Bartonella Infection in Animals: Carriership, Reservoir Potential, Pathogenicity, and Zoonotic Potential for Human Infection

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INTRODUCTION

When historians examine the events that have contributed to the many rapid advances in knowledge related to “emerging infectious diseases” during the latter portion of the 20th century, it is probable that research contributions related to Bartonella infections will prove to be of substantial comparative medical importance. Knowledge derived from studies of the biologic behavior of Bartonella spp. in animals, as compared to behavior in the human host, should provide important insights into the biological behavior and immunopathogenesis of these diseases. It is becoming increasingly obvious that Bartonella organisms are highly adapted to facilitate intracellular persistence in a wide variety of animals, including human beings. For example, Bartonella bacteremia can be found in up to 50% of the domestic and feral cat populations in regions where fleas are endemic (42). In addition, cats can be coinfected with more than one Bartonella sp. (41). In the southeastern United States and the United Kingdom, Bartonella spp. have been isolated from the blood of 42 and 62% of rodents, respectively (11, 64). Strikingly, nearly 100% of deer sampled at a single point in time in either California or France were found to have bacteremia (20; B. B. Chomel, R. W. Kasten, K. Yamamoto, C. Chang, T. E. Honadel, and Y. Kikuchi, Abstr. First Int. Conf. Bartonella Emerg. Pathogens, Main Speaker, p. 31).

In recent years, there have been several excellent reviews (2, 25, 73, 75, 87, 88, 100) and a text (98) detailing the clinical, diagnostic, pathophysiologic, and microbiologic aspects of Bartonella infection, with particular emphasis on human infection. However, collective recent observations have begun to support a role for Bartonella spp. as animal as well as human pathogens. This review will focus on Bartonella infection in animals, particularly as it relates to carriership, reservoir potential, pathogenicity, and the zoonotic potential for human infection. As information related to these concepts is in a rapid state of development, substantial advancement in our understanding of Bartonella infection in animals is anticipated in the future.

SPECTRUM OF ANIMAL INFECTIONS

Bartonella Species

Although continuing to expand rapidly, the genus Bartonella is currently composed of 16 species. In 1993, Brenner and colleagues (17) proposed that previously designated Rochalimaea species be united with the genus Bartonella and renamed Bartonella quintana, B. vinsonii, B. henselae, and B. elizabethae.
This reclassification also resulted in the transfer of these organisms from the family Rickettsiaceae to the family Bartonellaceae, which included Bartonella bacilliformis, and removed the family Bartonellaceae from the order Rickettsiales. In 1995, Birtles and colleagues (12) proposed the unification of the genus Grahamella with the genus Bartonella, which resulted in five additional Bartonella species: Bartonella talpae, B. peromysci, B. grahamii, B. taylorii, and B. doshiae. In effect, these reclassifications eliminated the previous genera Rochalimaea and Grahamella. Historically, Grahamella species were characterized as arthropod-transmitted hemotropic gram-negative bacteria of small mammals, fish, and birds, which remain of no known pathogenic consequence for higher mammals. In 1996, Bartonella vinsonii subsp. berkoffii, isolated from dogs, was designated a new subspecies (57), and Bartonella clarridgeiae, isolated from a cat, was characterized as a new species (70). Bartonella tribocorum was isolated from the blood of wild rats and characterized in 1998 (43). Bartonella washoensis (GenBank accession no. AF070463) was isolated from a patient with cardiac disease, and a rodent reservoir was implicated. During the first 11 months of 1999, Bartonella koehlerae, isolated from the blood of domestic cats (32); Bartonella alsatica, isolated from the blood of wild rabbits (44); Oryctolagus cuniculus and Bartonella vinsonii subsp. arupensis, isolated from the blood of a cattle rancher (106); and Bartonella weissi, isolated from a cat (GenBank accession no. AF199502), were added to the growing list of Bartonella species and subspecies. The phylogenetic relationship of these Bartonella species, based upon the 16S rRNA gene, is depicted in Fig. 1.

Microscopically, all Bartonella spp. are gram-negative bacilli or cocccobacilli. The isolation and characterization of Bartonella spp. have been reviewed in substantial detail elsewhere (96, 97). Bartonella bacilliformis and B. clarridgeiae have flagella, which in the case of B. bacilliformis facilitates erythrocyte invasion. Polar structures resembling fimbriae have been observed on B. tribocorum. Other Bartonella species, such as Bartonella henselae, appear to lack flagella (Fig. 2). Based upon in vitro studies using human umbilical vein endothelial cells, B. henselae is internalized by an actin-dependent invasome-mediated mechanism of cellular invasion (30).

As a genus, Bartonella are catalase, oxidase, urease, and nitrate reductase negative. Those members formerly classified as Grahamella and B. quintana (Fuller strain) are uniquely positive in the Voges-Proskauer test. Biochemical profiles of Bartonella spp. are fairly neutral except for the production of peptidases, which varies slightly among species (97). 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 (9), or four variants (variants I to IV) on the basis of several alternative DNA fingerprinting methods (94, 96). Although the same B. henselae subtype can be identified in cat scratch disease (CSD) patients and the blood of their cats, definitive correlations between subtypes and specific disease manifestations have not yet been reported (95).
Role of Insect Vectors

In general, knowledge related to vector transmission of Bartonella organisms is very incomplete. Several insects have been implicated in Bartonella transmission, including sand flies (36), the human body louse (93), the cat flea (23, 34, 45), the vole ear mite (3), and ticks (81, 106) (Table 1). Fleas (Xenopsylla cheopis) collected from rats in downtown Los Angeles were commonly infected (61%) with Bartonella strains, including B. elizabethae (L. Beati, B. A. Ellis, M. Rood, S. Eldaieef, and R. L. Regnery, First Int. Conf. Bartonella Emerg. Pathogens, 1999, abstr. 13, p. 34). Certain Bartonella spp. appear to be more highly adapted to cause persistent infection in rodents (28, 106; Beati et al., abstr. 13). Infection with rodent Bartonella spp. is very incomplete. Several insects have been implicated in Bartonella transmission to specific-pathogen-free endocarditis and febrile illness, respectively (28, 106). Bartonella henselae is more highly adapted to cause persistent infection in rodents than B. quintana, the most frequent cause of CSD, remains unclear, though as yet unproven, it is now generally accepted that B. henselae was transmitted by transferring B. henselae-infected cat fleas that had fed upon B. henselae-infected cats (23). In the same study, oral transmission, using fleas or flea feces, was not accomplished. Cats fed fleas that had fed upon B. henselae-infected cats and 45 mg of fresh flea feces from B. henselae-exposed fleas did not seroconvert or become bacteremic. Although as yet unproven, it is now generally accepted that B. henselae transmission from cats to humans is similar to transmission of B. quintana to humans. Mechanical transmission occurs when louse feces containing B. quintana are inoculated by scarification of the skin or through contact with conjunctival membranes. Although the exact mode of transmission of B. henselae, the most frequent cause of CSD, remains unclear, contamination of the claws or teeth with infected flea feces may be required for transmission. When uninfected SPF cats were housed with B. henselae-infected bacteremic cats in an ectoparasite-free environment, there was no evidence of Bartonella transmission (23). Elimination of flea and tick infestation, as potentially demonstrated in a household containing three pets, one each persistently infected with B. henselae, B. clarridgeiae, and B. vinsonii subsp. berkhoffii, may interrupt the transmission cycle (11). Although tick transmission has seldom been proposed as a means of Bartonella transmission to humans, substantial evidence is mounting to support tick transmission of these agents among animals (46, 81, 99; Chomel et al., Main Speaker, p. 31). Ixodes scapularis ticks in The Netherlands appear to be frequently infected with Bartonella spp. (46, 99).

TABLE 1. Insects associated with the transmission of Bartonella spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Insect vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. bacilliformis</td>
<td>Sand flies (Lutzomyia sp.)</td>
</tr>
<tr>
<td>B. clarridgeiae</td>
<td>Cat flea (Ctenocephalides felis)</td>
</tr>
<tr>
<td>B. henselae</td>
<td>Vole ear mite (Trombicula miroti)</td>
</tr>
<tr>
<td>B. henselae subsp. vinnsonii</td>
<td>Ticks (species unknown)</td>
</tr>
<tr>
<td>B. henselae subsp. anupensis</td>
<td>Deer tick (Ixodes scapularis)</td>
</tr>
<tr>
<td>B. quintana</td>
<td>Human body louse (Pediculus humanis)</td>
</tr>
<tr>
<td>B. quintana subsp. vinnsonii</td>
<td>Sand flies (Lutzomyia sp.)</td>
</tr>
<tr>
<td>B. quintana subsp. clarridgeiae</td>
<td>Cat flea (Ctenocephalides felis)</td>
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</tr>
<tr>
<td>B. quintana subsp. anupensis</td>
<td>Ticks (species unknown)</td>
</tr>
</tbody>
</table>

Host Specificity

Because all Bartonella spp. are believed to be vector-transmitted, blood-borne, intracellular organisms, vector preference for certain hosts could influence the transmission of these organisms. Presumably, this vector preference is partly responsible for the more frequent association of a given Bartonella sp. with a specific host, i.e., B. henselae, B. clarridgeiae, and B. koehlerae in cats, B. vinsonii subsp. berkhoffii in dogs and coyotes (Canis latrans), B. alsalica in wild rabbits, and B. quintana in human beings. However, preferential infectivity in specific hosts also appears to play a role in determining which animals will become infected with a particular Bartonella sp. For example, Bartonella isolates obtained from three Peromyscus species were all in the same phylogenetic cluster, whereas isolates from cotton rats (Sigmodon hispidus) from the same area or from more distant locations were contained within three clusters (64). In the United Kingdom, the same Bartonella sp. was isolated from five different species of small woodland mammals (11). Following experimental inoculation, dogs do not become bacteremic with B. henselae and cats do not become bacteremic with B. vinsonii subsp. berkhoffii (B. B. Chomel, R. W. Ermel, R. W. Kasten, K. Yamamoto, C.-C. Chang, R. Keller, D. Weber, A. Poland, Y. Piemont, H. J. Boulouis, and N. C. Pedersen, Int. Conf. Rickettsiae Rickettsial Dis. Am. Soc. Rickettsiol. 14th Sesquiannual Joint Meet., 1999, abstr. 41). At least in cats (41, 42, 62) and rodents (64), simultaneous infection with more than one Bartonella sp. (B. henselae and B. clarridgeiae in cats) has been documented.

Other as yet undetermined factors are presumably involved in determining host specificity. For example, although the cat flea, C. felis, frequently infests dogs within the same household that contains cats infected with B. henselae or B. clarridgeiae, to date these organisms have not been isolated from the blood of a dog. However, cats infected with a B. clarridgeiae-like strain obtained from a coyote subsequently developed bacteremia (Chomel et al., abstr. 41). Although host specificity may be responsible for the association of B. henselae with the canine reservoir, an alternative explanation may be a failure to isolate B. henselae from dog blood due to a low concentration of bacteria in the blood. Recently, B. henselae DNA was amplified and sequenced from the liver of a dog with peliosis hepatis (53), a lesion associated with B. henselae or B. quintana infection in human patients, particularly in those who are immunocompromised (54, 88). Unfortunately, blood was not available from this dog for culture or PCR analysis. Isolation of Bartonella spp. from embryos of naturally infected rodents suggests the possibility of transplacental transmission (65). Congenital transmission could also contribute to a predilection for a given Bartonella sp. to more frequently infect a specific host species. As with other infectious agents, differences in placentalization among different animal species could serve as a rate-limiting barrier for transplacental transmission.

Seroepidemiologic Studies

In veterinary medicine, the most extensive Bartonella seroepidemiologic studies have involved domestic and feral cats (21, 22, 24, 35, 37, 49, 104). Based upon more recent observations, there may be at least three limitations to the accurate interpretation of seroprevalence data derived from these cat studies (21, 22, 24, 35, 37, 49, 104). First, the patterns of cross-reactivity among the four Bartonella spp. isolated from cats have not been delineated. Second, the extent to which other organisms such as Chlamydia spp. and Coxiella burnetti, which have been associated with serological cross-reactions in people (69, 74), falsely influence seroprevalence data derived from
cats is not known. Third, an unknown number of cats will not have detectable antibodies despite being bacteremic at the time of sample collection (24, 59). Despite these limitations, seroprevalence surveys indicate that a remarkable number of cats throughout the world appear to have been exposed to *Bartonella* spp. Thus, cats have the potential to act as a substantial reservoir for human infection. Seroprevalence to *B. henselae* antigens is much higher (40 to 70%) in cats that live in warm, humid regions of the world in which severe flea infestation is expected. In the United States, regional seroprevalence varied from 3.7 to 54.6%; only the northern Rocky Mountain-Great Plains region had a seroprevalence rate of less than 10% (49). Of potential public health concern, feral cats are more likely to be seroreactive or bacteremic than pet cats from the same region (21–24, 104). However, seroprevalence in bobcats, mountain lions, and large cats maintained at zoological parks can be comparable to the prevalence found in the domestic cat population in the same geographic area (92; Chomel et al., abstr.). As the nocturnal opossum is frequently infested with *C. felis*, this animal as well as others in nature may be responsible for transporting infested fleas to repopulate different sites within a community.

Seroprevalence in cattery cats is generally bimodal. Foley et al. (35) found either that most cats within a given cattery had serologic evidence of exposure or that very few or none were exposed. Of note, flea infestation was the most important risk factor associated with high *Bartonella* seroprevalence in cattery cats. Seroprevalence in cats was 47% in Hawaii (31), 15% in Japan (104), 54% in Indonesia (71), 40% in Israel (4), 11% in Egypt (22), 7% in Portugal (22), 48% in Singapore (77), 21% in South Africa (51), and 24% in Zimbabwe (51). When sera from cats in North Carolina and Israel were tested by indirect fluorescent antibody (IFA) to both *B. henselae* and *B. quintana* antigens, antibodies to *B. quintana* were significantly higher in cats from Israel (4). However, absorption of sera with *B. henselae* or *B. quintana* antigens supported exposure to *B. henselae* rather than *B. quintana*. These results suggest that in Israel, cats may be exposed to one or more antigenically different *Bartonella* species, subspecies, or strains that, immunologically, more readily recognize *B. quintana* antigens.

Due to the relatively recent recognition that dogs can be infected with *B. vinsonii* subsp. *berkhoffii* and potentially other *Bartonella* spp., seroprevalence data are limited. Seroprevalence was determined in 1,920 clinically ill dogs from North Carolina and surrounding states that were evaluated at a veterinary teaching hospital (81). Using a cutoff reciprocal titer of ≥64, 3.6% of the dogs had antibodies to *B. vinsonii* subsp. *berkhoffii*. Risk factors that could be associated with seroreactivity included heavy tick infestation (odds ratio [OR], 14.2), cattle exposure (OR, 9.3), rural rather than urban environment (OR, 7.1), and heavy flea infestation (OR, 5.6). These data indicated that exposure to *B. vinsonii* subsp. *berkhoffii* was more likely in dogs in rural environments that were allowed to roam and likely to have a history of heavy tick and flea infestation. Using sera from dogs experimentally infected with *R. rickettsii* or *Ehrlichia canis*, cross-reactivity to *Bartonella* antigens was not detected; however, 36 and 52% of serum samples derived from dogs naturally infected with *Ehrlichia canis* or *Babesia canis*, respectively, were reactive to *B. vinsonii* antigens (81). As both *E. canis* and *B. canis* are transmitted by *Rhipicephalus sanguineus*, this tick may be involved in the transmission of *B. vinsonii*, particularly in kennels with severe tick infestation problems. The possibility of tick transmission was further supported by two additional studies (15, 63) involving dogs from the same geographic region infected with one or more *Ehrlichia* spp. Seroreactivity to *B. vinsonii* subsp. *berkhoffii* antigens was detected in 4 of 12 (33%) dogs diagnosed with ehrlichiosis (15) and in 23 of 27 (85%) Walker hounds in a kennel with severe tick infestation (63). Seroprevalence, using *B. vinsonii* subsp. *berkhoffii* antigens, was 10% (4 of 40 dogs) in dogs with suspected tick-borne illness from Israel (5). Using an enzyme-linked immunosorbent assay, 35% of 869 serum samples derived from coyotes in California contained antibodies to *B. vinsonii* subsp. *berkhoffii* antigens (19). Antibodies to *B. vinsonii* subsp. *berkhoffii* were detected in 66 of 483 (14%) sera from military working dogs stationed in southern France or Africa (B. Davoust, M. Drancourt, M. Boni, D. Parzy, J. Seigent, V. Roux, and D. Raoult, Int. Conf. Rickettsiae Rickettsial Dis. Am. Soc. Rickettsiol. 14th Sesquiannu. Joint Meet., 1999, abstr. 232B) and in 163 of 1,873 (9%) sera from U.S. government-owned dogs (Chomel et al., Main Speaker, p. 31).

**PREVALENCE, PERSISTENCE, AND PATHOGENICITY**

**Prevalence of Bacteremia**

The high prevalence of antibodies to *Bartonella* spp. in animals supports the possibilities of frequent exposure, persistent infection, and recurrent infection. In addition to seroprevalence data, a substantial number of recent studies have utilized blood culture to detect *Bartonella* bacteremia. *Bartonella* spp. can be isolated from blood more efficiently following lysis of erythrocytes and leukocytes (85, 96). This can be achieved by using a lysis solution (Isolator blood lysis tubes; Wampole, Cranbury, N.J.) or by freezing the EDTA-treated blood samples at −70°C for 24 h prior to inoculation of a blood agar plate. Detection of *Bartonella* species in conventional automated blood culture systems is less reliable (96). As most *Bartonella* species are highly fastidious, prolonged incubation periods (4 to 6 weeks) at low CO₂ concentrations (5 to 10%) maintained at 35°C can be required for visualization of colonies.

The prevalence of bacteremia within rodent populations can be quite high. In the United Kingdom and in the southeastern United States, 23 of 37 (62.2%) rodents and 119 of 279 (42.2%) rodents, respectively, were bacteremic (11, 64). In the southeastern United States, rodent *Bartonella* isolates cluster within four phylogenetic groups, based on homology of the citrate synthase gene (11). Despite the high prevalence of bacteremia, most infected rodents have low or undetectable levels of serum antibodies by IFA. *Bartonella* spp. were visualized in blood smears of shrews (10% of *Sorex cinererus*, 20% of *Sorex fumens*, and 14% of *Blarina brevicauda*) collected from Pennsylvania (66).

*Bartonella* spp. have also been isolated from the blood of a diverse group of wild animals from the western United States, including 2 of 7 mountain lions, 5 of 13 bobcats, 17 of 54 coyotes, 4 of 6 gray foxes, 17 of 100 elk, and 39 of 42 black-tailed deer (19, 20; Chomel et al., Main Speaker, p. 31, and abstr. 41). Although genetically similar, isolates from pumas and bobcats differ from *B. henselae* isolates obtained from domestic cats (Chomel et al., abstr. 41). *B. alsatICA* was isolated from 9 of 30 blood samples from wild rabbits in France (44). Recently, *Bartonella* bacteremia was reported in 47 (89%) of 53 beef cattle from Oklahoma and 11 (17%) of 63 dairy cattle from California (20). Tick, louse, or other vector transmission of *Bartonella* species in deer or cattle would also seem more likely, as fleas infrequently infest these species.

*Bartonella* spp. have been isolated from the blood of cats from San Francisco (54), North California (52), Hawaii (31), Japan (72), Sydney, Australia (13, 33), New Zealand (50), the
Netherlands (10), Nancy, France (42), and Indonesia (71) (Table 2). In Nancy (42), 17 isolates (34%) were \( B. \) \textit{henselae} type I (identical to the Houston I type strain) (85), 19 isolates were \( B. \) \textit{henselae} type II (identical to the BA-TF strain) (90), and 15 isolates (30%) were identical to the type strain (ATCC 51734) (70) of \( B. \) \textit{clarridgeiae} (42). In Indonesia, six \( B. \) \textit{henselae} and three \( B. \) \textit{clarridgeiae} isolates were obtained (71). \( B. \) \textit{tonella} subsp. were isolated from six of seven domestic cats belonging to four patients diagnosed with bacillary angiomatosis and from 17 of 19 domestic cats associated with CSD patients (54, 56). \( B. \) \textit{clarridgeiae}, isolated from a kitten, was implicated as the cause of CSD in a veterinarian (58). The isolate, designated 94-F40 (nucleotide sequence accession number U64691), was initially differentiated from other type strains by PCRrestriction fragment length polymorphism (RFLP) (Fig. 3).

When using the currently recommended microbiologic techniques, there appears to be considerable variation in the degree of difficulty associated with the isolation of \( B. \) \textit{tonella} spp. from the blood of different animal species. For example, in our laboratory, isolation of \( B. \) \textit{henselae} from the blood of naturally or experimentally infected cats is frequently successful; however, isolation of \( B. \) \textit{vinsonii} subsp. \textit{berkhoffii} from seroreactive dogs or dogs from which \( B. \) \textit{vinsonii} subsp. \textit{berkhoffii} DNA has been amplified from the same processed EDTA-treated blood sample is rarely successful, even when tissue culture isolation is employed (16). Retrospectively, long-term administration of immunosuppressive doses of corticosteroids for a presumptive diagnosis of systemic lupus erythematosus may have facilitated the isolation of the original type strain of \( B. \) \textit{vinsonii} subsp. \textit{berkhoffii} from a dog with endocarditis (14). Three other isolates of \( B. \) \textit{vinsonii} subsp. \textit{berkhoffii} that could be differentiated from the type strain by restriction digestion were cultured from a dog with hyperadrenocorticism, a healthy blood donor, and a healthy pet (57, 61). \( B. \) \textit{vinsonii} subsp. \textit{berkhoffii} has been isolated from the blood of 17 of 54 (32%) coyotes in California (19). Ease of isolation from the blood of an individual cat may be of particular relevance if, hypothetically, \( B. \) \textit{henselae} proves to be considerably easier to culture than \( B. \) \textit{clarridgeiae} or \( B. \) \textit{koehlerae}. \( B. \) \textit{koehlerae} appears to be more fastidious and requires inoculation on chocolate agar for isolation (32). In cats, accurate isolation data will be important to assess vaccine efficacy, as heterologous challenge with a different \( B. \) \textit{clarridgeiae} sp. does not confer protection (107).

Persistence of Bacteremia

Several lines of evidence support the conclusion that \( B. \) \textit{clarridgeiae} generally cause persistent infection in the susceptible host. When sampled at a single point in time, the high percentage of bacteremic animals that are detected indirectly supports persistent infection in several animal species for which long-term isolation studies in vector-free environments have not been performed. Prolonged periods of bacteremia have been documented in naturally (sequentially sampled for at least 15 months) (56) and experimentally (sampled for at least 454 days) (59, 60, 62) infected SPF cats. However, given current difficulties in documenting low levels of bacteremia, the maximum period of persistence in most animal species is unknown. Within an experimental group, individual cats can have highly variable patterns of relapsing bacteremia (59, 62). Sequential sera and \( B. \) \textit{clarridgeiae} isolates obtained from the same cat at identical time points failed to identify obvious differences in sodium dodecyl sulfate-polyacrylamide gel electrophoresis or Western immunoblot patterns, indicating that alterations in surface antigenic structure, as have been demonstrated for relapsing fever borreliae (91) in people, are not responsible for the relapsing pattern of bacteremia in cats (62).

Subclinical persistence of \( B. \) \textit{vinsonii} subsp. \textit{berkhoffii} has been documented in a healthy dog for 14 months (61). Allowing for potential difficulties associated with the isolation of \( B. \) \textit{vinsonii} from dog blood (16) or any \( B. \) \textit{clarridgeiae} sp. from the blood of immunocompetent people (2, 25, 73, 75, 88), culture may represent an insensitive means to confirm bacteremia. In support of persistent infection in people, \( B. \) \textit{bacilliformis} has been inadvertently transmitted by blood transfusion and \( B. \) \textit{quintana} has been repeatedly isolated from people over extended periods of time (18, 36). Brouqui et al. recently de-
scribed the isolation of *B. quintana* from the blood of 10 (14%) out of 71 homeless people, 5 of whom had chronic bacteremia for at least several weeks (18). Collectively, these observations support persistent infection with *Bartonella* spp. in the blood of cats, dogs, and human beings.

**Pathogenicity**

With respect to experimental infection studies in cats, the source of inoculum may substantially influence several experimental outcomes. For example, when cultured *B. henselae* organisms are used as the inoculum, clinical manifestations are generally absent, the duration of bacteremia is short (<3 months), seroconversion is followed by a rapid decline in immunoglobulin G (IgG) antibodies, and a relapsing pattern of bacteremia is not reported (1, 8, 38–40, 80, 89, 107). Presumably, culture on artificial medium results in attenuation of *B. henselae* strains, perhaps due to downregulation of genes that influence virulence (1, 8, 38–40, 80, 89, 107). These observations are in contrast to the results of studies in which cats are infected by intravenous transfusion or intramuscular administration of blood from chronically infected feline donors (59, 62) or by exposure to infected fleas (24). Following intravenous or intramuscular administration of blood obtained from bacteremic donor cats that had induced CSD in their owners, bacteremia in recipient cats was detectable by day 11, was relapsing in nature, and persisted in some cats for the 454-day duration of the study (59, 62). There was substantial variation in both the pattern of bacteremia and the pattern of IgG antibody production among recipient cats that received blood from the same donor cat. Following flea transmission, bacteremia can be detected within 2 to 4 weeks and IgG antibodies can be detected within 4 to 5 weeks (23). Subsequently, bacteremia, which was relapsing in one kitten, was accompanied by stable IgG antibody titers that were detected throughout the 22-week period of observation (23). There are also obvious limitations to using *Bartonella*-containing blood or fleas to experimentally infect cats: (i) the inoculum cannot be critically standardized, (ii) more than one *Bartonella* sp. can be unknowingly transmitted, and (iii) there is always the potential for inadvertent transmission of other known or unknown blood-borne or flea-borne pathogens (i.e., *Rickettsia felis*). From a clinical perspective, transmission of *Bartonella*-infected blood to an anemic sick cat may result in side effects that historically have been unknowingly attributed to a transfusion reaction. Although *Bartonella* spp. are subtle pathogens during chronic infection of a healthy cat, acute infection of a debilitated cat might induce more serious consequences.

**EXPERIMENTAL INFECTION STUDIES**

**Rodents**

Although much of the data has not yet been published, several laboratories have been actively pursuing experimental infection studies with rodents. Perhaps not surprisingly, immunocompetent rodent strains appear to be more resistant to infection with nonrodent *Bartonella* species. When female C57BL/6 mice were infected with 9.1 × 10^7 CFU of *B. henselae* (Houston 1 strain), cultivatable organisms were rapidly cleared; however, DNA could be detected by nested PCR throughout the duration of the study (94 days) (84). In additional experiments, mice infected with at least 5 × 10^7 *B. henselae* developed a granulomatous hepatitis with a predominance of CD4^+ lymphocytes or CD11b^+ monocytes. Significant lesions were not detected in other organs. The authors noted that “a key element in establishing this infection model was probably the passage of bacteria through mice prior to experimental infection.” Subcutaneous inoculation into the hind foot pad induced poxlike lymphadenopathy in female C57BL/6 mice (84).

Genetic susceptibility, age-associated immunocompetence, and pregnancy are factors that can influence *Bartonella* infection. When a *Bartonella* strain isolated from a field mouse (*Apodemus sp.*) was inoculated intravenously, BALB/c mice developed a significantly higher mean level of bacteremia than C57BL/6 or Swiss mice (F. Barrat, D. Bermond, C. Boullin, C. Gandon, D. Thibault, B. Chomel, R. Heller, Y. Piemont, and H. J. Boulois, First Int. Conf. *Bartonella* Emerg. Pathogens, 1999, abstr. 22, p. 47). Older BALB/c mice had significantly higher levels of bacteremia than younger mice, and the level of bacteremia was amplified in gravid mice compared to that in virgin mice. In utero transmission to the fetus was documented by the 18th day of gestation (Barrat et al., abstr.). A recombinant strain of *B. tribocorum* expressing green fluorescent protein has been used to study the kinetics of erythrocyte infection in rats. Following infection, bacteria are rapidly cleared from the blood within hours but subsequently reappear in erythrocytes on day 4 postinfection (R. Schülein, C. Gille, A. Seubert, Y. Hansmann, R. Heller, Y. Piemont, S. Andersson, and C. Lanz, First Int. Conf. *Bartonella* Emerg. Pathogens, 1999, Main Speaker, p. 51). Within erythrocytes, green fluorescent protein-expressing *B. tribocorum* reach a limiting cell density of 10 to 15 bacteria per erythrocyte. Similar to results derived from cats (55, 76) and rabbits (43), only 1 in 1,000 to 5,000 rat erythrocytes became infected throughout the entire 10-week bacteremic phase of infection. The factor(s) that limits infection of cat, rabbit, and rodent erythrocytes remains to be determined. In contrast, nearly 100% of human erythrocytes can be infected with *B. bacilliformis*, potentially leading to a severe hemolytic crisis associated with Oroya fever (36). When CD-1 mice were inoculated with *B. henselae* or *B. quintana*, immune sera with a high degree of species specificity were produced (102).

**Cats**

Findings derived from several experimental infection studies involving cats are difficult to compare as there has been variability in the type and size of inoculum, the route of infection, and the strain of *B. henselae*. In a comparative study, intradermal inoculation of culture-propagated *B. henselae* was more likely to induce infection than intravenous inoculation (1). Strain variability among *B. henselae* isolates may contribute to enhanced pathogenicity in experimentally infected cats (80). In contrast to what occurred with mice, transplacent or perinatal transmission was not found in cats experimentally infected with *B. henselae* (40). Cats may also serve as a useful species to study the efficacy of antibiotics for treatment of *B. henselae* or *B. clarridgeiae* infections (8, 38, 60, 89).

**Dogs**

Following experimental inoculation of SPF dogs with culture-grown *B. vinsonii* subsp. *berkhoffii*, there was sustained suppression of peripheral blood CD19 lymphocytes, accompanied by an altered cell surface phenotype and an increase in CD4^+ lymphocytes in the peripheral lymph nodes during the 149-day period of study (B. L. Pappalardo, T. Brown, M. Tompkins, and E. Breitschwerdt, Int. Conf. Rickettsiae Rickettsial Dis. Am. Soc. Rickettsiol. 14th Sesquicenn. Joint Meet., 1999, abstr. 175). As the clinical manifestations of natural *Bartonella* infection in dogs are similar to those associated with
human disease, the immunopathologic consequences of infection in dogs and people may prove to be similar. Experimentally, dogs inoculated with *B. henselae* did not become bacteremic. However, inoculation of dogs with a *B. clarridgeiae*-like strain isolated from a coyote induced bacteremia (Chomel et al., abstr. 41).

**Nonhuman Primates**

Few contemporary studies involving nonhuman primates have been reported. Historical accounts of experimental infection of nonhuman primates with *B. bacilliformis* have been reviewed (36). *Bartonella vinsonii* subsp. *vinsonii* was not isolated from two monkeys using yolk sac isolation, and there were no signs of illness or increased temperature (3). When simian immunodeficiency virus strain mac (SIVmac)-infected cynomolgus macaques were inoculated intradermally or subcutaneously with in vitro-cultured *B. henselae*, *B. quintana*, or *Afipia felis*, only the *B. quintana*-inoculated monkeys became bacteremic and seroconverted (8). Clinical abnormalities were not apparent. Two cynomolgus macaques inoculated subcutaneously with *B. henselae*-infected blood derived from an experimentally infected SPF cat developed fever and subcutaneous purple-red spots at the inoculation site; however, bacteria did not grow from regional lymph node samples obtained 7 to 9 weeks following inoculation and *B. henselae* antibodies were not detected (8).

**CLINICOPATHOLOGIC CORRELATIONS**

**The Cat**

Although *B. henselae* and *B. clarridgeiae* can remain a part of the intravascular microbial flora of healthy cats for months to years, recent evidence suggests that cats may pay a biologic price (i.e., development of chronic insidious disease manifestations) for persistent infection. *B. henselae* has been observed within feline erythrocytes by electron (Fig. 4) and laser confocal (55, 76) microscopy. For reasons that remain unclear, less than 1% of erythrocytes appear to be susceptible to infection (76). In a seroepidemiologic study involving 170 cats from Japan that were tested for antibodies to both canine immuno-deficiency virus (FIV) and *B. henselae* antigens, there was a significant increase in the incidence of lymphadenopathy and gingivitis in cats with serologic evidence of infection with both organisms (105). As FIV causes a progressive decrease in CD4⁺ lymphocytes, these results suggest that coinfection with *B. henselae* in an immunodeficient cat can induce specific disease manifestations. Of potentially unique importance to our future understanding of *Bartonella* spp. as pathogens in cats, a seroepidemiologic survey involving 728 healthy and sick cats from Switzerland identified a statistical correlation between the presence of antibodies to *B. henselae* antigens and stomatitis, as well as a variety of renal and urinary tract abnormalities (37). It is also of interest that in cats greater than 7 years of age, only clinically ill cats (17 of 110 clinically ill cats) were *Bartonella* seroreactive. As the seroprevalence in cats from Switzerland is substantially lower (8.3%) than in cats from warmer and more humid climates, detection of statistical associations with renal or urinary tract disease seem more feasible. Since chronic renal failure of undetermined etiology is a major cause of morbidity and mortality in older cats, clarification of the role of *Bartonella* spp. as a potential cause of chronic renal failure could prove to be of considerable importance. Recently, based upon detection of antibodies in serum and aqueous humor, *Bartonella* infection was proposed as a cause of uveitis in an immunocompetent cat (67). In a subsequent study, ocular production of *Bartonella* IgG in aqueous humor was detected in 7 of 49 cats with uveitis, 0 of 49 healthy shelter cats, and 4 of 9 cats experimentally infected with *B. henselae* (68).

In addition to the above seroepidemiologic and clinical observations, findings derived from experimental infection studies also have begun to support a pathogenic role for *Bartonella* spp. in cats. Historically, as earlier studies indicated that *Bartonella* could be isolated from the blood of many naturally infected healthy cats, many experimental infection studies, including those from our laboratory (59, 62), were not designed to critically address the role of *B. henselae* as a pathogen in cats, and in several earlier studies, histopathologic evaluation was not performed. Detailed histopathologic studies should be a more important consideration in future studies. Abnormalities reported in cats experimentally infected with *B. henselae* or *B. clarridgeiae* include fever, mild transient anemia, eosinophilia, lymphadenomegaly, cholangitis, cardiac and renal lesions, neurologic dysfunction, and reproductive failure. Fever, lymphadenomegaly, and anemia inconsistently accompany initial infection (39, 59, 80). Although perinatal transmission was not successful, the same group of investigators reported reproductive failure, characterized by failure to conceive, fetal involution and resorption, and delayed conception in cats inoculated intradermally with *B. henselae* (40). Transient neurologic dysfunction, characterized by lethargy, disorientation, and lack of response to environmental stimuli, has been reported in cats infected with blood- or culture-derived inocula (40, 59, 80). Focal myocardial inflammation, consisting predominantly of mononuclear cells, has been observed in cats experimentally infected with *B. henselae* or *B. clarridgeiae* (39, 59). Although presumably unrelated to *Bartonella* infection, cataracts developed, without explanation, in SPF cats obtained from a commercial vendor supplying cats without historical problems with cataracts within 1 year following *Bartonella* infection (59, 62).

In cats infected with blood inocula containing *B. henselae* (type II) and/or *B. clarridgeiae* and monitored for 454 days, a substantial number of unexpected histopathologic lesions, in-
cluding lymph node hyperplasia, splenic follicular hyperplasia, lymphocytic cholangitis, lymphocytic hepatitis, lymphocytic plasmacytic myocarditis (Fig. 5), and lymphocytic interstitial nephritis were identified (62). Bartonella DNA was amplified from the blood or tissues of individual cats regardless of bacteremia status. An important conclusion from this study, given the endemicity of feline bartonellosis in the natural cat population, is that the definition of normal pathology in healthy or sick cats may require reexamination with regard to the Bartonella infection status. This situation in cats could be analogous to the differences in the interpretations of gastric histopathology in human patients before and after the association of Helicobacter pylori with chronic gastritis and ulcer disease.

The Dog

The spectrum of disease associated with Bartonella infection in dogs is currently unknown. However, from a comparative medical perspective, dogs infected with Bartonella spp. can develop disease manifestations that are similar to lesions reported in human patients, including endocarditis (14, 16), granulomatous lymphadenitis, granulomatous rhinitis (83), and peliosis hepatis (53). With the exception of a single case report of B. henselae-associated peliosis hepatis (53), B. vinsonii subsp. berkholffii has been implicated on a molecular basis in all other disease processes identified in dogs to date. Endocarditis associated with B. vinsonii occurs in large-breed dogs with a potential predisposition for aortic valve involvement. Intermittent lameness or fever of unknown origin can precede the diagnosis of endocarditis in these dogs for several months. In addition to endocarditis, multifocal areas of severe myocardial inflammation can be found in dogs with B. vinsonii endocarditis. Potentially, myocarditis without endocarditis can result in cardiac arrhythmias, syncope, or sudden death (16). Similarly, myocarditis was identified in a 60-year-old male from Sweden with serologic and molecular evidence of infection with a Bartonella spp. who died suddenly during a running competition (47). Considering these preliminary and incomplete observations derived from experimentally infected cats, naturally infected dogs, and the patient from Sweden, the role of Bartonella spp. as a cause of myocarditis in animals and people deserve future research consideration. On the basis of seroreactivity, visualization of Warthin-Starry, silver-staining bacteria within the lymph node and PCR amplification followed by Southern blot hybridization, granulomatous lymphadenitis due to B. vinsonii subsp. berkholffii was diagnosed in a dog. The source of infection was believed to be a tick that was removed from the ear 7 days prior to the onset of illness (83). Although the sources of infection differed, the historical course and clinicopathologic findings for this dog were comparable to those of CSD lymphadenitis in human patients. B. henselae was amplified and sequenced on two independent occasions from the liver of a dog with peliosis hepatis (53). Although not identified, extrapolation from the frequent association of peliosis hepatis with HIV-infected individuals supports the possibility of an unidentified cause of immunosuppression in the dog.

Coinfection

Recent serologic and molecular evidence indicates that coinfection of dogs with Ehrlichia, Babesia, and Bartonella spp. may be more frequent than previously realized (15, 63, 81). Therefore, the extent to which infection with Bartonella influences the pathophysiology of ehrlichiosis or babesiosis, diseases of much longer historical venue, deserves critical reappraisal. Of similar potential concern in human medicine is the finding of cosegregation of Borrelia burgdorferi, Babesia microti, and a Bartonella sp. in Peromyscus leucopus mice in the north-central United States (46). In The Netherlands, Ixodes ricinus ticks can simultaneously contain B. burgdorferi (sensu lato), Ehrlichia spp., and Bartonella spp. (99). Potentially, Ixodes scapularis ticks, feeding upon infected mice, could subsequently transmit B. burgdorferi, B. microti, Ehrlichia equi (the putative agent of
human granulocytic ehrlichiosis), or a rodent Bartonella sp., which are rarely considered pathogenic in human beings (46, 106). These recent observations illustrate the potential difficulty in establishing causation associated with a single pathogen in dogs or people that may be coinfected with multiple tick-transmitted pathogens. As certain Borrelia, Ehrlichia, Babesia, and Bartonella spp. can cause chronic, subclinical infection in dogs, the relative role of each of these organisms to the pathogenesis of specific disease manifestations in a sick, naturally infected dog will remain difficult to establish.

ZOONOTIC POTENTIAL FOR HUMAN INFECTION

Routes of Transmission

Considering the extensive animal reservoirs and the large number of insects that have been implicated in the transmission of Bartonella spp., both animal and human exposure to these organisms may be more substantial than is currently believed. This statement is supported in part by seroprevalence data from healthy human blood donors as well as individuals with more frequent animal contact. Seroprevalence in healthy human blood donors has ranged from 2 to 6% (48, 86, 108) in the United States and 4% in Sweden (47). In Seattle, seroprevalence was 20% among an indigent, inner-city clinic population (48), and in Marseilles, 30% of homeless patients had high antibody titers to B. quintana (18). Two seroprevalence studies involving veterinary professionals from the United States and veterinarians from Europe have reported seroprevalence to B. henselae antigens as 7.1 and 51.1%, respectively (both studies used IFA testing) (78, 79). In addition to exposure to known and putative insect vectors, the high levels of bacteremia currently being documented in numerous dog exposures to known and putative insect vectors, the high levels of bacteremia currently being documented in numerous domestic and wild animal species indicate that there is a tremendous animal reservoir for these organisms in nature. In this regard, transmission without an insect vector, although perhaps infrequent, seems plausible. For example, as a high percentage of wild rabbits, deer, and beef cattle can be bacteremic, inadvertent animal transmission might occur during the butchering process.

Risk of Transmission

Based upon animal studies as well as recent and historical accounts, the risk of transmission of B. quintana or B. bacilliformis (in regions of South America where bartonellosis is endemic) through human blood transfusion may prove to be a legitimate medical concern. As fleas may be necessary for the transmission of feline Bartonella species to humans, rigorous flea control should be recommended by health care workers, particularly when advising immunocompromised individuals on risks related to pet ownership. Fortunately, several safe and highly effective products have been introduced in recent years that can eliminate or substantially reduce flea infestation in cats and dogs. Although vector competency has not been clearly established for tick species, circumstantial evidence suggests that tick control measures should also be emphasized to avoid the potential of Bartonella transmission to humans. Infrequently, dogs have been implicated in the transmission of Bartonella spp. following a bite or a scratch (52, 103). In future case studies implicating transmission from dogs, definitive documentation of the causative Bartonella sp. in people will be important, as B. vinsonii subsp. berkhoffii is the only species isolated from dogs to date. Children and immunocompromised individuals should be cautioned to avoid behaviors that increase the risk of animal bites or scratches.

CONCLUDING REMARKS

Not too long ago, many were taught during microbiology courses (or medical school training) that blood is generally a sterile medium. Increasingly, this assertion must be qualified with regard to Bartonella spp. as well as other intracellular pathogens that have coevolved with humans and animals to persist in circulating blood cells such as erythrocytes or macrophages for months to years and perhaps longer. As establishing a cause-and-effect relationship for bartonellosis infectious diseases can be extremely difficult, particularly for cats, among which the prevalence of infection is high, substantial effort will be required to clarify the role of Bartonella spp. as a cause of chronic insidious disease in animals and people. On an evolutionary basis, animals with substantial exposure to vector-borne intraerythocytic Bartonella have presumably developed a highly sophisticated immunologic response to organisms that can persist for long periods within the vasculature. Elucidation of the mechanisms associated with immunologic adaptation to intravascular persistence on a comparative basis may more readily unravel the complexity of this process and thereby benefit humans as well as animals. Despite substantial and rapid progress during the past several years, Bartonella spp. still have many secrets to share with investigators in the future.

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