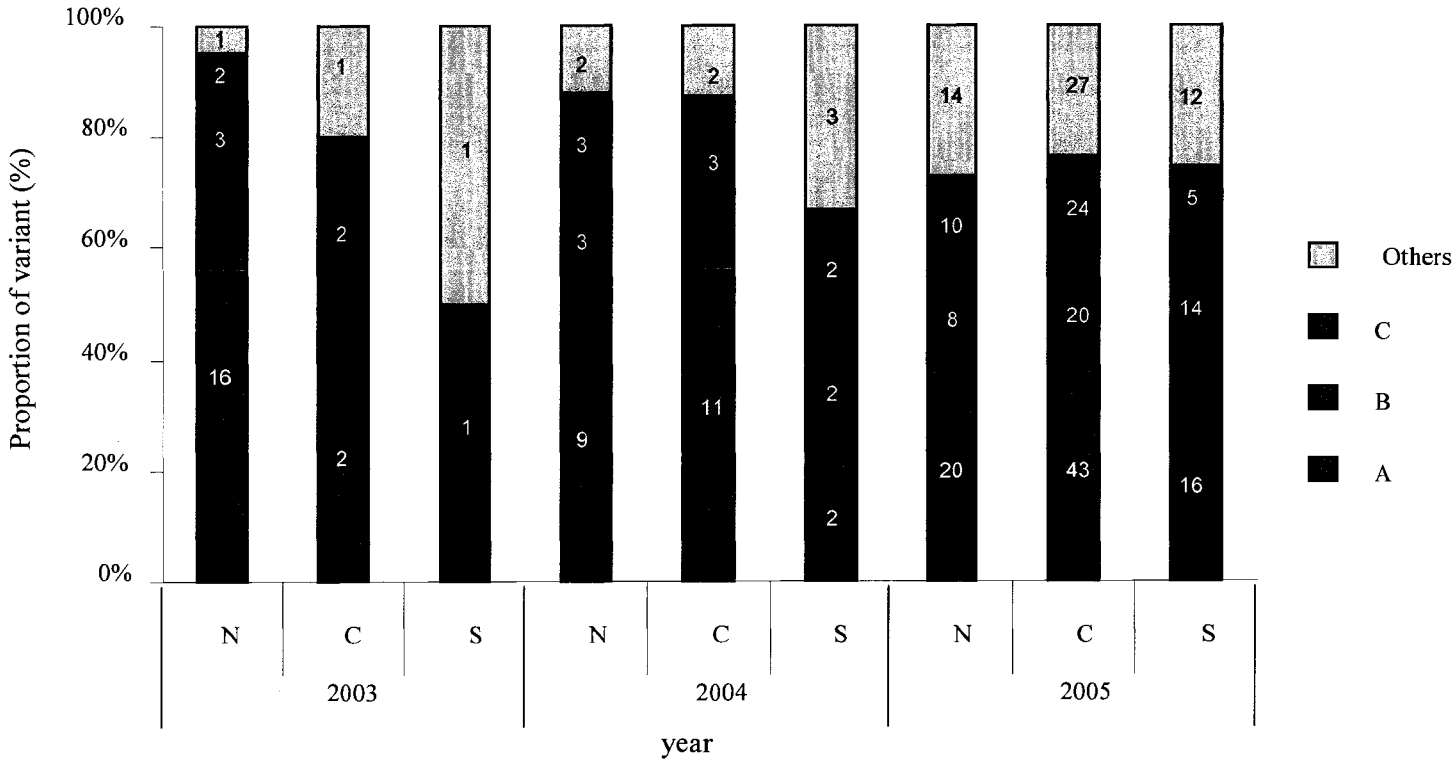

Figure 6-6 Temporal pattern of regional structure of *Bartonella* variants among black-tailed prairie dog colonies divided into three areas: northern (N), central (C), and southern (S), showing the composition of *Bartonella* variants in each area. The bars represent *Bartonella* variants that are classified into four categories: A, B, C, and Others (combining variant D-I). Each category is distinguished by color. The numbers shown in the bars represent the frequency of one particular variant detected at that area.

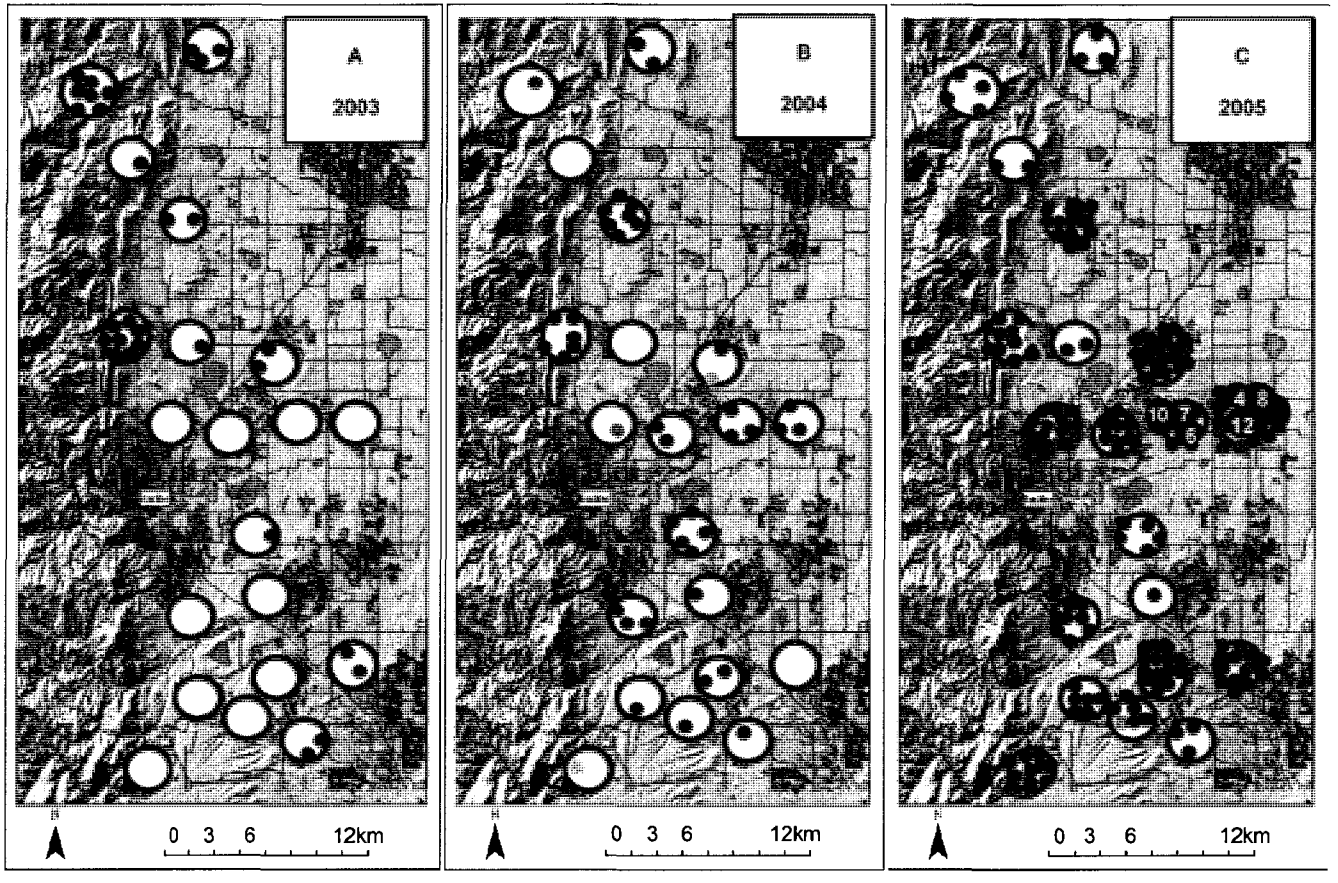

Figure 6-7 Spatial distribution of *Bartonella* variants in 20 black-tailed prairie dog colonies in 2003 (A), 2004 (B), and 2005 (C). *Bartonella* variants are distinguished by color. Each dot within a colony represents one sequence assigned to that variant, except in colony 13A and 16A, the frequency of sequences assigned to the variant is denoted by the number shown in the dots in 2005.

Table 6-2 *Bartonela* variant and frequency at each of 20 black-tailed prairie dog colony, Boulder, Colorado, 2003-2005

colony	A				B				C				D			E			F	G			H			I
	y03	y04	y05	T	y03	y04	y05	T	y03	y04	y05	T	y03	y05	T	y04	y05	T	y05	y04	y05	T	y04	y05	T	y05
1A	2	1	1	4	1		1	2		1		1							1							
2A	4			4	1		1	2	1		1	2	1		1				1	1	1	2				
3A			3	3			3	3	1		3	4	1	1	2				2		2	2				
4A	1		1	2										1	1											
5A	1	5	4	10		1	3	4	1	1	2	4		2	2		1	1			2	2				
6A			2	2	1			1		1	1	2	1		1											
7A			5	5		1	7	8		1		1		1	1				1							1
8A			1	1	1		1	2																		
9A	1	3	2	6			1	1		1	2	3								1		1				
10A		1	1	2																						
11A		1	2	3			2	2			1	1														1
12A		1	3	4			2	2						1	1					2	1	3				1
13A		2	7	9			6	6		1	10	11									1	1				1
14A			2	2			3	3			1	1				1		1	1		2	2				
15A	1	1	13	15			2	2	1		2	3				1		1	3							
16A		2	12	14			8	8		1	4	5		3	3				3		7	7				3
17A			8	8			1	1			4	4							1				1	1	2	1
18A		2	5	7							3	3												1	1	
19A	8	3	5	16		2	1	3		1	3	4					1	1			1	1				
20A			2	2		1		1			2	2					1	1								1
Total	18	22	79	119	4	5	42	51	4	8	39	51	3	9	12	2	3	5	13	4	17	21	1	2	3	9

y03 = year 2003, y04 = year 2004, y05 = year 2005, T = total

positive BTPDs (Figure 6-6). Within colony 19A, where the highest number of positive BTPDs (8) was found, variant A was the only variant detected; colony 2A was the only one where all 4 variants were detected, with total number of infected BTPDs was 7 (Figure 6-7A). In other colonies from the area, the number of infected animals ranged from 1 to 3, with 1 or 2 variants found. In the central area, variants A, C, D infected 2, 2, and 1 BTPD, respectively. Variant B was not detected in this area. In the southern study area, variants B and D each infected one BTPD that was captured in colony 6A (Figure 6-7A; Table 6-2). Variants A and C were not found in the area.

Distribution of Bartonella variants in 2004

In 2004, bartonella diversity and the number of BTPDs infected by bartonellae were higher than they were in 2003. Six variants were identified among 42 sequences. Variant A (52.4%, 22/42) was distributed in most colonies of the study area, but was the most prevalent in the central area (11/16, 68.8%). In the southern area, variant A was detected for the first time and accounted for 22.2% (2/9) of sequences obtained in this area. In contrast, the proportion of variant A in the northern area declined (9/17, 52.9%) from 2003 (Figure 6-6). Similar to 2003, variant B (11.9%, 5/42) was found in the northern and southern areas but not in the central area; variant C (19.0%, 8/42) was found in all three study areas; variant G, a novel variant, was detected in BTPDs of a few colonies that located in each part of the study area. In the northern area, 5 *Bartonella* variants were detected in 17 BTPDs. In colony 19A, variants B (2) and C (1) appeared in addition to the previously detected variant A (3). Within colony 5A, increasing bartonella diversity was also shown, with 3 variants among 7 infected animals. However, in colony 2A, variants found in 2003 were not detected. Instead, the new variant, G, was detected in the

only infected BTPD. In colony 17A, where no infections were detected in 2003, one BTPD was infected with variant H. In the central area, four variants were detected among 16 BTPDs. Variant A, the dominant type, was evenly distributed in the area. Two new variants, E and G, were found in one animal each in colonies 15A and 9A. In the southern area, five genetic variants, A, B, C, E, and G, were detected among the 9 infected BTPDs (Figure 6-7B; Table 6-2).

Distribution of Bartonella variants in 2005

In 2005, bartonellae of high genetic diversity were detected in large number of BTPDs. Nine variants were identified among 213 sequences. Most variants were widely distributed within the entire study area but were more prevalent in the central area (Figure 6-7C). Compared to that in previous years, the proportion of variant A continuously increased in the southern study area but decreased in the northern and central areas, with a similar proportion in each area (34.0%-37.7%, Figure 6-6). Variants B and C were presented in all three parts. Although frequency differed, the proportion of the variants A, B, and C in each area was close (73.1%-76.3%, Figure 6-6). The number of variants detected in BTPDs of each colony ranged from 1 to 7. The highest density was observed at colony 16A with 7 variants present. In colony 10A, located in the central area and adjacent to highway 36, only one BTPD was infected that with *Bartonella* variant A (Table 6-2).

Differentiation of bartonella subpopulations at selected colonies

The genetic diversity of bartonella subpopulation (number of variants) and the proportion of each variant in the subpopulation were compared between 10 selected

colonies, 3A, 5A, 7A, 13A – 19A, where ≥9 bartonella isolates were obtained in 2005 (Figure 6-8). The number of variants per colony ranged from 3 (18A) to 7 (16A). Variant A was found in all 10 colonies, with proportions ranging from 21.4% (3/14, 3A) to 65% (13/20,15A). The highest proportion, 65%, within colony 15A, was significantly higher than those within colonies 16A (30%, 12/40), 5A (28.6%, 4/14), 13A (28%, 7/25), 14A (22.2%, 2/9), and 3A (21.4%, 3/14) (Fisher's exact test, p=0.01-0.04). Variant B was detected at all 9 colonies, except for 18A, with subpopulations varying in proportion from 6.3% (1/16, 17A) to 46.7% (7/15, 7A). The highest proportion (46.7%), within colony 7A, was significantly higher than those within colonies 16A (8/40, 20%), 15A (2/20, 10%), 17A (1/16, 6.3%), and 19A (1/11, 9.1%) (Fisher's exact test, p=0.01-0.05). Variant C was detected at all 9 colonies except for 7A; proportions varied from 10% (2/20, 15A; 4/40, 16A) to 40% (10/25, 13A). Proportions were significantly lower at colonies 15A and16A than at colony 13A (Fisher's exact test, p<0.025), there was no evident difference between proportions within other colonies. Other variants in BTPDs from the compared colonies accounted for 8% (13A) to 40% (16A) of the total isolates.

Co-infections with two *Bartonella* variants in BTPDs

Concurrent infections with two variants in the same BTPD were observed in 10 BTPDs in 2005. Variants A, B, C, and G, the most frequently detected variants in the study, were detected in co-infections as: A-B (1), A-C (2), A-G (2), B-C (2), and B-G (3). Co-infections were not detected in BTPDs samples in other years.

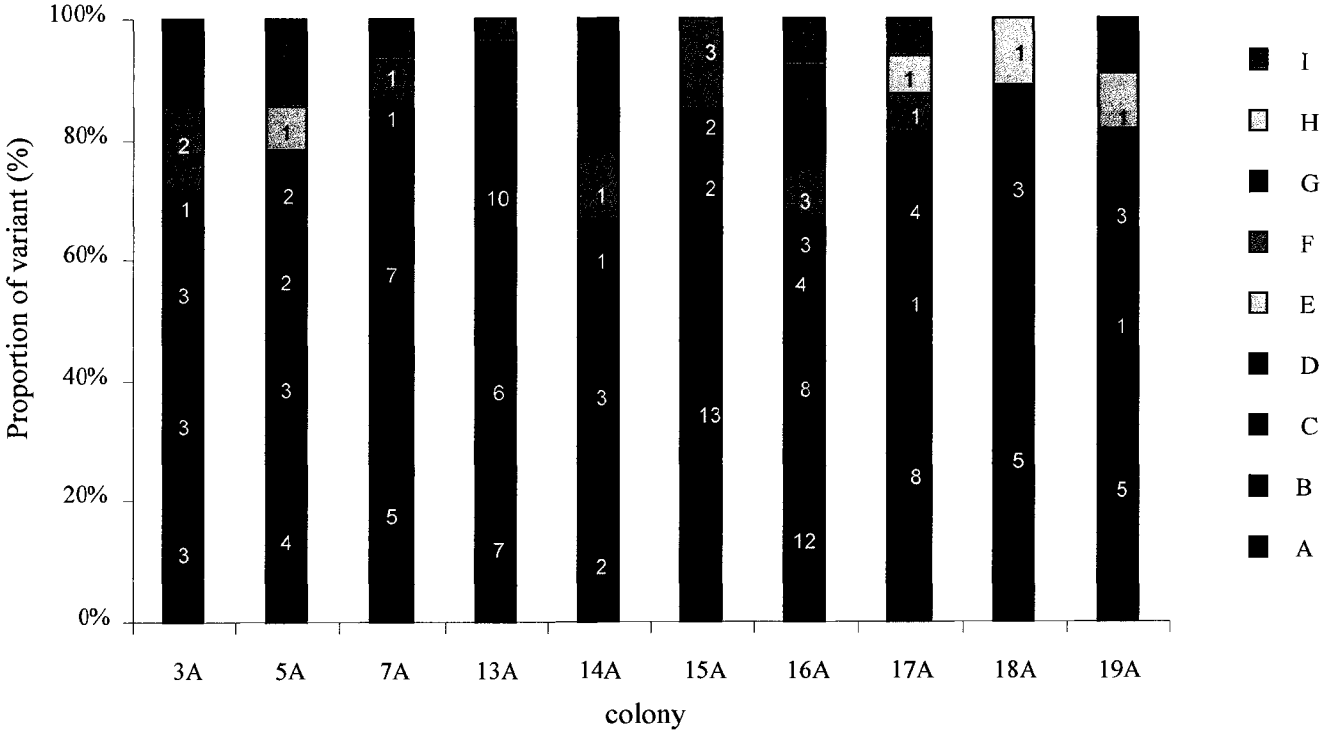

Figure 6-8 Structure of bartonella subpopulations at 10 selected black-tailed prairie dog colonies in 2005, showing the composition of *Bartonella* variants at each colony. The bars represent *Bartonella* variants and are distinguished by color. The numbers shown in the bars represent the frequency of a particular variant detected in black-tailed prairie dogs of that colony. The different levels of diversity and frequency indicate differentiation of bartonella subpopulations in black-tailed prairie dogs of particular colonies.

DISCUSSION

This study demonstrated that bartonellae of diverse genetic variants frequently infect BTPDs. Beginning in the northern area, bartonella infection in BTPDs spread with increasing prevalence and diversity over time. The spatial distribution of bartonellae in BTPDs showed the agent spread from northern to central and southern areas and from west to east over three years. Presumably, BTPDs contracted infection within their natal colonies, then dispersed to other colonies and served as a source of the infection. Bartonellae may have been spread directly by dispersal of infectious hosts, or by movement of infected fleas carried by dispersing hosts. Indeed, the observations of spread of bartonella reflected the directions of BTPD dispersal.

Significant spatial-temporal variation in prevalence and diversity of bartonella was observed among BTPDs of different colonies. Although bartonella activity might be site-specific and, local site effects might be important for determining prevalence, it is likely that the landscape characteristics in a particular area restricts or benefits movement of BTPDs and concomitant trafficking of bartonella. The variation of bartonella prevalence between colonies suggests different rates of BTPD dispersal. In 2005, high number of infected BTPDs and a high prevalence were observed in colonies 13A, 15A, and 16A, located in the central area. These colonies were in close proximity to each other. In contrast, many fewer infected individuals and low prevalence were found in the more isolated colonies 9A and 10A, although these colonies were located in the central area as well. The results suggest that BTPDs likely disperse to nearby colonies. The results support the hypothesis that geographic distance between colonies strongly influenced BTPD dispersal (Martin et al., personal communication). BTPDs in colony 17A, located

at the very southern end of the northern area, were free or rarely infected with bartonella in 2003 and 2004 but became heavily infected in 2005, with the bartonellae presumably coming from colony 19A, where infected BTPDs may preferentially traffic along roadways (Knowles, 1985; Reading and Matchett, 1997) between the two colonies. This observation supported the hypothesis that roads and trails could facilitate movement (Knowles, 1986; Garrett and Franklin, 1988) by acting as primary dispersal corridors (Knowles, 1985). Bartonella prevalence and density was at a very low level at colony 8A through the years of this study. Compared to 17A, 8A was closer to 19A but lacked road access. In addition, streams and ponds between colonies 19A and 8A also may be barriers to movement of animals that traffic bartonellae (Collinge et al., 2005). These landscape features together isolated BTPDs in colony 8A from BTPDs in other colonies. Urbanization as a barrier to prairie dog dispersal has been reported (Avashia et al., 2004). In the present study, dispersal of BTPDs from colony 17A (adjacent to Boulder) towards the northern area was blocked by urban environment. In response, these animals might have moved east into colony 18A by crossing highway 119. The lower prevalence and diversity of bartonella in 18A than in 17A indicated that the highway might have been a barrier to prairie dog dispersal but had less effect than did urbanization, as some BTPDs successfully crossed the highway. Alternatively, prairie dog dispersal did not occur. Infected fleas might have been transported by carnivores that crossed highways and spread bartonella. The high prevalence of bartonella observed in some colonies in the southern part of the area suggests the possibility of long-distance dispersal. However, it is perhaps more likely that bartonella movement across highways occurs through human-generated movement, i. e., the relocation program that involved moving animals across

several major roadways, done by Boulder County personnel in recent years (Collinge, personal communication).

The only published study on spatial distribution of *Bartonella* variants was that by Jardine and her colleagues (Jardine et al., 2006a). They detected four genotypes of *Bartonella* in Richardson's ground squirrels captured at 11 sites over three years, with the dominant genotype accounting for 87% of all sequences. Compared to Richardson's ground squirrels, BTPDs from the present study harbors a great diverse assemblage of bartonellae, with nine genetic variants identified, but only a few of them well established. Variant A, accounting for 41.9% of the total sequences obtained, was the most prevalent type, but the proportion decreased over time. Variants B and C contributed equally to the population and maintained similar levels through the years. Variant D, seemed not to be favored by natural selection, only represented a small portion of the population. New *Bartonella* variants emerged during the period of observation. These variants, tightly clustered with variant A, likely were lineages that emerged from variant A, suggesting a clonal structure of *Bartonella*. The increasing frequency of new variants suggests that genetic drift had occurred in the population. Described as Candidatus *Bartonella washoensis* subsp. *cynomysii* (Bai et al., 2008), these variants were not found in rodents of other species from the same area but were typical of BTPDs, indicating a strong host-specificity of bartonella and BTPDs. Although a diverse assemblage of *Bartonella* lineages has been identified in the study, the actual genetic diversity of bartonellae infecting the BTPDs may have been underestimated due to undetectable infections and unsuccessful sequencing. For example, in the present study, variant D was detected in 2003 but not in 2004. Also, the variants circulating in members of the population may

vary seasonally. The present study might not have detected some variants, since all trappings were done in the same season. A larger sample size from each colony is needed to obtain more precise estimates of diversity of BTPD-associated *Bartonella* variants.

Bartonella variants were not site-specific and seemed to appear randomly. However, in BTPD colonies, spatial distribution of *Bartonella* variants varied with regard to diversity and frequency, suggesting the fragmented structure of bartonella subpopulations and some level of bartonella subpopulation differentiation might have reflected the structure of BTPD population in an unrecognized manner. The change of diversity might have been driven by evolutionary forces and non-evolutionary factors simultaneously. At least three hypotheses could explain the observations. First, gene flow or BTPD migration occurring between colonies may have resulted in introduction of new genetic variants to the established gene pool of a particular species or population. Theoretically, gene flow occurs more often between BTPDs in colonies with shorter distances than between BTPDs in colonies with longer distances. The high diversity observed in colonies 13A and 16A, likely was the result of frequent gene flow or migration between the colonies. In the Richardson's ground squirrel study, bartonella diversity also varied among sites and the authors proposed that limited dispersal of the ground squirrels was one of the reasons causing low diversity (Jardine et al., 2006a). Gene flow not only could increase diversity within a subpopulation, it also could homogenize the entire population. The similar proportions of the three most prevalent variants (A, B, and C) in the northern, central and southern areas in 2005 suggested the bartonella population had been homogenized, likely by frequent gene flow. Noting that the number of infected BTPDs was high in these colonies, the present study suggests that diversity could be density-

dependant. Second, Mutation could be another factor creating variations in the gene pool, and causing an increase of the diversity. Certain colonies, such as 3A and 14A, are relatively isolated from other colonies. It is less likely that gene flow occurred between these colonies and others. The high diversity observed in these colonies could have been the result of high rates of mutation. If not caused by relocation, that variant B was not detected in the central area but was detected in the other two areas in the first two years also support the hypothesis of mutation. This is because long distance migration (from northern to southern), accomplished by BTPD crossing the urbanized area and the two highways, is unlikely to have occurred. Finally, the social system of BTPDs leads to genetic differentiation not only between colonies but also between coteries within colonies (Chesser, 1983; Dobson et al., 1997). This differentiation of coterie structure within a colony might support the circulation of different *Bartonella* variants. Greater diversity would be expected in a larger colony within which more coteries could be established. A more detailed sampling of known family groups within colonies is necessary to test this hypothesis.

There is no clear explanation for the extremely high bartonella activity in 2005, as compared to previous years. However, this increased activity may explain the increased bartonella diversity of the same subpopulation over time. When bartonella became highly prevalent, evolutionary pressures would result in a diverse and differentiated subpopulation. Nevertheless, some subpopulations, such as those from colonies 8A and 10A, were not highly variable. These subpopulations were isolated from other subpopulations and remained static.

The co-existence of *Bartonella* variants observed in 2005 might have been related to the plague epizootic that occurred in the study area in that year. A high rate of mixed bartonella infections was observed in an early investigation of bartonella in BTPDs during an outbreak of plague in Adams County, Colorado in 2001 (Kosoy et al., unpublished data). Mixed bartonella infections could be the result of a succession of independent horizontal transmission events. In chapter 4, it is mentioned that bartonella infection was never found in a recaptured BTPD, I explained as the result of acquired immunity. This immunity provides cross-protection from infection of any variants. However, this might be true only under normal conditions. When the conditions are changed, for example by heavy flea infestation leading to plague, the vertebrate host might be more vulnerable to infection and the immunity acquired from having had a previous infection may not be enough to protect the host from a subsequent infection with a different variant. Other ecological factors, such as change of climate, may also have contributed to the change. The finding of prevalent variants as source of co-infection may suggest that these variants were more pathogenic as a result of selection.

Recently, renewed and increased attention has been focused on the role of bartonellae as threats to human health (Anderson and Neuman, 1997; Breitschwerdt and Kordick 2000; Comer et al., 2001). Bartonellae of many species are potentially pathogenic to humans (Daly et al., 1993; Ellis et al., 1999a; Welch et al., 1999; Kosoy et al., 2003). Sharing landscape with other mammals, human populations have closer and more frequent contact with wild animals, which increases the opportunity for inter-species transfer of agents (Mills, 2005). In this study, certain colonies that had high prevalence of bartonella (17A and13A) were very close to residential areas. This may

increase the risk of BTPDs transmitting bartonella to humans. The finding of Candidatus *B. washoensis* subsp. *cynomysii* in a high proportion of BTPD colonies living in close proximity to residential areas suggests the potential significance of these bacteria as agents of human illness. Further investigation is needed to determine the potential public health significance of this subspecies.

CONCLUSIONS

The temporal dynamics and spatial distribution of bartonellae in BTPDs was described, based on analyses of the samples collected during a 3-year study conducted in 20 BTPD colonies in Boulder County, Colorado. The distribution pattern suggested a trend of spread of bartonella infection among BTPDs with increasing number of infected colonies from north to south within the study area. Given that bartonellae occurred at very low prevalence in a spatially restricted region of the study site in 2003, the results suggest epizootic spread. These data may provide an opportunity to investigate amplification dynamics during an early phase of development for this host-agent system. From an ecological standpoint, the study of bartonella infections in BTPDs can provide information useful for testing theoretical models of infectious disease dynamics within naturally susceptible populations.

The present study provided information on the diversity and spatio-temporal distribution of bartonellae circulating within black-tailed prairie dog populations. Spatial genetic structure of bartonella among BTPD colonies not only revealed a fragmented distribution of the organism, but also reflected the dispersal of the vertebrate hosts.

Highly informative, bartonellae hold great potential as genetic tags of their hosts.

They can serve as good ecological markers to retrospectively describe the dispersion of BTPDs from their local origin and to measure the geographical and ecological isolation of separate BTPD colonies. However, artificial efforts, such as relocation of BTPDs, may have overwhelmed the landscape effects on BTPD dispersal pattern and influenced the distribution of the bartonella population within and among colonies, partly or wholly. Patterns observed in the present study may not be generally applicable. Additional analyses, such as effect of colony size and comparisons of bartonella genetics with host genetics, might better clarify the structure of the host population. The diversity data reported here could serve in constructing models for BTPD dispersal as well as co-evolution of bartonellae.

CHAPTER SEVEN

CONCLUDING REMARKS

The importance of some bartonellae as emerging pathogens has been widely recognized during the past decade. In addition to the well-known pathogens *Bartonella bacilliformis*, *B. quintana*, and *B. henselae*, at least four rodent-associated *Bartonella* species (*B. elizabathe*, *B. washoensis*, *B. vinsonii* subsp. *arupensis*, and *B. grahamii*) also have been linked to human illnesses. Following such observations, interests in bartonella ecology in their natural hosts have increased, and have led to a variety of investigations of bartonellae in rodents worldwide. These investigations have collected a great deal of information on the ecology of bartonellae; however, most of those studies were limited to screenings for evidence of infections in rodent, without raising other important biological questions. Previously reported data showed a ubiquity of bartonellae in rodents of diverse taxonomic groups with an exceedingly wide range of prevalence (0-100%) and extremely high bartonella genetic diversity with controversial results regarding host-specificity. These results raised the question as to whether having additional information on bartonellae in rodents would facilitate our understanding of the natural cycle of these organisms and help us develop criteria useful in elucidating the epidemiology of bartonellosis.

My dissertation study was designed to determine how rodent-associated bartonellae are associated with their hosts in terms of community diversity, population structure, and spatial distribution, based on the hypothesis that under natural conditions, dynamics and

diversity of rodent-borne bartonellae populations are regulated by the rodent community structure and the density of the host population. The intent then was to apply the results to other fields, including public health, to fulfill its epidemiological significance.

The data on bartonella prevalence in various rodent communities and rodent populations and on bartonella genetic diversity were obtained by analyzing large number of rodent samples (>4,500) and bartonella cultures (>1,450) using ecological, microbiological, molecular biological and genetic approaches. The present study also showed that the prevalence of bartonella in rodents is generally high but varies widely between rodent communities and rodent populations. Recognizing the complex association with rodent community diversity, population structure, and other factors, it is clear that bartonella prevalence is the result of multiple factors. Questions of the effects of biodiversity on disease risk have received considerable attention since it was proposed that there is "dilution effect" for the infection rate of *Borrelia brugdorferi*, the pathogen of Lyme disease. Having demonstrated a negative correlation between bartonella prevalence and rodent community diversity, the current analysis supports this "dilution effect" hypothesis and provides valuable data to help understand the mechanisms explaining this effect. Such results suggest that information on the composition of rodent species at a given biotic community can be useful epidemiological criteria in predicting bartonella prevalence at the community.

It is, perhaps, remarkable that bartonella prevalence varied so widely in rodents of different species. Persistency of bartonella infection results in extremely high prevalence in some rodents, such as deer mice. Whereas rodents of some species, such as pocket mice, may be more resistant to bartonella infection than are others, resulting in almost

zero prevalence of bartonella in them. Such a hypothesis requires further experimental testing.

Rodent population parameters, including density, seasonal dynamics, age, and sex structures were evaluated as possible predictors in this study. Rodent age was found to be the most valuable parameter, inversely affecting prevalence of bartonella infection in rodent populations; this may be related to the immune status of the hosts.

Genetic analyses of bartonella from rodents of the western United States in the present study demonstrated extraordinarily high diversity of these microorganisms. Detailed analyses of associations between bartonella strains and their rodent hosts demonstrated that host-specificity occurs and is mostly expressed at generic level of rodent hosts. Under most circumstances, one genogroup of bartonellae is typical of rodents of a single taxon. In some cases, multiple bartonella species can be specific to rodents of a single species. Occassionaly, bartonellae can be transferred interspeciesly, resulting in acquisition of non-specific bartonellae. Nevertheless, the "bartonella-rodent" system I studied in North America appear to be noticeably differed from that observed in Europe, which probably reflects the evolutionary histories of bartonellae and might stimulate new perspectives in understanding bacterial evolution. The apparent congruence between the phylogeny of bartonellae and that of the specific hosts they parasitize under most conditions observed in this study suggests that bartonellae have long been adapted to specific hosts and co-speciated with the hosts.

The present study provided information on the diversity and spatio-temporal distribution of bartonellae in black-tailed prairie dogs. Such information, especially the localization of unique genetic variants, demonstrated that spatial structure of the rodent

population could determine distribution and movement of these bacteria. This investigation also proved the usefulness of bartonella as a good biological marker for measuring connectivity between rodent colonies, which could be meaningful in conservative biology.

Results obtained in this study confirmed the complexity of "bartonella-rodent" parasitic systems and, more importantly, they offer helpful criteria for predicting the prevalence of bartonella infection in rodents and provide explanations of which *Bartonella* species or strains can be expected in specific rodent populaitons. Such data can be useful in epidemiological practice, when information on host specificity can help establish a link between homologous genetic variants from human cases and those found in a wide range of potential rodent reservoirs. Also, these results challenge conclusions of some previous publications about these bacteria. Finally, these data have contributed to the development of hypotheses whose testing will benefit concepts of microbial ecology, bacterial evolution, and field epidemiology.

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