Bartonella (*Rochalimaea*) *quintana* Infections

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HISTORICAL ASPECTS

Trench fever was the first clinical manifestation of *Bartonella* infection to be recognized. The name "trench fever" was coined because the disease came to prominence as an infection of troops in both the German and Allied armies during World War I. The disease was characterized by a 5-day relapsing fever, with severe and persistent pain in the shins, and although rarely fatal, it resulted in prolonged disability. It has been estimated that trench fever affected over 1,000,000 people, including some nonmilitary personnel (47), with epidemics of the disease most frequently reported in Russia and on the Eastern, Central, and Western European fronts.

Trench fever has also been referred to as 5-day fever (because of its typical clinical presentation) and Wolhynian fever (because the disease was first observed by German medical officers on the East German front in Wolhynia). Although cases of trench fever were simultaneously reported on two fronts, the disease was supposedly conveyed from the eastern front to the western front by German soldiers in 1914 having been originally acquired from endemically infected Russian populations (60, 143, 151). British troops were also responsible for transferral of the disease, carrying it with them to Mesopotamia and Salonika (17, 25).

Reports of trench fever do, however, predate this period, and the disease may have been known as long ago as the Middle Ages (60). A disease with the same clinical presentation as trench fever was recognized in Northern Ireland in the first half of the nineteenth century (67), and a similar disease was described in Russia (143). However, only after the outbreak of large-scale epidemics of trench fever during World War I was the importance of the disease recognized. After the conclusion of the war, the incidence of trench fever and thus medical interest in the condition fell dramatically. However, with the outbreak of World War II, trench fever reemerged and large-scale epidemics of the disease were again reported, particularly among German troops (60), who carried the infection to Norway, Finland (11), and Yugoslavia (85).

Because early investigators were unable to induce trench fever in laboratory animals, most studies of the disease were carried out on human volunteers. In the earliest extensive report on the subject, McNee et al. (78) demonstrated that the

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agent of the disease was contained in whole blood by inducing trench fever in volunteers given injections of blood collected from patients. McNee et al. (78) were the first to suggest a role for lice in the transmission of trench fever, although they were unable to demonstrate this experimentally; it is likely that their reasoning was influenced by the work of Nicolle et al. (98, 99), who had earlier demonstrated the role of lice in the transmission of epidemic typhus caused by *Rickettsia prowazekii*. Delousing procedures were introduced and were an important part of the control of both epidemic typhus and trench fever. In 1917, Pappenheimer and Mueller (cited in reference 60) succeeded in transmitting the disease to one of three human volunteers by allowing lice to feed first on several trench fever patients and then upon the volunteers. Topfer, Schminke, and later Kuczynski (cited in reference 60) were among the first to propose a rickettsia-like organism, named *Rickettsia quintana*, as the etiologic agent of trench fever. Subsequently, the American Red Cross Commission and the British scientist Byam (18, 60) further explored the etiologic importance of these rickettsia-like organisms and investigated their occurrence in both lice and humans.

Although trench fever was most commonly associated with "campaign" conditions, the disease has also been reported, albeit intermittently, between and after the epidemics of the World Wars. After World War I, cases of the disease were reported in Spain, Sweden, Ukraine, Georgia, and Russia (60). In 1936, Mosing (88), despite proposing the alternative name of *Rickettsia weigli* for the rickettsia-like etiologic agent he observed, described a patient who had a laboratory-acquired illness with the same symptoms as those of trench fever. In 1939, Sparrow (138) reported the presence of *Rickettsia quintana* in ticks that had fed on North African patients suffering from trench fever-like illness. Later, similar syndromes were reported in Algeria (105), Egypt (6), and in a typhus laboratory in Addis Ababa, Ethiopia (84). The ubiquity of trench fever has been further demonstrated, with cases being reported in Japan (103), China (44), and, more recently, Mexico (148, 149).

Prior to successful cultivation of the trench fever agent, the poor characterization of rickettsia-like organisms observed or isolated from lice led to several names being proposed. In addition to the names *R. quintana*, proposed by Schminke et al. (cited in reference 60), and *R. weigli* (88) discussed above, others including *R. volhynica* (by Jungmann and Kuczynski, cited in reference 60), *R. pediculi* (cited in reference 60), and *R. rocha-limae* (158) were proposed. In 1961, Vinson and Fuller (152) reported the first successful cultivation of the agent of trench fever, which had been reclassified as *Rochalimaea quintana*, on cell-free media. The authors went on to fulfil Koch's postulates by inducing trench fever in human volunteers following their inoculation with suspensions of the isolated organisms (153). The report also emphasized that different clinical manifestations of trench fever were observed in different volunteers inoculated with the same strain. Despite these findings, medical interest in trench fever and *R. quintana* waned for the next 25 years because the disease was only very rarely encountered.

Recent investigations have, however, led to the reemergence of *R. quintana* as an organism of medical importance. In 1983, Stoler et al. (140) described a newly recognized syndrome in an AIDS patient. The disease, subsequently termed bacillary angiomatosis (BA), was initially characterized by the appearance of multiple cutaneous lesions. It was assumed to be infectious, because these lesions contained bacilli that stained with the Warthin-Starry stain (3, 65) and the lesions resolved on antibiotic treatment (23). In 1990, Relman et al. (118) partially characterized the observed bacillus by PCR, incorporating primers that hybridized to sites in the 16S rRNA gene of a broad range of gram-negative organisms and amplifying a partial 16S rRNA gene fragment from the bacillus directly from biopsy material of one heart transplant recipient and three human immunodeficiency virus (HIV)-infected patients with BA. By comparing the base sequences of these amplified fragments, they provided evidence that the same etiologic agent was present in three of the four patients and that it was closely related to *R. quintana*. The new organism represented by this sequence was named BA-TF. On the other hand, in one HIV patient, the 16S rRNA gene sequence differed from BA-TF at four positions and may have corresponded to that of *R. quintana*, although attempted cultivation of this organism had failed. Concurrent with the paper by Relman et al. (118), Perkocha et al. (107) reported a new AIDS-related syndrome of the liver, termed peliosis hepatitis (PH), and Slater et al. (131) reported the isolation of a previously uncharacterized bacterium from the blood of five patients with bacteremia. Subsequent collaboration between the three groups demonstrated that the agents of all three syndromes were indistinguishable by comparison of partial 16S rRNA gene sequences (116). In addition, the presence of endothelial cell proliferation on pathological examination was reported in cutaneous biopsy specimens and liver sections from patients with BA or PH, respectively. The organism was first isolated and subcultured from the lesions of patients with BA by Koehler et al. (58), and following its characterization, the organism was confirmed as a new species and named *Rochalimaea henselae* (165). Moreover, *R. quintana* was also cultured from skin lesions of BA patients by Koehler et al. (58), confirming an earlier report from Relman et al. (118) and demonstrating clearly that both *R. henselae* and *R. quintana* are etiological agents of the disease.

R. quintana, which has again been the subject of taxonomic reclassification and is now named *Bartonella quintana*, has been associated with other clinical syndromes. Drancourt et al. (30) reported the isolation of *B. quintana* from three homeless patients with endocarditis in France, and Spach et al. (137) reported *B. quintana* bacteremia in a similar group of patients in Seattle. *B. quintana* has also been associated with chronic lymphadenopathy (110).

Although *R. henselae* (now renamed *Bartonella henselae*) has also been implicated in other infections, including cat scratch disease (CSD), at present it has been isolated only in the United States. *B. quintana* has been isolated from patients on both sides of the Atlantic. However, this finding may not be a true indication of the likely distribution of the organisms, because DNA likely to be derived from *B. henselae* has been amplified from clinical material in Europe, including from a liver biopsy specimen of an HIV-positive patient from France with PH (70), from the lesions of an immunocompetent child with disseminated cat scratch disease in Switzerland (155), and from pus aspirates of patients with CSD in the Netherlands (10).

The present review focuses primarily on *B. quintana* and aspects of the infections caused by this species.

TAXONOMY

The 1984 edition of *Bergey's Manual of Systematic Bacteriology* (163) divided the order *Rickettsiales* into three families, namely, *Rickettsiaceae*, *Bartonellaceae*, and *Anaplasmataceae* (163). *R. quintana* and *Rochalimaea vinsonii* (162, 163), the only two members of the genus *Rochalimaea*, together with members of the genera *Rickettsia* and *Coxiella*, made up the

tribe *Rickettsiae* within the family *Rickettiaceae*. The species *Bartonella bacilliformis* was the only member of its genus (120), which together with the genus *Grahamella* formed the family *Bartonellaceae*.

Although the members of the *Rickettsiales* are almost exclusively strict obligate intracellular organisms, members of the genus *Rochalimaea* and the family *Bartonellaceae* can be grown on nonliving media. The differences between this group of organisms and other members of the order is also apparent in genotypic comparisons. The "true rickettsia" species (those belonging to the genus *Rickettsia*) have a very low percentage of guanine and cytosine (G+C) in their genome (28.5 to 33.3 mol%), whereas *Rochalimaea* and *Bartonella* species have a significantly higher level (39.0 to 40.0 mol%) (15). However, the classification of the *Rochalimaea* spp. close to the *Rickettsia* spp. was supported by DNA-DNA hybridization data reported by Myers et al. (93–95). Their studies had found significant levels of relatedness (27 to 31%) between *R. quintana* and *R. prowazekii* and between *R. quintana* and *R. typhi* (levels of relatedness of 36 to 53% were reported between different *Rickettsia* species in this study).

In 1992, membership in the genus *Rochalimaea* increased with the description of two new species, *Rochalimaea henselae* (112) and *R. elizabethae* (28). Their characterization was part of a renewed medical and microbiological interest in this group of organisms. The availability of modern taxonomic methods, such as DNA hybridization and 16S rRNA gene sequence comparison, which had been used in the characterization of these new species, also allowed an effective reappraisal of the classification of the established *Rochalimaea* and *Bartonella* species.

Using whole-genome DNA-DNA hybridization, attempts were made to determine the interrelatedness of *Rochalimaea* and *Bartonella* species. Welch et al. (165) and Daly et al. (28) demonstrated a level of relatedness of 55 to 62% between *R. quintana* and *R. vinsonii* and levels of relatedness of 49 to 71% between the four *Rochalimaea* species. The same authors reported levels of relatedness of 32 to 47% between *B. bacilliformis* and the *Rochalimaea* species (28, 165). These data indicate that *Rochalimaea* and *Bartonella* species share a very high level of genomic interrelatedness. In a recent reappraisal of the level of DNA-DNA homology between *R. quintana* and the *Rickettsia* species, Brenner et al. (15) were unable to detect the significant levels of relatedness previously reported by Myers and Wisseman (93). Instead, only very low levels (6 to 14%) of homology were found, suggesting a far less significant relationship between members of the genera *Rochalimaea* and *Rickettsia*. These new findings were, however, more consistent with the reported differences in G+C moles percent between the two genera.

Brenner and colleagues went on to propose the taxonomic unification of the genera *Bartonella* and *Rochalimaea* (15). The proposal was based not only on the revised DNA hybridization results but also on the findings of other taxonomic studies that used phylogenetic, genotypic, and phenotypic methods of comparison. Brenner et al. (15) also emphasized the extensive differences between the *Bartonella*/*Rochalimaea* group and other members of the *Rickettsiales* and proposed the removal of the unified genus from the order. The unified genus, which retained the name *Bartonella*, thus contained five species: *Bartonella bacilliformis*, the type species and agent of Carrion's disease, and the four former *Rochalimaea* species, *B. quintana*, *B. vinsonii*, *B. henselae*, and *B. elizabethae*. The genus was defined as containing gram-negative, oxidase-negative, fastidious, aerobic, rod-shaped bacteria which grew best on blood-enriched media in an atmosphere containing 5% carbon diox-

ide. After the proposal by Brenner et al., Birtles et al. (13) used a polyphasic approach as the basis of a proposal for the unification of the genera *Bartonella* and *Grahamella*; the two established *Grahamella* species, *G. talpae* and *G. peromysci*, were therefore renamed as *Bartonella* species. In addition to this unification, Birtles et al. described three new *Bartonella* species, *B. grahamii*, *B. taylorii*, and *B. doshiae*, all of which were isolated from woodland mammals. At present, therefore, the genus *Bartonella* contains 10 species.

Several phylogenetic studies, based on comparison of 16S rRNA gene sequences, have been completed on the *Bartonella* species (12, 102, 112, 116–118, 159, 160); several of these studies were cited by both Brenner et al. (15) and Birtles et al. (13) in the taxonomic proposals discussed above. Comparison of sequences indicated extremely high homology between the four former *Rochalimaea* species (99.1 to 99.7%) and between these species and *B. bacilliformis* (97.9 to 98.8%) (15). All members of the genus belong to the alpha-2 subdivision of the *Proteobacteria*, whereas the *Rickettsia* species lie on a deeply diverging lineage in the alpha-1 subdivision. Interestingly *Coxiella burnetii*, another organism considered to be closely related to both *Rickettsia* species and the former *Rochalimaea* species, has been shown to be phylogenetically remote from both groups, belonging to the evolutionarily distant gamma subdivision of the *Proteobacteria*. Within the alpha-2 subdivision, the *Bartonella* species are most closely related to *Brucella abortus* and *Agrobacterium tumefaciens* (160). *Afipia felis*, one of the proposed agents of CSD, is also classified within the alpha-2 subdivision. Phylogenetic trees showing relationships of species belonging to the alpha-1, alpha-2, and gamma subdivisions of the Proteobacteria are presented in Fig. 1, whereas relationships of *B. quintana* among the *Bartonella* species are presented in Fig. 2.

The reclassification of *Rochalimaea* species into the genus *Bartonella* also highlights similarities in the pathology of the diseases they cause; *B. henselae* and *B. quintana* can induce both bacteremia and cutaneous angioprolific lesions in infected patients, both presentations similar to Carrion's disease, the manifestation of *B. bacilliformis* infection. Interestingly, the evolutionary homology between *Bartonella* species and *Agrobacterium tumefaciens* may also be reflected in their pathogenicity, because the latter species induces angioproliferative tumors in plants.

BACTERIOLOGY

This section will discuss the bacteriological characteristics of *B. quintana* and compare these with the characteristics of other members of the unified *Bartonella* genus (15).

The 1984 edition of *Bergey's Manual of Systematic Bacteriology* describes only two of the *Bartonella* (formerly *Rochalimaea*) species, namely, *Bartonella* (*Rochalimaea*) *quintana* and *Bartonella* (*Rochalimaea*) *vinsonii* (163). Both are defined as short rods, 0.3 to 0.5 μm wide and 1.0 to 1.7 μm long, often curved, gram negative, and closely resembling *Rickettsia* species in morphology and staining properties (Fig. 3 and 4). Catalase and oxidase reactions are negative. *Bartonella* (*Rochalimaea*) cells can be grown on axenic media, either on blood-enriched agar or in broth enriched with amino acids, yeast extract, and fetal bovine serum (161). Succinate, pyruvate, and glutamine or glutamate, but not glucose, can be utilized as sources of energy (46, 162). Growth is enhanced by increased carbon dioxide pressure and by fetal calf serum (71) and is hemin dependent (90, 92). *B. quintana* also requires sodium bicarbonate as a source of carbon dioxide, whereas *B. vinsonii* does not.
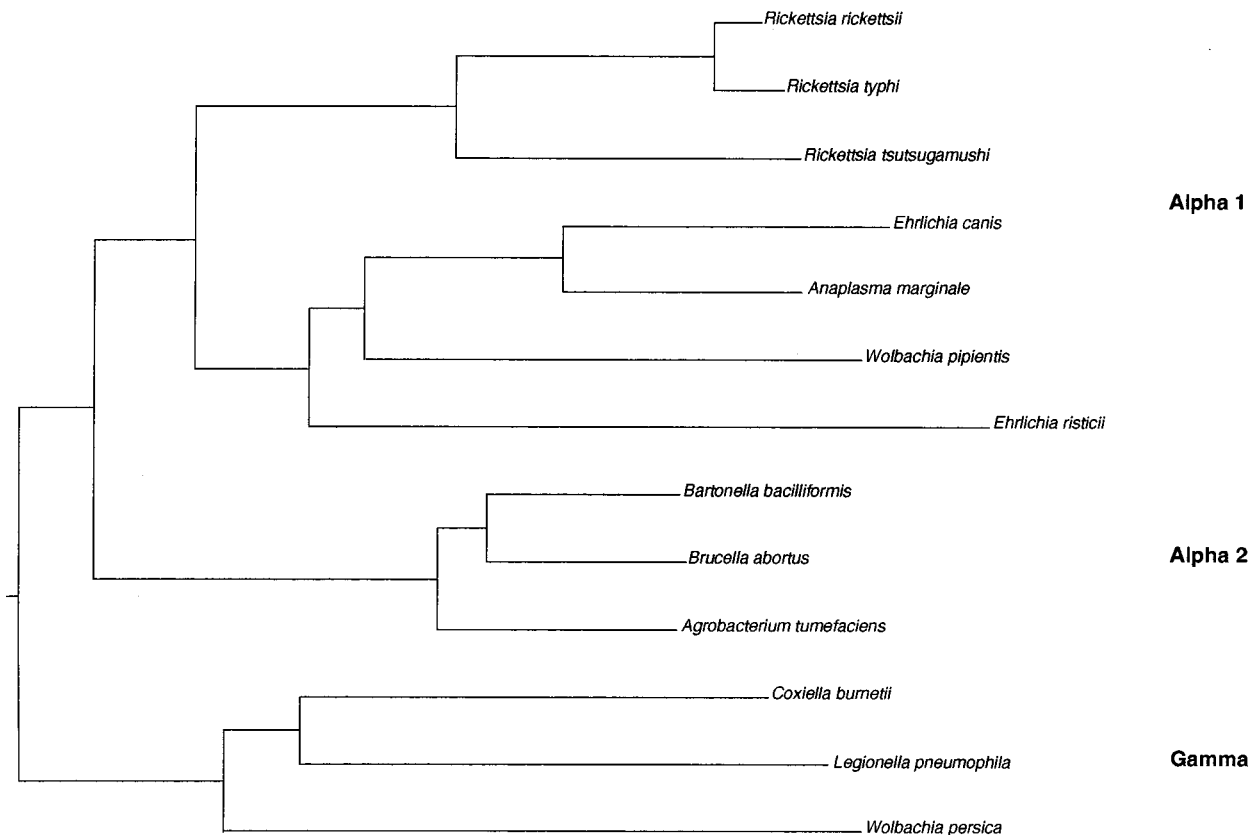
FIG. 1. Phylogenetic tree showing relationships of alpha-1, alpha-2, and gamma subdivisions of the *Proteobacteria*, with *B. bacilliformis* being representative of the *Bartonella* species. The tree was constructed by the neighbor-joining method (124) with 16S rRNA gene sequences.

When grown on blood agar, rough colonies, deeply embedded in the agar, are obtained on primary isolation, typically after 12 to 14 days of incubation at 37°C. Incubation periods required for primary isolation may, however, be as long as 45

FIG. 2. Phylogenetic tree of the alpha-2 subdivision of the *Proteobacteria*, showing the relationships of *B. quintana* with other *Bartonella* species. The tree constructed by the neighbor-joining method (124) with 16S rRNA gene sequences.

days (76). First subcultures of an isolate are difficult to obtain, with colony formation after 10 to 15 days. Repeated subcultures soon reduce this time to only 3 to 5 days, and colonies become smooth, shiny, and less adherent. In our experience, the description of *B. quintana* colonies as round and translucent in *Bergey's Manual* (163) or smooth and nonpitting by Koehler et al. (56) better corresponds to their appearance after several subcultures. Cell culture of *B. quintana* has been described; the bacterium grows on the surface of eukaryotic cells such as mouse L cells (80).

The genome size of *B. quintana* was estimated by Reschke et al. (119) to be 1,700 kb (compared with 2,100 kb for that of *B. vinsonii*). More recently, these sizes have been corroborated by Roux and Raoult (122), who used pulsed-field gel electrophoresis to calculate the genome sizes of all the former *Rochalimaea* species to be between 1,700 and 2,174 kb.

The natural reservoir of *B. quintana* remains uncertain. The human body louse is the only proposed vector for *B. quintana*, although the intimacy of the relationship between bacterium and arthropod is not known. Humans remain the only proven animal host for the bacterium in vivo, and human infection is characterized by diverse manifestations including BA, bacteremia, endocarditis, and chronic lymphadenopathy (30, 58, 76, 110, 135, 137). The typical clinical manifestations of *B. quintana* have not been reproduced in laboratory animals, apart from a report of an induced bacteremia in a rhesus monkey (87).

The species *B. vinsonii* is represented by only a single isolate, obtained from a *Microtus pennsylvanicus* vole trapped on Grosse Island in Quebec, Canada (8). The species has never been associated with human disease. It is reported that *B.*
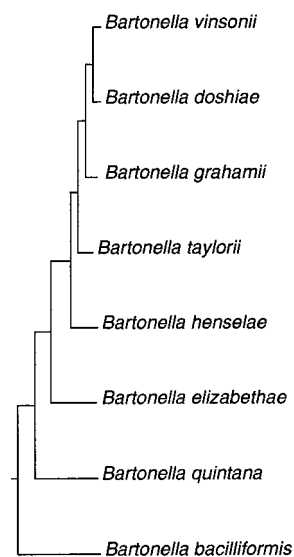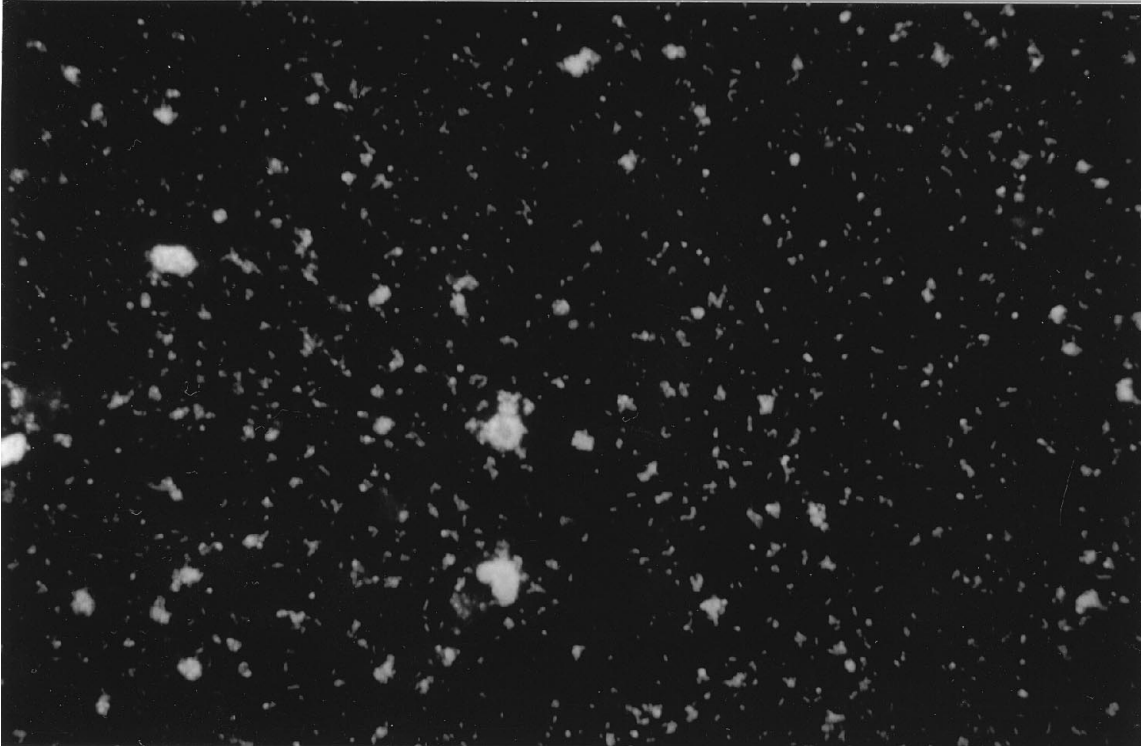
FIG. 3. *B. quintana* revealed by the direct immunofluorescence assay with mouse anti-*B. quintana* antibodies. Magnification, ×400.

vinsonii is not as dependent on the presence of carbon dioxide for growth as is *B. quintana* (163).

The species *B. henselae* was first described (as *R. henselae*) by Regnery et al. (112) in 1992. The microbiological characteristics of this species are very similar to those of *B. quintana*. As with *B. quintana*, *B. henselae* has been associated with multiple clinical manifestations. In immunocompromised patients (58, 107, 112, 116, 118, 131, 133, 165), the infection presents as BA, PH, or bacteremia, and in immunocompetent persons (40, 68, 145), it presents as BA, meningitis, bacteremia, or endocarditis. Importantly, *B. henselae* is also considered the main etiological agent of CSD (29, 113, 170).

FIG. 4. Scanning electron micrograph of *B. quintana* colonies grown on blood agar after 5 days of incubation.

B. elizabethae, like *B. vinsonii*, is represented by only a single isolate, obtained from a patient with endocarditis. The species was first described in 1992 by Daly et al. (28), who based their proposal on DNA homology and 16S rRNA gene sequen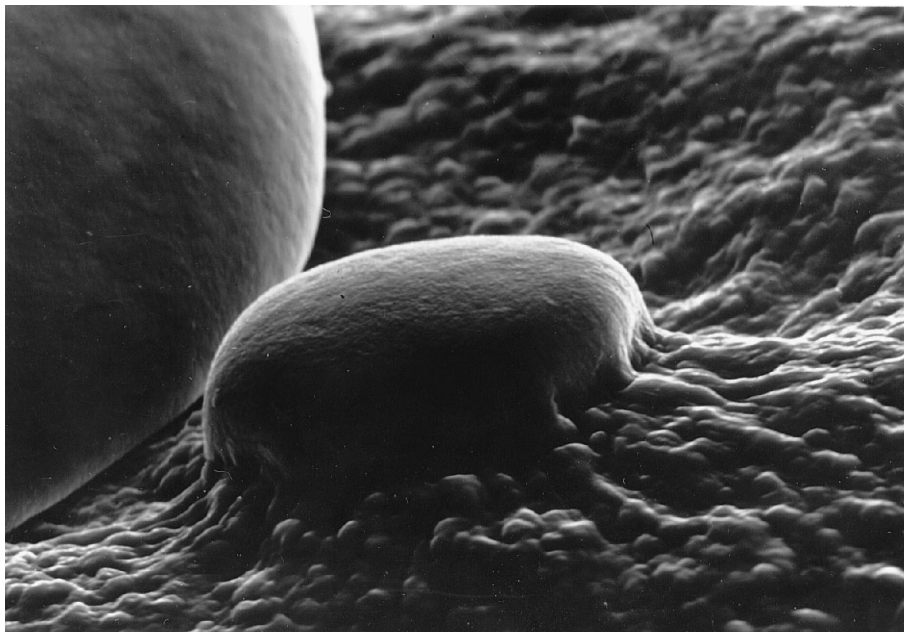ce comparison of their new isolate with the established *Rochalimaea* species. Phenotypically, *B. elizabethae* most closely resembles *B. henselae* but can be differentiated from this species because it gives only incomplete hemolysis on rabbit blood agar.

B. bacilliformis was the earliest member of this group of organisms to be described (120). In 1907, the Peruvian scientist Alberto Barton recognized what he considered to be infectious particles in the erythrocytes of Carrion's disease patients. The disease itself has been recognized since pre-Columbian times, involving the native population of, and visitors to, the Peruvian and Ecuadorian Andean region of South America. Carrion's disease is unusual in that it has two distinct clinical manifestations: a progressive, severe febrile anemia (termed Oroya fever) usually precedes the development of angioprolific cutaneous lesions (termed verruga peruana), which may persist for several months. Humans are the only proven reservoir for the disease, which is thought to be transmitted between hosts by arthropod vectors, including *Lutzomyia* species, the vectors of *Leishmania* in the region. As with the diseases caused by other *Bartonella* species, Carrion's disease has not been successfully reproduced in laboratory animals, although verruga-like lesions were induced in monkeys (120). Microscopically, *B. bacilliformis* organisms appear as small, pleomorphic, often curved bacteria. Unlike other *Bartonella* species in vitro, it possesses unipolar flagella (108), although their presence on bacteria in infected tissue has not been observed. *B. bacilliformis* is usually cultivated at 28°C on media supplemented with blood from either rabbits, sheep, horses, or humans. The organism has also been grown in the chorioallantoic fluid or yolk sac of chicken embryos.

The two former *Grahamella* species, *B. talpae* and *B. peromysci*, are defined in *Bergey's Manual* as gram-negative rods, sometimes curved, which resemble *B. bacilliformis* but do not possess flagella (120). Like *B. bacilliformis*, they parasitize erythrocytes and thus require hemoglobin for growth in vitro. Both species were isolated from small mammals; *B. talpae*, the type species, was isolated from the mole, and *B. peromysci* was isolated from the deer mouse. Neither species has ever been associated with human disease. However, no isolates of either of these species remain extant, and their validity has been questioned.

Birtles et al. (13) have recently described three new *Bartonella* species, namely, *B. grahamii*, *B. taylorii*, and *B. doshiae*. All three species have the growth and morphological characteristics typical of the *Bartonella* genus. The species were isolated from a number of different species of small mammals, primarily voles of the genera *Clethrionomys* and *Microtus*, and mice of the genus *Apodemus*. None of these three species has yet been implicated in infections in humans; however, since they are able to infect different species of small mammals, the possibility of human susceptibility cannot be ruled out.

The spectrum of clinical manifestations induced by *Bartonella* infections is startling. For example, although *B. quintana* infection typically presents as cutaneous lesions in BA, the symptoms of trench fever seldom involve the skin. Similarly, whereas trench fever appears to be a superficial, self-limiting illness, *B. quintana* can also cause life-threatening endocarditis. One possible explanation is that different diseases result from infection by different "subspecies" within each *Bartonella* species, perhaps with different subspecies exhibiting different virulence factors and pathogenicity. Studies have demonstrated

intraspecies differences within a number of isolates of *B. henselae* and *B. quintana*. Recently, Roux and Raoult (121, 122) obtained the nucleotide base sequences of the intergenic spacer regions of seven *B. quintana* isolates, four *B. henselae* isolates, *B. vinsonii*, and *B. elizabethae*. Each of the four *B. henselae* isolates had a unique base sequence, whereas only two distinct sequences were found amongst the *B. quintana* isolates. Restriction fragment length polymorphism (RFLP) analysis of PCR amplicons derived from intergenic spacer regions allowed differentiation of *Bartonella* isolates at the species level. Roux and Raoult (122) also studied genotypic heterogeneity among the same group of isolates by pulsed-field gel electrophoresis (PFGE) analysis. Specific profiles were obtained for each of the four *Bartonella* species studied. By using a number of different restriction enzymes (*Eag*I and *Sma*I), PFGE analysis also discriminated between strains of the same species, although intraspecies differences were small. However, no correlation was found between the PFGE profile groups into which the *B. quintana* strains fell and either their geographical distribution or their clinical manifestation. Interestingly, a recent *B. quintana* isolate from a French HIV-positive patient with BA was shown to be very closely related to the type strain of the species that was isolated from a trench fever patient in Yugoslavia almost 50 years ago (76).

EPIDEMIOLOGY

Humans are the only host in which infection by *Bartonella* species leads to profound disease. Attempts to experimentally induce disease in laboratory animals through inoculation with either *B. quintana* (87) or *B. bacilliformis* (100, 101) have succeeded only when primates were used. It is unlikely that *Bartonella* infections are directly transmitted, and arthropod vectors have often been implicated. Sand flies of the genus *Lutzomyia* are likely vectors of *B. bacilliformis* infections (43), whereas the human body louse (*Pediculus humanis*) has been associated with the transmission of *B. quintana* infections (18, 30, 60, 137). Fleas have been proposed as the vectors of the former *Grahamella* species, and infection by these species has been induced in laboratory animals following inoculation with preparations of crushed fleas collected from infected hosts. The presence of *B. henselae* in cats and cat fleas (*Ctenocephalides felis*) has also been demonstrated (20, 52, 53, 111), and one epidemiological survey has indicated that the ownership of kittens with fleas is the common predisposing factor in cases of CSD, the agent of which is *B. henselae* (113). Epidemiological factors supposedly associated with *Bartonella* infections are summarized in Table 1. Not all of this table is based on true epidemiologic data.

Arthropod Vectors

Most human diseases caused by members of the order *Rickettsiales* are arthropod borne. Within the family *Bartonellaceae*, *B. bacilliformis* and *B. quintana* are transmitted by the sand fly and louse, respectively. The life cycles of other members of the order involve a wide range of vector species. Ticks are the vectors for most of the spotted-fever group of *Rickettsia* species, whereas lice are responsible for the transmission of *R. prowazekii*. Fleas are the vectors of *R. typhi*, and mites are the vectors of scrub typhus.

Role of lice as vectors of *B. quintana*. Soon after the first cases of trench fever were recognized in France in 1915, medical officers realized that the disease occurred primarily when large numbers of people lived together in cramped, unhygienic circumstances. The role of lice in the transmission of human

TABLE 1. Epidemiological factors supposedly associated with *Bartonella* infections

Species	Disease[a]	Vector	Cat contact	Home-less	Alco-holic	HIV
B. quintana	TF	Body louse		+	+	
	BA	Body louse	+	+		+
	BACT	Body louse	+	+	+	
	ENDO	Body louse	+	+	+	+
	LA		+			
	BA		+	+		+
B. henselae	PH		+	+		+
	BACT		+			+
	CSD		+			
B. bacilliformis	CrD	Sand flies				

[a] TF, trench fever; BACT, bacteremia; ENDO, endocarditis; LA, chronic lymphadenopathy; CrD, Carrion's disease.

disease had already been demonstrated prior to World War I. In 1909, Nicolle et al. (98, 99) observed that patients with typhus were no longer infective to other patients if their clothing was removed and thoroughly washed. From this observation, they proposed a role for the body louse, which was found in the patients' clothes, in the transmission of typhus. The authors subsequently infected macaque monkeys with blood from a typhus patient and demonstrated transmission of the disease between monkeys by means of body lice. Observation of the heavy infestation of the uniforms of World War I troops with the same body louse led to the proposal of its involvement in trench fever as well as typhus (60). It was demonstrated that the lice tended to migrate away from fevered hosts, thus spreading the infection to people in close contact with the ill soldier. The spread of lice between individuals was encouraged by the cramped living conditions in the trenches, and the dispersion of lice was especially enhanced during the winter, when the cold weather necessitated the wearing of multiple layers of underclothes for long periods and kept people indoors and in especially close contact. Washing and delousing procedures were introduced as methods of effective transmission control.

Both the body louse and the head louse are capable of experimental transmission of trench fever, although the role of the body louse is more firmly established. Lice infected with *B. quintana* appear to remain so until they die, although their life span apparently does not decrease. Since Rocha Lima (cited in reference 60) described the extracellular position of the bacterium in the lumen of the louse intestine, it seems likely that the relationship between *B. quintana* and the louse is one of commensal and host in which the bacterium neither invades the host tissues nor causes it any serious detriment. The association between *B. quintana* and the human host is less well understood; although the bacteria are usually present in a patient's blood only during the febrile stages of trench fever, infection may persist long after the disappearance of all clinical signs of the disease. Importantly, the possibility of a persistent bacteremia would facilitate the spread of trench fever by an arthropod vector (19, 60, 85, 86, 152, 153, 166).

Strong et al. (141) demonstrated the presence of *B. quintana* in blood plasma, although clear serum, produced by centrifugation of coagulated blood, contained no organisms. Infecting bacteria were also frequently found in the urine of trench fever patients, although never in feces.

The belief that trench fever was solely a wartime disease, associated only with "campaign conditions," was disproved by

the recognition of postwar cases of the disease. As described by Kostrzewski (60), cases were presented by Pena-Yanez in Spain, Laurell in Sweden, Braslawski in Kiew, Swinkina in Leningrad, and Juszancew in Tiflis. In Poland, Mosing described a laboratory outbreak of disease caused by a new rickettsia-like organism under the name *Rickettsiaemia weigli* (88); the organism was later shown to be *B. quintana* (60). A description of a similar outbreak was given by Herzig in 1939 (cited in reference 60), and Sparrow (138) reported the presence of *B. quintana* in lice that fed on infected patient volunteers in North Africa. Parrot (105) diagnosed a case of trench fever in Algeria in 1945, and the disease was also reported in Egypt (6) and in Addis Ababa (84). Trench fever has also been recognized in East Asia, with cases being reported in Japan (103) and China (44). The most recent report of trench fever describes cases in Mexico City (148, 149).

Despite these occasional accounts, trench fever is widely considered a disease of the past, a syndrome whose medical importance disappeared with the abandonment of the military tactics from which it took its name. However, recent reports have indicated a reemergence of *B. quintana* infections, and although the organism is no longer as big a threat to the health of servicemen, its association with disease among homeless people in the modern cities of industrialized nations has once again made it an organism of medical significance (115, 139). Such manifestations of *B. quintana* infection have been reported in both Europe and the United States. For example, a 48-year-old louse-infested homeless patient was hospitalized in Marseille, France (139), with a history of relapsing fever, headaches, and pain in the leg bones. The patient presented with stupor, fever, and dehydration, all of which resolved after rehydration therapy. Later, the patient suffered a relapse and was readmitted to hospital. Laboratory data demonstrated a massive hyperleukocytosis, and *B. quintana* infection was diagnosed serologically. The patient was successfully treated with a 4-week course of doxycycline.

B. quintana bacteremia (137) has also recently been reported among homeless patients in Seattle. Infection was characterized by relapsing fever, although headache and bone pain were not reported. The major predisposing factors for these *B. quintana* infections included poor living conditions and chronic alcoholism. These risk factors are also common to HIV-positive patients who develop BA (76), suggesting that *B. quintana* bacteremia may also be arthropod transmitted.

The squalid, unhealthy, and stressful living conditions experienced by homeless people make them susceptible to a range of diseases not usually associated with the inhabitants of industrialized nations. The increase in the homeless population has already led to a striking rise in the incidence of tuberculosis in Europe and North America; the likelihood of a significant rise in the number of cases of *B. quintana* infection must also be expected.

Potential role for ticks as vectors of *Bartonella* infections. Various species of ticks have been implicated in the transmission of rickettsial diseases, and tick bites have been associated with *Bartonella* infections (68). However, at present, there is no evidence that *Bartonella* species infect ticks; clearly, more epidemiological data are required to validate or negate these implications.

Role of cat fleas in transmission of bacillary angiomatosis. Cat fleas (*Ctenocephalides felis*) have been proposed as vectors of *Bartonella* species (56, 145, 146). Although Tappero et al. (144) reported finding no association between insect bites and BA or PH, Koehler et al. (56) used PCR to demonstrate the presence of *B. henselae* in the tissues of cat fleas obtained from

a cat owned by a BA patient. The presence of *B. quintana* in cat fleas has not, as yet, been demonstrated.

The Domestic Cat as a Potential Reservoir for *Bartonella* Species

The domestic cat has been proposed as a potential vector and reservoir of *Bartonella* species, infecting humans either directly through scratches, bites, or licks or indirectly via an arthropod vector. A role for the cat in the life cycle of *Bartonella* species was proposed following the discovery of *B. henselae* as the etiologic agent of CSD. Regnery et al. (113) were the first to demonstrate anti-*B. henselae* antibodies in the serum of patients with CSD, and subsequently Dolan et al. (29) cultured *B. henselae* from infected lymph nodes. DNA derived from *B. henselae* has also been amplified from the tissues of infected patients by PCR (2, 106). Involvement of the cat in the life cycle of *B. henselae* was confirmed when the organism was isolated from the blood of almost half the cats tested. In a recent survey carried out by Regnery et al. (111) in San Francisco, most of the cats from which *B. henselae* was isolated appeared healthy, although infection was associated with illness in some animals. Other *B. henselae*-related disorders, including BA (49, 58, 81, 118, 144, 145) and PH (66, 144), are also thought to be zoonoses, acquired from cats.

Cat contact or cat scratches were reported in *B. quintana*-infected patients (30, 58, 110, 135, 137). As yet, no animal reservoir has been demonstrated for *B. quintana*, although seven of the *Bartonella* species have been isolated from apparently healthy animal reservoirs. Additionally, two of the three *Bartonella* species newly described by Birtles et al. (13) were also shown not to be host specific, being isolated from at least two different species of small mammals. If cats are implicated in the life cycle of *B. quintana*, these pets may be the source not only for CSD and BA but also for other syndromes including bacteremia, endocarditis, and chronic lymphadenopathy.

CLINICAL MANIFESTATIONS

The clinical manifestations of *Bartonella* infections are summarized in Table 2.

Trench Fever

Trench fever, also known as 5-day fever, quintana fever, or Wolhynia fever, is defined as infection of human blood by *B. quintana*. The disease is communicable by means of *Pediculus humanus*, the human body louse. Infection is thought to be conveyed from lice to humans via the arthropod excreta, which enters the body through broken skin. We have summarized in the following section the various clinical aspects of trench fever described by Byam et al. (18).

The incubation period for trench fever is between 15 and 25 days. This incubation period was reduced to less than 9 days when infection was experimentally induced in volunteers following their inoculation with a large volume of a preparation of crushed infected lice. Reduction in the volume of the preparation inoculated resulted in a longer incubation time of 16 days for the disease.

Clinical manifestations of trench fever may range from asymptomatic infection to severe, life-threatening illness. "Classical" trench fever, the presentation most often reported among troops, corresponds to a febrile illness of acute onset and of a periodic nature often accompanied by severe headache and pain in the long bones of the legs.

However, the sudden development of a wide range of symptoms can indicate the onset of trench fever. Such symptoms

TABLE 2. Clinical characteristics of *Bartonella* infections

Characteristic	Presence in[a]:				
	TF	BEA	BACT	ENDO	LA
Clinical samples for diagnosis					
Blood	+	+	+	+	+
Skin		+			
Bone marrow		+			+
Lymph node		+			+
Cardiac vegetation				+	
Leukocyte count					
Leukocytosis	+ (Major [PMN])				
Leukopenia		+		+	+
Normal		+	+	+	
Pathologic changes					
Capillary proliferation		+			
Granulomatous reaction					+
Extensive valve destruction				+	
Presence of *Bartonella* antibodies					
Non-HIV patients	+	±	±	+++	−
HIV patients		−	−		
Prognosis					
Resolution	+				
Relapses	+	+	+	+	+
Possibly fatal		+	+	+	

[a] TF, trench fever; BEA, bacillary epithelioid angiomatosis; BACT, bacteremia; ENDO, endocarditis; LA, chronic lymphadenopathy; PMN, primarily neutrophils.

include headache, weakness, pain in the legs, malaise, dyspnea, giddiness, pain in the loins, shivering, abdominal pain, diarrhea, constipation, anorexia, nausea, frequent micturition, restlessness, and insomnia. The prodromal period may last for 2 days or more. The severity of symptoms increases gradually over the first few days of disease. Headache is most often severe, especially at the front of the head and behind the eyes. When occipital, it is often accompanied by a stiffness of the neck, and symptoms may therefore suggest meningitis. Pain may spread to the back and limbs, with leg pain being the most severe. This pain is often felt in the bones, specifically in the tibia. The patient will suffer regular cycles of profuse sweating and then shivering. On examination, the tongue is often slightly furred, and conjunctival congestion and a decrease in the pulse rate in relation to the severity of the fever may be present. Areas of tenderness are associated with the pains involving muscles, tendons, bones, and joints. The spleen often becomes palpable.

The pyrexia associated with trench fever is often periodic, although the cycles may be of irregular duration. The level of the pyrexia is also variable, and there may be a relationship between the degree of pyrexia and its duration. The interval between attacks of pyrexia is usually between 4 and 8 days, with 5 days being the most commonly observed period. The term "quintan fever" refers to the 5-day recurrences. Usually each succeeding attack is less severe than its predecessor, although in profound cases the patient becomes weaker and leg pains become more persistent.

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 continues for 4 to 6 weeks. A minority of illnesses will become chronic, during which time the following signs and symptoms may develop: exhaustion, headaches, recurrent limb pains, irritability, nervous manifestations such as depression, abnormal response to stimuli, tendency to sweat, coldness of extremities, fever, anemia, and loss of weight. The chronically ill patient often also complains of breathlessness on exertion, palpitations, pain over the precordium, giddiness, and disordered activity of the heart. Damp weather exacerbates all pain. In some cases, the infection is very persistent and acute febrile lapses occur months after quiescence. Byam et al. (18) defined chronic trench fever as "a state of marked debility, with or without attacks of slight fever and aching, and characterized by a hyperexcitability of the nervous system in general."

Bacillary Angiomatosis

BA and epithelioid angiomatosis are the terms given to a vascular proliferative disease most often involving the skin and first described in HIV-infected patients (23, 140) and organ transplant recipients (49, 131). More recently, BA has also been described in immunocompetent patients (21). Both *B. quintana* and *B. henselae* are considered etiological agents of BA (58, 118, 134). BA may be accompanied by disseminated visceral disease in both immunocompromised (49, 57, 81) and immunocompetent (21) patients. Several organs may be involved, including the liver, spleen, bone marrow, and lymph nodes. Although patients with BA usually respond to an antibiotic regimen, it may be life threatening if untreated.

BA most often involves infection of the skin (22, 59, 134, 157). Cutaneous lesions may be solitary or multiple; they may be 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. Primary lesions correspond to papules, which gradually increase in size to form nodules. Oral, anal, conjunctival, and gastrointestinal mucosal surfaces may be involved. When multiple, the distribution of cutaneous lesions may be widespread. A characteristic feature is that skin lesions may bleed profusely when punctured. Deep lesions often involve underlying bone, varying from simple cortical erosions to extensive geodes (54, 57). Leukopenia and $CD4^+$ cell counts less than $100/mm^3$ were most often reported in HIV-infected patients with BA (14, 59).

The clinical differential diagnosis of BA includes pyogenic granuloma, hemangioma, various subcutaneous tumors, and Kaposi's sarcoma (22, 59, 134, 157). Demonstration of the presence of bacteria in histologic skin biopsy specimens may allow distinction of BA from these other conditions. Distinction of BA from *B. bacilliformis*-induced verruga peruana is likely to be very difficult, although the latter disease is limited to the Andean valleys of Peru and Ecuador (4). BA has yet to be reported in South America.

As outlined above, cutaneous BA may be accompanied by involvement of bone marrow, spleen, liver, or lymph nodes (23, 49, 54, 57, 63, 69, 81). The presence of skin lesions and enlarged lymph nodes in BA patients has been extensively reported (49, 58, 69, 81, 107, 118, 125). In most patients, enlarged lymph nodes corresponded to the regional lymphatic drainage of skin lesions. Axillary or epitrochlear lymphadenopathies have been noted in patients with unilateral cutaneous lesions of the elbow (49, 118) or hand (81, 125), and inguinal or femoral lymphadenopathies have been reported in patients with BA lesions of the legs (58, 69). Abdominal nodes, together with hepatic and splenic enlargement, were found in one patient suffering from BA and Kaposi's sarcoma (107). Involvement of the skin does not always occur in BA; patients in whom only the spleen, liver, or lymph nodes were affected have been described (49, 63, 107, 130).

In HIV-infected patients, BA may be associated with PH (27, 39, 107, 128), a syndrome characterized by cystic, blood-filled spaces in hepatic parenchyma. Prior to its association with BA, PH was reported in patients suffering from wasting diseases such as tuberculosis and advanced cancers (42, 50, 168, 169) and in association with the use of drugs such as anabolic steroids (7, 38, 96, 97). Histologic examination of hematoxylin-and-eosin-stained liver sections from patients with BA associated with PH reveals dilated, blood-filled spaces in the fibromyxoid stroma containing inflammatory cells, dilated capillaries, and clumps of granular purple material. As observed in BA skin lesions, this granular material is revealed as masses of bacteria when stained with Warthin-Starry stain. Because lesions with similar histologic appearance have also been reported in the spleen of HIV-infected patients with BA (49, 54, 145, 165), perhaps the less specific term "parenchymal peliosis" is more suitable, allowing reference to PH-like symptoms in other organs of the body. To date, *B. henselae* remains the only confirmed agent of the syndrome (107, 133, 165).

Bacteremia

Persistent bacteremia has long been a recognized symptom of *B. quintana* infection, being reported among a number of trench fever patients during the World Wars (19, 60, 85, 86, 166). More recently, both *B. quintana* and *B. henselae* have been demonstrated to cause bacteremia in both immunocompromised and immunocompetent patients.

In 1990, Slater et al. (131) were the first group to report the reemergence of *Bartonella*-associated bacteremia; they obtained isolates of the previously unrecognized species *B. henselae* 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. More widespread adoption of the blood culture procedures described in this study has led to an increasing number of isolates of *Bartonella* species being obtained over the last 5 years. Bacteremia due to *B. quintana* has also now been reported in patients with BA (76) and endocarditis (30, 135).

Many of the recent patients with *B. quintana* infections are from deprived backgrounds, usually being homeless; the poor underlying state of health of these people and the unhygienic conditions in which they live are clearly comparable to the lifestyles of trench fever sufferers. In the most complete study to date, 10 cases of *B. quintana* bacteremia in Seattle were presented and discussed by Spach et al. (137). Of the 10 patients, 8 were homeless. None had risk factors for HIV infection, and all six of those tested had no detectable anti-HIV antibodies. Three patients reported previous cat scratches, five were parasitized by scabies, and one was louse infested. No consistent symptoms were recorded other than fever and weight loss. Two patients had splenomegaly with a mean leukocyte count of $9,600/mm^3$. Thirty-four blood cultures collected from the 10 patients yielded *B. quintana*. The bacteremia persisted for over 10 days in four of the patients and for over 8 weeks in one who refused antibiotic therapy. Only 1 of
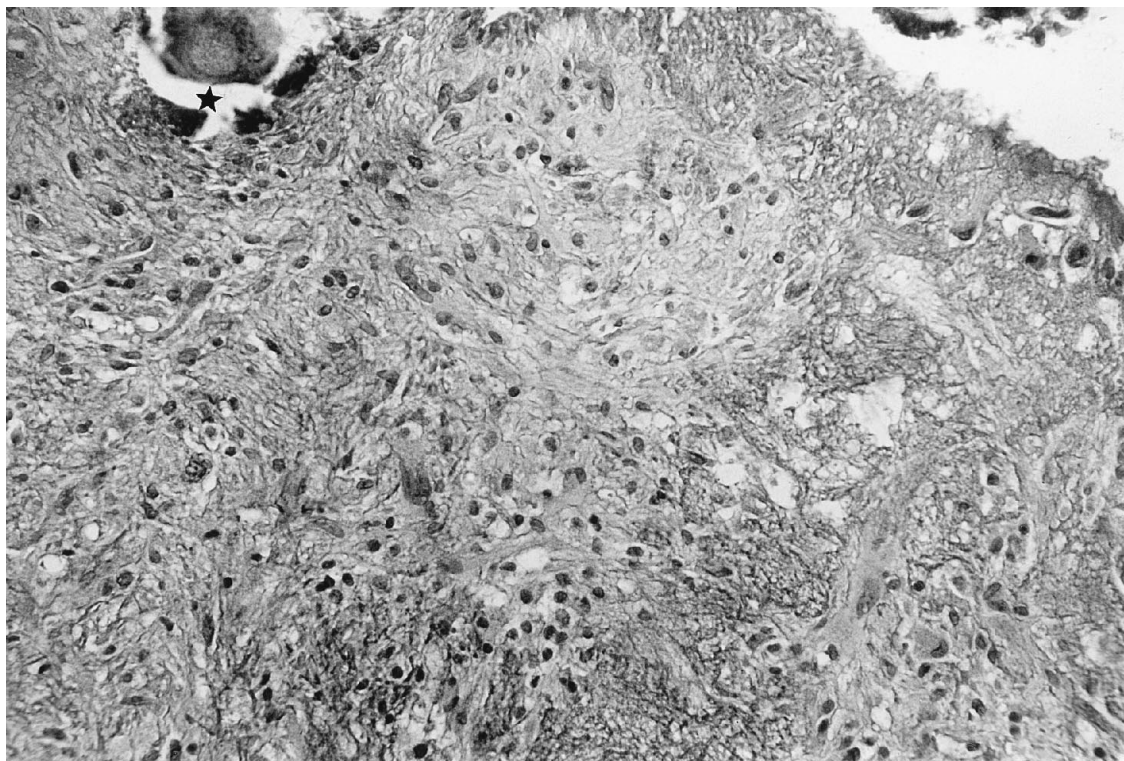
FIG. 5. Section of an aortic valve from a patient with *B. quintana* endocarditis. Notice the vegetation and focal calcifications (★) on the valve surface. There is extensive destruction of valve tissue, which is diffusely infiltrated with mononuclear inflammatory cells. A few neutrophils are also present. The valve stroma is fibric and endothelialized. Hematoxylin-phloxin-saffron stain; magnification, ×250.

the 10 patients died, presumably from a concurrent *Streptococcus pneumoniae* bacteremia.

Endocarditis

B. quintana (30, 135, 136), *B. henselae* (40, 68), and *B. elizabethae* (28) have recently been isolated from patients suffering from bacterial endocarditis, with only *B. quintana*-induced endocarditis being reported on more than one occasion in both the United States and Europe. Spach et al. (135) were the first to report the syndrome, affecting an HIV-infected homosexual man who presented with a swollen erythematous left palm and a holosystolic murmur. An echocardiogram showed the presence of an echogenic mass on the aortic valve. *B. quintana* was grown from blood cultures after 28 to 42 days of incubation. An epidemiologic investigation found that the man owned flea-infested cats that frequently scratched him.

More recently, *B. quintana* endocarditis was reported in three non-HIV-infected men in France (30). Congestive heart failure with cardiac murmur was noted when all three patients were examined. Leukocytosis occurred in one patient, thrombopenia was present in two, and anemia and elevated erythrocyte sedimentation rate were present in all three. Echocardiography revealed the presence of an echogenic mass on the mitral valve of one patient, on the aortic valve of the second patient, and on both valves of the third patient; all patients required valve replacements. Pathologic investigation revealed extensive destruction of the heart valves and confirmed the diagnosis of endocarditis in all cases (Fig. 5). Blood samples from all three patients were inoculated onto blood agar and into endothelial cell culture lines. Blood agar culture yielded *B. quintana* from one patient, whereas cell culture yielded *B.*

quintana from the other two. Antibody estimations were attempted on sera from all three patients by using antigen derived from both plate-grown and cell culture-grown *B. quintana*. Antisera at dilutions of 400 to 800 reacted with plate-grown antigen, whereas dilutions as high as 6,400 and 12,800 were reactive when cell cultured antigens were used. The presence of *B. quintana* was also demonstrated in the removed cardiac valves by using both immunofluorescence and PCR-based RFLP techniques on citrate synthase genes. Surprisingly, *B. quintana* could not be cultivated from any of the removed valves, although extensive antibiotic therapy of patients prior to the removal of their heart valves may explain this failure. Of the 10 *B. quintana* bacteremic patients described by Spach et al. (137), 1 also possessed an aortic valve vegetation. *B. quintana* was detected by PCR-based methods in the valve following its surgical removal.

B. quintana infection must now be suspected when diagnosing patients with endocarditis, especially if regular blood cultures remain sterile despite the absence of antibiotic therapy and if antibody assays for *C. burnetii*, *Brucella* species, or *Francisella tularensis* are negative. In such cases, strategies for the isolation of *B. quintana*, bearing in mind the highly fastidious nature of the organism, must be considered. Assays for the detection of specific anti-*B. quintana* antibodies should become part of routine microbiological investigations of endocarditis patients. It was necessary for all the patients identified in the study by Drancourt et al. (30) to undergo heart valve replacement; such surgery may be avoided in the future if clinicians and clinical scientists can be made more aware of the potential role of *B. quintana* in endocarditis.

The more widespread use of assays for the estimation of *Bartonella*-specific antibodies has led to problems with the tests

being used. Significant cross-reactions between the sera of patients with culture-proven *B. quintana* and *Chlamydia* antigens have been reported by Drancourt et al. (30); immunoglobulin G (IgG) titers of 256 were found against *Chlamydia pneumoniae* antigens, and titers of ≥64 were found against *Chlamydia psittaci* and *Chlamydia trachomatis* antigens. When serum samples were absorbed with *B. quintana* antigen, all activity against *Chlamydia* antigens was eliminated. Western blotting (immunoblotting) also indicated significant cross-reactivity between the organisms. More recently, experiments performed in our laboratory have shown that most of the cases of chlamydial endocarditis previously diagnosed by Etienne et al. (33) may, in fact, have been due to *Bartonella* spp. (75). In fact, cross-reactions between *Bartonella* and *Chlamydia* species have been reported previously (32, 55). This cross-reactivity is clearly a problem when diagnosing infective endocarditis, because both *B. quintana* and *Chlamydia* species are recognized agents of the syndrome (33, 129).

Chronic Lymphadenopathy

B. quintana was isolated from a non-HIV-infected, 30-year-old woman with afebrile chronic cervical and mediastinal adenopathy (110). The patient had first noted the cervical adenopathy in 1991 but did not seek medical advice until 1993, when the involved node became enlarged. Further examination by computed tomography also demonstrated the presence of mediastinal lymphadenopathy. The patient's leukocyte count was normal, but lymphopenia was noted. The enlarged node was removed, and samples of blood and bone marrow were also collected. Histologic examination of both the node and the bone marrow showed a granulomatous reaction. Regular cultures of blood samples and the lymph node remained sterile; however, two blood specimens inoculated into cell culture systems, including human embryonic lung cell and human endothelial cell lines, yielded bacterial growth after 10 weeks of incubation. The bacteria were subcultured onto Columbia blood agar, and the subsequent colonies were identified as *B. quintana* by serological methods. Comparison of the base sequence of the 16S rRNA of the bacterium with that of another *B. quintana* strain demonstrated that they were identical. The isolate could, however, be distinguished from other *B. quintana* strains by the PCR-based RFLP technique on the citrate synthase gene. *B. quintana* could not be isolated from the patient's lymph node, and no *B. quintana*-specific antibodies could be detected in the patient's serum.

More recently, *B. quintana* was isolated in our laboratory from a 37-year-old woman with Goujerot-Sjögren disease and receiving low doses of steroids. She had been undergoing renal dialysis since early 1994 and became ill in February 1994, presenting with fever, dry cough, and weakness. A thoracic CT scan revealed the presence of mediastinal lymphadenopathy. The patient's condition worsened despite establishment of an antituberculous regimen. The combination of undiagnosed lymphadenopathy and a history of cat contact led to the suspicion of *Bartonella* infection. Histologic examination of the removed mediastinal lymph node showed that it was comparable to lymph nodes in patients with CSD. *B. quintana* was grown from a bone marrow biopsy specimen but not from the lymph node. Cultures for *Mycobacterium tuberculosis* remained sterile. The patient's treatment regimen was altered to include an increased level of steroids and gentamicin, which resulted in her dramatic recovery.

PATHOPHYSIOLOGY

Microscopic observation of the lesions characteristic of *B. quintana*- or *B. henselae*-induced BA reveals tumor-like capillary lobules (64). These lobules are rounded aggregates of capillaries and proliferate rapidly (22, 64). The proliferating endothelial cells, which often demonstrate nuclear atypia, may protrude into or even occlude the vascular lumina. Similar tumor-like cell proliferation is observed in PH, which is characterized by blood-filled spaces and vascular proliferation (107). *B. bacilliformis* also induces cutaneous lesions, referred to as verruga peruana, in which histologic findings are similar to those of BA (4, 5).

Interestingly, *Agrobacterium* species, which are known to share a close evolutionary link with *Bartonella* species (102), are also associated with tumor induction but in plants rather than animals. *Agrobacterium* species induce cortical hypertrophy of the roots of many species of plants, and molecular studies have shown that the tumor-forming ability of these organisms is related to the transfer and integration of bacterial DNA into the infected plant cell genome (51, 61, 156, 167). The integrated DNA then induces tumor formation by stimulating the overproduction of plant growth hormone.

The interactions between members of the genus *Bartonella* and eukaryotic cells have been known about and investigated for a long time. The ability of *B. bacilliformis* to invade cells was first demonstrated by Pinkerton and Weinman (109), using mesenchymal cells from the tunica vaginalis of guinea pigs. More recently, Benson at al. (9) investigated the interaction between *B. bacilliformis* and erythrocytes, demonstrating that the binding of bacteria to the erythrocyte surface led to the formation of indentations and deformation of the cellular membrane and, ultimately, entry into the cell within large vacuoles. Other workers have demonstrated that *B. bacilliformis* will 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, 77). As discussed above, the specific interaction between *B. bacilliformis* and endothelial cells forms the basis of lesion formation in verruga peruana. The interactions between other species of *Bartonella* and eukaryotic cells have not yet been investigated in detail, although the improved recovery of *B. henselae* and *B. quintana* from clinical material by cell lysis procedures or cell culture systems (30, 58, 68, 110) intimate in vivo relationships. Recent work in our laboratory corroborates these findings; experiments suggest that *B. quintana* is phagocytosed by endothelial cells in vitro and exists intracellularly in vacuoles (16) (Fig. 6).

The association of *Bartonella* species with neovascularization and the regression of lesions when antimicrobial agents are administered strongly suggest that the microorganisms themselves stimulate the angiogenesis seen in the lesions of BA (22, 64) and verruga peruana (4, 5). Work has already demonstrated that proliferation of endothelial cells is induced in vitro in the presence of either intact or homogenized *B. bacilliformis* cells (36). Furthermore, a *B. bacilliformis* protein effective in endothelial cell proliferation in vitro and in proliferation of new blood vessels in vivo has been identified and partially characterized (35). Similarly, an extracellular product of *B. bacilliformis*, termed deformation factor, has been shown to induce excess indentations and trenches in erythrocyte membranes (79).

Koehler et al. (58) recently reported that when *B. quintana* or *B. henselae* is 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
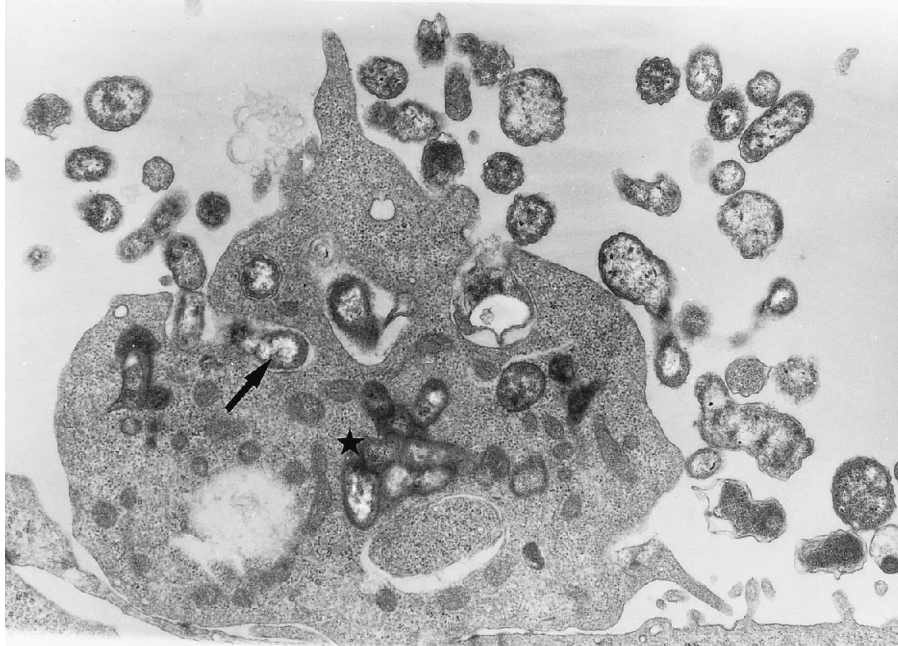
FIG. 6. Transmission electron micrograph of endothelial cells (human endothelial cell line ECV 304,9) infected with *B. quintana*. Phagocytosed bacteria are seen within vacuoles (arrow). Clumps of bacteria are also visible (★).

seen in endothelial cells exposed to angiogenic factors (34). In vitro cocultivation of *B. henselae* with human umbilical vein endothelial cells (HUVEC) is also reported to enhance proliferation of the HUVEC (24). Similar results were found in our laboratory when either *B. quintana* or *B. henselae* was cocultivated with HUVEC and when the proliferating HUVEC were monitored by quantitative microscopy (104). Cell growth was clearly enhanced in infected cultures when compared with uninfected cell cultures, and infected cells became larger and more spindle shaped. Topographical analysis of HUVEC revealed that infected cells were more "ordered" than uninfected controls. As this in vitro model reproduced some of the histologic findings associated with BA, the authors suggest that *B. quintana* may induce neovascularization in BA lesions through the secretion of angiogenic factors, as previously proposed for *B. bacilliformis* (104).

An extracellular round to icosahedral particle with a diameter of 40 nm has been detected in the supernatant collected from cultures of *B. henselae* (1). The particle contained a 14-kb linear DNA segment, which was also found within *B. henselae* cells as an extrachromosomal element. A similar 14-kb DNA element has also been found in *B. bacilliformis* cells and may correspond to a bacteriophage-like particle previously observed in *B. bacilliformis* by electron microscopy (147). The role of this particle in the pathophysiology of *Bartonella* infections remains unknown, and its presence has not yet been demonstrated in *B. quintana*.

Although some pathophysiological mechanisms for the induction of BA lesions by *B. quintana* have been proposed, angioprolific lesions have not been reported to occur either in trench fever patients (despite their remaining untreated and closely observed for long periods) or in modern-day patients with *B. quintana*-induced endocarditis or persistent bacteremia. Indeed, the increasing spectrum of *B. quintana*-associated clinical manifestations is striking; why, for example, has *B. quintana*-induced endocarditis only recently been demonstrated? Because there have been no reports of increased in-

cidence of endocarditis in World War I troops, despite the vast numbers who developed trench fever, perhaps this is indeed a new manifestation of *B. quintana* infection, possibly resulting from changes in the virulence of the species. Evolutionary divergence within the species may also explain the range of *B. quintana* disease manifestations observed today, although attempts to demonstrate genetic variation between isolates have so far failed to identify a correlation between a specific genotype and a specific clinical manifestation (72, 76, 122).

DIAGNOSIS

Histology

Diagnosis of BA is most often determined by histologic examination of skin biopsy specimens (Fig. 7). In hematoxylin-and-eosin-stained sections, BA is characterized by lobular capillary proliferations (64), with the lobules being rounded aggregates of capillaries. The stroma surrounding the lobules is edematous in early lesions and becomes fibrotic in later stages. The endothelial cells of BA may protrude into vascular lumina, which may become occluded. Nuclear atypia of endothelial cells may be seen. Amphophilic granular aggregates are highly indicative of BA. Such aggregates are revealed to be masses of bacteria by using Warthin-Starry stain, electron microscopy, or immunofluorescence. Neutrophils frequently cluster around these aggregates of bacteria.

Culture

Following the isolation and characterization of *B. henselae* (118, 131), workers recognized that the routine blood culture methods in use at the time would not allow the detection of slowly growing *Bartonella* species. New procedures were therefore recommended, including the use of enriched media, prolonged incubation times, cell lysis centrifugation, and cell culture systems. Indeed, continuing improvements in routine culture and isolation techniques are probably responsible for
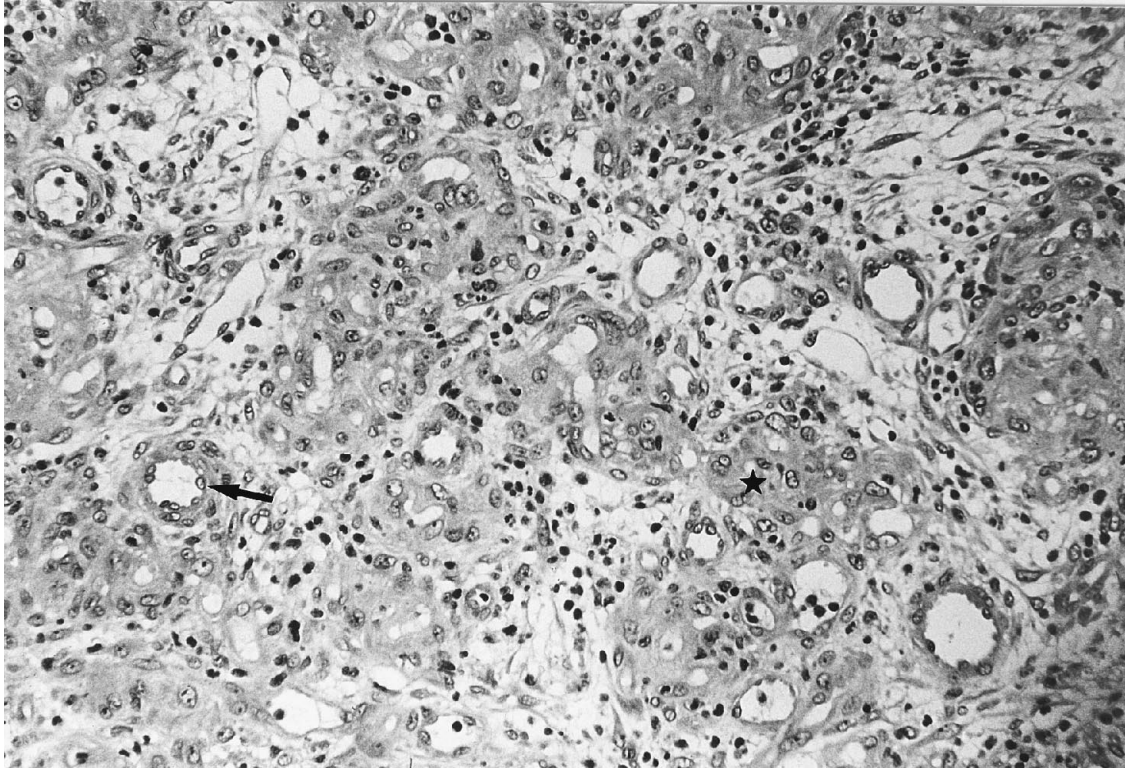
FIG. 7. Cutaneous bacillary angiomatosis showing lobular proliferation of small, round blood vessels in the dermis (★). The vascular channels are lined with epithelioid endothelial cells that protrude into vascular lumina (arrow). A mixed inflammatory cell infiltrate, with neutrophils predominating, is scattered throughout the lesion. Hematoxylin-phloxin-saffron stain; magnification, ×250.

the increased number of *Bartonella* isolates obtained over the last 5 years. There remains, however, debate over the optimum conditions required for the isolation of bartonellae. The two most widely used methods are direct plating onto solid media (30, 62, 76, 137) and cocultivation in cell culture (30, 58, 110, 137), although even groups using the same methods describe differing levels of success (30, 58, 62, 76, 110). A combination of the two methods may be useful for optimizing the recovery of *Bartonella* spp. (30). Differences have also been reported when considering the mean incubation time necessary to visualize the growth of bacteria and the difficulty of performing subcultures. These variations may not be due solely to different laboratory practices; the timing of specimen collection and the exact nature of the patient's illness may be equally important. Similarly, intraspecies heterogeneity among *Bartonella* isolates, implying variations in optimum conditions of growth, may necessitate alternative methods for their cultivation and isolation; differences between strains of *B. henselae* have already been demonstrated by whole-cell fatty acid analysis (155) and PFGE of genomic DNAs (122).

Culture on blood agar. On blood-enriched agar, *Bartonella* species are best cultivated in a humid, CO_2-rich (5%) atmosphere (90, 112, 150). Primary isolation from the blood of infected patients may require up to 45 days of incubation before colonies become apparent. Thus, the use of a moist chamber to maintain high humidity (over 80%) during incubation is required. Horse and rabbit blood are reported to be more effective supplements than sheep blood (58, 165). Freshly prepared blood-supplemented agar plates must be used.

Vinson and Fuller were the first to report the isolation of *B. quintana* by using blood-enriched agar (152). They demonstrated that growth depended on the presence of erythrocytes

but that serum factors (which were not characterized) were also important (150). These findings were subsequently confirmed by Myers et al. (90). More recent investigations have demonstrated the hemin dependence of *Bartonella* species (31, 127), with optimum growth resulting from media containing hemin at 50 to 250 μg/ml (31, 127). At a higher concentration (500 μg/ml), hemin inhibits growth. Several attempts have been made to develop blood-free media for the cultivation of *Bartonella* species, although no growth was achieved when established media such as tryptic soy agar, MacConkey agar, Schaedler broth, or brain heart infusion broth were tested (31). A recently described liquid medium consisting of brucella broth supplemented with 6 to 8% Fildes enrichment and 250 μg of hemin per ml does, however, allow the successful cultivation of *B. henselae* (127), as does a solid medium of brucella agar incorporating the same supplements.

The use of lysis centrifugation has been shown to enhance the recovery of *Bartonella* spp. from blood (165). This procedure has previously been recommended for the isolation of other facultative intracellular organisms such as *Mycobacterium tuberculosis* and *Cryptococcus neoformans*. Detection of fastidious organisms such as *Bartonella* spp. in conventional blood cultures remains difficult, in part because the organisms produce little or no CO_2 or visible growth. Thus, systematic detection of bacteria in blood cultures by using an acridine orange dye staining procedure was proposed by Larson et al. (62) as an approach to improving the recovery of *Bartonella* species. Briefly, acridine orange, a fluorescent compound which interacts with the DNA of organisms, was used to stain an aliquot from each aerobic bottle before it was discarded. When organisms with *Bartonella* morphology were seen upon acridine orange staining, a subculture was made on chocolate

agar and incubated until growth was visible. The method, which has been demonstrated to be more sensitive than Gram staining, was recently used by Spach et al. (137) in isolating *B. quintana* from 34 blood cultures within a 6-month period.

Cell culture systems. *B. quintana* was first isolated from a BA patient by Koehler et al. (58) following the cocultivation of cutaneous biopsy material with a bovine endothelial cell line. Subsequently, this and other endothelial cell culture systems have been shown to yield isolates when direct plating of clinical material onto a number of solid axenic media has not. Recently, Drancourt et al. (30) reported using this approach when isolating *B. quintana* from the blood of three homeless patients with endocarditis, and similar cultivation methods led to the successful isolation of *B. quintana* from the blood of a woman with chronic lymphadenopathy and lymphopenia (110).

Identification. *Bartonella* species 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* species have been described, ranging from the comparison of the biochemical reactivities of isolates to more complex genotypic and phenotypic analyses (12, 13, 15, 28, 30, 31, 48, 72, 76, 102, 112, 116–118, 122, 132, 159, 160, 164, 165).

Primary cultures of *Pasteurella bettyae* (formerly HB5), *Actinobacillus actinomycetemcomitans*, and the *Capnocytophaga* species *C. sputigena* (formerly DF-1) and DF-3 may be confused with those of *Bartonella* species, but the application of simple biochemical tests will allow differentiation: all the above species ferment glucose, whereas *Bartonella* species do not; *P. bettyae* also produces indole and *A. actinomycetemcomitans* reacts positively in the catalase test. *Bartonella* species have been reported to be biochemically inert in almost all conventional biochemical tests, although the use of the Microscan rapid anaerobe panel has been reported for the identification of bartonellae to species level (164). More recently, Drancourt and Raoult (31) demonstrated that the introduction of 100 µg of hemin per ml to the test medium led to reactivity in a number of biochemical tests in which *Bartonella* species had previously been considered inert. This supplement allowed the effective differentiation of *B. quintana*, *B. henselae*, and *B. vinsonii* from one another. *B. quintana* exhibited a positive Voges-Proskauer reaction, leucine arylamidase and pyrazinamidase activity, weak esculin hydrolysis, and fermentation of maltose and saccharose, whereas *B. henselae* exhibited a positive Voges-Proskauer reaction, leucine arylamidase activity, esculin and hippurate hydrolysis, and lactose fermentation, and *B. vinsonii* exhibited alkaline phosphatase and pyrazinamidase activity and hippurate hydrolysis.

Perhaps the most convenient and clearest way of differentiating *Bartonella* species is through the use of polyvalent antisera (76, 132). A strong antibody response can be induced in laboratory animals by intraperitoneal inoculation of whole, killed *Bartonella* cells. The reactivities of these antisera, as determined by either immunofluorescence tests, enzyme-linked immunosorbent assays, or immunoblotting (76, 132), are highly species specific, and each antiserum reacts well with different strains of the same species.

Immunoblotting has revealed a size range of 10 to 65 kDa for the immunoreactive proteins of *B. quintana*, *B. henselae*, and *B. vinsonii* (76). Although each species had a unique protein profile, a dominant strongly immunoreactive 48.5-kDa protein was found in all three.

Cell wall fatty acid analysis has been attempted on the *Bartonella* species (28, 30, 165), which are characterized by the presence of large amounts of *cis*-11-octadecanoate (C18:1ω7c),

hexadecanoate (C16:0), and octadecanoate (C18:0). All species also contained C17:0 and traces of C16:1ω7c, C17:1ω6c, C18:2, and C18:1ω9c (28). No hydroxy or cyclopropane acids were found. Although very small differences were noted between *Bartonella* species, these cannot be considered suitable markers for the confident differentiation of species.

Members of the genus *Bartonella* have been differentiated through the application of an extensive range of genotypic analyses, including 16S rRNA gene sequencing (12, 76, 102, 112, 116–118, 159, 160), citrate synthase gene analysis (30, 48, 76, 112, 118), DNA hybridizations (15, 28, 165), and PFGE of genomic DNA (76, 122). Further genotypic methods have also been used to demonstrate intraspecies differences (72, 122).

Comparison of all the *Bartonella* species (including former *Rochalimaea* species and *B. bacilliformis*) by DNA-DNA hybridization, the "gold standard" of species identification, has now been completed. Although distinct, the species exhibit a very high level of interrelatedness, and their taxonomic position within the same genus is clearly without doubt (15, 28, 165). A very recent taxonomic reassessment, which included DNA-DNA hybridization assessments, also demonstrated the high level of interrelatedness of members of the genera *Bartonella* and *Grahamella*, and thus the unification of these genera was proposed (13). This unification led to the expansion of the *Bartonella* genus to include five new species: *B. talpae*, *B. peromyscii*, *B. grahamii*, *B. taylorii*, and *B. doshiae*.

The 16S rRNA gene sequences of all eight of the *Bartonella* species so far sequenced are unique but very similar (15, 28, 76, 112, 117, 118). Interestingly, it was through the analysis of 16S rRNA gene fragments amplified directly from infected tissue from patients with BA that Relman et al. (118) first identified *B. henselae* in 1990.

Birtles et al. have recently exploited the known variation in 16S rRNA gene sequences of *Bartonella* species to present a method for their differentiation (13). Amplified 16S rRNA genes are subjected to RFLP with enzymes known to be active in regions of the 16S rRNA gene where the greatest interspecies diversity occurs. All eight extant species can be differentiated from one another with a combination of *Dde*I and *Mnl*I. A different PCR-based RFLP scheme for the differentiation of *Bartonella* species, based on the exploitation of variation in the citrate synthase gene, has also been described (76, 112). Use of the enzymes *Taq*I and *Mse*I to digest an approximately 400-bp fragment of this gene allows differentiation of *B. quintana*, *B. henselae*, *B. elizabethae*, and *B. vinsonii*. The benefit of both of these schemes over serological or biochemical identification methods is that they require only a very minute amount of biomass of what are known to be fastidious, slowly growing organisms.

Joblet et al. (48) described the use of PCR-based RFLP on the citrate synthase as part of a scheme for the rapid differentiation of *Bartonella* species from other fastidious gram-negative bacilli that may be responsible for endocarditis (Fig. 8). No amplification product was obtained when most species (including *Capnocytophaga* species except *C. ochracea*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella kingae*, *Haemophilus* species, *Brucella abortus*, and strains DF-3 and HB-5) were incorporated into this PCR. Of the non-*Bartonella* species, only *Capnocytophaga ochracea* yielded a citrate synthase gene amplification product. Sequencing of this product, together with those derived from each of the *Bartonella* species, revealed sequence heterology in the products, and the restriction enzymes *Taq*I and *Aci*I were used to exploit this variation, allowing differentiation of all species. Joblet et al. (48) proposed that the use of this scheme on acridine orange-staining blood cul-
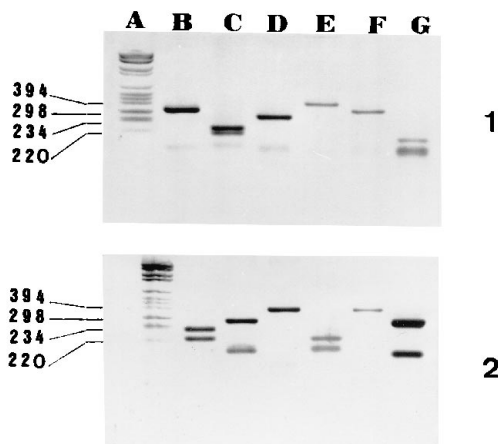
FIG. 8. Restriction profiles obtained after *Taq*I (panel 1) and *Aci*I (panel 2) digestion of a portion of the citrate-synthase gene amplified from *Bartonella* species and *Capnocytophaga ochracea*. Lanes: A, molecular size markers (base pair values are indicated in the left margin); B, *B. quintana*; C, *B. henselae*; D, *B. vinsonii*; E, *B. elizabethae*; F, *B. bacilliformis*; G, *C. ochracea*.

ture supernatants would allow the rapid diagnosis of *Bartonella* bacteremia or endocarditis.

Bartonella species can also be differentiated by PFGE, incorporating the restriction enzyme *Sma*I, *Eag*I, *Hin*dIII, *Hae*III, or *Taq*I (76, 122).

Serology

Laboratory diagnosis of trench fever originally relied on a passive hemagglutination test, using tanned sheep erythrocytes sensitized with soluble antigen from *B. quintana* (26). With this assay, antibodies could be detected at titers of between 20 and 640 in trench fever patients either following primary infection or during relapses. Both IgM and IgG could be detected, although IgG predominated. The specificity was estimated to be >99%, although the authors described a cross-reaction with 1 of 11 sera taken from Q fever patients.

Other methods of specific antibody detection and estimation are now used in the diagnosis of *Bartonella* infections, including the immunofluorescent-antibody test and enzyme-linked immunosorbent assays. It must, however, be noted that in most HIV-infected patients, a group particularly prone to *Bartonella* infections, a significant antibody response to infection is not mounted. Thus, antibody estimation cannot be relied on in the diagnosis of *B. quintana*-induced infections in this group. In contrast, in a recent study of immunocompetent individuals with *Bartonella*-induced endocarditis, very high levels of antibodies were detected (30). The titer to which antibodies are detected, however, depends on the method by which the antigen used in the assay is prepared. In the above study (30), antigen prepared from plate-grown *Bartonella* species reacted with antisera to dilutions of 1:400 to 1:800. When the same sera were tested against antigens prepared from organisms cocultivated with cell monolayers, titers of 6,400 to 12,800 were obtained. *B. quintana* infection has also been diagnosed by demonstration of seroconversion (139). In one reported instance, for example, a patient's antibody titer rose from 400 to 1,600 over the 15 days following presentation. Even among immunocompetent individuals, specific antibodies may not always be detectable during a *B. quintana*-induced illness. In a patient with chronic lymphadenopathy, no antibodies were detected despite isolation of *B. quintana* from blood cultures (110).

The specificity of antibody estimation tests has been ques-

tioned by several groups. First, current serologic tests may not reliably distinguish *B. quintana* antibody responses from *B. henselae* responses. On the other hand, a cross-reactivity between *Bartonella* species and other organisms, including *C. burnetii* (26) and *Chlamydia* species (30), has been reported. Prior to the implication of *B. henselae* in CSD, Emmons et al. (32) demonstrated that antisera from CSD patients reacted with *C. trachomatis* antigens. Cross-reaction between *B. bacilliformis* and *C. psittaci* has also been reported, with a common surface epitope being characterized as part of the lipopolysaccharide (LPS) (55); the authors thus advocated that to improve the specificity of *B. bacilliformis* antigens, the LPS should be removed. More recently, Drancourt et al. reported that patients with *B. quintana*-induced endocarditis had IgG titers of greater than 256 against *C. pneumoniae* and 64 against *C. trachomatis* and *C. psittaci* (30). Absorption of the sera with *C. pneumoniae* did not reduce the high antibody titers against *B. quintana*, but absorption with *B. quintana* eliminated reactivity with *C. pneumoniae* antigen. This cross-reactivity was confirmed by immunoblotting. The susceptibility of the cross-reacting antigens to proteinase K indicates their protein nature. Clearly, the cross-reactivity of antigens of these two species is of diagnostic importance, because both are potential etiologic agents of infective endocarditis (33, 129).

A study investigating the immunochemical properties of the outer membrane-associated LPS and proteins of *B. quintana* demonstrated the presence of phenol-soluble LPS containing 2-keto-3-deoxyoctulosonic acid and heptose (45). This LPS may be similar to the phenol-soluble LPS reported for the typhus, spotted fever, and scrub typhus groups of rickettsiae (126) and is clearly different from the aqueous soluble LPS of the members of the *Enterobacteriaceae*. The study also demonstrated that the LPS elicits a precipitin response in infected humans and that the antibody response is directed against the polysaccharide moiety. The protein component of the outer membrane is also antigenic, inducing antibody responses in both humans and other animals.

Since most *Bartonella*-related infections have only recently been described and the number of cases identified is still relatively small, the titer at which antibody levels become significant (i.e., the "cutoff" value) for diagnosis of infection has yet to be determined. Over the past 17 months, over 12,000 serum samples have been tested in our laboratory for the presence of *B. quintana* antibodies by using the immunofluorescent-antibody test and antigen from plate-grown organisms. IgG titers of >100 have been found in 70 of these serum samples, with titers of >400 in 31 of these 70. At present, we would consider an IgG titer of >100 to be significant.

DNA Amplification

The 16S rRNA gene (58, 110, 112, 118) and the citrate synthase gene (30) have been the two DNA fragments on which efforts have been focused in attempts to develop specific PCR-based assays for the diagnosis of *Bartonella* infections. Anderson et al. (2) have described an alternative approach of heat shock gene amplification followed by hybridization.

Relman et al. (118), in their seminal report in 1990, were the first to design *Bartonella*-specific PCR primers based on unique regions of the 16S rRNA gene. As described above, these primers were used to amplify a 280-bp fragment of the *Bartonella* 16S rRNA gene from clinical material from three BA patients. The primers, p24E and p12B, have subsequently been used in several different studies, in which their specificity for the 16S rRNA gene of *Bartonella* species has been substantiated by subsequent sequencing of PCR products (58, 110, 112,

118). *B. quintana* 16S rRNA has been successfully amplified from a variety of clinical material, including cutaneous lesions of BA patients (58, 118) and the lymph node of a patient with chronic lymphadenopathy (110).

Amplification of citrate synthase gene fragments, coupled with RFLP, was first described for the detection and identification of rickettsiae (114), and the scheme was later extended to the former *Rochalimaea* species. The primers used in the amplification are not *Bartonella* specific, although RFLP of amplification products does permit discrimination of *Bartonella* species from other bacteria. By using this approach, *B. quintana* has been identified from surgically removed cardiac valves of endocarditis patients (30).

More recently, Anderson et al. (2) described a PCR assay involving degenerate primers that allowed amplification of a 414-bp fragment of DNA from either *B. quintana* or *B. 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 *B. elizabethae*, *B. vinsonii*, *B. bacilliformis*, or *Afipia felis*. The technique was applied to 16 fresh lymph node tissue biopsy specimens and 9 node lymph aspirates from patients with CSD (2). The 414-bp fragment was amplified from 21 of 25 samples, and in all cases the 414-bp fragment hybridized with a *B. henselae*-specific probe.

TREATMENT

In Vitro Antibiotic Susceptibility

There are very few data on the antibiotic susceptibility of *Bartonella* species in axenic media (74, 91), largely because so few strains have yet been isolated. Recently, however, our laboratory has evaluated the antibiotic susceptibilities of nine *B. quintana* isolates, three *B. henselae* isolates, one *B. vinsonii* isolate, and one *B. elizabethae* isolate, using horse blood-supplemented Columbia agar as the assay medium (73). The results indicate that all isolates were highly susceptible to beta-lactams, with the MIC for 90% of isolates (MIC_{90}) of penicillin G and amoxicillin being 0.06 μg/ml, that of ticarcillin and cefotaxime being 0.25 μg/ml, and that of imipenem being 0.5 μg/ml. The MIC_{90}s of oxacillin and cephalothin were 4 and 16 μg/ml, respectively. The MIC_{90}s of aminoglycosides ranged from 1 to 4 μg/ml, with gentamicin being more effective than tobramycin and amikacin. Erythromycin, doxycycline, and rifampin displayed MIC_{90}s of 0.12, 0.12, and 0.25 μg/ml, respectively. The MIC_{90}s of roxithromycin, azithromycin, and clarithromycin were 0.06, 0.015, and 0.015 μg/ml, respectively. Clindamycin was rather less effective, with an MIC_{90} of 8 μg/ml. Large variations were noted in susceptibility to the fluoroquinolone compounds, with the MIC_{90}s of pefloxacin, ciprofloxacin, and sparfloxacin being 8, 2, and 0.12 μg/ml, respectively. MIC_{90}s of trimethoprim-sulfamethoxazole were 1 and 5 μg/ml, and the MIC_{90} of fosfomycin was 64 μg/ml. The MIC_{90} of colistin and vancomycin was 16 μg/ml, a level of susceptibility surprising for gram-negative bacteria. Among this group, vancomycin susceptibility has previously been reported only for *Eikenella corrodens*, *Capnocytophaga* species, and *Flavobacterium meningosepticum* (142, 154). It should be emphasized that in vitro MIC determination for the fastidious *Bartonella* species must be considered cautiously, and the true relevance of in vitro susceptibilities to clinical care and outcome remains to be established.

The bactericidal activities of antibiotics against *B. henselae*, both when cocultivated with the murine macrophage-like cell line ATCC TIB63 and the human endothelial cell line ECV

304,9 and when cultivated on an axenic Schaedler medium supplemented with vitamin K_3, 5% defibrinated sheep blood, and 300 μg of hemin per ml, have also been studied (89). Only the aminoglycosides (gentamicin, tobramycin, and amikacin) were bactericidal on either axenic or cell line-cultured organisms. The other antibiotics tested, which did not display any significant bactericidal activity, included amoxicillin, cefotaxime, ceftriaxone, doxycycline, erythromycin, rifampin, and ciprofloxacin.

Clinical Efficacy of Antibiotics

Because most cases of trench fever were reported prior to the antibiotic era, published observations of antibiotic therapy efficacy are scarce. Successful treatments with tetracycline (83) or chloramphenicol (153) have been reported, although the authors do not indicate if such compounds could prevent recurrence of the disease.

Data on the efficacy of antibiotics in the treatment of the newly recognized *Bartonella*-induced infections are more widely available. A number of failed regimens have been described for the treatment of BA, including nafcillin (57), dicloxacillin (57), and cephalexin (131). The inefficacy of these antibiotics is consistent with the results of in vitro susceptibility testing, which demonstrated poor activity of both the penicillin M compounds and narrow-spectrum cephalosporins against *Bartonella* species. Clinical data suggest that amoxicillin (68), aminoglycosides (68, 107), and trimethoprim-sulfamethoxazole (21, 131) may be suitable for treatment, even though several patients being treated with these regimens relapsed. Doxycycline has been reported to be both effective (112) and ineffective (57, 68, 112, 165) in treating the illness and preventing relapse. Regnery et al. (112) reported an HIV-infected patient with BA who relapsed after a 4-week course of doxycycline therapy but in whom the illness resolved after an 8-week regimen of the same drug.

A wide range of other antibiotics has been reported to successfully treat *Bartonella*-induced BA. Lesions resolved in two patients treated with ceftriaxone (68, 131), and macrolide compounds have been reported to be effective in controlling primary *B. henselae* infections (49, 57, 65, 107, 118, 123, 131, 165). Successful therapy of three patients with BA with norfloxacin and ciprofloxacin (68, 131) has also been reported. A regimen of erythromycin (250 to 500 mg, four times a day) has been reported to be the most effective method of treating cutaneous BA, although relapses have been reported once antibiotic treatment was withdrawn, particularly in HIV-infected patients being given short courses (<15 days) of treatment (131). The duration of therapy may be more important than the choice of antibiotic; fewer relapses have been noted in patients treated with either tetracycline or the macrolide compounds for more than 1 month. Antimycobacterial drugs are also reported to be effective in eradicating BA (41, 54, 57, 65, 107).

Data on the requirements for the effective treatment of *Bartonella*-induced endocarditis are scarce, again because so few cases have yet been reported. Successful treatment has been reported with complex regimens consisting of intravenous amoxicillin combined with gentamicin (30); intravenous vancomycin with ofloxacin and netilmicin, followed by oral therapy with rifampin, ofloxacin, and pristinamycin (30); and intravenous ceftriaxone followed by long-term erythromycin (135). Endocarditis due to *B. elizabethae* did not respond to treatment with nafcillin and gentamicin but resolved after cardiac valve replacement and a regimen of vancomycin and imipenem (28). Intravenous antibiotic therapy was continued for 6 weeks, and oral trimethoprim-sulfamethoxazole was administered for a

TABLE 3. Proposed antibiotic regimen for diseases caused by *Bartonella* species

Disease[a]		Regimen[b] for:			Duration (wk)
		Adults	Pregnant women	Children	
TF		Gentamicin, 2 mg/kg bid i.m.,		Gentamicin, 2 mg/kg bid i.m.	2
	or	doxycyline, 200 mg od p.o.	Erythromycin, 500 mg qid p.o.	Erythromycin, 25 mg/kg tid p.o.	4
BEA, LA		Gentamicin, 2 mg/kg bid i.m.,		Gentamicin, 2 mg/kg bid i.m.	2
	and either	erythromycin, 500 mg qid p.o.,	Erythromycin, 500 mg qid p.o.	Erythromycin, 25 mg/kg tid p.o.	4[c]
	or	doxycycline, 200 mg od p.o.			4[c]
BACT, ENDO			Erythromycin, 500 mg qid i.v./p.o.		8
		Gentamicin, 2 mg/kg bid		Gentamicin, 2 mg/kg bid	2
	and either	ceftriaxone, 2 g od i.v.		Ceftriaxone, 200 mg/kg od i.v.	4
	or	doxycycline, 200 mg od p.o.			4[c]

[a] TF, trench fever; BEA, bacillary epithelioid angiomatosis without bacteremia; LA, chronic lymphadenopathy; BACT, bacteremia; ENDO, endocarditis.

[b] od, once a day; bid, twice a day; tid, three times a day; qid, four times a day; po, per os; i.v., intravenous; i.m., intramuscular.

[c] 8 weeks in HIV-infected patients.

further 5 weeks. It should be noted that *Bartonella*-induced endocarditis usually results in extensive valve damage, requiring replacement (28, 30).

The treatment of *B. quintana* patients with bacteremia has also been described (137). Of the 10 patients studied by Spach et al. (137), 5 were treated with ceftriaxone for 7 days followed by either oral erythromycin or azithromycin for 3 weeks. Follow-up was possible for only three patients, in all of whom the bacteremia has resolved.

The poor efficacy of the beta-lactams in the treatment of *Bartonella*-induced illness is surprising, because these drugs demonstrate high anti-*Bartonella* activity in vitro. However, this discrepancy has also been noted for other bacteria, including *Mycoplasma* species (82) and *Borrelia burgdorferi* (37), and may be due to the intracellular position of these bacteria in vivo. As discussed above, several observations and experiments have indicated that *Bartonella* species are also found intracellularly in infected tissue (16, 80).

Although a wide variety of antibiotics have been shown to inhibit the growth of *Bartonella* species, a bactericidal activity may be more important in vivo, especially in immunocompromised patients or patients with chronic infections or endocarditis. The relatively large number of patients with *Bartonella*-induced infections who relapse is perhaps indicative of the failure of most prescribed antibiotics to kill the infecting bacteria. Indeed, a very recent study investigating the bactericidal activity of antibiotics on *Bartonella* species in vitro found only aminoglycosides to be bactericidal on *B. henselae* grown either in axenic broths or in cocultivation with eucaryotic cell lines (89). The use of these drugs may therefore be the most advisable in treatment of *Bartonella* infections.

Antibiotic Treatment Proposal

The wide spectrum of different clinical manifestations of *Bartonella* infections, together with the immunological status of the patient, complicates the formulation of a standard antibiotic regimen for the treatment of these infections. Prolonged treatments must be advocated for immunocompromised patients. In Table 3, we have attempted to establish antibiotic treatment recommendations according to current knowledge of both antibiotic susceptibility of *Bartonella* spp. in vitro and the antibiotic therapy efficacy in vivo, although, overall, the usefulness of specific regimens can be evaluated only when more clinical experience has been gained. The recommendation of an aminoglycoside, especially gentamicin, in the treatment of *Bartonella* infections seems reasonable, although data on gentamicin and *B. quintana* infections are very limited.

PREVENTION AND RISK FACTORS

As the cycle of the *Bartonella* species in nature remains unclear, likely predisposing epidemiological factors also remain uncertain, and thus it remains difficult to establish valid recommendations for prevention of *Bartonella*-induced illnesses.

For trench fever, the role of the human body louse (*Pediculus corporis*) as a vector and potential reservoir of *B. quintana* has often been proposed (18, 60). Transmission of *B. quintana* by this vector is also suspected in homeless people suffering from bacteremia (137) and endocarditis (30). As advocated during World War I, the best method for reducing louse infestation is by the regular changing or washing of clothing. The World Health Organization has also issued recommendations for the large-scale control of louse infestation by using insecticides; permethrin (1%) dusting powder is the compound of choice. This powder should be applied in a dose of 30 to 50 g per adult (125 to 205 mg/m^2 of clothing) by means of an air compressor with multiple duster heads. All clothing should be dusted inside and out, and bedding should also be treated. All lice should succumb to the insecticide within a few hours, after which all clothing should be rinsed in cold water. The treatment should be repeated every 6 weeks.

There is now both epidemiological and microbiological evidence for the involvement of domestic cats in the life cycles of *Bartonella* species, particularly *B. henselae*. *Bartonella*-specific antibodies have also been detected in 81% of 48 cats living in households reporting CSD in Connecticut (170), whereas only 46% of 13 cats from non-CSD households had detectable antibodies. In a recent study by Koehler et al. (56), *B. henselae* was isolated from the blood of all seven cats to which four BA patients had prolonged exposure. The study also found *B. henselae* in the blood of 41% of 61 apparently healthy pet or impounded cats in the San Francisco area. *B. henselae* was also detected by both direct culture and PCR in several cat fleas infesting these bacteremic cats. Immunocompromised persons, including those infected with HIV, should avoid contact with cats. On the other hand, antibiotic treatment of infected cats and control of flea infestations have been advocated as potential strategies for prevention of *Bartonella* infections, especially when *B. henselae* was considered (56).

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