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REVIEW

Bartonella Species, an Emerging Cause of Blood-Culture-Negative Endocarditis

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SUMMARY Since the reclassification of the genus *Bartonella* in 1993, the number of species has grown from 1 to 45 currently designated members. Likewise, the association of different *Bartonella* species with human disease continues to grow, as does the range of clinical presentations associated with these bacteria. Among these, blood-culture-negative endocarditis stands out as a common, often undiagnosed, clinical presentation of infection with several different *Bartonella* species. The limitations of laboratory tests resulting in this underdiagnosis of *Bartonella* endocarditis are discussed. The varied clinical picture of *Bartonella* infection and a review of clinical aspects of endocarditis caused by *Bartonella* are presented. We also summarize the current knowledge of the molecular basis of *Bartonella* pathogenesis, focusing on surface adhesins in the two *Bartonella* species that most commonly cause endocarditis, *B. henselae* and *B. quintana*. We discuss evidence that surface adhesins are important factors for autoaggregation and biofilm formation by *Bartonella* species. Finally, we propose that biofilm formation is a critical step in the formation of vegetative masses during *Bartonella*-mediated endocarditis and represents a potential reservoir for persistence by these bacteria.

KEYWORDS *Bartonella*, blood-culture-negative endocarditis, emerging infections, trimeric autotransporter adhesins, biofilm

INTRODUCTION

Just over a century ago, an emerging disease plagued almost a million frontline troops during World War I, rendering them unfit for duty for months at a time. The disease became known as "trench fever," and it was subsequently shown to be caused by the louse-borne bacterium now known as *Bartonella quintana* (1). Interestingly, at that time many soldiers affected by trench fever were also reported to have cardiac involvement, and a complication called "disordered action of the heart" was described (2, 3). Diseases caused by bacteria in the current genus *Bartonella*, which once plagued the soldiers of World War I as trench fever, remained somewhat obscure until appearing as opportunistic infections in AIDS patients and homeless patients in urban areas in the early 1990s. Now characterized as reemerging, bacteria in the genus *Bartonella* are fastidious, Gram-negative, facultative intracellular pathogens with a unique intraerythrocytic lifestyle. Bartonellae usually exist in two specific habitats: the gut of the obligately bloodsucking arthropod vector, where they are exposed to toxic concentrations of heme, and the bloodstream of the mammalian host with deprivation of access to heme and iron (4). The ability of these bacteria to be transmitted by bloodsucking arthropods facilitates survival and dispersion while avoiding the host immune system. Over the past 20 years, there has been a rapid increase in the number of *Bartonella* species, with 45 species now designated and with some species containing more than one subspecies (Table 1). New species and subspecies are constantly being proposed, as evidenced by the description of *Bartonella vinsonii* subsp. *yucatanensis* as a distinct new taxon (5). Additionally, *Bartonella* isolates and candidate species from a wide range of animal reservoirs have been described but not yet assigned new species designations and will undoubtedly further expand this growing genus of bacteria. Bartonellae

TABLE 1 Currently designated *Bartonella* species, their hosts, and associated human disease

Species	Host(s)	Human disease association
B. acomydis	Golden spiny mouse (*Acomys russatus*) (398)	
B. alsatica	Rabbits (39)	Endocarditis (40)
B. ancashensis	Human patient (41)	Verruga peruana (41, 399)
B. apis	Honeybee symbiont (400)	
B. australis	Kangaroos (58)	
B. bacilliformis	Human (26, 401)	Oroya fever, verruga peruana, Carrion's disease (26)
B. birtlesii	Mice (402)	
B. bovis	Dairy cattle (403)	
B. callosciuri	Plantain squirrel (398)	
B. capreoli	Deer (403)	
B. chomelii	French cattle (404)	
B. clarridgeiae	Cat (187)	Lymphadenopathy, fever, papule, CSD (44, 187)
B. coopersplainsensis	Rat (58)	
B. doshiae	Voles (405)	
B. dromedarii	Camels (406)	
B. elizabethae	Rats (24)	Endocarditis, neuroretinitis (18, 407)
B. florenciae	Shrew, mouse (408)	
B. fuyuanensis	Field mouse (409)	
B. grahamii	Rodents, voles (405)	Neuroretinitis, CSD (51, 53)
B. heixiaziensis	Vole (409)	
B. henselae	Cat (31, 140)	CSD, endocarditis, bacillary angiomatosis, bacteremia (140)
B. jaculi	Greater Egyptian jerboa (398)	
B. japonica	Mice (410)	
B. koehlerae	Cat (411)	Endocarditis (19)
B. koehlerae subsp. *bothieri*	Bobcat (412)	
B. koehlerae subsp. *boulouisii*	Mountain lion (412)	
B. mayotimonensis	Bats (55)	Endocarditis (20)
B. melophagi	Sheep (413)	
B. naantaliensis	Bats (55)	
B. peromysci	Mouse (405)	
B. pachyuromydis	Fat-tail gerbil (398)	
B. phoceensis	Rat (414)	
B. queenslandensis	Rats (58)	
B. quintana	Human (415)	Trench fever, endocarditis, bacteremia, bacillary angiomatosis
B. rattaustraliani	Rats (416)	
B. rattimassiliensis	Rats (414)	
B. rochalimae	Foxes, raccoons, coyotes (57, 417)	Bacteremia, splenomegaly (57)
B. silvatica	Mice (410)	
B. schoenbuchensis	Deer (418)	
B. senegalensis	Tick (419)	
B. talpae	Moles (405)	
B. tamiae	Rodents, humans (58)	Fever (58, 59)
B. taylorii	Rats (405)	
B. tribocorum	Rats (420)	
B. vinsonii subsp. *arupensis*	Mice (65)	Endocarditis (21)
B. vinsonii subsp. *berkhoffii*	Dog, coyotes (181, 421)	Endocarditis (23)
B. vinsonii subsp. *vinsonii*	Voles (24)	
B. vinsonii subsp. *yucatanensis*	Rodents (5)	
B. weissii	Cat (181)	
B. washoensis	Dog (422)	

are zoonotic bacteria transmitted from host to host by a diverse range of hematogenous arthropod vectors, including fleas, lice, ticks, and sandflies (6). The association of *Bartonella* species with new vectors such as sheep keds has been recently reported (7). Likewise, the association of *Bartonella* species with vertebrate host reservoirs, including cats, rodents, and humans, has long been established, but a steadily expanding range of new animal reservoirs has been reported, including marine mammals (8), terrestrial herbivores such as camels (9), and wild carnivores, including lions, bears, and foxes (10). The emergence of *Bartonella* in a wide range of hosts and environments and the association of these bacteria with disease are mirrored by a steady increase in the number of articles about *Bartonella* which have been published in the last 2 decades compared to earlier time periods (Fig. 1A).

The role of *Bartonella* species in causing endocarditis was first reported in 1993

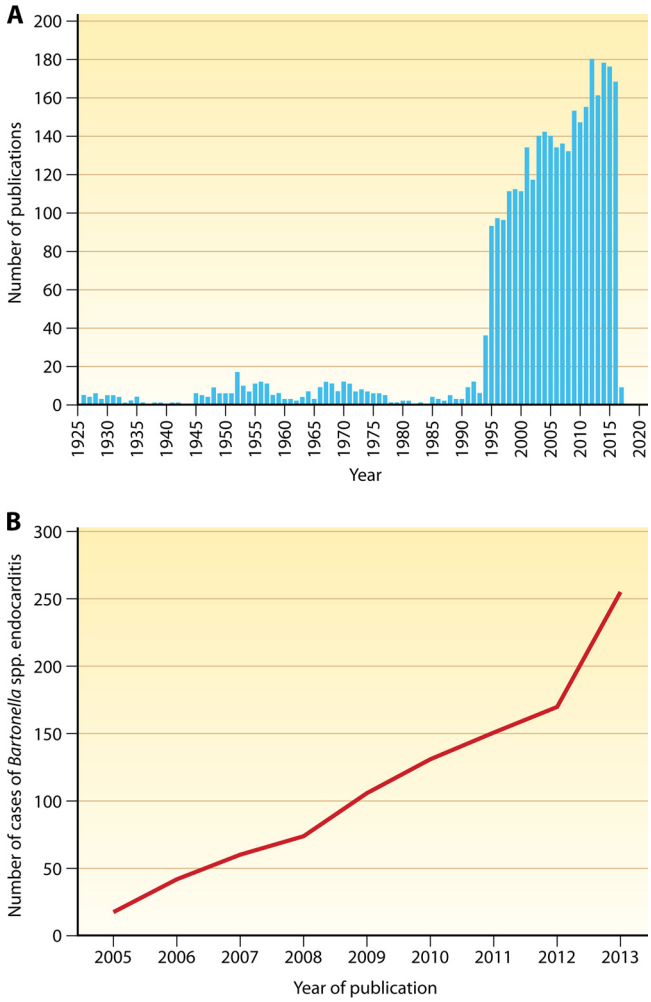

FIG 1 (A) Number of publications on *Bartonella* in PubMed. Source: https://www.ncbi.nlm.nih.gov/pubmed/?term=bartonella. (B) Increase in reported *Bartonella* endocarditis cases. (Adapted from reference 177 with permission.)

when *B. quintana* was identified in a patient with HIV infection (11). Soon thereafter, *B. quintana* was also isolated from several homeless patients with chronic alcoholism, some of whom were immunocompetent and had been diagnosed as having blood-culture-negative endocarditis (BCNE) (12–14). In those cases, specialized isolation techniques were used to isolate *B. quintana* from the patient's blood and/or PCR was used to confirm the etiology. That same year, *Bartonella henselae* was also shown to be responsible for a case of "culture-negative" endocarditis (15) and also in a second immunocompetent patient with endocarditis who owned a cat from which he most likely acquired the bacterium (16). Since that time, the number of cases of endocarditis and blood-culture-negative endocarditis that have been attributed to *B. quintana* and *B. henselae* has steadily increased (Fig. 1B). While these two species represent the vast majority of endocarditis cases attributed to *Bartonella* species, several other species, including *B. alsatica* (17), *B. elizabethae* (18), *B. koehlerae* (19), *B. mayotimonensis* (20), and *B. vinsonii* subsp. *arupensis* and *berkhoffii* (21–23), have been associated with endocarditis in humans. In this review, we summarize the current knowledge of the human-pathogenic *Bartonella* species, focusing on the two species, *B. henselae* and *B. quintana*, which are most commonly associated with endocarditis.

TAXONOMY OF THE GENUS *BARTONELLA*

Taxonomic History

Despite the recent rapid expansion of the genus *Bartonella*, *B. bacilliformis* was the

only recognized species in the genus until 1993 (24). *B. bacilliformis* is the agent of the biphasic Carrion's disease, which includes the acute hemolytic anemia phase known as Oroya fever and the chronic phase known as verruga peruana (see reference 25 for a recent review). The skin lesions in patients with verruga peruana are unique in that they are highly vascularized nodules with evidence of angiogenesis (26). *B. bacilliformis* is restricted to certain regions in the Andes Mountains because of the distribution of the sandfly vector (25). Despite the unique pathology observed in patients with Carrion's disease, the study of *B. bacilliformis* was limited until the last 25 years, perhaps due to its vector-restricted geographic distribution. Similarly, the agent of trench fever was first known as *Rickettsia quintana* due to its cell association and difficulty in culturing, similar to the rickettsiae (1). In 1965, *Rickettsia quintana* was grown in axenic medium in the absence of host cells (27, 28), and it was subsequently reclassified as *Rochalimaea quintana*.

Interest in these bacteria increased greatly in the early 1990s when *Rochalimaea quintana* and a new species, *Rochalimaea henselae*, were first described in HIV-infected patients and subsequently in immunocompetent patients (29–33). Both species were established as etiologic agents of bacillary angiomatosis, which also exhibits angiogenic lesions similar to verruga peruana (33). Furthermore, *Rochalimaea henselae* was recognized as the primary etiologic agent of cat scratch disease (CSD) (34, 35). DNA relatedness studies and rRNA gene analysis showed that *B. bacilliformis* and members of the genus *Rochalimaea* were closely related, and so these two genera were merged, establishing the current genus *Bartonella*, and the family *Bartonellaceae* was removed from the order *Rickettsiales* (24).

Current Status

The genus *Bartonella* contains aerobic or microaerophilic, fastidious, Gram-negative bacilli belonging to the alpha-2 subgroup of the class *Proteobacteria*. Out of the 45 *Bartonella* species listed in Table 1 which infect animals, 13 have been implicated in human diseases. In addition to the currently recognized species, numerous subspecies exist, as do isolates from animal reservoirs that have not yet been fully characterized and named (candidate species). Thus, the genus is expanding rapidly in real time and very likely includes more distinct species than those listed in Table 1. It should also be recognized that not all of the species listed in Table 1 have been validated, but they are included here for the sake of completeness and because they have become established in the literature. Currently, there are 35 *Bartonella* species/subspecies with standing in nomenclature (http://www.bacterio.net/bartonella.html). Several recent studies/reviews have described the phylogenetic relationships among *Bartonella* species, strains, and isolates using different gene loci, but these are not addressed in this review (25, 36–38).

Bartonella Species Known To Infect Humans

Human infections caused by several different *Bartonella* species have been reported, and the list of potential human pathogens in the genus continues to grow. However, currently the vast majority of infections in humans are attributed most probably to *B. bacilliformis*, *B. henselae*, or *B. quintana*. The association of other *Bartonella* species with human disease relies on substantial information in the literature for some species and very limited information or even single case reports for other species. Isolation of the *Bartonella* species from diseased tissues is described in some reports, while in others, serology or molecular diagnostics supports the etiologic role. Accordingly, the strength of the association of each *Bartonella* species with human disease must be considered variable. Regardless, it has been proposed that any *Bartonella* species found in animals may be capable of infecting humans (20). *Bartonella* species that have been associated with human disease include the following.

B. alsatica. *B. alsatica* was initially isolated from the blood of wild rabbits (39). *B. alsatica* was isolated from a patient diagnosed with BCNE who also had a preexisting valve lesion (40). A subsequent report implicated *B. alsatica* in a second case of BCNE

in a patient who was a rabbit breeder (17), suggesting that rabbits may serve as the reservoir for transmission of *B. alsatica* to humans.

***B. ancashensis*.** *B. ancashensis* is a recently identified new species of *Bartonella* that was isolated from two patients with verruga peruana in the Ancash region of Peru (41). Initially, these patients were thought to be infected with *B. bacilliformis*, until molecular analysis indicated that their isolates were a distinct new species. This finding raises a question: do other *Bartonella* species infect patients in areas of South America where only *B. bacilliformis* is thought to be endemic?

***B. bacilliformis*.** Acute and chronic symptoms of *B. bacilliformis* infection are known as Oroya fever and verruga peruana, respectively, and are collectively referred to as Carrion's disease. Oroya fever is an acute life-threatening hemolytic anemia that is geographically limited to the high Andes, may result in death for more than 80% of infected patients in the absence of antibiotic treatment, and is increasing at an alarming rate in the pediatric population (42). The chronic form of *B. bacilliformis* infection results in angiogenic lesions on the skin called verruga peruana. The diverse clinical presentation of Carrion's disease suggests adaptation by *B. bacilliformis* to facilitate immune evasion in the human host to maintain the reservoir state for vector transmission (43).

***B. clarridgeiae*.** At least three *Bartonella* species, *B. henselae*, *B. clarridgeiae*, and *B. koehlerae*, are associated with cats. *B. clarridgeiae* was isolated from two different immunocompromised patients reported to have CSD (44, 187). Symptoms reported include severe headache, fever, lymphadenopathy, chills, sweating, and malaise (44). There is also evidence of coexistence of *B. henselae* and *B. clarridgeiae* in populations of cats and their fleas (46). Evidence of *B. henselae* and *B. clarridgeiae* DNA has also been reported in saliva of cats and dogs, and it has been suggested that *B. clarridgeiae* is a minor cause of CSD (47).

***B. elizabethae*.** *B. elizabethae* has been reported to cause human illness, and strains of this bacterium have been isolated from small mammals in Asia (48). It was originally isolated by Daly et al. (18) from a patient with endocarditis, and human serologic evidence of *B. elizabethae* infection has been reported in Thailand (49). Clinical characteristics may include headache, lethargy, muscle pain, conjunctival suffusion, and anemia. Almost 70% of patients with evidence of *B. elizabethae* infection also recorded exposure to rats, while the rest had cat exposure (50).

***B. grahamii*.** The first human isolate of *B. grahamii* was from an immunodeficiency virus-negative patient presenting as a case of neuroretinitis, proving that *B. grahamii* is pathogenic to humans (51). It has been reported as one of the most prevalent species in rodents (52) and has been reported as being a causative agent of CSD-like illness in an immunocompromised patient (53).

***B. henselae*.** First isolated in 1992 from a febrile patient infected with HIV (31), *B. henselae* commonly infects domestic and feral cats (*Felis catus*) causing long-term bacteremia. *B. henselae* is the primary etiologic agent of CSD and is the second most common *Bartonella* species causing endocarditis. *B. henselae* also causes bacteremia and bacillary angiomatosis.

***B. koehlerae*.** *B. koehlerae* was detected in heart valve tissue resected from a patient with BCNE (19). In an additional case, the patient reported depression and anxiety, headaches, joint stiffness, and hallucinations as a result of persistent infection with *B. koehlerae* that resolved following antibiotic treatment (54).

***B. mayotimonensis*.** *B. mayotimonensis* was isolated from the aortic valve tissue of a patient from the United States with infective endocarditis (20). The patient lived on a farm in Iowa and reported owning a cat prior to his illness and possible exposure to mouse fecal droppings. The authors of that study suggest the possibility that any *Bartonella* species can cause human infection and BCNE (20). A subsequent report isolated *B. mayotimonensis* from the blood of bats and detected *Bartonella* species in their ectoparasites (55).

***B. quintana*.** *B. quintana* is the cause of louse-borne trench fever; the bacterium is also recognized as the causative agent of bacteremia, bacillary angiomatosis, chronic lymphadenopathy, and endocarditis. It is one of the two species of *Bartonella* with a

human reservoir. A recent review identified *B. quintana* as the most frequent cause of vector-borne infections in homeless and marginalized populations in the United States and Europe (56). *B. quintana* is the most common *Bartonella* species causing endocarditis.

***B. rochalimae*.** *B. rochalimae* has been reported to be a cause of bacteremia, fever, and splenomegaly in a patient who traveled to Peru (57).

***B. tamiae*.** Three isolates of *B. tamiae* were recovered from patients in Thailand who had fever (58, 59). *B. tamiae* DNA has since been detected in chigger mites and ticks, suggesting that these may serve as possible vectors for the transmission of this *Bartonella* species (60).

***B. vinsonii*.** *B. vinsonii* has been isolated from patients with endocarditis, arthritis, neurological disease, and vasoproliferative neoplasia (61, 62). Both *B. vinsonii* subsp. *arupensis* (21) and *B. vinsonii* subsp. *berkhoffii* (22, 23) have been associated with endocarditis. Vector transmission of *B. vinsonii* subsp. *berkhoffii* is suspected among dogs and wild canines (63), but cats have also been implicated as possible reservoirs (64). *B. vinsonii* subsp. *arupensis* is carried by rodents (65).

MICROBIOLOGY OF THE GENUS *BARTONELLA*

Growth Properties

Bartonella species are Gram-negative pleomorphic rods that stain poorly with the Gram stain but better with the Gimenez stain (66). *Bartonella* species are fastidious, relatively slow-growing bacteria with a requirement for heme. This growth requirement is met by growth supplements, including hemoglobin, erythrocytes, or hemin added to agar bases such as heart infusion agar, Columbia agar, brucella agar, or Trypticase soy agar. Additional supplements such as IsoVitaleX are used by some laboratories. *Bartonella* species grow best at 35 to 37°C with 5% supplemental CO_2, with the exception of *B. bacilliformis*, which grows best at 28°C in the absence of supplemental CO_2. Liquid medium has more recently been shown to support the growth of *Bartonella* species and has proven useful in both clinical and research laboratories (67, 68). Unique protein profiles, fatty acid composition, enzymatic activities, restriction fragment length polymorphisms, and PCR with and without DNA sequencing are all techniques used to identify *Bartonella* to the species level and are presented in depth in other reviews (66, 69).

Some *Bartonella* species (*B. bacilliformis*, *B. clarridgeiae*, and *B. rochalimae*) possess flagella. All *Bartonella* species, except *B. bacilliformis*, are thought to have a VirB/VirD4 type IV secretion system (T4SS), and most are thought to have a surface-localized Trw T4SS. It has been hypothesized that the presence and functions of flagella and the Trw T4SS are mutually exclusive (70). All *Bartonella* species possess surface appendages that were initially described as type IV pili (71) but later shown to be comprised of trimeric autotransporter adhesins (TAAs) (72). The size of the appendages and the molecular mass of native TAAs have been shown to vary with species and are thought to be over 1 million Da for the protein trimer of the Brp TAA homologue in *B. vinsonii* (73). Expression of TAA genes is also highly variable among different species, within strains of the same species (72), and even under different conditions (74). The expression of TAA genes correlates with autoaggregation and has also been recently shown to play an important role in biofilm formation by *B. henselae* (75). It is well known that these autoaggregative properties are more apparent in recent low-passage-number isolates. The initial report of the isolation of *B. henselae* Houston-1 noted adherent colony morphology of the primary isolate (Fig. 2), which was lost upon serial subculture, resulting in more rapidly growing bacteria (31, 76). Spontaneous mutants lacking expression of the TAA gene in *B. henselae* have been reported (77) (see Pathogenesis of *Bartonella* Species), but it is not clear if this conversion from the highly autoaggregative phenotype to the nonaggregative phenotype occurs in nature or if it is only a result of laboratory growth and passage.

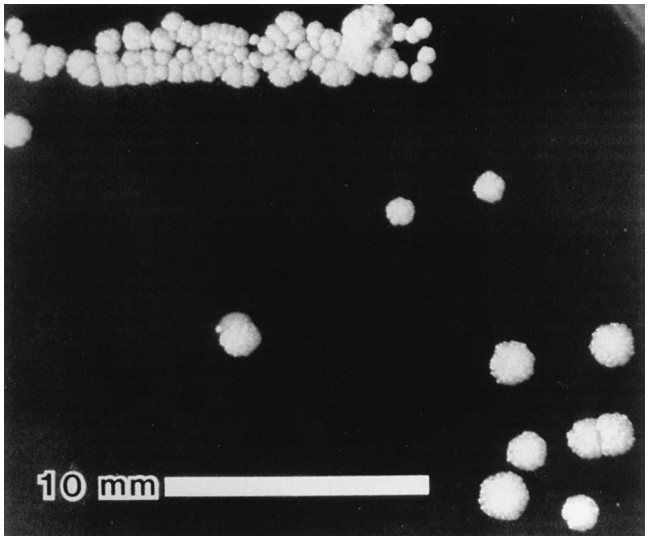

FIG 2 Colony morphology of low-passage-number Houston-1 type strain of *B. henselae* (ATCC 49882). A highly adherent colony phenotype was observed in this isolate which has subsequently been attributed to expression of *badA*. (Reproduced from reference 31.)

Genetics and Genome Organization

Bartonella species possess a single circular chromosome that varies in size from 1.45 Mbp for *B. bacilliformis* to 2.64 Mbp for *B. tribocorum* (36, 66). Based on the *Bartonella* genomes sequenced to date, the genome size loosely correlates with host specificity, with rodent-associated species having larger genomes and the human-specific species *B. bacilliformis* having the smallest. Rodent-associated *Bartonella* species show more evidence of horizontal gene transfer and gene duplication than do the human-specific *Bartonella* species. Of particular relevance to this review, it appears that the genomes of the rodent-associated species harbor more host adaptability factor genes such as the T4SSs as well as TAA, transporter, and adhesin genes than do the human-restricted species (78).

Some *Bartonella* species, including *B. grahamii* and *B. tribocorum*, possess plasmids (36, 78). The functions of plasmid-borne genes in these species are not well characterized but include putative small regulatory RNAs (75). Additional episomal DNA elements are the linear DNA fragments of a uniform 14-kb size that were observed in the cells of *B. henselae* (79). These linear fragments of DNA were shown to be a result of random packaging of genomic DNA into 40- to 50-nm icosahedral bacteriophage-like particles (BLPs) (Fig. 3) (79). A similar BLP was previously observed in *B. bacilliformis* and was described as a 40-nm icosahedral particle with a 16-nm tail (80). Subsequently, the *B. bacilliformis* BLP was shown to consist of specific proteins but nonselectively packaged genomic DNA (81). Initial experiments to demonstrate gene transfer by the BLPs of both *B. henselae* and *B. bacilliformis* were not successful (79, 81). The genes directing synthesis of components of the *Bartonella* bacteriophage-like particles have not definitively been identified; however, integrated in most of the *Bartonella* genomes sequenced to date are genes annotated as phage-related genes or prophage genes (36, 37, 78, 82–84). The BLP has also been described in *B. grahamii*, and it has been proposed as a gene transfer agent with successful *in vitro* particle-mediated transfer of genes being reported (37, 78). It has been further proposed that *Bartonella* BLPs have properties that are intermediate between those of gene transfer agents and transducing bacteriophages (85). Regardless, the function of these novel particles in packaging and exporting genomic DNA provides evidence that they play an active role in horizontal gene transfer and the evolution of *Bartonella* species.

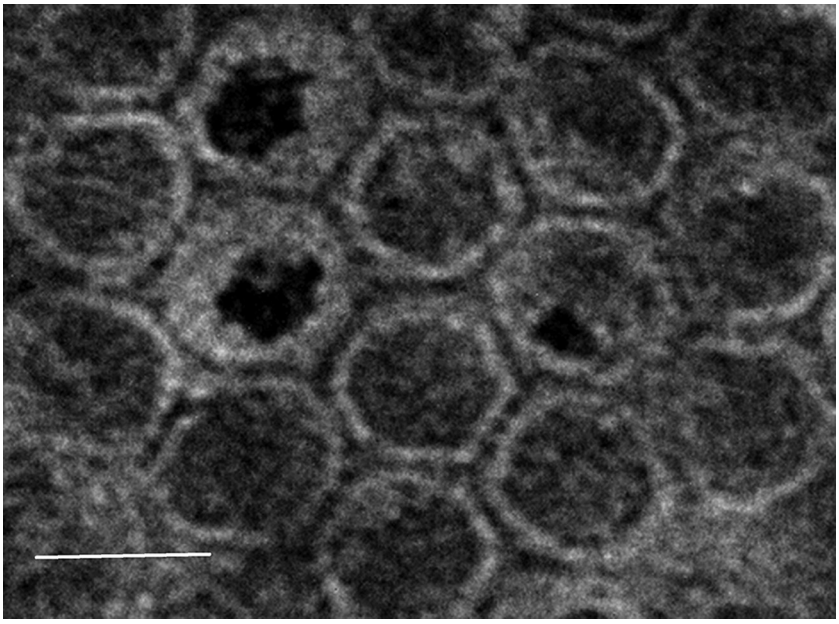

FIG 3 Transmission electron micrograph of the bacteriophage-like particles of *B. henselae* stained with uranyl acetate. White bar, 50 nm.

LABORATORY DIAGNOSIS OF INFECTIONS CAUSED BY *BARTONELLA*

Isolation and Culture

B. henselae and most likely the other newly recognized *Bartonella* species associated with human disease avoided detection by routine blood culture methods for many years before it was appreciated that primary isolation requires specific growth medium and extended incubation times. Endocarditis has traditionally been diagnosed based on a positive blood culture. However, blood culture methods have sensitivity as low as 20% for diagnosing *Bartonella* endocarditis, while tissue culture of surgically excised valves has a similarly low sensitivity of 30% (86). In general, direct plating of blood or tissue homogenates is preferable, and several different agar base formulas, including heart infusion, Trypticase soy, brucella agar, and Columbia agar supplemented with 5% rabbit blood or 5% hemoglobin, have been used successfully for primary isolation (66). For primary isolation, extended incubation times of up to 21 days may be required (33). Plates for primary isolation are incubated in 5% CO_2 at 35 to 37°C, except for the isolation of *B. bacilliformis*, which prefers ambient CO_2 and temperatures of 26 to 28°C. It has been reported that the lysis centrifugation method for sample preparation increases isolate recovery (29). Successful isolation of *Bartonella* species from automated blood culture systems and liquid culture media has also been reported (67, 87), as has isolation using cell culture systems (33).

Special Stains

Histopathology of valve tissue stained with hematoxylin-eosin typically reveals marked inflammation, fibrosis, and calcification compared to endocarditis not caused by *Bartonella* (88). Warthin-Starry silver staining has been a frequently employed method for the detection of *Bartonella* species and reveals small, dark-staining bacteria in the fibrotic areas of the affected valve in over 75% of *Bartonella* endocarditis cases (89), but this stain is not specific for *Bartonella* species. Other special stains include Giemsa and Gimenez stains, which can be used on valvular tissue for initial diagnosis, but these techniques are also not specific for *Bartonella* species (66). Acridine orange has also been used to nonspecifically detect *Bartonella* species in culture (90). Immunohistochemical staining of affected tissues has employed both monoclonal and polyclonal antibodies with various degrees of success and affords higher specificity

than Warthin-Starry staining (91). Regardless, due to the low yield of blood culture and lack of specificity of specialized stains, current diagnosis of *Bartonella* endocarditis relies heavily on serology and/or molecular testing of blood or valvular tissue specimens.

Serology

Serology has played a critical role in diagnosis of *Bartonella* infections and, in fact, was crucial in the establishment of *B. henselae* as the etiologic agent of CSD (34). That initial serologic assay used *B. henselae* cocultivated with Vero cells as an antigen in an indirect fluorescent antibody assay (IFA) to test for IgM and/or IgG antibodies in the patient's serum. A positive titer was considered >16 for IgM and >64 for IgG, with a 4-fold rise in titer for IgG between acute-phase serum and convalescent-phase serum samples (collected at least 2 weeks apart) preferable for definitive diagnosis (34, 92). Further reports of *Bartonella* species cultivated in the absence of host cells or prepared using other approaches resulted in wide variations in both specificity and sensitivity (93, 94). It is very likely that the highly autoaggregative nature of most *Bartonella* species, subsequently attributed to expression of the TAAs on the surface of the bacterium, contributed to this variation, since the TAAs are antigenic and recognized by serum from patients infected with *Bartonella* species (77, 95). Additionally, the variable expression of the TAA genes in different *B. henselae* isolates may have further contributed to this problem (72). The IFA is not regarded as species specific, and there is considerable cross-reactivity between *B. henselae* and *B. quintana* and perhaps other *Bartonella* species as well (96). A similar IFA was used to study 22 cases of BCNE, and a positive predictive value of close to 90% was reported, but very high antibody titers (>1,600) were found in these patients, perhaps due to their subacute or chronic infection with *Bartonella* species (97).

Serologic assays employing enzyme-linked-immunosorbent-assay (ELISA)-based approaches have also been used for several years, but similar problems have been reported for these assays as well, including cross-reactivity and lack of specificity (98). One approach to serologic diagnosis to reduce cross-reactivity is to substitute specific antigens for whole bacterial antigens. An example is the use of the VirB5 17-kDa antigen recombinant protein expressed in *Escherichia coli*. In that case, the 17-kDa recombinant protein was examined in Western blot assays and shown to have reactivity with human sera from patients with CSD, very similar to the IFA (99). The recombinant 17-kDa antigen has subsequently been adapted to an ELISA-based assay to detect IgG antibody against *B. henselae* (100) and for an IgM capture assay where high sensitivity and specificity were reported (101). Another approach to serologic diagnosis is to employ subcellular fractions as an antigen in an ELISA to detect IgG for the diagnosis of CSD (102). Cross-adsorption and Western immunoblotting techniques also have been reported with high specificity and sensitivity in detecting *Bartonella* endocarditis; the cross-adsorption technique was used to overcome false positivity from cross-reactivity with other bacterial species, especially with *Chlamydia* species, which could also be a causative agent of endocarditis (86, 103–106).

Molecular Tools for Detection of *Bartonella*

PCR is not only one of the mainstays for diagnosing *Bartonella* infections, but it has played a critical role in fulfilling molecular Koch's postulates to associate *Bartonella* with new disease syndromes. The detection of *Bartonella* 16S rRNA gene sequences in the lesions of patients with bacillary angiomatosis provided the first link of *Bartonella* with this condition (32). Similarly, the detection of *B. henselae* 16S rRNA gene sequences in skin test antigens used to diagnose CSD helped resolve a longstanding mystery about the etiology of CSD (35). PCR alone or coupled with restriction fragment digestion to detect polymorphisms, enrichment broth culture, or DNA sequencing has all been used to identify *Bartonella* isolates (see reference 89 for a recent review). Many different primer pairs and techniques have been described to detect *Bartonella* DNA in clinical specimens by PCR (see reference 89 for a recent review). When specifically applied to *Bartonella* endocarditis, amplification of *Bartonella* DNA from valvular tissue by PCR has

been shown in multiple case series to have higher sensitivity and specificity, ranging from 72 to 98% (86, 97, 107, 108). PCR testing can also be performed on whole blood, plasma, or serum samples, with studies reporting sensitivity of 58% and specificity of 100% (109). For a recent review of laboratory diagnostic procedures for *Bartonella* species, see the work of Gutierrez et al. (69).

EPIDEMIOLOGY OF *BARTONELLA* INFECTIONS

Natural Reservoirs and Arthropod Vectors

Bartonella species have been isolated or detected in a wide range of animal species, including terrestrial animals, rodents, bats, and marine animals, such as beluga whales and sea turtles (8, 110). In most cases, the presence of *Bartonella* species in the blood of these infected animal reservoirs does not result in serious disease. Thus, this vast range of infected animals serves as a ubiquitous reservoir for potential zoonotic infection. Only two species of *Bartonella*, *B. bacilliformis* and *B. quintana*, are known to infect humans as their reservoir host. These two species, together with *B. henselae*, cause the vast majority of human disease attributed to *Bartonella* species (111).

There are several examples illustrating how different *Bartonella* species have evolved with their mammalian hosts. This would include *B. henselae* with cats, *B. vinsonii* subsp. *berkhoffii* with dogs, *B. bovis* in cows, *B. melophagi* in sheep, and *B. australis* with kangaroos in Australia (112). However, the list of rodent-adapted *Bartonella* species is growing exponentially, as exemplified by species that have evolved with multiple types of squirrels: ground squirrels (*B. washoensis*), gray squirrels ("*Candidatus* Bartonella durdenii"), flying squirrels ("*Candidatus* Bartonella volans"), and even groundhogs ("*Candidatus* Bartonella monaxi") (113, 114). Most recently, bats have been identified as the reservoirs of diverse and novel species of *Bartonella*. In addition to infected bats in eastern Africa (Kenya) and Guatemala, Peru has a similar overall prevalence (24.1%) but presents a greater variety of prevalence by species: for example, more than half of the population of common vampire bats in Peru is infected (113). The high prevalence is most likely due to the relatively long lifespan of bats, an average of 10 to 20 years. There is a theoretical possibility, yet to be confirmed, that transmission of *Bartonella* species, in addition to transmission via ectoparasites, may occur via direct bat bite, as is the case with rabies transmission. Because some bat species (especially *Carollia* and *Glossophaga* bats) share roosts with other species, there is a potential for both intra- and interspecies transmission of infections. Bats are frequent hosts to a wide variety of ectoparasites such as fleas, bat flies, soft ticks, and mites (115). Studies from Egypt and the United States have shown that arthropods have a role as vectors of *Bartonella* species to other wildlife, with humans being incidental hosts (116, 117).

Bartonella species have been labeled as emerging pathogens, and yet they have been detected in the dental pulp of humans dating back to antiquity (118). *Bartonella* was first recognized as an agent of endocarditis in 1993 (11). Transmission of *Bartonella* species may be via arthropod vectors or direct inoculation, depending on which species is involved. In the case of *B. bacilliformis*, disease incidence follows geographic boundaries that are limited by the distribution of its vector, the sandfly (*Lutzomyia verrucarum*), to 1 to 3 km of altitude in the Andes Mountains in Peru. Its presence in Ecuador and Colombia supports an argument for yet another vector or mode of transmission (119). While *L. verrucarum* is its most important arthropod vector, other phlebotomine sandflies—*L. maranonensis* and *L. robusta*—may serve as vectors in areas devoid of *L. verrucarum* (120). Humans are the only established reservoir of *B. bacilliformis*, and several reports examining nonhuman reservoirs, including plants (121), rodents (122, 123), and domesticated animals (124), were inconclusive. Infection with *B. bacilliformis* is biphasic, with both an acute hemolytic anemia (Oroya fever) and a chronic form with vascular proliferative lesions (verruga peruana) (125).

B. henselae is endemic worldwide, and transmission to humans has been linked to cats by both serology and epidemiologic studies (34, 92, 98, 126). Healthy cats bacteremic with *B. henselae* are associated with bacillary angiomatosis and CSD in their human contacts. The major vector of transmission between cats is the cat flea (*Cteno-*

cephalides felis) (127, 128), with about 50% of cats bearing signs of previous or current infection (129). Cat-to-cat transmission of the organism by the cat flea, with no direct contact transmission, has been demonstrated (130). *B. henselae* has been experimentally detected in oral swabs from infected cats (131, 132) and is able to replicate in the gut of the cat flea, is shed in the feces, and is also shown to live in flea feces for up to 3 days postexposure (133–135). Possible mechanisms of cat-to-cat transmission include flea bite and ingestion of fleas and flea feces (136). Of note, asymptomatic but bacteremic cats are more likely to harbor fleas, therefore leading to persistence in the flea vector. *Bartonella* species has been detected in other types of fleas, as well as ixodid and *Dermacentor* ticks (109, 137, 138), but no definitive transmission studies have shown that these vectors transmit *B. henselae* (139). No flea-to-human transmission has been identified as of yet (127, 131, 140). Rather, transmission of *B. henselae* occurs indirectly, primarily by contaminated flea feces that are inoculated by a cat scratch (141) and rarely through a cat bite (131). *Bartonella clarridgeiae* and *B. koehlerae* are widespread in cats but are uncommon causes of illness in humans (142–144). *B. elizabethae* has been isolated in humans, while its DNA has been amplified from the blood of dogs (145). The majority of CSD cases occur in children aged 5 to 9 years and those living in the southern United States. The estimates show 22,000 diagnoses of CSD each year in the United States with about 2,000 hospitalizations (146). The incidence of CSD varies by season, with most cases occurring during the fall and early-winter months, September through January. Some attribute the seasonal prevalence to the breeding patterns of cats, peak time of domestic cat adoptions, and the temporal presence of fleas on cats, which spread the bacteria among the cat population (146).

Unlike *B. bacilliformis*, *B. quintana* (trench fever) has worldwide distribution often associated with war zones and poor sanitation predisposing to infestation with the human body louse (*Pediculus humanus*), the only known vector for transmission (147). The disease presents with fever, rash, bone pain, and splenomegaly lasting for about 4 to 5 days, thus its name of quintan or 5-day fever. On rare occasions, the symptoms persist or recur as multiple paroxysms. World War I brought this relatively rare disease to light, since about 1 million troops were thought to have been infected. Now, the disease is seen mostly in alcoholic and homeless populations and has been dubbed "urban trench fever" (148, 149). The 1990s and the HIV epidemic brought the resurgence of the disease presenting with fever and bacteremia with and without endocarditis (107). Although the human body louse *P. humanus* is the main vector for its transmission, *B. quintana* has been detected in cat fleas, monkey fleas, and cat dental pulp, suggesting potential methods of transmission other than infestation and bite by the body louse (150, 151). *B. quintana* is transmitted by lice through its feces, and its mode of transmission is well researched (152). Much like *B. henselae*, it is also found to replicate in the gut of the vector (134). In a state of prolonged bacteremia, it is found in the erythrocytes (153), and nonhemolytic intracellular colonization of erythrocytes preserves the pathogen for efficient transmission by lice while protecting it from the host immune response and decreasing antimicrobial efficacy (153). It is also proposed that *B. quintana* could present a risk in blood transfusion, since undetected bacteria could be present in erythrocytes of blood donors (153). Recently, *Bartonella* species were detected in 3.2% of asymptomatic blood donors from Brazil (154). In its classic form of causing endocarditis, *B. quintana* multiplies in the louse intestine and is excreted in the louse's feces and deposited on human skin. Entry across the skin occurs when a pruritic area of the skin is scratched and abraded (155).

Epidemiology of *Bartonella* Endocarditis

B. henselae, when combined with *B. quintana*, accounts for over 90% of *Bartonella* endocarditis cases (107). *Bartonella* species in general have wide geographic distribution, possibly due to the geographic specificity of their respective hosts and vectors (156–162). For example, DNA of several species of *Bartonella* was isolated from bat flies and bats in Africa, Asia, Europe, and both North and South America (163). *B. henselae* and *B. quintana*, the two species most commonly associated with endocarditis, are also

known to occur worldwide (58, 164–166). While the majority of cases of *Bartonella* endocarditis reported are from Europe and the Americas, cases have also been reported from Asia, East and West Africa, and Australia, suggesting worldwide distribution of *Bartonella* endocarditis (167–171). There is a clear preponderance of the male gender in the reported *Bartonella* endocarditis cases, with males accounting for 60 to 85% of cases (97, 107, 172). It is not clear whether there is a biologic basis for this difference other than the difference in demographic factors (such as homelessness or alcohol abuse) that exists between the genders. *B. quintana* endocarditis was originally reported in a patient with HIV; however, subsequent reports have shown that *B. quintana* endocarditis occurs in people without known immunodeficiency (11, 13). Some of the most frequently recurring epidemiologic associations with *B. quintana* endocarditis were homelessness, alcoholism, and exposure to body lice. These epidemiologic associations are likely to be interrelated and probably are surrogate markers for low socioeconomic status rather than each factor being individually associated with the risk of *B. quintana* endocarditis. However, interestingly the majority of these patients with *B. quintana* endocarditis did not have previously known valvular diseases, which would be expected in people of low socioeconomic status. *B. henselae* endocarditis accounted for about 25% of all *Bartonella* endocarditis and usually occurred in people who had previous valve diseases and had a history of exposure to cats or cat fleas (107, 173, 174). *B. henselae* also has been implicated as a coinfecting agent in a patient with other bacterial etiologies such as staphylococcal endocarditis (173). Even though this was a single case report, the possibility that the virulence and pathogenic features of *Bartonella*, such as endothelial proliferation, may prime the valve for subsequent infection by other, more commonly encountered bacteria such as staphylococci is an area for future investigation. Patients with *Bartonella* endocarditis generally tend to have a lower average age than patients with other types of bacterial endocarditis, with observed geographic variability in age possibly reflecting regional differences in demographics and socioeconomic status (175–177).

BARTONELLA AND DISEASE

Disease Syndromes Associated with *Bartonella* Infection

B. quintana was identified in human dental tissue dating as far back as 4,000 years, and relics of the Inca empire depict features of the disease verruca peruana, which is now known to be caused by *B. bacilliforms* (118, 178). However, until only a few decades ago only one other human disease; namely, trench fever, was attributed to bacteria in the current genus *Bartonella*. Since the early 1990s, several species and subspecies of *Bartonella* have been characterized, and the spectrum of natural reservoirs, vectors, and human diseases caused by *Bartonella* species has significantly expanded (179–181). Below is a brief description of some of the major human diseases caused by *Bartonella* species. Even though these diseases may be distinct from endocarditis in some ways, it is increasingly evident now that many of them are accompanied by intraerythrocytic (bacteremic) phases which may lead to endocarditis (182).

Carrion's disease. Carrion's disease (bartonellosis) is caused by *B. bacilliforms* and transmitted by sandflies of the species *L. verrucarum*. It is endemic in higher-altitude areas of Peru, Colombia, and Ecuador, but sporadic cases have been reported in people returning from visits to areas of endemicity (57). The classic manifestation of Carrion's disease is described as having two phases, the first an acute febrile illness (sometimes known as Oroya fever) characterized by fever, generalized lymphadenopathy, myalgia, headache, jaundice, and severe hemolytic anemia with fatality rates of as high as 90%. Meningeal and cerebral involvement can occur in up to 20% of patients with Carrion's disease and manifests as delirium, paralysis, and seizures. A subsequent chronic and cutaneous eruptive phase of the disease is characterized by development of verrucous dermal eruptions that result from proliferation of vascular endothelial cells (183, 184).

Trench fever. Trench fever is so named because it classically occurred in the trenches of World War I among various European armies' troops but is also linked with a more recent epidemic that has been reported among homeless people in impover-

ished urban areas of several countries (148, 185). Trench fever is caused by *B. quintana* and is transmitted person to person by the body louse, *P. humanus*. It is characterized by a sudden onset of high-grade fever, retro-orbital headache, myalgia, and bone pain, especially over the pretibial area. It is classically described as a 5-day relapsing fever, hence its other name, febris quintana, from the Latin for five. The disease can take a chronic course and last several weeks, during which bacteremia is common and cardiac involvement may complicate the course with insidious onset of endocarditis (186).

CSD. Cat scratch disease (CSD) is caused primarily by *B. henselae* and is transmitted by the scratch or, less likely, by the bite or lick of cats (150). Other *Bartonella* species have been implicated in CSD-like disease in individual case reports (44, 53, 187). Even though more than 50% of domestic cats may be carriers of *Bartonella* as shown in some studies, they usually do not show signs or symptoms of infection (140, 188, 189). *B. henselae* has also been isolated in fleas recovered from infected cats (140). The classic description of CSD involves a scratch by a cat followed by local inflammation 10 to 14 days later and significant enlargement of regional lymph nodes. Systemic symptoms such as fever and malaise typically develop and could last for several weeks. Most cases run a benign course and resolve spontaneously, although serious complications such as meningitis, osteomyelitis, encephalitis, and endocarditis are known to occur (190, 191). Oculoglandular syndrome (also known as Parinaud's oculoglandular syndrome) is an ocular manifestation of cat scratch disease of granulomatous conjunctivitis with pre- and postauricular lymphadenopathy; a recent case report also highlights an expanding spectrum of ocular involvement by CSD presenting as an optic nerve granuloma (192, 193). Transmission between cats is mainly by the cat flea, although other arthropods, mainly ticks of the genus *Ixodes*, have been proposed as possible vectors (194).

BA. Bacillary angiomatosis (BA) is a proliferative disease of vascular epithelia manifesting as a solitary or multiple papulonodular cutaneous lesions. The etiologic agents are both *B. henselae* and *B. quintana* (32, 33, 195). BA was originally described in patients with HIV and other immunocompromised status such as organ transplant recipients on immunosuppressive therapy; however, it has since been described in immunocompetent hosts (196). The cutaneous lesions are highly vascular, bruising or bleeding easily, due to the underlying effect of these species of *Bartonella* to cause abnormal vascular endothelial cell proliferation and neovascularization (183, 197). The skin lesions could be superficial or deep in the subdermal structures, at times even involving the bones. Regional lymphadenopathy and involvement of mucous membranes of the mouth, conjunctivae, and the gastrointestinal tract, including the perianal area, have also been described. Visceral involvement is also known to occur involving the liver, spleen, lymph nodes, and the bone marrow, with other reports documenting isolated visceral involvement in the absence of cutaneous lesions (198, 199).

Peliosis hepatis. Peliosis hepatis is characterized by multiple vascular, hemorrhagic parenchymatous and cystic lesions of the liver ranging from a few millimeters to 3 cm in size. Peliosis was originally described in a case report associated with tuberculosis in 1916 (200). Several subsequent reports have shown association with many other pathological conditions, including multiple infectious and noninfectious diseases such as neoplastic processes and exposure to toxins and anabolic steroids (201, 202). In more recent years, peliosis has been distinctly associated with HIV infection (203, 204). Histologically, the lesions of peliosis show dilated capillaries, vascular hyperplasia, and inflammatory cells much as in the case of BA (30, 198, 205). *B. henselae* is the species most often associated with peliosis hepatis, and affected patients usually present with abdominal pain, fever, and weight loss. Hepatomegaly is usually present, and some patients may have concomitant cutaneous lesions of BA (206).

***Bartonella*-related mimics of autoimmune disease.** Juvenile arthritis and myositis associated with high serum titers for *B. henselae* that rise and fall with disease activity have been reported in children (207, 208). Although causal association has not been proven, increased rates of seropositivity for *B. henselae* have been described in patients with leukocytoclastic vasculitis and Henoch-Schönlein purpura (209, 423) as well as in a case of Coombs-positive autoimmune hemolytic anemia (210). Cases of uveitis

associated with HLA-B27 seropositivity have also been described in *B. henselae*-infected patients presumed to have ocular involvement by *Bartonella* (211). It should be noted that the association of *Bartonella* species with autoimmune disorders has been largely limited to serology-based testing and could be compromised by cross-reacting antibodies.

***Bartonella* as a risk factor for atherosclerosis.** Several infectious agents, including *Chlamydophila pneumoniae*, *Helicobacter pylori*, cytomegalovirus, and periodontal pathogens, have been reported to contribute to atherosclerotic vascular disease (see reference 212 for a review). Antimicrobial activity of statins has been reported, and it has been suggested that this activity may, at least in part, be responsible for the reduction in cardiovascular mortality associated with the use of these drugs (213). However, antibiotic treatments have largely failed to significantly reduce cardiovascular mortality (214). *B. henselae* has been shown to infect human $CD34^+$ hematopoietic progenitor cells, and it was proposed that these cells may serve as the primary niche of infections (215). A subsequent report showed that endothelial progenitor cells were infected with *B. henselae*, resulting in damage counteracted by nitric oxide as demonstrated by the administration of L-arginine (216). It has been proposed that *B. henselae* infection of endothelial progenitor cells could reduce both the number of these cells and their functionality (216). In so doing, the natural repair role for endothelial progenitor cells would be diminished, thereby indirectly contributing to the growth of atherosclerotic plaque (217). Further study is needed to support the role of the interaction of *Bartonella* with both endothelial progenitor cells and endothelial cells. The association of *Bartonella* species with the onset and progression of atherosclerosis remains speculative at this point.

Myocarditis. Myocarditis associated with both *B. henselae* and *B. quintana* infection has been reported (218–220). While myocarditis is not a common clinical presentation of *Bartonella* infection, it warrants mention for the severe disease course reported to manifest in one specific affected group. During the period of 1979 to 1992, 16 sudden, unexplained cardiac deaths were reported in elite Swedish orienteers; orienteering is a popular activity in Sweden with extreme physical demands, extended outdoor exposure, and interaction with nature (221). Of the 16 fatal cases, myocarditis was the most common diagnosis; heart tissue from five orienteers was tested by PCR, and *Bartonella* species was detected in four (221). Additionally, four of the five patients' sera were tested and shown to have antibodies to *Bartonella* species (221). A subsequent retrospective study on 1,136 sera from orienteers showed that 31% had antibodies to *Bartonella* compared to 6.8% in time-matched healthy blood donor control sera (222). The authors of that study concluded that antibodies to *Bartonella* species in Swedish orienteers may be indicative of risk factors associated with the development of myocarditis and sudden unexpected cardiac death in these athletes who were previously in good health (221, 222).

Bacteremia. Invasion of erythrocytes and prolonged intraerythrocytic presence in their respective reservoir hosts (intraerythrocytic bacteremia) are one of the hallmarks of inoculation of a reservoir host by *Bartonella* species (223, 224). *Bartonella* species have been shown to employ several molecular mechanisms as a basis for invasion of erythrocytes and evasion of the immune responses that would otherwise typically trigger symptoms in bacteremic hosts (225–228). Such asymptomatic bacteremia has been reported in various nonmammalian and mammalian hosts, including humans (8, 110, 229, 230). Asymptomatic bacteremia has been shown to persist from a few weeks to several months in studies conducted in healthy human volunteers (231). In humans, a small proportion of the erythrocytes, usually no more than 1%, is infected by *B. quintana*, and such low-level bacteremia can persist for months to years with no or only subclinical symptoms (153, 224, 232). In a rat model of infection with *B. tribocorum*, B-cell-deficient rats had a more prolonged bacteremia than immunocompetent rats (232, 233). It is plausible that low-level bacteremia may precede the pathogenesis and eventual clinical development of endocarditis in humans; however, this has not been epidemiologically or experimentally confirmed (234).

BCNE. Blood-culture negative endocarditis (BCNE) is generally defined as endocarditis where the microbial etiology cannot be established after at least three different blood samples in a standard blood culture system fail to grow an organism after at least 5 days of incubation (235). The incidence of reported BCNE varies dramatically, ranging from 2.5% to 76% of all infective endocarditis cases (236–238). One of the reasons for such broad variability of the incidence of BCNE is the geographic variation in the reported rates of culture-negative endocarditis, probably reflecting the differences in availability of testing alternatives. For instance, reports from South Africa, Algeria, and Pakistan put the rates of culture negativity at about 50% of all endocarditis cases (238–240), whereas case series from Japan, France, and the United Kingdom report BCNE rates of 12 to 20% (235, 241, 242). In addition to geographic variations, earlier reports may have overestimated the incidence (of culture negativity) partly because of the limited testing capability in the past, even in developed nations, with ever-expanding contemporary options for serologic and molecular testing. It is now estimated that BCNE accounts for around 5% of all endocarditis cases (236, 241, 243–245). There are various reasons why a blood culture may be negative in the face of endocarditis (hence presumed bacteremia). The main reasons for culture negativity are pretreatment with antibiotics prior to the blood culture; nonbacterial etiologies for the endocarditis (such as fungal etiology, for instance); right-sided endocarditis, which may not be as bacteremic in systemic circulation; and the presence of implanted devices such as pacemakers or implantable defibrillators (246–248). The microbial etiologic agent of the endocarditis being fastidious and thus not easily grown on standard culture medium is one of the most important reasons why blood culture may be negative in the case of endocarditis (86, 177, 249). *Bartonella* is also thought to account for 3 to 4% of all cases of endocarditis (97, 177). While *B. quintana* and *B. henselae* account for the majority of cases of endocarditis, several other species of *Bartonella* have been shown to cause endocarditis, as shown in Table 1 (18, 86, 250, 251).

The majority of patients with *Bartonella* endocarditis have clinical presentations similar to other cases of subacute bacterial endocarditis. Nonspecific symptoms such as fever, fatigue, and weight loss predominate in the clinical picture. In one series of 348 cases of BCNE from France, *Bartonella* species accounted for 28% of the cases (86). Almost all of the patients had fever as a presenting symptom, whereas about 50 to 70% had symptoms of heart failure such as exertional dyspnea and about 50% had insidious weight loss (86, 252–254). Physical examination findings typically include cardiac murmur, and the aortic valve either in isolation or with another valve is the most frequently affected valve, including in the pediatric age group (255–258). In a detailed report, a 33-year-old man with a known bicuspid aortic valve was shown to have BCNE confirmed by echocardiography that was shown to be caused by *B. henselae* (258) (Fig. 4).

While the majority of the valves affected were native valves, prosthetic valve involvement has been reported, and prosthetic valve involvement by *Bartonella* seems to take a more aggressive course with valve perforation and rapid development to heart failure (12, 259, 260). Additional physical exam findings include splenomegaly, which has been reported in up to 40% of *Bartonella* endocarditis cases in one series; thromboembolic phenomena; digital clubbing; and hepatomegaly (86, 175, 177). The most commonly observed laboratory abnormalities include elevated inflammatory markers such as erythrocyte sedimentation rate (76 to 83%), anemia (55 to 68%), thrombocytopenia (33 to 50%), elevated liver enzymes (20%), evidence of renal failure (40 to 50%), leukocytosis, and positive rheumatoid factor (86, 175, 177). Earlier reports showed *Bartonella* endocarditis with significant mortality rates of 7 to 30%; however, more recent studies report mortality rates in the lower range, probably signifying improved diagnostic and therapeutic measures, including improved surgical techniques (255, 261, 262).

Currently, there is a lack of criteria for the diagnosis of endocarditis caused by *Bartonella* species (177). The use of traditional blood culture methods is hampered by the low rate of culture positivity and the need for prolonged incubation time, use of

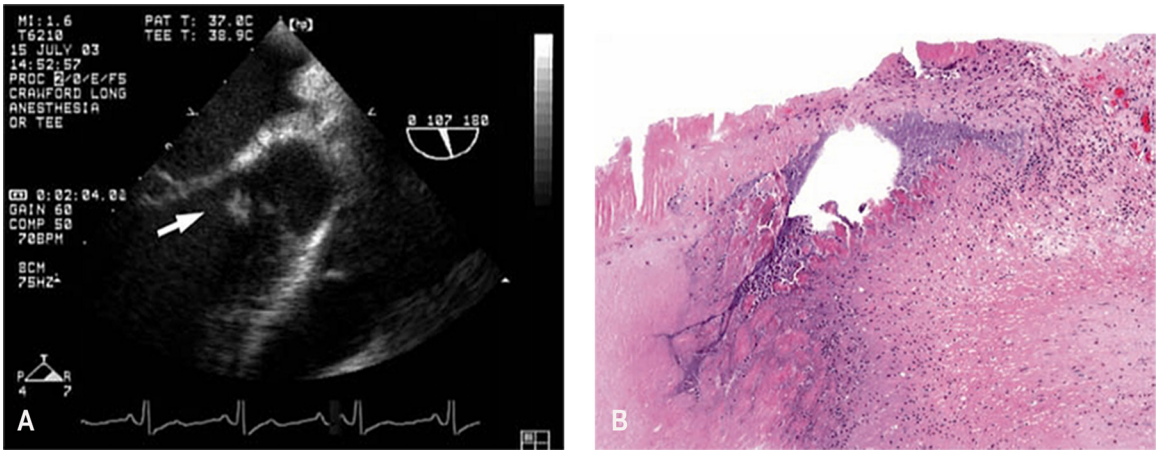

FIG 4 (A) Transesophageal echocardiogram from a patient with BCNE caused by *B. henselae*. Bicuspid aortic valve with left coronary leaflet almost entirely replaced by a large vegetation (arrow). (B) Giemsa stain of the patient in panel A showing extensive fibrosis and coccobacilli on the aortic valve that were confirmed to be *B. henselae*. (Both panels reproduced from reference 258 with permission from Elsevier.)

specialized media, and special growth conditions. Despite measures to optimize the yield, blood cultures are likely to remain negative, with some estimates showing that up to 75% of *Bartonella* endocarditis cases could be culture negative (90, 97, 263). Therefore, diagnostic criteria that rely on blood culture positivity, such as the Duke criteria, are likely to miss a significant proportion of *Bartonella* endocarditis cases. Moreover, due to the indolent nature of *Bartonella* infections, the other major Duke criterion of echocardiographic evidence of vegetation may not be as readily apparent as it is for the other types of endocarditis. Thus, the utility of the Duke criteria to diagnose *Bartonella* endocarditis using blood culture and echocardiographic evidence as major criteria has been in question (264). Serologic testing for IgG antibodies to either *B. henselae* or *B. quintana* using microimmunofluorescence techniques has been used for the diagnosis of *Bartonella* endocarditis in several studies. A *Bartonella* IgG titer of $\geq$1:800 is recommended as the cutoff for a positive test, offering high sensitivity, specificity, and positive predictive value (177, 265, 266). Likewise, PCR testing of blood, plasma, or serum samples taken from confirmed cases of *Bartonella* endocarditis was shown to have a sensitivity of about 58% and specificity of 100% (267). The use of serology and PCR testing has been proposed and eventually added as major Duke criteria for *Coxiella burnetii* infection, which is epidemiologically and demographically closely related to *Bartonella* endocarditis (268, 269). Similarly, incorporating a positive *Bartonella* serology or PCR test as a major Duke criterion for the diagnosis of *Bartonella* endocarditis has been proposed (177, 241, 264, 265, 270–273). Thus, available evidence advocates including *B. henselae* or *B. quintana* IgG serology at $\geq$1:800 or a positive PCR as a major Duke criterion for the diagnosis of *Bartonella* endocarditis.

(i) *B. quintana* endocarditis. *B. quintana* accounts for about three-fourths of *Bartonella* endocarditis cases. After transmission by the human body louse *P. humanus*, bacterial entry across the skin occurs and adherence to and infection of erythrocytes and endothelial cells ensue. The resulting bacteremia can persist for prolonged periods, at times lasting for years. *B. quintana* has evolved with variably expressed outer membrane proteins (Vomps) that are believed to be essential for infectivity as well as evasion of detection by the host immune system (74, 274). *B. quintana* has also been shown to induce intracellular signals that lead to decreased apoptosis and increased proliferation of vascular endothelial cells (see Pathogenesis of *Bartonella* Species), attributes believed to enhance its capacity to cause chronic infection and intracellular aggregation in endothelial cells, including valvular endothelium (275). Vascular and valvular endothelial cells are also targets of *B. quintana* colonization and production of cytokines and mitogenic factors, leading to endothelial proliferation and cytoskeletal rearrangement

(228, 276). Thus, *B. quintana* employs several strategies to evade host immunity and cause a prolonged and asymptomatic bacteremia that culminates in the development of endocarditis. These pathogenic mechanisms of *B. quintana* underlie the insidious clinical presentation and subtle variations in clinical findings of endocarditis caused by *B. quintana*.

(ii) *B. henselae* endocarditis. *B. henselae* accounts for about one-fourth of *Bartonella* endocarditis cases. The majority, but not all, of *B. henselae* endocarditis cases have a history of contact or interaction with a cat. *B. henselae* shows tropism for endothelial cells and replicates and persists in the endothelium similarly to *B. quintana*. Pathogenic mechanisms include *Bartonella* adhesin A (BadA) and the TAA, which is expressed in *B. henselae* as well as in *B. quintana* (70, 277, 278).

(iii) Treatment considerations for *Bartonella* endocarditis. By virtue of their pathogenic and virulence mechanisms, namely, intraerythrocytic propagation and ability to persist in a primary niche, *Bartonella* species are in general endowed with a substantial capacity to evade the host immune system and to resist antimicrobial agents (156, 181, 224, 279, 280). Earlier studies have reported widespread *in vitro* susceptibility of several *Bartonella* species to various classes of antibiotics, including penicillins, beta-lactams, macrolides, and aminoglycosides (280–282). However, subsequent studies and clinical experience have shown that treatment failures of *Bartonella* infections are a significant problem despite seemingly low MICs suggesting susceptibility (282–284). Moreover, several of the antimicrobial classes tested against *Bartonella* species exhibit only bacteriostatic properties, with the exception of aminoglycosides such as gentamicin (283, 285, 286). The issue of host defense evasion, potential biofilm formation, and hence resistance to antimicrobials is even more acutely important in cases of *Bartonella* endocarditis where cultures are likely to be negative and diagnosis delayed. Based on experience from the past 2 decades in the treatment of *Bartonella* endocarditis, multiple reports and recommendations advocate the use of at least two antibiotics, one of them being an aminoglycoside (172, 287, 288). The recommended duration of therapy is generally for a minimum of 4 weeks in native valve disease and a minimum of 6 weeks in prosthetic valve endocarditis. Aminoglycosides are recommended at least for the first 2 weeks of therapy, and the duration of combined aminoglycoside use and the total duration of therapy correlate with a beneficial clinical outcome (98). Thus, current recommendations for treatment of *Bartonella* endocarditis stress the use of an aminoglycoside at least for the first 2 weeks of therapy in conjunction with a two-drug regimen, the second drug being a beta-lactam, a macrolide, or a tetracycline depending on the specific concomitant clinical considerations (289, 290). The combination of gentamicin and doxycycline has been suggested in a recent report (291).

PATHOGENESIS OF *BARTONELLA*

Human Infection

Pathogen-associated molecular patterns (PAMPs) are molecules associated with specific pathogen groups that are recognized by cells of the innate immune system. To detect invading pathogens such as bacteria and viruses, the immune system is equipped with pattern recognition receptors that are specialized in their recognition, including the Toll-like receptor (TLR). Following intravenous or intradermal inoculation with *Bartonella*, the bacteria evade the host innate immune system. This has been attributed to the inability of the TLR to identify the lipopolysaccharide on the outer membrane of the bacteria as a result of the reduced endotoxic activity of the lipopolysaccharide of *Bartonella* (292). Moreover, *B. quintana* employs strategies to dampen the host inflammatory response through overproduction of the anti-inflammatory interleukin-10 (IL-10) and antagonizing proinflammatory factors such as Toll-like receptor 4 (293, 294). *B. bacilliformis*, which possesses flagella, structures that are recognized by the TLR, is also known to evade the innate immune system because of a primary amino acid sequence change in the flagellin which promotes its evasion (295). After innate immune system evasion, the bacteria are cleared from the circulating bloodstream for their primary niche, most likely the endothelium, where they grow and seed back to reinfect

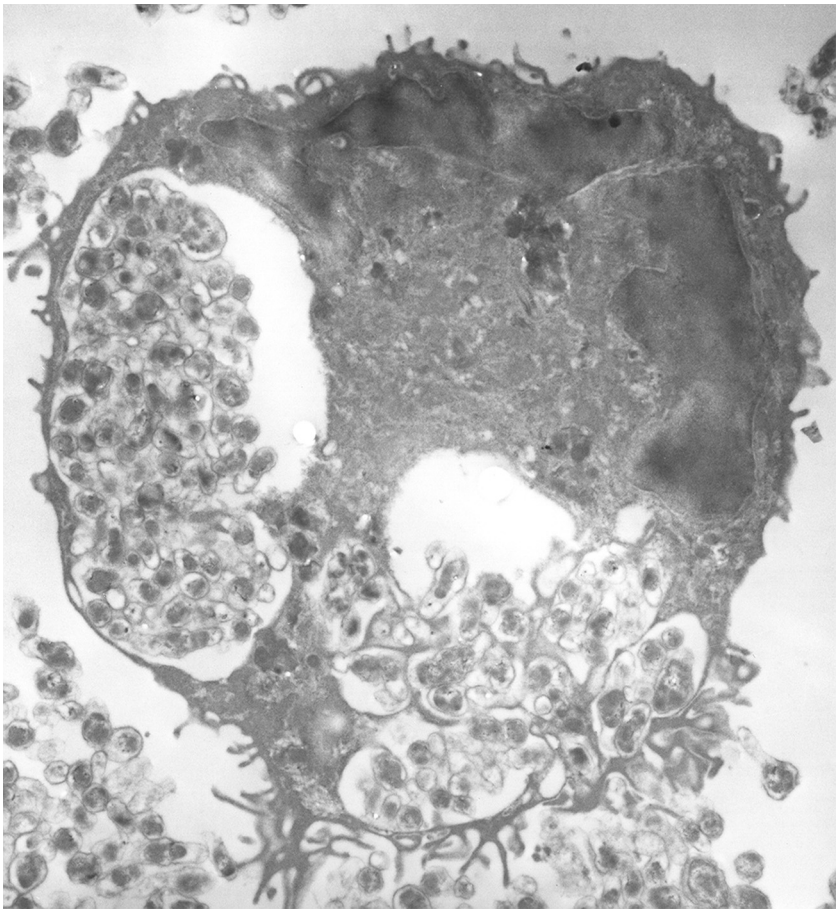

FIG 5 Transmission electron micrograph of a *B. henselae* invasome after internalization into an endothelial cell. Magnification, ×12,000.

the blood, causing bacteremic relapses in cats, mouse models, and rhesus macaques (74, 127, 224, 296). *Bartonella* is known to infect a range of host cells, but the endothelial cell is thought to be the primary niche location (296). This hypothesis stems from the evidence shown in the verruga peruana of *B. bacilliformis* characterized by tumors from endothelial cell proliferation (297) and studied *in vitro* (183, 298). *B. quintana* and *B. henselae* have also been shown to invade endothelial cells (299, 300). *B. henselae* has been shown to invade human endothelial cells *in vitro* by both a VirB/VirD4 T4SS-dependent mechanism resulting in intracellular invasion of large aggregates of bacteria termed invasomes (Fig. 5) and a VirB/VirD4 T4SS-independent mechanism (275, 277). While *B. henselae* has been shown to invade human endothelial cells *in vitro* by these two different mechanisms, the role of intracellular growth in facilitating human disease is not clear (301, 302).

Regardless of the proposed role of endothelial cells in serving as the primary niche of infection by *Bartonella* species, it is clear that the interaction with the endothelium results in a host response that is unique to these bacteria. This unique angiogenic host response results from infection by each of the three major human pathogens in the genus *Bartonella*, *B. bacilliformis*, *B. henselae*, or *B. quintana.* Angiogenesis caused by *B. henselae* is induced by production of proangiogenic factors such as vascular endothelial growth factor (VEGF), by promoting endothelial cell proliferation, and by suppressing apoptosis of vascular endothelial cells (303–306). VEGF is also a potent mitogen and tumor angiogenesis stimulator, and during *B. henselae* infection, production of VEGF is shown to be increased in microvascular endothelial cells (300). The production of VEGF by *B. henselae* has been shown to be regulated by the TAA-dependent activation of

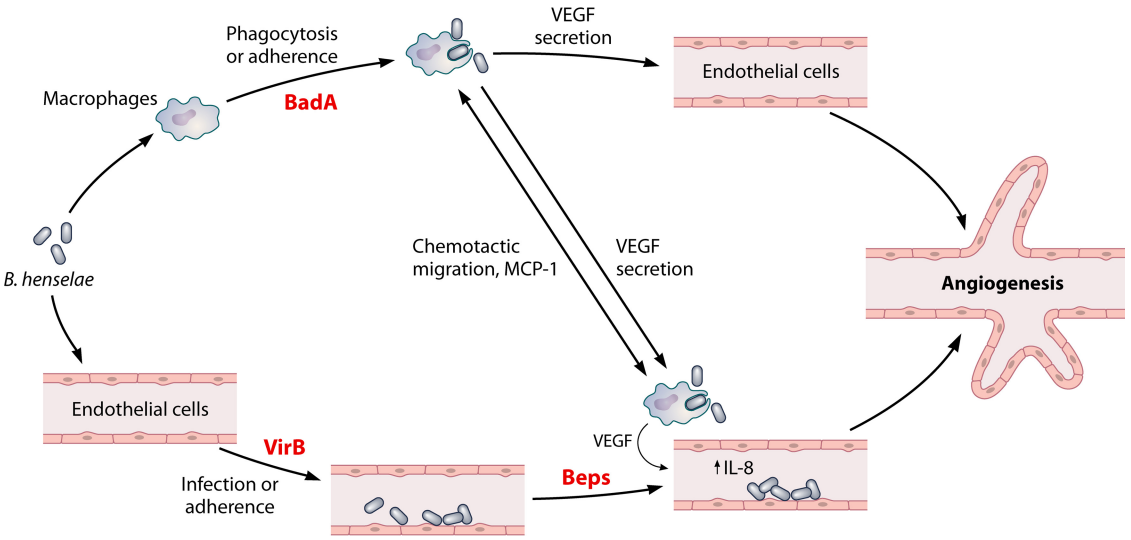

FIG 6 Paracrine angiogenic loop model for *B. henselae*. The role of BadA, VirB, and the cognate effectors (Beps) in inducing the angiogenic host response that is unique to *Bartonella* species is shown. (Adapted from reference 312.)

hypoxia-inducible factor 1 (Hif-1) (77, 307). *B. henselae* has also been shown to reprogram human endothelial progenitor cells (308). *B. bacilliformis* infection shows evidence of endothelial cell proliferation in its vascular tumor (Peruvian warts) evident during the chronic phase, and *in vitro* models have shown that it stimulates endothelial cells and also induces angiogenesis (183). The production of VEGF during *B. henselae* infection has been shown to require BadA and occurs through a hypoxia-inducible factor 1-dependent mechanism (77). A similar role has been proposed for the Vomps of *B. quintana* (309). In contrast, the inhibition of apoptosis has been shown to be a result of delivery of the *Bartonella* effector proteins (Beps), namely, BepA, by the VirB/VirD4 T4SS of *B. henselae* (310). Thus, in the case of *B. henselae*, the coordinated efforts of BadA and the VirB/VirD4 T4SS are thought to be required for the angiogenic host response induced by this bacterium (311). A paracrine angiogenic loop model has been proposed to explain this unique host response in *B. henselae* (Fig. 6) (312). However, it should be noted that *B. bacilliformis* is able to induce dramatic angiogenic lesions in infected patients, but this species has no known VirB T4SS encoded in its genome. Thus, the role of the VirB/VirD4 T4SS appears to be dispensable for at least some *Bartonella* species to induce angiogenesis. Alternatively, it is possible that *B. bacilliformis* has as-yet-undefined factors that function in a capacity similar to that of the Beps to augment the role of the TAAs in eliciting a proangiogenic host response. Mitogenic activity associated with the *B. bacilliformis* GroEL chaperone for human umbilical vein endothelial cells has been demonstrated, suggesting a possible role for this protein in *Bartonella*-induced angiogenesis as well (313).

Virulence Factors

Every bacterium carries virulence factors specifically adapted to its needs for invasion, colonization, replication, and survival in the host cell, and *Bartonella* species are no exception. With the exception of virulence-associated surface-exposed proteins, most bacterial virulence factors are delivered either to the extracellular environment or directly into host cells (314). The virulence factors described to date for *Bartonella* species fall into these two categories. While many virulence factors for these bacteria have been described, infection by *Bartonella* species is facilitated by the presence of two major virulence factors, the TAAs and the T4SSs.

The TAA family of proteins. The TAAs of Gram-negative bacteria are a family of proteins that are considered type V secretion systems. While there is considerable variability in TAA size, sequence conservation, and the number of gene copies in the genomes of different *Bartonella* species, all *Bartonella* species appear to have at least one TAA gene

(315). The TAA family of proteins is found in many other Gram-negative bacteria (316). The TAA protein family is termed BadA in *B. henselae* (77), Vomps in *B. quintana* (74), and *Bartonella* repeat proteins (Brps) in *B. vinsonii* (73). The role of BadA and the Vomps in virulence in the genus *Bartonella* is the best-studied facet of these TAAs.

(i) BadA. BadA has been ascribed several functions, the first of which is adherence to host extracellular matrix proteins or target endothelial cells. BadA is a monomer of 328 kDa that forms filaments that are about 240 nm long on the surface of *B. henselae* (315). Other bacteria possess TAAs with similar characterized adherence functions such as *Yersinia* (YadA) (317) or *Neisseria meningitidis* (NadA) (318). TAAs form extracellular filaments composed of head and stalk domains assembled on a C-terminal membrane anchor, forming a "lollipop-like" structural architecture (315, 319, 320). During assembly, the TAA is secreted into the periplasm while the membrane anchor builds a homotrimeric 12-stranded beta-barrel in the outer membrane. This trimerization is required to maintain the stability and adhesive property of the protein (319). The trimer barrel forms a pore which transports the head and stalk domains to the cell surface, and the C-terminal part of the stalk clamps the pore (321). Previous experiments with mutant strains show that while the BadA head is responsible for adherence to the extracellular matrix and autoagglutination (AAG), the stalk is required for fibronectin adherence (322). BadA prevents bacteria from being phagocytized and also stimulates endothelial cell proliferation by inducing a proangiogenic host cell response through activation of Hif-1, a crucial transcription factor for angiogenic cytokine secretion (77, 323, 324). A Δ*badA* mutant of *B. henselae* was shown to exhibit reduced replication and a weakened proangiogenic host response in a zebrafish embryo model (325).

Even within *B. henselae*, the size of the BadA protein is variable, and it is thought that much of this variation is due to the different size of the repeating neck/stalk regions (72). Likewise, the amount of surface-localized BadA is also highly variable, ranging from no detectable BadA in the Berlin-1 and ATCC 49793 strains to very high expression in the Marseille strain (72). Regulation of the *badA* gene has been attributed to the BatR/S two-component regulatory system (326) and the general stress response system (327). More recent studies have shown that a family of nine unannotated and highly transcribed RNAs designated *Bartonella* regulatory transcript (Brt1 to Brt9) found upstream of a putative transcriptional regulator protein are important in the regulation of *badA* (75).

Considerable confusion exists in the literature with regard to the genome sequence (accession no. BX897699) of the *badA* locus for the Houston-1 type strain of *B. henselae* (ATCC 48892), which reports a 1-bp deletion in the anchor of the *badA* gene (BH01510) (82). This results in the BadA anchor region being annotated as a separate 67-amino-acid-protein-encoding gene (BH01520) which partly overlaps the 3′ end of BH01510 (77). Riess et al. subsequently reported that the *B. henselae* Houston-1 strain exhibits surface-localized BadA and does not contain this mutation in the membrane anchor region of BadA (72). Independent sequence analysis of low-passage-number *B. henselae* Houston-1 in our laboratory shows BH01510 and BH01520 as a single merged open reading frame (unpublished data). Furthermore, immunoelectron microscopy shows a surface-localized BadA, indicating a functional membrane anchor domain (Fig. 7), suggesting that the genome sequence reported by Alsmark et al. (82) may reflect a higher-passage-number variant with a laboratory-derived mutation in the *badA* gene. It should be noted that other strains or laboratory-derived variants have also been shown to be defective for either *badA* expression or BadA surface localization (72).

(ii) Vomps. Vomps are a multigene family of TAA proteins found in *B. quintana* that have a similar modular structure (head-neck-membrane anchor) as BadA of *B. henselae* (74, 328). There are four Vomps (A to D), which, like BadA, function as adhesins mediating host cell adhesion and autoaggregation in *B. quintana* (74). Expression of the *vomp* genes varies in the host and is thought to suppress the host immune response, favoring adaptive interaction (74). The Vomps are closely related to the afimbrial adhesin YadA, a TAA of *Yersinia enterocolitica*. The surface-expressed Vomps contain conserved structural features of YadA, including collagen-binding motifs (74, 329). VompC confers

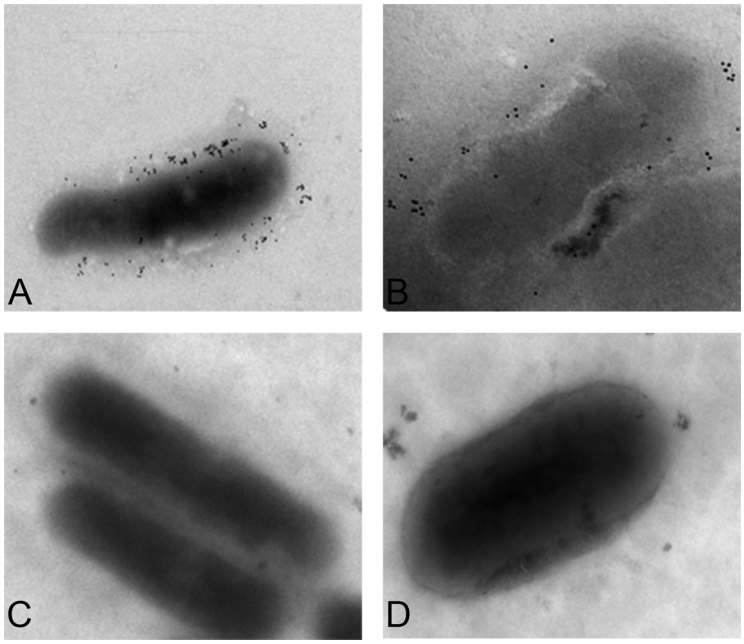

FIG 7 Expression and surface localization of BadA in *B. henselae*. Houston-1 (A) and Marseille (B) strains were reacted with rabbit anti-BadA antibody, followed by goat anti-rabbit IgG conjugated to 10-nm colloidal gold particles. Cells were washed, suspended in phosphate-buffered saline, transferred onto a copper-coated grid, air dried, and imaged using a JEOL JEM 1400 microscope. Surface localization of BadA can be seen in both the Houston-1 and Marseille strains but not the isogenic *badA* deletion mutants (Houston-1 Δ*badA* mutant [C] and Marseille Δ*badA* mutant [D]). The markerless, nonpolar in-frame Houston-1 deletion mutant was constructed as previously described (325). The Marseille deletion mutant was constructed by the same approach (unpublished data). Rabbit anti-BadA antibody was raised to the stalk region of the BadA protein (77) and was generously provided by Volkhard Kempf.

the ability to bind collagen IV, and VompA is necessary and sufficient for autoaggregation (74). *In vivo*, Vomp genes are differentially expressed, and gene deletions were shown to occur during prolonged bloodstream infection (74).

VirB/VirD4 T4SS. Bacterial T4SSs are present in many Gram-negative bacteria, including *Helicobacter pylori*, *Coxiella burnetii*, *Agrobacterium tumefaciens*, and *Legionella pneumophila* (224, 330–332). In *B. henselae*, the VirB/VirD4 T4SS is the best-characterized T4SS among the *Bartonella* species (see reference 223 for a review). The *B. henselae* VirB/VirD4 T4SS is comprised of a multiprotein system (VirB2 to VirB11) which translocates Beps to target cells through a contact-dependent process that is thought to require a pilus-like surface-protruding filament that is believed to consist of VirB2 and VirB5 (303, 333–336).

Postadherence, host cell signaling interruption is mediated via the translocation of seven unique *Bartonella* effector proteins (BepA to -G) (337), all encoded immediately downstream of the VirB/VirD4 T4SS on the genome, by using the *B. henselae* VirB/VirD4 T4SS for delivery. VirB T4SSs consist of a substrate translocation channel which spans the two membranes of Gram-negative bacteria and a surface filament which extends from the bacterial envelope and establishes contact with target cells. This translocation channel extends to the host cell membrane and facilitates translocation of substrate. Bacteria gain entry into human endothelial cells either as a single bacterium using a VirB/VirD4 T4SS-independent zipper-like mechanism (302) or through an invasome-mediated uptake requiring the VirB/VirD4 T4SS (Fig. 5) (275). The VirB/VirD4 T4SS delivers BepG for invasome-mediated uptake (338) and/or BepC and BepF to extensively rearrange the actin cytoskeleton (339). This rearrangement produces bacterial aggregation and ultimately engulfment by host cell membranes and entry into endothelial cells via the invasome (275). The presence of the bacterium causes a proinflammatory response activating NF-κB, and cytokines that promote inflammation such as tumor necrosis factor alpha (TNF-α) are released. Activation of

NF-κB induces the secretion of interleukin-8 and the expression of intercellular adhesion molecule 1 (ICAM-1) and E-selectin.

This VirB/VirD4 T4SS is also crucial for the inhibition of endothelial cell apoptosis (340) and intraerythrocyte infection; a mutant defective in *virB4* and *virD4* was not bacteremic in a model system (332). On delivery into the endothelial cell, BepA also localizes to the plasma membrane to induce production of second messenger cyclic AMP conveying an antiapoptotic property to the cells (341). *Bartonella* infection is known to suppress early and late events in apoptosis, namely, caspase activation and DNA fragmentation, respectively (304). The translocation of the Beps into endothelial cells, together with the induction of VEGF production, which is dependent on the presence of BadA on the surface of *B. henselae*, is thought to work together to induce the angiogenic host response (Fig. 6). The interplay of BadA and the VirB/VirD4 T4SS has been studied, and it was shown that BadA can affect effector translocation (311). The contributions of each of these two virulence factors in inducing angiogenesis are not yet entirely clear, and study has been hampered by the lack of a practical animal model.

Trw T4SS. Asides from VirB/VirD4, a second type of T4SS, Trw, has been identified as a molecular determinant of host-specific erythrocyte infection (342). It is a set of virulence genes that were laterally acquired, and the gene products are effective in promoting prolonged bacteremia through erythrocyte adhesion and infection (342–344). Trw does not translocate any known effectors but produces multiple variant pili involved in the attachment and invasion of host erythrocytes (345). The presence of Trw has been shown to correlate with loss of flagella, which represents a major pathogenicity factor for erythrocyte invasion by *B. bacilliformis* and probably other flagellated *Bartonella* (70). Variable expression of some Trw protein genes appears to correlate with the presence or absence of other Trw proteins. The Trw T4SS has several components: of more importance are the adherent components, TrwL and TrwJ, which are surface-exposed components that mediate host specificity (342). TrwL and TrwJ have been referred to as anchoring and pilus proteins, respectively (346). In *in vitro* adhesion and invasion assays, the TrwJ and TrwL transposon mutants showed reduced adhesive properties and lacked the ability to invade the erythrocyte (342).

Hbps. Heme is essential for *Bartonella* survival. In the gut of their arthropod vector, *Bartonella* species are exposed to toxic concentrations of heme, which is otherwise confined to the bloodstream of the mammalian host. Thus, one of the roles of the multigene family of hemin binding proteins (Hbps) is to facilitate survival in the vector, as is the case for *B. henselae* in the cat flea (347). It is thought that *Bartonella* uses HbpA for iron acquisition and to bind hemin required for bacterial growth (348). Five different Hbp genes have been identified in both *B. henselae* (349) and *B. quintana* (350). The Hbps also serve as adhesins and have been shown to bind fibronectin and facilitate entry into endothelial cells (351). HbpC, in contrast to HbpA, is sensitive to environmental changes such as those in temperature and hemin and is overexpressed in the arthropod gut. HbpC is hypothesized to bind hemin to prevent access to the bacterial cell and counter the resultant heme toxicity (4). Deletion of the *hbpC* gene also affected the ability of *B. henselae* to infect the cat host. HbpA and HbpC were also observed on the outer membrane vesicles produced by *B. henselae*, and constitutive expression of HbpC increased the amount of hemin associated with the vesicle. It should be noted that Pap31, originally described as a protein associated with the BLP of *B. henselae* (352), is homologous to the multigene Hbp family of *Bartonella*, Omp31 of *Brucella*, *Agrobacterium tumefaciens* Omp25, and opacity proteins of *Neisseria* species (350). Pap31 is also thought to be involved in heme acquisition and virulence and is also said to bind fibronectin and heparin, possibly on the same domain (349, 350, 353).

Fhas. The filamentous hemagglutinins (Fhas) are a family of type V secretion system proteins encoded in the genome of some *Bartonella* species, including *B. henselae*, but not all species. Fhas are known to be a virulence factor in other Gram-negative bacteria like *Bordetella pertussis* (354, 355) and *Pasteurella multocida* (356) by facilitating adhesion. Nine copies of full-length and truncated *fhaB* genes (*fhaB1* to *-9*) are found on the *B. henselae* genome with each gene copy being immediately downstream of or in close

proximity to the hemolysin activator gene (*hecA*); hemolysin activator is a separately encoded protein that is thought to serve as a transporter domain for FhaB (315). While the Fhas represent additional potential pathogenicity factors, it is not clear which of the Fha gene loci are expressed in *B. henselae*. Proteomics studies have shown the presence of antibodies in cat sera that are reactive with the FhaB2 protein of *B. henselae*, providing indirect evidence that at least one of the FhaB loci is expressed *in vivo* (357). It has been proposed that the Fha proteins may play a supporting role for adherence when BadA is not expressed in *B. henselae* (315). Regardless, the function of the Fha proteins in *Bartonella* adherence and virulence remains unknown.

Other *Bartonella* adhesins and outer membrane proteins. Surface labeling of *B. henselae* demonstrated several surface-localized proteins, a subset of which were shown to bind human endothelial cells (358). One of those, Omp43, functions as an adhesin during *B. henselae* interaction with endothelial cells (359), binds fibronectin (360), and bears homology to the Omp2 family of porins from *Brucella* spp. Omp43 was shown to be immunogenic in cats, with sera from most infected cats being reactive to this protein (361). Another putative adhesin of *B. henselae* is Omp89, an immunogenic surface protein containing a zinc metalloprotease domain that has been shown to bind fibronectin (360). Other membrane-associated proteins, GroEL, HtrA, and Omp89, are also thought to be involved in protein folding or degradation (360–362). In *B. bacilliformis*, several outer membrane proteins between 11.2 and 100 kDa have been identified (363, 364). Six of these have been shown to mediate bacterial interactions with erythrocytes (363). A 43-kDa immunogenic lipoprotein, which is a homologue of LppB proteins of *Haemophilus* spp. that are associated with virulence, has been reported (365). *B. bacilliformis* also possess a flagellin, a 42-kDa protein subunit of the flagella which has been shown to be resistant to protease or trypsin treatment and provides the bacteria with motility (43, 366).

Invasins. Invasion-associated locus A and B genes (*ialAB*) of *B. bacilliformis* were first described by Mitchell and Minnick (226). These genes are necessary for both the invasive phenotype and its erythrocyte parasitism (367). In *B. bacilliformis*, IalB is localized to the inner membrane, but in *B. henselae*, it is localized on the outer membrane (226, 361, 367). IalB has been implicated in invasion of erythrocytes in both *B. bacilliformis* and *B. henselae*. In *B. bacilliformis*, IalB is produced in increasing amounts as the temperature drops below 37°C (368), but it has also been noted that other environmental changes like those in iron availability and pH have an effect on expression (367). In *B. henselae*, IalB and Omp43 elicit an antibody response that varies between cats (226, 361, 367). IalB has also been shown to be required for intraerythrocytic bacteremia (367). In *B. birtlesii*, IalB was localized to the outer membrane and shown to be required for invasion of erythrocytes but not adherence (342). In *B. henselae*, IalB is thought to facilitate entry into erythrocytes, and recombinant IalB protein has been reported to be immunogenic and proposed as a possible diagnostic tool for an immunoassay (369). Genome analysis of *B. elizabethae* and *B. grahamii* also shows the presence of an *ialB* locus (370, 371).

Growth in Biofilms

A biofilm may be defined as a cluster of bacterial cells embedded in a matrix, which is more tolerant of most antimicrobials and host defenses than are free-floating single cells (372). Surface protein adhesins facilitate substrate adherence and autoagglutination (AAG). Adherence has been described as a two-phase process: phase 1, in which the bacterial cells first behave as dormant colloidal particles making reversible contact with surfaces according to their physiochemical properties, and a subsequent phase in which biologically produced adhesins anchor the cells more irreversibly to the surface (373). AAG activity is a known signal for host cell interaction and virulence in several Gram-negative bacteria, and outer membrane proteins of these bacteria have been demonstrated to be autoagglutinins (374). Once autoagglutinated, bacteria communicate with one another using chemical signal molecules, a process called quorum sensing, which allows bacteria to monitor the environment and to change behavior depending on conditions in the community. Quorum sensing is critical for

biofilm formation (375). Biofilms are formed when bacteria irreversibly adhere to a surface, communicate, and excrete a protective matrix. Production of an extracellular polymeric substance (EPS) matrix, reduced growth rates, and alternating regulation of specific genes distinguish biofilm sessile aggregates from their planktonic counterparts (376). Biofilms are characterized by their stability, chronic bacterial infections, and increased resistance to antibiotics. Biofilm EPS consists of proteins, DNA, polysaccharides, and excreted cellular components (377). It ensures that the bacteria are protected from host immune responses such as macrophage engulfment, antibiotics, or host immune defenses while sequestering valuable enzymes and nutrients, exchanging genetic information, and recycling lysed cell components (378).

Despite the well-known role of both BadA of *B. henselae* and the Vomps of *B. quintana* in facilitating autoaggregation and adherence, which are critical initial steps in biofilm formation, very little information about the growth of *Bartonella* species in biofilms and the role that the TAAs play in the process has been reported. In the case of *B. henselae*, growth in biofilms was first reported by Kyme et al. (76). In that report, the autoaggregative nature was described as phase variation, which has subsequently been shown to be attributable to the presence of BadA on the surface of *B. henselae* (72). Studies show that BadA accumulates as a dense surface layer of long hair-like structures (~240 nm) and that the absence of BadA prevents AAG (379). The role of TAAs in biofilm formation has been proposed or experimentally demonstrated for a wide range of Gram-negative bacteria, including *Acinetobacter baumannii* (380), *Burkholderia* species (381–383), uropathogenic *Escherichia coli* (UPEC) (384), enterohemorrhagic *E. coli* (EHEC) (385), and *Salmonella enterica* (386). Not surprisingly, we have shown that BadA plays an important role in biofilm formation by *B. henselae*, as a *badA* deletion mutant has an impaired ability to form biofilms (Fig. 8) (75). When our laboratory constructed nonpolar deletion mutants of the *badA* gene in both the Houston-1 type strain and the Marseille strain of *B. henselae*, they were shown to have a reduced ability to form biofilms. Scanning electron micrographs show that the Δ*badA* strains have reduced adherence and biomass accumulation, whereas the wild-type parental strains for both Houston-1 and Marseille have the ability to form well-structured biofilms (unpublished data) (Fig. 8).

The autoaggregative nature of low-passage-number *Bartonella* species grown under laboratory conditions is well known, and large aggregates of bacteria have been observed in histopathological specimens from patients with BA (387), verruga peruana (388), and CSD (389). The ability of *B. henselae* (75, 76) and perhaps other *Bartonella* species to aggregate and form biofilms suggests that these bacterial communities are an integral component of the vegetative masses reported for endocarditis caused by *Bartonella*. Molecular studies and histopathology of heart valves from cases of *Bartonella* endocarditis demonstrate the presence of these bacteria on the surface of damaged valves (97, 177). A damaged heart valve could serve as the substrate on which *Bartonella* biofilms form and establish infections that are resistant to antibiotic treatment, as is the case for BCNE.

It is also tempting to speculate on a possible role of *Bartonella* biofilms in colonization of their arthropod vectors. Biofilm formation by *Yersinia pestis* in the proventriculus of the rat flea vector has been shown to promote transmission (390). *Y. pestis* apparently evolved from non-vector-borne *Yersinia* species for rat flea transmission by three loss-of-function mutations that enhanced biofilm formation in the flea foregut (391). It may also be possible that biofilm formation plays a limited role in transmission of *Y. pestis* by the cat flea (392). Five different *Bartonella* species have been shown to persist in the cat flea vector (393). *B. henselae* replicates in the flea gut and is excreted in the flea feces (128, 135). It has been proposed that *B. henselae* forms a biofilm in the flea gut and possibly on the flea feces to persist before transmission on the claws of cats (394). It has also been hypothesized that *B. quintana* may also form a biofilm on louse feces (133). Thus, it is possible that the biofilm formation in the arthropod vectors of *Bartonella* species and/or their feces enhances transmission of these bacteria, much as it does for *Y. pestis*.

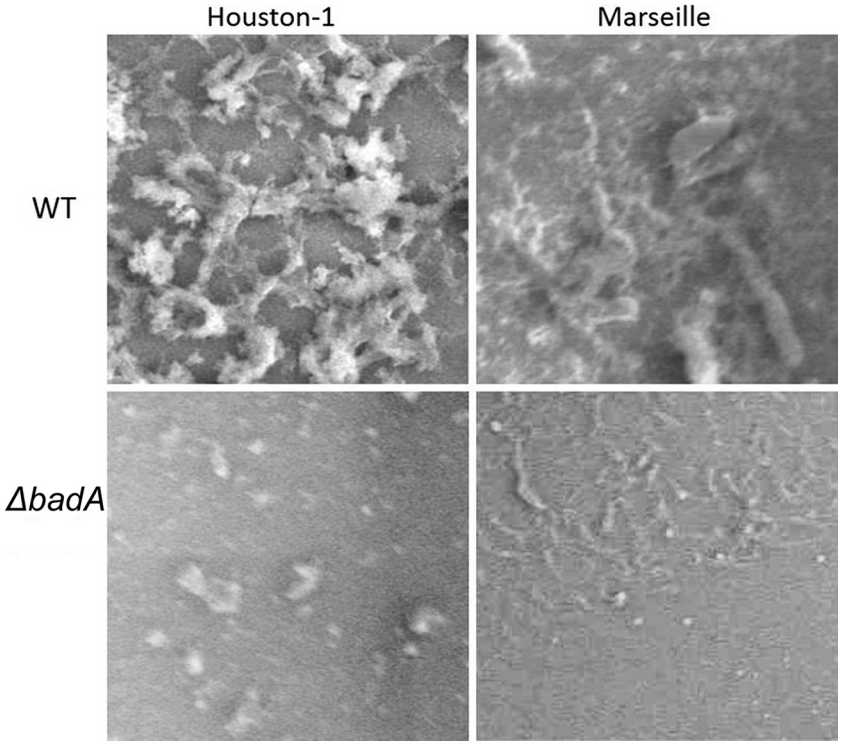

FIG 8 Biofilm formation by *B. henselae*. Scanning electron microscopic images of the adherent cells for both the Houston-1 and Marseille wild-type (WT) strains compared to reduced adherence, autoaggregation, and biofilm formation for the isogenic mutants in which the *badA* gene is deleted (Δ*badA*). Bacterial cells (10^5) were inoculated onto a coverslip in a six-well plate and grown for 24 h in Schneider's liquid medium at 37°C and 5% CO_2. Cells were fixed with 2% paraformaldehyde plus 2% glutaraldehyde and 0.15% alcian blue (to preserve the polysaccharide moieties found in the EPS of biofilms [396, 397]) in 0.2 M sodium cacodylate buffer, pH 7.2. Samples were washed, postfixed for 90 min in 1% OsO_4, and dehydrated. Samples were air dried overnight; the coverslip was mounted on adhesive carbon film, coated for 20 s with Au/Pd (60:40) at 16.40 g/cm and 25 mA, and examined using a JEOL JSM6490LV microscope operated at 3-kV low vacuum; and secondary images were collected as JPEG files. The Δ*badA* mutants were the same strains as those described in the legend to Fig. 7.

Bartonella species share the ability to infect erythrocytes in their natural hosts, and it has been speculated that a primary niche exists to allow persistence of these bacteria in the host and to seed the bloodstream for subsequent transmission by the arthropod vector. The preponderance of evidence supports the role of the endothelium in serving as this primary niche (296), but it has also been suggested that endothelial progenitor cells may also function as a niche reservoir (215–217, 308). Intracellular survival in endothelial cells would afford an opportunity for immune evasion and may explain the stealthy nature of these pathogens (223). However, in humans bartonellae are not widely reported inside endothelial cells, suggesting that this may be a laboratory-generated phenomenon of unclear clinical relevance (203, 301, 387, 388). Similarly, an immunocompromised mouse model (SCID/BEIGE) using *Bartonella taylorii* was developed and shown to establish a chronic infection with the bacteria localized as extracellular aggregates embedded within the collagen matrix (395). An alternate explanation is that *Bartonella* species form stable biofilms (rather than carrying out intracellular invasion of endothelial cells), which allow immune evasion and persistence in their hosts. Extracellular attachment of bartonellae to endothelial cells or extracellular matrix proteins may provide the substrate for the formation of a focus that becomes a structured biofilm for seeding of the bloodstream and persistence in the face of both antimicrobial treatment and the host immune response.

CONCLUSIONS

The genus *Bartonella* is a rapidly expanding group of ubiquitous bacteria that are found in a diverse array of animal reservoirs. Three species of *Bartonella*, *B. bacilliformis*,

B. henselae, and *B. quintana*, are responsible for the vast majority of human disease caused by this group of bacteria. In particular, *B. henselae* and *B. quintana* are frequent causes of BCNE that often go undiagnosed due to technical challenges in diagnosing infections caused by *Bartonella* species. Despite this challenge, the number of reported cases of *Bartonella* endocarditis is rapidly rising, and it represents a significant cause of infective endocarditis that remains understudied. The role of the TAAs (BadA and Vomps) and the T4SSs of *Bartonella* in the interaction of this bacterium with the vascular endothelium has been well studied, and they certainly play critical roles in the establishment of endocarditis. Furthermore, the ability of *Bartonella* to form biofilms is likely to be a crucial step in infection of heart valves and therefore in causing endocarditis. Additionally, the role that biofilms play in colonization and transmission in the arthropod vectors responsible for transmission of *Bartonella* species and persistence in both the natural and incidental hosts should also be considered.

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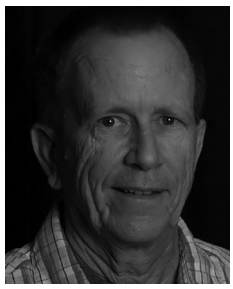

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