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Current Knowledge of *Bartonella* Species

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Bartonella species are now considered emerging pathogens. Of the 11 currently recognized species, four have been implicated in human disease, although only two have been encountered in Europe. *Bartonella quintana* infections are now being diagnosed among the urban homeless and deprived, manifesting as trench fever, and *Bartonella henselae* has been shown to be the causative agent of cat scratch disease. Both species also cause a variety of HIV-associated infections, including bacillary angiomatosis. However, perhaps the most significant presentation of bartonellae infection is culture-negative endocarditis. The epidemiologies of *Bartonella* infections are poorly understood; most *Bartonella henselae* infections are probably acquired from infected cats, either directly by contact with a cat or indirectly via fleas. No animal reservoir has been implicated for *Bartonella quintana*; however, infection can be transmitted via the human body louse. Diagnosis of *Bartonella* infections can be made using histological or microbiological methods. The demonstration of specific antibodies may be useful in some instances, although certainly not in all. Cultivation of *Bartonella* is difficult, as the bacteria are extremely fastidious. Polymerase chain reaction-based or immunological methods for the detection of bartonellae in infected tissues have proven useful. Clinical relapse is often associated with *Bartonella* infections despite a wide range of prescribed regimens. Only aminoglycosides display in vitro bactericidal activity against intracellular *Bartonella* species; therefore, they are recommended for treatment of *Bartonella* infections.

Human infections due to *Bartonella* species are widely considered emerging diseases. They include long-recognized diseases such as Carrion's disease (classic bartonellosis), trench fever, and cat-scratch disease and newer clinical manifestations such as bacillary angiomatosis, peliosis hepatitis, septicemia, endocarditis, chronic lymphadenopathy, and neurologic disorders. New molecular biology techniques, mainly based on 16S rRNA gene amplification and analysis, have allowed recognition of the role of *Bartonella* (formerly *Rochalimaea* species in a number of these pathological conditions. The association of *Bartonella henselae* infection with bacillary angiomatosis is an example.

The availability of specific techniques to diagnose *Bartonella* infections has led to the description of new *Bartonella* species and recognition of a broadened disease spectrum due to these microorganisms. The most striking pathological feature of *Bartonella* infection is the apparent ability of these bacteria to produce angioproliferative lesions in immunocompromised patients, such as those infected with HIV. Capillary and endothelial cell proliferations are characteristic histologic findings of bacillary angiomatosis, peliosis hepatitis, and classic bartonellosis. Bartonellae are the only known bacteria with the ability to produce angiogenic tumors in humans, although *Agrobacterium* species, which belong to the same phylogenic group as *Bartonella* species, produce tumors in plants.

The present review focuses on the epidemiological and clinical aspects of infection due to the *Bartonella* species presently recognized as human pathogens: *Bartonella bacilliformis*, *Bartonella quintana*, *Bartonella henselae*, and *Bartonella elizabethae*.

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History of *Bartonella*

Before recent taxonomic proposals, *Bartonella bacilliformis* was the only member of the genus

Bartonella. This bacterium, first recognized in Peru in 1909 by Alberto Barton, infected the erythrocytes of patients with bartonellosis (or Carrion's disease). Reports of this disease, which presents as one of two distinct manifestations, are limited to the Andean region of South America and predate Columbus. *Bartonella quintana* was recognized during World War I as the causative agent of trench fever, but culture of the organism was not reported until 1966 (1). Trench fever afflicted several thousand troops during the war (2, 3), but it is likely that trench fever-like syndromes were recognized much earlier (4). *Bartonella vinsonii* has been isolated only once, from a field vole on Grosse Island in the St. Lawrence Seaway, Canada, in 1943 (5); however, a recent report details the isolation of a subspecies of *Bartonella vinsonii* from an apparently healthy dog and from one with endocarditis (6).

The two former *Grahamella* species were also first described more than 50 years ago: *Bartonella talpae* was observed in the blood of moles captured near Cambridge, UK, in 1905, and *Bartonella peromysci* was isolated from a deer mouse in New England, USA, in 1942. However, unlike *Bartonella vinsonii*, the precise identity of these two species is unclear, as their original characterizations were vague and representative strains no longer exist. The remaining six *Bartonella* spp. have all been described within the last five years, although the medical syndromes now attributed to *Bartonella henselae* have been recognized for much longer.

The identification and characterization of *Bartonella henselae* as an organism of medical importance resulted from a protracted search that began with the recognition of cat scratch disease in France in 1950 (7) and was ultimately resolved following study into the etiology of an AIDS-related disease, bacillary angiomatosis, which was first observed in 1983 (8). Bacillary angiomatosis was initially characterized by multiple skin lesions that were considered infectious because histological examination of biopsy material revealed Warthin-Starry-staining bacilli. Furthermore, the lesions resolved with antibiotic treatment (9). However, it was not until 1990 that these bacteria were identified. Relman et al. (10) used polymerase chain reaction (PCR) to amplify bacterial 16S rRNA gene fragments directly from biopsy material taken from four patients with bacillary angiomatosis. The nucleotide base sequences of these amplification products were identical for all four patients; thus, all four were infected by the same organism. When this sequence was compared with those available for other bacteria, it was found to be very similar to, but distinct from, that of *Bartonella quintana*.

Concurrent with this work, peliosis hepatitis, a disease previously reported in patients suffering from wasting diseases or in association with the use of anabolic steroids, was first characterized in AIDS patients (11), at about the same time a previously uncharacterized bacterium was isolated from five HIV-infected patients with bacteremia (12). Subsequent collaboration demonstrated that the agents of all three syndromes were indistinguishable by comparison of partial 16S rRNA gene sequences (13). Isolation and subculture of the organism from the lesions of patients with bacillary angiomatosis were subsequently achieved (14), and following its characterization, the organism was confirmed as a new species and named *Rochalimaea henselae* (15).

Following the isolation and identification of *Bartonella henselae*, specific methods for detection of the bacterium were developed, which led to increased medical awareness of its potential pathogenicity. The species has subsequently been implicated in other clinical syndromes occurring among immunocompetent as well as immunocompromised patients, including cat-scratch disease. Interestingly, improved diagnosis of bacillary angiomatosis led to the implication of *Bartonella quintana* in several cases reviewed recently (16). The spectrum of modern illnesses associated with both organisms continues to increase.

Bartonella elizabethae has been isolated only once, from the blood of an immunocompetent patient suffering endocarditis (17). Three other newly described species, *Bartonella grahamii*, *Bartonella taylorii*, and *Bartonella doshiae*, were characterized following their isolation from the blood of small mammals in the UK in 1994 (18). The sixth new species, *Bartonella clarridgeiae*, was isolated from an American cat and was first described in 1996 (19).

Bacteriology

Following recent taxonomic proposals (18, 20), the genus *Bartonella* presently includes the 11 validated species as described above. A second serogroup (Marseille) has been identified among *Bartonella henselae* isolates (21), and a subspecies of *Bartonella vinsonii, berkhoffi*, has been described re-

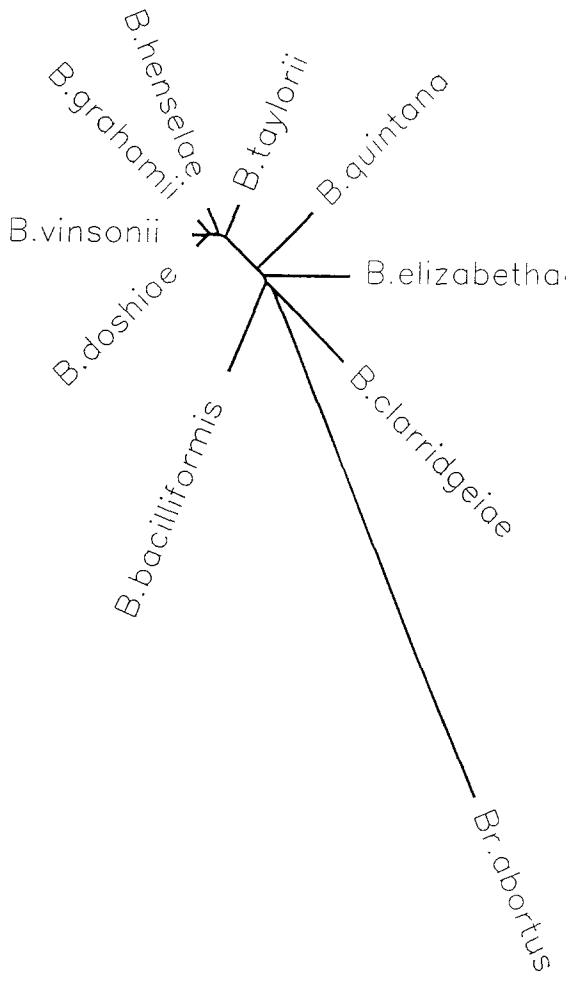

Figure 1: Phylogenetic tree showing relationships between *Bartonella* spp. The tree was inferred from an alignment of partial (c 1000 base pair) 16S rRNA gene sequences using the DNADIST and the NEIGHBOR (UPGMA) programs within the PHYLIP suite (Felsenstein, University of Washington, USA). The bar represents approximately 1% sequence divergence. *Brucella abortus* is included as an outgroup.

cently (6, 22). Other, as yet unnamed species isolated from small mammals have also been discussed (23–25), (R. Regnery et al., 12th Sesqui-Annual Meeting of the ASRRD, Pacific Grove, California, 1996, Abstract no. 49) but are not yet fully characterized. Historically, the genera that now comprise *Bartonella* were all considered members of the order *Rickettsiales*; however, with the application of modern phylogenetic comparative methods came the demonstration of evolutionary diversity within the order (26).

Although both *Bartonella* and *Rickettsia* lie within the alpha subdivision of the *Proteobacteria*, they are phylogenetically remote; *Bartonella* spp. demonstrate far closer evolutionary homology with members of the genus *Brucella* and the plant-associated genera *Agrobacterium* and *Rhizobium* (Figure 1). Formal proposal for the removal of *Bartonella* from the *Rickettsiales* has been made (20). Within the genus, *Bartonella* spp. exhibit high levels of evolutionary homology with each other. Comparison of 16S rRNA gene sequences (on which most phylogenetic inferrals have been based) demonstrates that species share more than 98% sequence similarity, a value that may be observed between strains of the same species in other genera. This close proximity is supported by DNA:DNA homology calculations; some species share only slightly less than 70% homology, which is widely accepted as being the cut-off value in bacterial species definition (15, 18, 20, 26–28).

Members of the genus *Bartonella* are defined as gram-negative, oxidase-negative, fastidious, aerobic, rod-shaped bacteria. Growth may be obtained in axenic medium, either on blood-enriched agar (rabbit blood or horse blood are more effective than sheep blood) or in broth (29, 30). Study of the nutritional requirements of *Bartonella quintana* and *Bartonella bacilliformis* has demonstrated that growth could be obtained using succinate, pyruvate, glutamine or glutamate, but not glucose, as sources of energy (31). Growth was enhanced by increased carbon dioxide pressure and fetal calf serum and was found to be hemin dependent (32, 33). *Bartonella* spp. grow best in vitro at 37°C, except for *Bartonella bacilliformis*, which grows best at 28°C. Unlike other *Bartonella* spp., *Bartonella bacilliformis* and *Bartonella clarridgeiae* express flagella.

Growth of all *Bartonella* is slow; when using blood agar, primary isolates are typically obtained after 12 to 14 days, although prolonged incubation periods of up to 45 days are sometimes necessary (34). First subcultures of an isolate are also difficult to obtain, with colony formation again taking ten to 15 days. Repeated subculture, however, reduces this time to only three to five days, although colonial morphology is significantly affected. Colonies are rough, adherent, pit the agar on primary isolation, and become smooth and less adherent after several passages.

In vivo, *Bartonella* spp. appear to exist in close association with host cells. *Bartonella bacilliformis* infects human erythrocytes and human endothelial cells (35–37), and the former *Grahamella* spp. are generally considered intraerythrocytic parasites. The intraerythrocytic presence of *Bartonella henselae* in cat erythrocytes has also been observed recently (38).

Table 1: Epidemiological factors and clinical manifestations associated with the four *Bartonella* species identified as human pathogens.

	B. bacilliformis	*B. quintana*	*B. henselae*	*B. elizabethae*
Reservoir	humans	humans	cats	unknown
Geographic distribution	Peru, Ecuador Columbia	worldwide	worldwide	unknown
Trench fever	–	+	–	–
Carrion's disease	+	–	–	–
Cat-scratch disease	–	–	+	–
Chronic lymphadenopathies	–	+	–	–
Bacillary angiomatosis	–	+	+	–
Peliosis hepatitis	–	–	+	–
Septicemia	+	+	+	–
Endocarditis	–	+	+	+
Neurologic disorders	–	+	+	–

+, known to cause the disorder listed; –, not known to cause the disorder listed.

Genotypic analysis has determined that the mol G+C% content of *Bartonella* spp. is low, ranging from 38.5 to 41% (18, 20). Macrorestriction analysis (39) has also been used to calculate the genome sizes of *Bartonella* (formerly *Rochalimaea*) spp., which were found to be relatively small, ranging from 1700 to 2174 kb.

Epidemiology and Life Cycle of Bartonellae

Reports of profound disease resulting from *Bartonella* infection are almost entirely limited to humans. Attempts to experimentally induce disease in laboratory animals through inoculation with either *Bartonella quintana* or *Bartonella bacilliformis* have succeeded only when primates were used (40–42). *Bartonella henselae*, however, is commonly isolated from cats, which appear to be the natural reservoir for the organism; thus, *Bartonella henselae* infections of humans can be considered zoonoses.

Reservoirs other than humans have not been convincingly demonstrated for any of the other *Bartonella* spp. pathogenic to humans (Table 1). Although transmission of *Bartonella* spp. between hosts appears to involve arthropods, human infection may not necessarily be acquired this way.

Arthropod Vectors. Sand flies of the genus *Lutzomyia* are vectors of *Bartonella bacilliformis*. In Peru, the distribution of the species *Lutzomyia verrucarum* corresponds to regions where bartonellosis is endemic, although the disease is found in areas where this sand fly does not exist (43). *Lutzomyia verrucarum* is not found in bartonellosis-endemic regions of Ecuador, although other closely related sand fly species exist there. The ability of *Bartonella bacilliformis* to infect and be transmitted by other arthropods has been demonstrated in vitro when infection was transmitted between rhesus monkeys by *Dermacentor andersonii* ticks.

The human body louse (*Pediculus humanis corporis*) has been demonstrated to transmit *Bartonella quintana*-induced trench fever during wartime conditions (44–46). Although the head louse (*Pediculus humanis capitis*) has been shown to transmit the disease under experimental conditions, its role in vivo has yet to be established. Lice do not die from *Bartonella quintana* infection but remain infected for life; it is not yet known how they acquire the infection. Following inoculation, humans are usually bacteremic only during the sporadic febrile stages of trench fever. Occasionally however, bacteremia may persist for a long period after the disappearance of all clinical signs of the disease, a circumstance that would facilitate transmission of *Bartonella quintana* to other lice. However, the role of lice in the transmission of modern-day *Bartonella quintana* infections is less clear.

During investigations into a recent outbreak of *Bartonella quintana* bacteremia among ten homeless, alcoholic persons in Seattle, USA (45), only one patient had lice at the time of presentation to the hospital. A subsequent serological survey conducted among 192 patients attending a downtown clinic in Seattle (47) identified 20% as being infected with the bacterium, but multivariate analysis demonstrated that only alcohol abuse was independently associated with seropositivity. Similarly, infesting lice were not observed on three homeless French men in whom endocarditis due to *Bartonella quintana* had been diag-

nosed (44). The largest study to date of *Bartonella*-induced endocarditis (22 patients) again demonstrated only homelessness and alcoholism to be specifically related to *Bartonella quintana* infection (48).

Cat fleas (*Ctenocephalides felis*) have been proposed as potential vectors of *Bartonella henselae* (49–51). Epidemiological studies have identified ownership of flea-infested cats as being a predisposing factor in cat-scratch disease and bacillary angiomatosis (52, 53). The presence of *Bartonella henselae* in *Ctenocephalides felis* has been demonstrated (54–57). More recently, experimental transmission of *Bartonella henselae* from cat-to-cat via the cat flea has been demonstrated (58). Although these arthropods are likely to transmit infection between cats, their precise role in the transmission of infection to humans remains debatable. A report of a case of *Bartonella quintana*-induced chronic lymphadenopathy identified cat ownership as the patient's only *Bartonella* risk factor (59). This finding, however, may have been merely coincidental.

Fleas have also been demonstrated to transmit *Bartonella* infection between small mammal hosts; the incidence of *Bartonella* bacteremia in small rodent populations shows a seasonal variability compatible with this mode of transmission (60). As *Bartonella* spp. show no specificity as to their mammalian host, infecting several different species that share ectoparasite fauna (61), and, since several different species of fleas exist in local small mammal populations, it would perhaps be pertinent to consider the extent of specificity demonstrated by *Bartonella* spp. towards their vector. A potential role for ticks as vectors of *Bartonella vinsonii* has also been tentatively proposed (62).

Natural Reservoirs. The cat is the likely reservoir of *Bartonella henselae*. Cats may infect humans either directly through scratches, bites, or licks, or indirectly via an arthropod vector. Epidemiological evidence that the cat may represent the natural reservoir of *Bartonella henselae* resulted from a large survey indicating that the ownership of kittens with fleas is the common predisposing factor among patients with cat-scratch disease and bacillary angiomatosis (53, 63). This evidence was confirmed by several studies in which *Bartonella henselae* was isolated from the blood of a significant number of domestic cats; the first of these studies, carried out in the San Francisco area, found that 41% (25/46) of cats tested were infected with *Bartonella henselae* (49). Most of the infected cats appeared healthy, although infection was associated with illness in some animals. In a subsequent study of 205 Californian cats (64), *Bartonella henselae* isolates were obtained from 39.5% (n = 81). Within this sample, impounded or former stray cats were almost three times more likely to be bacteremic than domestic cats. Similarly, young cats and cats infested with fleas were more likely to be infected. In addition, 81% of cats were seropositive for *Bartonella henselae*.

Other serological studies of *Bartonella henselae* infection in cats, based on sample sizes of between 600 and 700 animals, have reported much lower, although still significant, seroprevalences of between 15 and 27.9% (54, 65). However, seroprevalence in North America varied from 3.7% in the Rocky Mountain area to up to 54.6% in the southeastern USA (65). Whether seropositive but nonbacteremic cats are purged of *Bartonella henselae* infection is not clear. Longitudinal studies of infected cats have demonstrated that prolonged or recurrent bacteremia occurs; Kordick et al. (24) reported that *Bartonella henselae* could be cultured repeatedly from the blood of some cats for at least 12 months after initial testing; the same investigators made similar observations in experimentally infected cats (38). More recently, eight cats were experimentally infected with *Bartonella henselae* (66). All animals remained bacteremic for four to seven weeks, until they received doxycycline therapy. Although doxycycline administered for one week suppressed bacteremia in all cats, *Bartonella henselae* infection was cleared in only four of eight animals. Amoxicillin alone or combined with clavulanate (in 1 cat) cleared the infection in the remaining four animals. After the bacteremia was cleared, all animals were rechallenged with *Bartonella henselae*, but none developed new bacteremia, suggesting that antibody levels in these cats were protective.

For both *Bartonella bacilliformis* and *Bartonella quintana*, no reservoir other than humans has been demonstrated. Asymptomatic infection by *Bartonella bacilliformis* has been reported in several studies, including a recent survey by Herrer (67), who discovered asymptomatic infection in 8% of the inhabitants of the endemic Condebamba Valley. However, as bartonellosis can be acquired in regions that are uninhabited by humans, alternative reservoirs may exist. *Bartonella bacilliformis* infections can be induced experimentally in a range of other animals including squirrels, dogs, rabbits, and chickens. Most suffer only mild fevers or develop verrugas at the site of

inoculation. Even plants have been proposed as reservoirs.

As outbreaks of trench fever during Word War I coincided with the infestation of the trenches with voles, these animals have been circumstantially implicated as reservoirs of *Bartonella quintana*. However, surveys of small mammal populations have, to date, failed to identify any animals infected with this species (61); (R. Regnery et al., 12th Sesqui-Annual Meeting of the ASRRD, 1996, Abstract no. 49). That sylvian mammals could be the source of modern-day *Bartonella quintana* infections, which occur exclusively among inner-city alcoholic or homeless populations and the HIV seropositive, is less plausible.

The natural cycle of *Bartonella elizabethae* remains to be elucidated, as only a single isolate has been reported. However, recent studies have identified *Bartonella elizabethae*-like organisms in small mammals in the USA and Peru (25); (R. Regnery et al., 12th Sesqui-Annual Meeting of the ASRRD, 1996, Abstract no. 49). Although the precise taxonomic relationships between these isolates are yet to be investigated, the findings allow speculation that *Bartonella* infections can be acquired from small mammals. If this is the case, the *Bartonella* spp. associated only with these animals must be considered as potentially pathogenic to humans.

Pathogenesis of *Bartonella* Infections

Microscopic observation of the lesions characteristic of *Bartonella quintana* or *Bartonella henselae*-induced bacillary angiomatosis reveals tumor-like capillary lobules (68), which are rounded aggregates of capillaries with rapidly proliferating endothelial cells (68). Similar tumor-like cell proliferation is observed in peliosis hepatitis (11) and in the cutaneous lesions (verrugas) of Carrion's disease (69). Interestingly, species of the genera *Agrobacterium* and *Rhizobium*, which are known to share a close evolutionary link with *Bartonella* spp. (28), are also associated with induction of nodules or tumors in plants.

Rhizobium spp. induce on their leguminous hosts the formation of branching, deforming, and curling root hair (70). Within eukaryotic cells of these specialized plant organs, the bacteria convert atmospheric nitrogen into ammonia, which is used by the plant as a nitrogen source. Bacterial lipooligosaccharides called Nod factors are the active substances that change the plant development program. Nodulation genes (nod genes) are most often plasmidic. *Agrobacterium* spp., including *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, are gram-negative bacteria that normally infect plants, leading to the formation of neoplastic crown gall and hairy root diseases, respectively (71). Molecular studies have shown that the tumor-forming ability of these organisms is related to the transfer and integration of a segment of plasmidic DNA (Ti plasmid for *Agrobacterium*, Ri plasmid for *Rhizobium*) into the infected plant cell genome. Recently, Ti plasmid transfer to plant cells has been shown to occur via *Agrobacterium* pili (72). Integration of the T-DNA results in tumor formation by overproduction of or hypersensitivity to plant growth hormones.

Research has identified a number of determinants that are potentially involved in the invasion of erythrocytes by *Bartonella bacilliformis*. Benson et al. (35) demonstrated that the binding of *Bartonella bacilliformis* to the erythrocyte surface led to the formation of indentations and deformation of the cellular membrane, ultimately permitting entry into large vacuoles within the cell; an extracellular product, termed deformation factor, has been shown to induce these indentations and trenches in erythrocyte membranes (73). Initial attachment of the bacteria to the erythrocyte cell surface may be mediated by fimbriae, and preliminary data demonstrating their presence on *Bartonella bacilliformis* was recently presented (S.I. McAllister et al., 12th Sesqui-Annual Meeting of the ASRRD, 1996, Abstract no. 70).

That antiflagellin antibodies had a significantly detrimental effect on erythrocyte invasion has also been demonstrated (74). A two-gene loci of *Bartonella bacilliformis* associated with its ability to invade erythrocytes has now been characterized (75), and data on other genes coding for putative pathogenic determinants have also been preliminarily reported (A. Raji et al., 92nd Annual Meeting of the American Society for Microbiology, 1992, Abstract no. D-237). Recently, a two-gene loci in *Bartonella henselae* homologous to those found in *Bartonella bacilliformis* was identified and sequenced (G.J. Murakawa et al., 12th Sesqui-Annual Meeting of the ASRRD, 1996, Abstract no. 50).

Bartonella bacilliformis can interact in vitro with a variety of eukaryotic cell lines, including human dermal fibroblasts, Hep-2 cells, HeLa cells, and human umbilical vein endothelial cells (36, 37). The interactions between other species of *Bartonella* and eukaryotic cells is suggested by the improved

recovery of *Bartonella henselae* and *Bartonella quintana* from clinical material when cell lysis procedures or cell culture systems are used (14, 44, 59, 62). Merell et al. (76) first described the association of *Bartonella quintana* with eukaryotic cells. Recent work suggests that *Bartonella quintana* are phagocytosed by endothelial cells in vitro and exist intracellularly in vacuoles (37, 77). Recently, Brouqui et al. (77) developed a human endothelial cell system (ECV304.9) in which *Bartonella quintana* grew intracellularly. The bacteria were observed in vacuoles in which they multiplied, leading to the formation of morulae, as reported for *Chlamydia* and *Ehrlichia* spp. In older cultures, a large number of vesicle-like structures were found in vacuoles containing bacteria. These vesicles were identified as bacterial membrane blebs.

The association of *Bartonella* spp. with neovascularization and regression of lesions when antimicrobial agents are administered strongly suggests that the microorganisms themselves stimulate the angiogenesis seen in the lesions of bacillary angiomatosis (68) and verruga peruana (69). Work has already demonstrated that proliferation of endothelial cells is induced in vitro in the presence of either intact or homogenized *Bartonella bacilliformis* cells (78). Furthermore, a *Bartonella bacilliformis* protein effective in endothelial cell proliferation in vitro and in proliferation of new blood vessels in vivo has been identified and partially characterized (79). Koehler et al. (14) recently reported that when *Bartonella quintana* or *Bartonella henselae* are inoculated into bovine endothelial cells, the cell monolayers remain intact and viable for longer periods than do uninfected monolayers. The cells do, however, undergo morphological changes described as similar to those seen in endothelial cells exposed to angiogenic factors. In vitro cocultivation of *Bartonella henselae* with human umbilical vein endothelial cells (HUVEC) is also reported to enhance proliferation of the HUVEC (80). Similar results were found in our laboratory by monitoring *Bartonella quintana* or *Bartonella henselae* cocultivation with HUVEC by quantitative microscopy (81).

An extracellular round- to icosahedral-shaped particle of 40 nm diameter has been detected in the supernatant collected from cultures of *Bartonella henselae* (82). The particle contained a 14 kb linear DNA segment, which was also found within *Bartonella henselae* cells as an extrachromosomal element. A similar 14 kb DNA element has also been found in *Bartonella bacilliformis* cells and may correspond to a bacteriophage-like particle previously observed in *Bartonella bacilliformis* using electron microscopy (83). The role of this particle in the pathophysiology of *Bartonella* infections remains unknown, and its presence has not yet been demonstrated in other *Bartonella* spp. Preliminary data regarding the genetic and protein structure of this particle in *Bartonella henselae* have been reported (B. Anderson et al., 12th Sesqui-Annual Meeting of the ASRRD, 1996, Abstract no. 45).

Clinical Manifestations of *Bartonella* Infections

Trench Fever. Trench fever, also known as five-day fever, quintan fever, or Wolhynia fever, is defined as infection of human blood by *Bartonella quintana*. Detailed descriptions of trench fever that infected troops during World War I have been made (46, 84). The incubation period for the disease is between 15 and 25 days, with clinical manifestations ranging from mild or even asymptomatic infection to severe illness. The presentation most often reported among troops corresponded to a febrile illness of acute onset and periodic nature often accompanied by severe headache and pain in the legs. The headache may suggest meningitis, especially when it is occipital and associated with stiffness of the neck. Leg pain is often severe and felt specifically in the tibia. The term "quintan fever" refers to the five-day recurrences most often observed, although both the duration of recurrences and the level of the fever can vary. Usually, each succeeding attack is less severe than its predecessor, although in profound cases the patient can become weaker and the leg pains more persistent. The patient's tongue is often slightly furred, and conjunctival congestion as well as a slow pulse relative to the severity of the fever may be observed. Areas of tenderness are associated with muscle, tendon, bone or joint pain, and the spleen often becomes palpable. Major polymorphonuclear leukocytosis often accompanies the febrile stages of the disease. Anemia may also occur, especially in chronically ill patients.

Although trench fever often results in prolonged disability, no fatalities have been recorded. Patients are most profoundly ill during the early stages of the disease, which usually continue for four to six weeks. A minority of patients will suffer chronic infection, which Byam et al. (46) defined as "a state of marked debility, with or without attacks of slight fever and aching, character-

ized by hyperexcitability of the nervous system in general". Although trench fever was most predominant during wartime, sporadic cases were reported between and after the World Wars, including modern day cases (85, 86).

Bacillary Angiomatosis. Bacillary angiomatosis refers to a vascular proliferative disease that most often involves the skin, but may disseminate to other organs. Bacillary angiomatosis was first described in HIV-infected patients (8, 9, 87, 88) and organ transplant recipients (12) but may also affect immunocompetent patients (89). Both *Bartonella quintana* and *Bartonella henselae* are agents of bacillary angiomatosis (10, 14, 90).

Primary skin lesions correspond to papules, which gradually increase in size to form nodules. These lesions may bleed profusely when punctured. Cutaneous lesions may be solitary or multiple, superficial, dermal, or subcutaneous. Superficial lesions may be red, purple, or uncolored. Deep lesions are usually uncolored and either mobile or fixed to underlying structures, including bones. Oral, anal, conjunctival, and gastrointestinal mucosal surfaces may be involved (91). Bacillary angiomatosis is most often associated with leukopenia and CD4+ cell counts of less than 100/mm^3 in HIV-infected patients. The clinical differential diagnosis of bacillary angiomatosis includes pyogenic granuloma, hemangioma, various subcutaneous tumors, and atypical Kaposi's sarcoma; demonstration of bacteria in histologic specimens of skin biopsies clearly distinguishes bacillary angiomatosis from these other conditions. Differentiation of cutaneous bacillary angiomatosis from the skin lesions of Carrion's disease (verruga peruana) may not be possible in endemic areas.

Cutaneous bacillary angiomatosis may be accompanied by involvement of bone marrow, spleen, liver, brain, lymph nodes, or the bowel (92–94). The presence of enlarged lymph nodes corresponding to the regional lymphatic drainage of skin lesions has been reported extensively (10, 11, 14). Abdominal nodes, together with hepatic and splenic enlargement, have also been reported (11, 95). Involvement of the skin does not always occur in bacillary angiomatosis, and in some patients only the spleen, liver, or lymph nodes were affected (11, 96). According to Mohle-Boetani et al. (97), bacillary angiomatosis in HIV-infected patients may have three clinical presentations, which may eventually be combined: cutaneous lesions (in 50% of the patients affected), abdominal symptoms (as the sole clinical manifestation in 20% of patients affected), and fever and lymphadenopathy (as the sole manifestation in 20%).

Peliosis Hepatitis. Peliosis hepatitis is defined as a vascular proliferation resulting in blood-filled spaces in the liver. It was first reported in patients suffering from wasting diseases such as tuberculosis and advanced cancers and in association with the use of drugs such as anabolic steroids. *Bartonella henselae* has recently been recognized as a new cause of peliosis hepatitis in HIV-infected patients (11, 13, 98, 99). Peliosis hepatitis may be associated with bacillary angiomatosis lesions in HIV-infected individuals (13, 100, 101). Lesions that appear similar histologically to those observed in peliosis hepatitis have also been reported in the spleen of HIV-infected patients with bacillary angiomatosis (13, 50); thus, the less specific term "parenchymal peliosis" has been proposed. Peliosis hepatitis may also be considered a visceral manifestation of bacillary angiomatosis, and the term bacillary peliosis has been proposed.

Bacteremia. Persistent bacteremia was a recognized symptom of trench fever during World War I and is now one of the most frequently reported manifestations of re-emergent *Bartonella quintana* infections among the homeless and alcoholic populations of modern cities. In the most complete study to date, ten cases of *Bartonella quintana* bacteremia in Seattle, Washington, were reported by Spach et al. (45). No consistent symptoms were recorded in this group other than fever and weight loss. Two patients had splenomegaly with a mean leukocyte count of 9600/mm^3. Thirty-four blood cultures collected from the ten patients yielded *Bartonella quintana*. The bacteremia persisted for more than ten days in four patients and more than eight weeks in one who refused antibiotic therapy. One patient died from concurrent *Streptococcus pneumoniae* bacteremia.

Bacteremia due to *Bartonella henselae* was first reported in 1990 (12). Bacteria were isolated after prolonged incubation of blood cultures from five patients, including two HIV-infected patients, a bone-marrow transplant recipient, and two immunocompetent patients with febrile illnesses. Bacteremia due to *Bartonella quintana*, *Bartonella henselae*, or *Bartonella elizabethae* has also been reported in patients with bacillary angiomatosis, peliosis hepatitis, endocarditis, and cat-scratch disease.

Endocarditis. Bartonella quintana (44, 102–104), *Bartonella henselae* (62, 105, 106), and *Bartonella elizabethae* (17) have been associated with bacte-

rial endocarditis. Spach et al. (102) first reported the syndrome, which affected an HIV-infected homosexual man. One of the ten *Bartonella quintana* bacteremic patients described (45) in Seattle possessed an aortic valve vegetation, and *Bartonella quintana* DNA was detected in the valve following its surgical removal. *Bartonella quintana* endocarditis has also been reported in three non-HIV-infected, homeless men in France (44). All patients required valve replacements, and pathological investigation confirmed diagnosis of endocarditis. In all cases blood samples yielded *Bartonella quintana*, and antibodies directed against *Bartonella quintana* antigens were found in the patients' sera. The presence of *Bartonella quintana* was also demonstrated in the removed cardiac valves by immunofluorescence and molecular methods. In a further study the same group (21) isolated *Bartonella henselae* from infected valvular tissue of an endocarditis patient. Characterization of this isolate indicated that it belonged to a novel serogroup (Marseille) of the species.

Following these reports, Raoult et al. (48) diagnosed 22 cases of culture-negative endocarditis caused by *Bartonella* spp. Five cases were attributed to *Bartonella quintana*, four to *Bartonella henselae*, and 13 to *Bartonella* spp. (this latter group was diagnosed using serological assays that cannot clearly distinguish between the two species). All 22 cases were native-valve endocarditis. However, *Bartonella quintana* endocarditis could be differentiated from *Bartonella henselae* endocarditis mainly on the basis of associated epidemiological factors. *Bartonella quintana* endocarditis was most often observed in homeless, chronic alcoholic patients, without previous known valvulopathy. *Bartonella henselae* endocarditis most often occurred in patients with known valvulopathy who had contact with cats. The incidence of *Bartonella* endocarditis could be evaluated. Evidence of *Bartonella* infection was found in 3% of all cases of endocarditis diagnosed in patients tested in reference centers in three different countries. Therefore, *Bartonella* infection must be suspected when diagnosing patients with endocarditis, especially if routine blood cultures remain sterile despite the absence of antibiotic therapy. Such suspicion should allow more rapid diagnosis of *Bartonella* endocarditis and may prevent the need for valve replacements, which are often required only when extensive valve lesions are detected.

Cat-Scratch Disease. Cat-scratch disease was first described in 1950 (7). The most frequent clinical presentation is lymphadenopathy in nodes draining the site of the scratch (107). Local lesions at the site of the scratch may also develop. The lymph nodes involved usually regress over a period of weeks or months, although the lymphadenopathy may proceed to suppuration in approximately 10% of cases. Unusual presentations include the following: Parinaud's occuloglandular syndrome (108), with preauricular lymphadenopathy and conjunctivitis when bacterial inoculation occurs via the conjunctiva; uveitis and papillo-retinitis (109); encephalopathy, erythema nodosum; osteolytic lesions; and disseminated diseases with visceral involvement (110–112), especially in immunocompromised patients.

The disease was confirmed as being of bacterial origin in 1983 when Wear et al. (113) demonstrated the presence of bacilli in skin sections using Warthin-Starry silver staining. The presence of bacteria of identical morphology was later demonstrated at the site of inoculation in cat-scratch disease patients (114). In 1988 a gram-negative bacillus was isolated from the lymph nodes of infected patients (115). When this bacterium was characterized as a new species, *Afipia felis* (116), the mystery surrounding the etiology of cat-scratch disease appeared to be over. However, in a subsequent serological survey of cat-scratch disease patients (63), 88% of sera contained antibodies specific to *Bartonella henselae* rather than *Afipia felis* antigens. *Bartonella henselae* was later isolated from the lymph nodes of patients with cat-scratch disease (117–119), and *Bartonella henselae* DNA was amplified from pus preparations prepared from cat-scratch disease patients that were used for skin test antigen in diagnosis (120). Since then, a mass of epidemiological, serological, and molecular detection-based data implicating *Bartonella henselae* rather than *Afipia felis* as the main agent of cat-scratch disease has been produced, but very few clinical isolates have been obtained. Furthermore, in a minority of cases of typical clinical cat-scratch disease, no evidence of *Bartonella henselae* infection can be found, possibly indicating that, albeit less frequently, other etiologic agents including *Afipia felis* may be responsible for the disease (121). Alternatively, recently detected antigenic variations in *Bartonella henselae* strains (21) may explain the apparent lack of specific antigens in patients with typical clinical signs of cat-scratch disease. Recent data indicate the existence of two *Bartonella henselae* genotypes (122).

Chronic Lymphadenopathy Due to Bartonella quintana. Bartonella quintana has been isolated

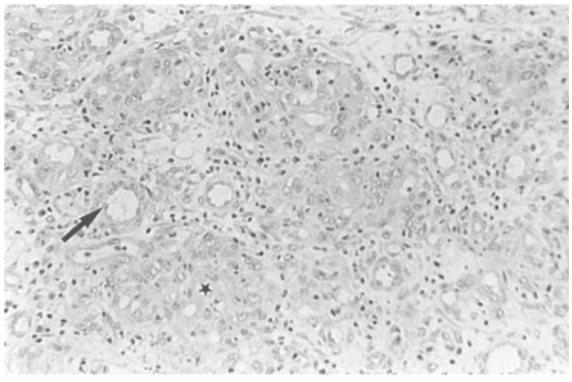

Figure 2: Cutaneous bacillary angiomatosis. The histologic preparation of the skin section reveals lobular proliferation of blood vessels in the dermis (*), with proliferating epithelioid endothelial cells lining the vascular channels. Some endothelial cells protrude into vascular lumina (arrow). (Hematoxylin-phloxin-saffron stain, original magnification x 250).

from a non-HIV-infected, 30-year-old woman with afebrile chronic cervical and mediastinal adenopathy (59). Histological examination of both the node and bone marrow showed a granulomatous reaction. Cell culture of blood specimens yielded *Bartonella quintana*, although isolates could not be obtained from the patient's lymph node, and no *Bartonella quintana*-specific antibodies could be detected in the patient's serum. More recently, *Bartonella quintana* was isolated from a 38-year-old woman with Goujerot-Sjögren disease who was receiving low doses of steroids (123). *Bartonella quintana* was grown from a bone-marrow biopsy, but not from the lymph node. Cultures for *Mycobacterium tuberculosis* were sterile. The patient's treatment regimen was altered to include an increased level of steroids and gentamicin, which resulted in dramatic recovery.

Miscellaneous Clinical Presentations. Other clinical presentations of *Bartonella* spp. infections have been described, although they represent anecdotal reports. They include pulmonary nodules (124), bacillary angiomatosis presenting as a malleolar ulcer (125), aseptic meningitis (126), encephalopathy with dementia in HIV-infected people (127, 128), and neuroretinitis (126).

Laboratory Diagnosis of *Bartonella* Infections

Historically, cat-scratch disease has been diagnosed using five criteria: the presence of a cutaneous inoculation site; chronic lymphadenopathy without specific diagnosis; cat scratches or cat contact; a granuloma on histologic examination of lymph node tissue biopsy; or a positive skin test. However, elucidation of the etiology of this syndrome and the others already discussed has allowed the development of more specific tests for the laboratory diagnosis of *Bartonella* infections.

Histology. Diagnosis of bacillary angiomatosis is most often determined by histological examination of skin biopsies (Figure 2). In hematoxylin-eosin stained sections, bacillary angiomatosis is characterized by lobular capillary proliferation (68), the lobules being rounded aggregates of capillaries. The stroma surrounding the lobules is edematous in early lesions, becoming fibrotic in later stages. The endothelial cells of bacillary angiomatosis may protrude into vascular lumina, which may become occluded. Nuclear atypia of endothelial cells may be seen. Amphophilic granular aggregates are highly indicative of bacillary angiomatosis. Such aggregates are revealed to be masses of bacteria using Warthin-Starry silver staining, electron microscopy, or immunofluorescence. Neutrophils frequently cluster around these aggregates of bacteria.

Histologic examination of hematoxylin- and eosin-stained peliosis hepatitis liver sections reveals dilated, blood-filled spaces in the fibromyxoid stroma containing inflammatory cells (Figure 3), dilated capillaries, and clumps of granular purple material that are revealed as masses of bacteria by Warthin-Starry silver staining.

In contrast to bacillary angiomatosis, histological examination of lymph nodes removed from cat-scratch disease patients reveals the presence of a nonspecific granuloma.

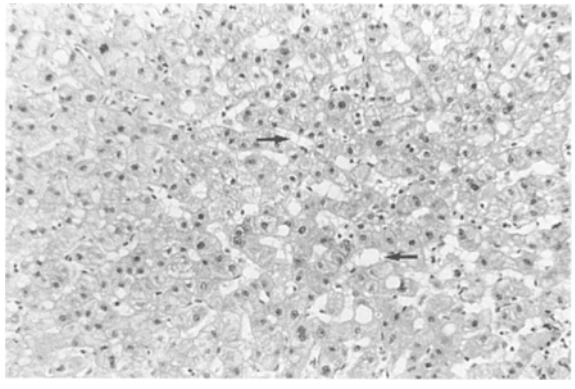

Figure 3: Peliosis hepatitis. The histologic preparation of the liver section shows the presence of cystic, blood-filled spaces (arrows) in hepatic parenchyma. (Hematoxylin-phloxin-saffron stain, original magnification x 250).

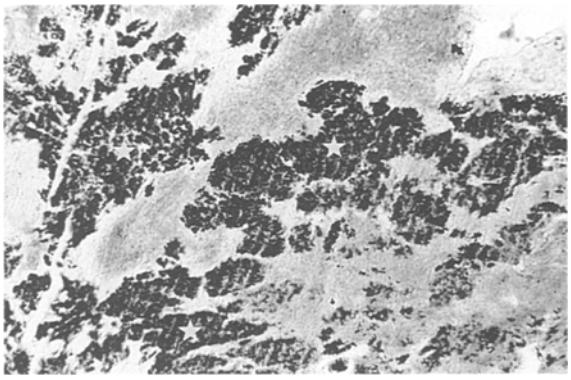

Figure 4: Warthin-Starry staining of a section of aortic valve from a case of *Bartonella henselae* endocarditis. Notice the numerous clumps of bacteria within the vegetation (*), stained by the Warthin-Starry method (original magnification x 400).

When cardiac valves were removed from patients suffering *Bartonella* endocarditis, histologic preparations revealed massive vegetations on the valve surface with extensive destruction of the underlying valve tissue and the presence of numerous bacteria on Warthin-Starry staining (Figure 4).

Culture. The two most widely used methods of culture are either direct plating onto solid media (34, 44, 45, 129) or cocultivation in cell cultures (14, 44, 45, 59), although groups using the same methods describe differing levels of success (14, 34, 44, 59, 129). Cell culture systems have previously been reported to be more sensitive and allow more rapid growth of bartonellae than the blood agar technique. This finding was verified in our experience, as some isolates were obtained only in cell cultures. Furthermore, subculture of bartonellae grown in cell culture systems onto blood agar was often difficult to obtain. In a few cases, however, only the blood agar technique allowed isolation of the bacteria. A combination of both methods is probably most useful for optimizing recovery of *Bartonella* spp. (44).

Blood Agar-Based Culture. On blood-enriched-agar, *Bartonella* spp. are best cultivated in a humid, CO_2-rich (5%) atmosphere (1, 15, 32). Primary isolation from the blood of infected patients may require up to 45 days of incubation before colonies become apparent. Horse and rabbit blood are more effective supplements than sheep blood (13, 14). Both the use of blood-enriched agar and the need for prolonged incubation of the plates increase the possibility of contamination, especially from fungi. Adding amphotericin B to the culture medium may therefore be beneficial. However, due to the high level of in vitro susceptibility of bartonellae to most antibiotics, their inclusion in culture medium in order to prevent bacterial overgrowth would also impair growth of bartonellae themselves. The use of lysis centrifugation has been shown to enhance the recovery of *Bartonella* from blood (13). Detection of fastidious organisms such as *Bartonella* in conventional automated blood culture systems remains difficult, as the organisms produce little or no CO_2 and yield little visible growth. Thus, systematic detection of bacteria in blood cultures using an acridine orange dye-staining procedure has been proposed (129) as an approach to improving recovery of *Bartonella* spp. The method was recently used (45) in isolating *Bartonella quintana* from 34 blood cultures within a six-month period.

Cell Culture Systems. Bartonella spp. can be grown in vitro in a number of cell culture systems, including endothelial cells, L929, and HeLa cells (14, 37, 44). Endothelial cell cultures, however, are considered most effective and have been reported to support bartonellae growth more efficiently. *Bartonella quintana* was first isolated from a patient with bacillary angiomatosis following cocultivation of cutaneous biopsy material with a bovine endothelial cell line (14). Recently, a similar approach was used when *Bartonella quintana* was isolated from the blood of three patients with endocarditis (44) and when *Bartonella quintana* was isolated from the blood of a woman with chronic lymphadenopathy and lymphopenia (59).

Identification. Bartonella spp. should be suspected when nonmotile, small, gram-negative bacilli are recovered following prolonged incubation of inoculated blood-enriched media in a CO_2-rich, humid environment at 30 to 37°C. Several methods for confirming the identity of presumptive *Bartonella* spp. have been described, ranging from the comparison of the biochemical reactivities of isolates (130) to more complex genotypic and phenotypic analyses. Bartonellae are virtually inert in all standard biochemical assays, although species have been differentiated on the basis of the presence of different preformed aminopeptidases (18, 20). Rabbit antisera raised against type strains of each species appear not to significantly cross-react with nonhomologous antigens (18) and provide a useful method of speciation.

A number of PCR-based differentiation methods have also been described, including repetitive element PCR (131) and various PCR-restriction fragment length polymorphism (RFLP) analyses exploiting variation within the RNA operon (10,

15, 26–28, 34, 132–134) or the citrate synthase (*gltA*) gene (10, 15, 34, 44, 135, 136). Determination of partial 16S rRNA or *gltA* sequences is also reported regularly. Macrorestriction analysis using pulse-field gel electrophoresis has been used to demonstrate intraspecies differences (39, 137).

Serology. Early laboratory diagnosis of trench fever relied on a passive hemagglutination test, using tanned sheep erythrocytes sensitized with soluble antigen from *Bartonella quintana* (138). Other methods of specific antibody detection and estimation are now used in the diagnosis of *Bartonella* infections, including the immunofluorescence antibody test (IFAT) and enzyme immunoassays (EIA) (139–141). It must be noted that in most HIV-infected patients, a group particularly prone to *Bartonella* infections, a significant antibody response to infection is not mounted. In contrast, up to 88% of patients with cat-scratch disease had specific serum antibodies to *Bartonella henselae* when tested by IFAT (63), and 95% had serum antibodies when tested by EIA (141).

A recent serological study of immunocompetent individuals with *Bartonella*-induced endocarditis detected very high levels of antibodies (44). The titer to which antibodies are detected, however, appears to depend on the method by which the antigen used in the assay is prepared, and even different batches of the same antigen preparation can yield different results (142). In the above study (44), antigen prepared from plate-grown *Bartonella* spp. reacted with antisera to dilutions of 1:400 to 1:800, whereas titers of 6400 to 12,800 were obtained when the same sera were tested against antigens prepared from organisms cocultivated with cell monolayers.

Genetic heterogeneity among *Bartonella henselae* strains was observed during preliminary characterization of the species (10), although subsequently, genotypic and phenotypic differences were observed among *Bartonella henselae* isolates obtained from cat blood (49). Drancourt et al. (21) characterized new *Bartonella henselae* strains from French patients with endocarditis and cat-scratch disease and from English and Swiss cats and demonstrated that they had antigenic differences from previously assessed isolates. Other phenotypic and genotypic differences were also noted, and it was proposed that these strains belonged to a separate serogroup of the species. Examination of 16S rRNA gene fragment sequences derived from *Bartonella henselae*-infected tissues of Dutch patients with cat-scratch disease identified two distinct groups of strains; the respective signature sequences of the 16S rRNA genes of the two groups matched those of the two serogroups proposed above (122). It therefore appears that a widely distributed group of serovariant strains exists. Drancourt et al. (21) were also able to demonstrate significant reactivities in 18 of 113 apparently negative serum specimens when a strain of the new serogroup was incorporated as antigen into a diagnostic IFAT. Antigenic variation among *Bartonella henselae* strains may therefore explain inconsistent results in the serological diagnosis of cat-scratch disease.

Since most *Bartonella*-related infections have only recently been described and the number of cases identified is still relatively low, the titer at which antibody levels become significant (i.e., the cut-off value) for diagnosis of infection has yet to be determined. Over 12,000 sera from patients with suspected *Bartonella* infections have been tested in our laboratory for the presence of specific antibodies using IFAT and antigen from plate-grown organisms. In 70 of these sera IgG titers of > 100 against *Bartonella quintana* have been found; titers were > 400 in 31 of these 70. At present, we consider an IgG titer of > 100 significant.

The specificity of antibody estimation tests has been questioned by several groups. Current serological tests may not reliably distinguish *Bartonella quintana* antibody responses from *Bartonella henselae* responses. Cross-reactivity between *Bartonella* spp. and other organisms, including *Coxiella burnetii* (138) and *Chlamydia* spp. (44), has also been reported. Emmons et al. (143) first demonstrated that antisera from cat-scratch disease patients reacted with *Chlamydia trachomatis* antigens. Cross reaction between *Bartonella bacilliformis* and *Chlamydia psittaci* has also been reported, with a common surface epitope being characterized as part of the lipopolysaccharide (144). Drancourt et al. (44) reported that patients with *Bartonella quintana*-induced endocarditis had IgG titers of > 256 against *Chlamydia pneumoniae* and titers of 64 against *Chlamydia trachomatis* and *Chlamydia psittaci* (44). Absorption of the sera with *Chlamydia pneumoniae* did not reduce the high antibody titers against *Bartonella quintana*, but absorption with *Bartonella quintana* eliminated reactivity with *Chlamydia pneumoniae* antigen. This cross-reactivity was confirmed using immunoblotting. The susceptibility of the cross-reacting antigens to proteinase K indicated their protein nature.

Recently, we tested eight sera from eight patients previously diagnosed as having chlamydial endocarditis based upon serological data. All sera reacted strongly against both *Chlamydia* and *Bartonella* antigens. Adsorption procedures and Western blotting confirmed cross-reactions between *Bartonella* and *Chlamydia* antigens and indicated that antibody reactivities were more compatible with the antibody response observed in *Bartonella*-infected patients. All patients were considered to have *Bartonella* endocarditis rather than chlamydial endocarditis. La Scola et al. (145) have also reported that more than 50% of patients with chronic Q fever had antibodies that reacted with *Bartonella henselae* antigen to a significant level. These reactions were confirmed by a cross-adsorption study and protein immunoblotting.

Clearly, the cross-reactivity between *Bartonella*, *Chlamydia*, and *Coxiella burnetii* is of diagnostic importance, as all are potential etiologic agents of endocarditis. However, because the levels of specific antibodies observed in *Bartonella*-induced endocarditis are extremely high, low-level cross-reaction with other antigens should not lead to misdiagnosis, provided serology for all suspected agents is performed.

PCR-Based Detection Methods. The 16S rRNA gene (10, 14, 15, 59, 146) and the citrate synthase gene (44) are the two DNA fragments on which efforts have been focused in attempts to develop specific PCR-based assays for the diagnosis of *Bartonella* infections. An alternative approach based on PCR amplification of a heat shock gene fragment has also been described (147).

Relman et al. (10) were the first to design *Bartonella*-specific PCR primers based on unique regions of the 16S rRNA gene. As described earlier, these primers were used to amplify a 280 bp fragment of the *Bartonella henselae* 16S rRNA gene from clinical material from three bacillary angiomatosis patients. The primers, p24E and p12B, have subsequently been used in several different studies in which their ability to amplify the 16S rRNA gene from different *Bartonella* spp. has been demonstrated by sequencing of PCR products (10, 14, 15, 59). *Bartonella quintana* 16S rRNA has been successfully amplified from a variety of clinical material, including cutaneous lesions of bacillary angiomatosis patients (10, 14), the lymph node of a patient with chronic lymphadenopathy (59), and pus aspirates of cat-scratch disease patients (148). However, these primers are not genus specific and also amplify 16S rRNA gene fragments from other members of the alpha-2 subdivision of the *Proteobacteria*. The production of a suitably sized fragment using this PCR cannot therefore be considered evidence of the presence of *Bartonella* DNA. Amplicons need to be further characterized, most effectively by sequencing or probing.

The amplification of citrate synthase gene (gltA) fragments coupled with RFLP was first described for the detection and identification of rickettsiae (149); the scheme was later extended to the former *Rochalimaea* spp. The primers used in the amplification are not *Bartonella* specific, although RFLP of amplification products does permit discrimination of *Bartonella* spp. from other bacteria. Using this approach, *Bartonella quintana* has been identified from surgically removed cardiac valves of endocarditis patients (44).

More recently, Anderson et al. (147) described a PCR assay using degenerate primers that allowed amplification of a 414 bp fragment of DNA either from *Bartonella quintana* or *Bartonella henselae*. Internal oligonucleotides were used as hybridization probes and allowed rapid differentiation of these two species. Conversely, the 414 bp DNA fragment was not amplified in the PCR assay of *Bartonella elizabethae*, *Bartonella bacilliformis*, *Bartonella bacilliformis*, or *Afipia felis*. The technique was applied to 16 fresh node tissue and nine node aspirates from patients with cat-scratch disease (147). The 414 bp fragment was amplified from 21 of 25 samples, and in all cases the 414 bp fragment hybridized with the *Bartonella henselae*-specific probe.

Treatment of *Bartonella* Infections

In Vitro Antibiotic Susceptibility. Few data on the in vitro antibiotic susceptibility of *Bartonella* spp. were available prior to 1990 (150), mainly because so few strains had been isolated. Recent development of *Bartonella*-specific cultures in routine laboratory practice has allowed more frequent isolation of *Bartonella* strains. In a recent study, the antibiotic susceptibilities of nine *Bartonella quintana* isolates, three *Bartonella henselae* isolates, one *Bartonella bacilliformis* isolate, and one *Bartonella elizabethae* isolate were assessed using horse blood-supplemented Columbia agar as the assay medium and an incubation of five days at 37°C (151). Although it should be emphasized that in vitro MIC determination for the fastidious *Bartonella* spp. must be considered cautiously, the results

indicated that all isolates were highly susceptible to beta-lactam agents, aminoglycosides, macrolides, tetracyclines, and rifampin. *Bartonella* spp. were less susceptible to penicillinase-resistant penicillins, first-generation cephalosporins, and clindamycin. Considerable variations were noted in the susceptibility to fluoroquinolone compounds. The bactericidal activity of antibiotics against *Bartonella henselae* was then evaluated in axenic medium and in cell culture, using either a murine macrophage-like cell line (ATCC TIB63) or a human endothelial cell line (ECV 304, 9) (152). Cocultivation of *Bartonella* spp. with eukaryotic cells did not change the bacteriostatic activity of antibiotics. However, only the aminoglycosides (gentamicin, tobramycin, and amikacin) were bactericidal on either axenic or cell-line cultured organisms. Interestingly, although *Afipia felis* is highly resistant to antibiotics in vitro, only the aminoglycosides display significant in vitro bacteriostatic and bactericidal activity both in axenic medium and in cell systems (153).

Clinical Data on Antibiotic Therapy of Bartonella Infections. Reports of successful treatment of trench fever using tetracycline or chloramphenicol have been scarce. Data on the efficacy of antibiotics in the treatment of the newly recognized *Bartonella*-induced infections, mainly bacillary angiomatosis, are more widely available.

A regimen of erythromycin (250 to 500 mg, q.i.d.) is reported to be the most effective method of treating cutaneous bacillary angiomatosis (10–13, 154), although relapses may occur after cessation of antibiotic treatment, particularly in HIV-infected patients on short (< 15 days) courses of treatment (12). Failures have been reported for the treatment of bacillary angiomatosis when using nafcillin, dicloxacillin, and cephalexin, and such data are consistent with in vitro results of antibiotic susceptibility. Clinical data suggest that amoxicillin (62), aminoglycosides (11, 62), doxycycline (15), and trimethoprim-sulfamethoxazole (12, 89) may be suitable for treatment, even though several patients on these antibiotics relapsed.

A wide range of other antibiotics have been reported to successfully treat *Bartonella*-induced bacillary angiomatosis. Lesions resolved in two patients treated with ceftriaxone (12, 62). Successful therapy of three cases of bacillary angiomatosis has been reported using norfloxacin (12) and ciprofloxacin (12, 62). Antimycobacterial drugs are also reported to be effective in eradicating bacillary angiomatosis, supposedly because of the rifampin component (11). It should be emphasized that the duration of therapy may be more important than the choice of antibiotic, especially in the immunocompromised host. Fewer relapses have been noted in patients treated for more than a month.

Data on the requirements for the effective treatment of *Bartonella*-induced endocarditis are scarce, again because so few cases have been reported. Successful treatment has been reported with a complex regimen consisting of intravenous amoxicillin combined with gentamicin (44); intravenous vancomycin with ofloxacin and netilmicin, followed by oral therapy with rifampin, ofloxacin, and pristinamycin (44); and intravenous ceftriaxone followed by long-term erythromycin (102). Endocarditis due to *Bartonella elizabethae* did not respond to treatment with nafcillin and gentamicin but resolved after cardiac valve replacement and a regimen of vancomycin and imipenem (17). Intravenous antibiotic therapy was continued for six weeks and oral trimethoprim-sulfamethoxazole was administered for a further five weeks. It should be noted that *Bartonella*-induced endocarditis usually results in extensive valve damage, necessitating valve replacement (17, 44).

The treatment of *Bartonella quintana* patients with bacteremia has also been described (45). Of the ten patients described by Spach et al. (45), five were treated with ceftriaxone for seven days followed by either oral erythromycin or azithromycin for three weeks. Only three patients could be followed up; bacteremia has resolved in all of them.

Cat-scratch disease typically does not respond to antibiotic therapy. Only the aminoglycosides, mainly gentamicin, are effective treatments for patients suffering from suppurative complications (155, 156). Anecdotal reports indicate that ciprofloxacin, rifampin, and sulfamethoxazole-trimethoprim may be active (157–159).

Considerations on Antibiotic Therapy and Prophylaxis of Bartonella Infections. The poor efficacy of beta-lactam agents in the treatment of *Bartonella*-induced illness and the frequency of relapses when antibiotic therapy is withdrawn despite high in vitro susceptibility of bartonellae to antibiotics are striking. As discussed earlier, several observations and experiments have indicated that *Bartonella* spp. are also found intracellularly in infected tissue (77). On the other hand, recent in vitro experiments have shown that most antibiotics except the aminoglycosides do not display any bactericidal activity against bartonellae (152), and bactericidal activity may be more im-

portant in vivo, especially in immunocompromised patients or patients with chronic infections or endocarditis. Although the formulation of a standard regimen for antibiotic treatment of *Bartonella*-related diseases remains hazardous, both the use of aminoglycosides and prolonged administration of antibiotic therapy should be advocated.

As the cycle of the *Bartonella* spp. in nature is still unclear, likely predisposing epidemiological factors are also uncertain. Thus, it remains difficult to establish valid recommendations for prevention of *Bartonella*-induced illnesses. Delousing procedures were recommended for prevention of trench fever and may apply to prevention of bacteremia (45) and endocarditis (44) in homeless people as well. As for *Bartonella henselae* infections, immunocompromised persons, including those infected with HIV, should avoid contact with cats.

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