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In Pursuit of a Stealth Pathogen: Laboratory Diagnosis of Bartonellosis

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Abstract

Members of the genus *Bartonella* cause illnesses in humans and animals. *Bartonella* spp. are gram-negative, fastidious, facultative, intracellular pathogens transmitted by the bites of certain arthropods or by bites and scratches from infected animals. While some of the illnesses are acute and self-limiting, others are chronic, debilitating, and difficult to diagnose. *Bartonella* spp. are able to invade host cells and modulate the host immune response, contributing to their success as stealth pathogens. This article provides an overview of diseases caused by members of the genus *Bartonella* and the mechanisms of pathogenesis. The laboratory diagnosis of bartonellosis relies on three primary methods. This article examines the evolution of culture techniques, serology, and nucleic acid amplification tests used to detect *Bartonella* spp. in clinical specimens and suggests areas for future research to improve laboratory diagnostics. In this way, a better understanding of the epidemiology of bartonellosis can be achieved.

Background

Members of the genus *Bartonella* have excelled as stealth pathogens, causing a variety of acute and chronic disease manifestations that vary not only with the infecting species, but also with the host species that becomes infected. Although bacteremia, which can be relapsing in nature and persistent in character, typifies the ecological behavior of *Bartonella* spp. within the host, the sites of bacterial localization, as well as the distribution of disease pathology, can vary substantially, even within the same host species. Members of the genus are gram-negative, facultatively intracellular, aerobic, fastidious, vector-borne bacteria that provide challenges, not only to those involved in patient management, but also to both translational and basic researchers.

Certain disease presentations caused by infection with a *Bartonella* spp. have been evident for decades; however, the pathogen remained elusive for many years. It was not until 1992 that the etiologic agent of bacillary angiomatosis, bacillary peliosis, and septicemia was established as either *Bartonella henselae* or *Bartonella quintana* (1-3), due in part to the emerging HIV epidemic. The current lineages of selected species assigned to the genus *Bartonella* are shown in Table 1 (4). As new disease presentations continue to be unveiled in humans and animals in association with known and novel *Bartonella* spp., establishing a diagnosis of bartonellosis is critical. In addition, diagnostic laboratories play an important role in ongoing efforts to define the epidemiology and disease spectrum associated with this etiologic agent. Following a brief introduction to illnesses caused by *Bartonella* family members and currently characterized mechanisms of pathogenesis, we review existing laboratory methods and what the future may hold for advances in laboratory diagnostic testing.

Overview of diseases associated with *Bartonella* spp.

Veterinary

A multitude of case reports have shown that bartonellosis occurs in a variety of animal species, causing a myriad of disease presentations. While

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B. henselae is most often found to be the etiologic agent, infections with other *Bartonella* spp. have been well documented (5). For example, dogs have presented with endocarditis caused by a variety of *Bartonella* spp., and both *B. henselae* and *Bartonella vinsonii* subsp. *berkhoffii* have been detected in pleural, pericardial, and abdominal effusions from dogs (5). While many reports involve infections in dogs with a variety of *Bartonella* spp., it is important to note that pathology is not limited to dogs. Examples include myocarditis and myositis in cats (6). In addition, sick foals and adult horses with musculoskeletal disease were shown to be infected with *B. henselae* and *B. vinsonii* subsp. *berkhoffii* (7). Some recent case reports include vasoproliferative diseases in dogs, cats, a horse, a red wolf, and a steer, implicating one or more species of *Bartonella* (8) as the etiologic agent(s).

Human

Bartonella bacilliformis, *B. quintana*, and *B. henselae* are the best-known species causing human illness. These pathogens cause Carrion's disease/Oroya fever, trench fever, and cat scratch disease (CSD), respectively, in the acute phase of illness. In the chronic state, vasoproliferative disorders, such as verruga peruana, bacillary angiomatosis, and bacillary peliosis, are observed, particularly in immunocompromised patients (9). Incidental infection of both immunosuppressed and immunocompetent humans with *B. henselae* and other *Bartonella* spp. can lead to a variety of disease presentations affecting most organ systems of the body. Several species of *Bartonella* cause endocarditis, lymphadenitis, persistent bacteremia, and neuroretinitis (5). It is becoming increasingly important to understand the role of *Bartonella* infections in neurological disease, as detailed in a recent review of several cases, one of which was fatal and all of which were debilitating (10). In a study of 8 immunocompetent patients with animal and arthropod contact and chronic illnesses (11), 7 experienced fatigue and pain, while 6 had insomnia. In addition, 5 of the 8 patients complained of headaches, and 4 reported irritability, muscle weakness, loss of sensation or numbness, and balance problems. All 8 patients were positive for *Bartonella koehlerae* by PCR, and 4 of the patients also had *B. vinsonii* subsp. *berkhoffii* (11). The role co-infections play in complex disease manifestations, and subsequent treatment challenges, remains an open question for health care providers managing patients with bartonellosis. Based on this brief overview of clinical presentations that can result from an infection with *Bartonella* spp., it is clear that these bacteria are important pathogens, causing a wide range of human and veterinary diseases, many of which are debilitating and some potentially fatal.

Pathogenesis

Several excellent recent reviews have summarized current knowledge of *Bartonella* pathogenesis (4,9,12). *Bartonella* spp., such as *B. henselae*, *B. quintana*, and *B. vinsonii* subsp. *berkhoffii*, have developed survival mechanisms that allow successful infection of the host and low-level persistence in the host, so that the organism is available for subsequent transmission to another vector and host. These mechanisms involve interactions with both nucleated and non-nucleated host cells and are dependent on a number of virulence factors. Studies have shown that *Bartonella* spp. may establish infection inside red blood cells in a species-specific manner, yet the ability to invade other cell types does not appear to be as restrictive (4,9,12). This is important because *Bartonella* spp. are able to hide in one or more protected host niche(s) and slowly infect red blood cells over time in order to facilitate a persistent infection within the host. The location of the protected host niche or niches remains a major clinically relevant question and has created some controversy among researchers.

Some of the best-characterized virulence factors are highlighted below. Apart from the initial adherence and invasion, these virulence factors collectively contribute to the pathogenesis of *Bartonella* infections by modulating the immune response to allow persistence, by forming new blood vessels via endothelial cell proliferation, and by potentially influencing other, yet to be determined host cell functions.

Type IV secretion systems (T4SS) (VirB/VirD4 and Vbh types) represent an elegant assembly of molecules that facilitates the efficient transfer of bacterial effector molecules to the host cell by acting as a translocation pore (4). Once translocated to the host cell, these *Bartonella* effector proteins (Beps) alter several host cell functions to the ultimate benefit of the bacteria. Beps promote invasome formation, allow the entry of *Bartonella* into the host cell, promote angiogenesis, and inhibit apoptosis of the infected host cell (12). Certain *Bartonella* spp. within lineage 4 (including *B. quintana*, *B. henselae*, and *B. vinsonii* subsp. *berkhoffii*) also have a Trw T4SS that is critical for bacterial adherence to red blood cells and determines host specificity (4,9,12). Much energy is required by the bacteria to form the translocation pore, and thus, a transcriptional regulatory system exerts control over the process. In *Bartonella* spp., the VirB T4SS is tightly regulated by the combination of a histidine kinase and a response regulator, known as the BatR/BatS two-component regulatory system (4).

Table 1. Selected *Bartonella* spp. and current lineage assignments

Lineage	Member(s)
1	*B. bacilliformis*
2	*B. bovis*, *B. chomelii*, *B. capreoli*, *B. schoenbuchensis*
3	*B. rochalimae*, *B. clarridgeiae*
4	*B. henselae*, *B. quintana*, *B. koehlerae*, *B. vinsonii* subsp. *berkhoffii*, *B. elizabethae*, *B. grahamii*, *B. alsatica*