Human infection with *Bartonella* species

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**ILLUSTRATIVE CASE HISTORY**

A 14-year-old Caucasian boy presented with a 4-week history of malaise, intermittent fever, tender lymphadenopathy of the axillary and cervical nodes, and fatigue which had been interfering with his school work. He had previously been healthy, with no developmental problems or past medical history, except for tonsillectomy at the age of 8 years. On direct questioning, he denied loss of appetite or a reduction in weight; there had been no rash or other type of cutaneous lesion. His father, mother and two younger siblings were well and asymptomatic. He had last been on holiday with the entire family 4 months previously in Greece. Family pets included his father's tropical fish, a hamster, and an aging cat.

Examination failed to detect any signs other than the lymphadenopathy. The patient was afebrile. Investigation revealed normal biochemistry, haemoglobin concentration and platelet count, but the total white cell count was raised, at $12.4 \times 10^9/L$, with a neutrophilia of 96%. The erythrocyte sedimentation rate was 39 mm/h. The chest X-ray was unremarkable.

Histologic examination of a lymph node demonstrated caseating granulomata, and a specific stain confirmed the diagnosis. Routine cultures of blood and node homogenate were negative at 7 days, and the specimens were discarded.

With appropriate management, the boy completely recovered within the next 2 months.

**Thoughts on the case**

- What was the most likely diagnosis?
- How could investigation have been optimized?
- What was the nature of the successful management?

**MULTIPLE-CHOICE QUESTIONS**

In each of the numbered questions, at least one, and up to five, of the individual entries are correct.

(The answers are at the end of this article.)

1. **Regarding cat scratch disease (CSD)**
   (a) The first reports of lymphadenopathy following animal contact date back to the 1880s. True/False
   (b) The causative organism is now generally accepted to be *Bartonella henselae*. True/False
   (c) The primary reservoir for the organism is the adult cat, especially if elderly or diseased. True/False
   (d) The vector of transmission of infection to humans has been shown to be the cat flea. True/False
   (e) Eighty per cent of cases of CSD occur at age less than 21 years. True/False

2. **Concerning the clinical features and natural history of CSD**
   (a) A history of contact with cats is essential to make the clinical diagnosis of CSD. True/False
   (b) It is not uncommon for the primary skin lesion not to be detected on clinical examination. True/False
   (c) Lymphadenopathy is almost always present. True/False
   (d) In the immunocompetent patient, CSD can lead to complications in almost every organ system. True/False
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(c) CSD requires treatment with oral antibiotics such as doxycycline or ciprofloxacin. True/False

3. Bartonella spp. are increasingly recognized as causes of human infection. The following statements are correct

(a) Four species have been implicated in causing human illness. True/False
(b) Prior to the 1990s, members of this genus (as now named) were associated only with the arthropod-borne infections Carrion’s disease and trench fever. True/False
(c) Bartonella spp. have been implicated in the etiology of a specific syndrome characterized by cutaneous lesions called bacillary angiomatosis. True/False
(d) Bartonella spp. are recognized causes of ‘culture-negative’ endocarditis. True/False
(e) Prolonged bacteremia with relapsing fever due to bartonella infection is seen only in immunosuppressed patients. True/False

4. Regarding the microbiology of Bartonella spp.

(a) Members of this genus are obligate intracellular pathogens. True/False
(b) B. henselae is an aerobic, non-motile, oxidase- and catalase-negative, Gram-negative rod. True/False
(c) The species causing human infections can be divided into two groups on the basis of differential phenotypic properties. True/False
(d) Identification to the genus level is relatively straightforward, but identification to the species level requires genotypic methods. True/False
(e) Acridine orange is a useful diagnostic stain. True/False

5. The following statements concerning the isolation of Bartonella spp. are correct

(a) Cell-culture systems are the proven optimal method for isolation of Bartonella spp. True/False
(b) Bartonella spp. can be cultured by inoculation of blood or lymph node aspirate directly onto standard agar media. True/False
(c) Bartonella spp. will not grow on MacConkey agar. True/False
(d) Detection of CO₂ production by standard automated blood culture systems should not be relied upon as a means of detecting growth. True/False
(e) On primary isolation, colonies often pit and strongly adhere to the agar. True/False

6. With respect to non-cultural diagnosis of bartonella infections

(a) Warthin–Starry silver staining of biopsy specimens is useful in demonstrating the organisms. True/False
(b) Granuloma formation is seen in the lesions of bacillary angiomatosis (BA) and CSD. True/False
(c) In CSD, intradermal injection of antigen results in a delayed-type hypersensitivity reaction in >90% of infected individuals. True/False
(d) Infection results in a species-specific antibody response detectable by indirect immunofluorescent antibody (IFA) assay. True/False
(e) Restriction fragment length polymorphism (RFLP) analysis of the citrate synthase gene is a useful means of identifying bartonellae to the species level. True/False

DISCUSSION

Question 1

(a) Parinaud in 1889 first observed the development of conjunctival inflammation and preauricular lymphadenopathy in patients following animal contact [1]. More specifically, in the 1930s, Foshay in the USA, and Debre in France reported cases of regional lymphadenopathy resulting from cat scratches, and in the following decade Hanger and Rose purified a skin test antigen from the lesion of such a patient [1]. The term cat scratch disease was eventually adopted in recognition of the syndrome by Debre et al in their publication of 1950, entitled ‘La maladie des griffes du chat’ [2].

(b) Rochalimaea henselae was declared a new species in 1992 [3,4] on the basis of 16S rRNA gene sequencing and DNA–DNA hybridization studies of Rochalimaea-like organisms which had been isolated from the blood of patients presenting with febrile illnesses, first described by Slater et al in 1990 [5]. Subsequently, the genus was merged with Bartonella (see answer to Q5a).

Although CSD has been a recognized clinical entity since Debre’s description, it was not until 1983...
that bacilli were actually demonstrated within affected tissue by Warthin–Starry silver staining of lymph nodes [6]. Modified Gram stain of these organisms showed them to be slender, pleomorphic, Gram-negative rods. Subsequently, identical organisms were identified in the skin at the primary inoculation site [7]. In 1988, English et al successfully cultured a fastidious Gram-negative bacillus from the lymph nodes of 10 patients of a group of 19 clinically diagnosed with CSD [8]. Subsequently, it was proposed that this organism, presumed to be the cause of CSD, should be named *Afpia felis* after the Armed Forces Institute of Pathology (AFIP), where the discovery of the organism had been made.

In the same year that Wear et al [6] demonstrated the presence of bacteria in lymph node tissue from patients with CSD, Stoler et al described a novel syndrome of angioproliferative subcutaneous nodules in association with fever, sweats, diarrhea and weight loss in an HIV-positive individual [9]. Staining of tissue sections of a nodule revealed argyrophilic bacteria throughout the interstitium. The nodules responded to erythromycin. Further reports of this syndrome, later to be named bacillary angiomatosis (BA), followed [10]. Subsequently, in the same year that English et al cultured their bacillus from CSD lymph nodes, LeBoit et al, using the Warthin–Starry silver stain on identical angioproliferative lesions from seven patients with AIDS [11], demonstrated bacilli with a staining pattern indistinguishable from that of the original organisms described by Wear et al. They also reported resolution of these nodules with antibiotics, either erythromycin or a combination of rifampicin and isoniazid. In the light of these discoveries, it became widely accepted that the causative agents of CSD and BA were the same organism, presumed to be *A. felis*.

In 1990, Relman et al [12], amplified and sequenced 16S rRNA gene fragments directly from tissue samples of BA lesions from four patients, and identified an organism which had 98.3% 16S rRNA homology with *Rochalimaea quintana*. In 1990 also, Slater et al [5], using EcoRV restriction endonuclease digestion of whole DNA of organisms cultured from the blood of five patients with febrile illnesses, and found to have whole cell fatty acid composition closely resembling that of *R. quintana*, demonstrated a common banding pattern between the isolates which differed from that of *R. quintana*. The organism was later named *Bartonella henselae* [3,4].

Further work has favored this organism, rather than *A. felis*, as the cause of CSD. Serologic studies have repeatedly demonstrated a lack of antibody response to *A. felis* in patients with CSD [13–15]. Between 84% and 88% of patients with CSD have a titer of 1:64 or greater in the immunofluorescent antibody test (IFAT) against *B. (R.) henselae*, compared with 3% of controls [16,17]. Polymerase chain reaction (PCR) has detected DNA in the lymph nodes of a similar percentage of CSD patients [18,19]. Antigen preparations for diagnostic skin testing have also been shown by PCR to contain the DNA of *B. henselae* rather than that of *A. felis* [20]. *B. henselae* has also been cultured from the lymph nodes of patients with CSD [21,22]. By contrast, there is no evidence to suggest that other species of *Bartonella* have a causal role.

However, the fact that evidence of *B. henselae* infection is lacking in a significant proportion of cases, and the fact that *A. felis* has been demonstrated in some lesions, means that debate persists as to the definite etiology [23]. It may be that CSD is caused by more than one agent, and that *A. felis* is simply less commonly implicated. Alternatively, *A. felis* may be the cause of a CSD-like syndrome.

(c) The role of cats in the transmission of CSD is well established [17,24–29]. Kordick et al [27] observed *B. henselae* bacteremia in 25% of a sample of 24 healthy cats, compared with 84% of 19 cats whose owners were diagnosed as having CSD. Similarly, Chomel et al [24] reported a bacteremia rate among 146 healthy cats of 37%, compared with seven out of 10 cats implicated in cases of CSD or BA. Thus bacteremia was determined to be a risk factor for CSD. It has also been demonstrated that kittens under 12 months of age are more likely than older cats (with serologic evidence of past infection) to be bacteremic [24,25], thereby implicating kittens rather than adult cats as the primary reservoir for infection. Zangwill et al [17] noted that patients with CSD were 15 times more likely than controls to own a kitten, 27 times more likely to have been bitten or scratched by a kitten, and 29 times more likely to have at least one kitten with flea. Demers et al directly and specifically link CSD with kittens [25]. In the illustrative case at the beginning of this article, the probable source of this patient’s infection was not his own cat, but the kitten which his best friend had recently been given!

(d) It has been shown that bacteremic cats are more likely to be infested with the cat flea (*Ctenocephalides felis*) than non-bacteremic cats [24]. Also, by using an artificial feeding device, it has been demonstrated that *B. henselae* is able to replicate and persist in the cat flea [30]. Experimental transmission between cats has been achieved using a flea vector [31], but direct transmission from cat to human has not been demonstrated. It is, of course, entirely possible [32], considering the known arthropod vectors involved in two other bartonella species...
infections (see answer to Q3b). There is some epidemiologic evidence linking *B. henselae* infection with tick bites [33] but no clear connection has been demonstrated.

(c) CSD is primarily a disease of children and young adults. Eighty per cent of cases occur below the age of 21 years [34,35], with a peak incidence between 2 and 14 years. In the USA, there are an estimated 24,000 cases of CSD per year [23]. Seasonal variation is noted, most cases occurring in the autumn and winter [34,36]. Clustering of cases has been documented within families, usually coincident with the acquisition of a new pet [23].

Question 2

(a) Before serologic and genotypic methods were available for the diagnosis of CSD, a clinical diagnosis was established if three of the four following criteria were fulfilled: (1) contact with a cat resulting in a primary lesion; (2) a positive skin test; (3) lymphadenopathy in the absence of any other cause; and (4) characteristic histopathology of a lymph node [37]. By definition, therefore, a history of contact with cats is not essential to the clinical diagnosis. A small proportion of patients provide no such history [37,38].

(b) Reports vary as to the percentage of patients presenting with primary skin lesions, from 25% [17] to 94% [37]. The lesion, a pustule or papule at the site of injury, may have resolved by the time medical attention is sought, but Carithers et al noted that many lesions may not be readily apparent, e.g. if situated on the scalp, and that a thorough search is often necessary [37]. A large study of the presenting features of 1174 cases of CSD over a 29-year period [36] cites the presence of an inoculation lesion in 58.6%.

(c) Lymphadenopathy occurs in 100% of cases [35,36], although between 5% and 20% of cases present because of other manifestations [23]. Lymphadenopathy is the sole presenting sign in approximately 45–50% [35,36]. Other clinical signs include: fever of 38–41°C and malaise in ~30%; anorexia and headache in ~15%; splenomegaly in ~10%; and rash and conjunctivitis in 4–6% [35,36].

(d) Typically, CSD is a benign infection which begins with the formation of a pustule or papule at the site of inoculation 4–6 days after a cat scratch or bite [1]. Regional lymphadenopathy, tender in most patients, develops within 7–50 days of the appearance of the primary lesion. Eighty per cent of the involved nodes are located in the head, neck, and axillary areas [1]. In approximately 15% of cases, the lymphadenopathy is suppurative [1]. Spontaneous resolution within 2–6 months is the rule [35,36].

Reports vary as to the incidence of complex CSD. Margileth et al [36] observed ‘typical’ infection in 88.4% of the cases studied, and ‘atypical’ or ‘complicated’ infection in 11.6%. The commonest complication was Parinaud’s ocuoglandular syndrome (POS), with an incidence of 6.4%. However, rates of POS have been reported from 2% up to 17% [39,40]. POS presents either as conjunctivitis with parotid swelling caused by lymphadenitis, or as an ocular granuloma. Recovery without sequelae in 2–4 months is the usual outcome [23].

Other complications include hepatic and splenic abscesses, pneumonia and pleural effusion, osteomyelitis, paravertebral abscess, and neurologic involvement (references cited in Anderson [23]). A recent article describes a case of CSD mimicking pancreatic malignancy [41].

Neurologic manifestations occur in 1–7% of patients, most commonly as an acute encephalopathy presenting with fever, agitation, confusion, twitching, and even status epilepticus and coma [23,34,42,43]. Children aged 7–12 years are at greatest risk [43,44]; intrathecal antibody production to *B. henselae* is detectable in the cerebrospinal fluid (CSF) [45]. Most patients recover rapidly, usually within 14 days [23]. Other neurologic complications include aseptic meningitis, neuroretinitis, cranial and peripheral neuropathies, myelitis, and meningencephalitis [22,23].

(e) Data concerning in vitro activity of antibiotics against *Bartonella spp.*, and their clinical efficacy, are sparse because of the limited number of isolates and cases. Maurin et al [46] determined the MICs of a wide range of antimicrobials for 14 *Bartonella* isolates by agar dilution. Table 1 summarizes the data for commonly used agents. The E-test has also been used to assess in vitro susceptibilities [47].

Another study, using a cell-culture bactericidal assay system with murine macrophage-like cells and human endothelial cells, demonstrated that, among many antimicrobials, only gentamicin, tobramycin and amikacin possessed bactericidal capability [48].

The use of antibiotics in the treatment of uncomplicated CSD is of questionable value, and is not generally recommended [23,35,49]. In the context of complicated infection, there may be some benefit to be gained. Margileth et al [36] reported in detail on 23 immunocompetent adults who had prolonged severe CSD with constitutional symptoms (e.g. malaise, fatigue, anorexia, weight loss) and lymphadenopathy for between 2–3 weeks and over 2 years. Eighteen patients had fever,
from 38.3° to 40.6°C, lasting from 2 days to 2 months. The remaining five were afebrile throughout. Five patients had systemic complications in the form of neuroretinitis, pleurisy, arthritis, splenic abscesses, and medastinal lymphadenopathy, and enlarged nodes at the head of the pancreas. Treatment with oral antibiotics such as ampicillin, co-trimoxazole, erythromycin and tetracycline was started in 18, and had no effect on the duration or severity of the illness. One patient who developed shock and a convulsion required the addition of tobramycin and cefotaxime, to which the patient is reported to have responded. All patients recovered completely.

Another paper reports the rapid response of three patients with complicated CSD (two had extensive hepatic involvement; one had marked, painful regional lymphadenopathy) to intravenous gentamicin [49]. Note that there is a distinction to be made between the syndrome of ‘complicated CSD’ in the immunocompetent patient, in which the usual outcome is spontaneous recovery, and the syndrome of relapsing fever in the immunocompetent due to prolonged bacteremia with either B. henselae or B. quintana, which would require treatment (see answer to Q3e). CSD in the immunosuppressed, on the other hand, can lead to disseminated and life-threatening infection necessitating treatment [50] (see answer to Q3c).

**Question 3**

(a) Until recently, the genus *Bartonella* contained only a single species, *B. bacilliformis*. In 1993, it was proposed to unite the genus with another, *Rochalimaea*, on the basis of DNA–DNA hybridization studies and 16S rRNA sequencing analysis [51]. At the time, perhaps the best-known species within the genus *Rochalimaea* was *R. quintana*, now *Bartonella quintana*. *R. henselae* had just been identified as a cause of bacteremia in patients with febrile illnesses, and was being mooted as the cause of CSD.

One other species is reported to have caused human infection: a single case of infective endocarditis caused by *B. elizabethae* has been reported [52]. The other members of the genus, *B. vinsonii* (the ‘Canadian vole agent’), *B. henselae*, *B. taylorii*, *B. doshiae*, *B. talpae* and *B. peromysci*, have not yet been associated with human disease [23].

(b) Carrion’s disease is caused by *B. bacilliformis*. It is found only in Peru, Ecuador and Columbia. The bacterium is transmitted by the Peruvian sandfly, *Lutzomyia verrucarum*, and infection results in a biphasic disease characterized by an acute stage called Oroya fever, which is a life-threatening hemolytic illness, followed by a chronic stage termed verruga peruana. This second phase is manifested by the appearance of vascular proliferative lesions of the skin [53].

Until recently, the only described illness associated with *B. quintana* was trench fever, also known as 5-day fever, or quintana fever. The first cases were described in France among allied troops in 1915. The infection is transmitted by the human body louse, *Pediculus humanus*, probably via excreta entering the bloodstream through broken skin [54]. Clinical manifestations include cyclic fever occurring every 4–8 days, in association with periodic rigors, intense retro-orbital headache, pain in the long bones of the leg and the loins, restlessness, weakness, and insomnia. Symptoms range from very mild to severe. Successive relapses tend to diminish in severity and, although the duration of illness may be prolonged, i.e. 4–6 weeks, no fatalities have been recorded [54].

(c) The two predominant *Bartonella* pathogens, *B. henselae* and *B. quintana*, are now known to cause a range of human infections. These species do not cause disease in any host other than the human [54]. Their spectrum of infection is wide, and has been described in both immunocompetent and immunocompromised individuals. Reported disease associations are summarized in Table 2.

**BA** is a vascular proliferative disorder which most commonly causes lesions of the skin [54], but which also results in extracutaneous manifestations. Both *B. henselae* and *B. quintana* have been reported as causative [55]. Despite the lack of a proven animal reservoir for *B. quintana*, Tappero et al [56] postulate that BA is a zoonosis, based on a case-control study of 48 cases and 94 matched controls. They demonstrated that, from a long list of environmental factors, only traumatic cat contact (i.e. bite or scratch) was associated with BA by bivariate analysis, conferring a four-fold increased risk. PCR of 22 case patient tissues identified a genus-specific 16S rRNA fragment for *Rochalimaea* in all 22.

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**Table 1**

<table>
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<th>Antibiotic</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/L)</th>
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<tr>
<td>Penicillin, amoxycillin</td>
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<td>Cefotaxime, cefazidime</td>
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<tr>
<td>Imipenem</td>
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<td>Gentamicin, tobramycin, amikacin</td>
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<tr>
<td>erythromycin</td>
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<tr>
<td>Clindamycin</td>
<td>8</td>
</tr>
<tr>
<td>Sparfloxacin, ciprofloxacin, pefloxin</td>
<td>0.12, 2, 8 respectively</td>
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<td>Co-trimoxazole</td>
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Stewart: Human infection with *Bartonella* species
The species were not identified. Furthermore, in HIV-infected patients, BA appears to be a manifestation of disseminated CSD [11,57–61].

Although the first reports of BA were in HIV-infected patients, subsequent reports have described identical lesions in organ transplant recipients [4,12,58,62,63], and the immunocompetent [56,64,65].

Cutaneous lesions are usually multiple. They are characteristically red vascular papules or nodules when situated superficially, and non-colored nodules if present in the dermis or subcutaneous tissues [54,66]. Superficial lesions are distinguishable from Kaposi’s sarcoma (KS) on the basis of physical characteristics and histology [66]. Unlike those of KS, the lesions of BA are redder and blanch with pressure; they bleed if punctured, and are often painful. The diagnosis is made by histopathologic appearances of a biopsy, and the presence of the organisms by Warthin–Starry stain [66]. The lesions are indistinguishable from those of verruga peruana, and, interestingly, BA has not yet been reported in South America [54].

BA may present with extracutaneous manifestations, ranging from lymphadenopathy to disseminated overwhelming infection. Involvement of the skin does not always occur with BA, but extracutaneous manifestations usually accompany skin lesions [54]. Lesions within the bone marrow, spleen, liver, respiratory and gastrointestinal mucosa, heart, bone, voluntary muscle, and brain have been reported (references cited in Anderson [23], Maurin and Raoult [54] and Adal et al [66]).

A role for B. henselae in HIV-positive patients presenting with neurologic signs, including encephalopathy, has been mooted. BA of the central nervous system in this group of patients has been shown to be associated with intrathecal synthesis of specific antibody by an ELA which utilizes whole B. henselae as the antigen for detection of reactive antibodies [45,67]. Schwartzman et al demonstrated a 32% seroprevalence rate of antibodies to B. henselae among 50 HIV-positive patients with neurologic symptoms, only 29% of whom had abnormal computed tomography (CT) or magnetic resonance image (MRI) appearances, compared with 4% of 51 HIV-positive controls without neurologic involvement [67]. The ratio of organism-specific antibody concentration in CSF to that in serum confirmed intrathecal synthesis. Three patients had detectable IgM in CSF (of whom two also had detectable levels in serum), and PCR of 16S rRNA fragments specific to B. henselae were positive in all three [67]. Symptoms ranged from cranial nerve palsies to ataxia, and from acute disorientation with hallucinations to progressive dementia. These findings suggest that B. henselae is a more common cause of neurologic disease in HIV-positive patients than is currently appreciated, particularly if CT and MRI imaging is normal. Initial experience suggests that prolonged therapy with erythromycin is effective [68,69].

Parenchymal bacillary peliosis is the broader term now used instead of bacillary peliosis hepatis (BPH) [54] to describe a syndrome which may occur as the sole manifestation of bartonella infection, or may be concomitant with BA or bacteremia [66]. Some authors consider it to be an extracutaneous manifestation of BA [23]. The syndrome is characterized by the development of cystic, blood-filled spaces within the parenchyma of, most commonly, the liver and also the spleen [54,62]. Patients present with gastrointestinal symptoms, such as nausea and vomiting, abdominal pain and distension, fever, chills, and hepatosplenomegaly. Diagnosis is by liver biopsy. Prior to its association with BA and HIV, bacillary peliosis had been described in patients with chronic diseases such as tuberculosis and terminal malignancies [54], and has now been described in immunocompetent patients [65]. To date, only B. henselae has been implicated in parenchymal bacillary peliosis [4,56,62,70].

Therapy is indicated for BA and parenchymal bacillary peliosis. Several agents have been reported to be effective, although it may be that the duration of therapy is more important than the choice of antibiotic. Erythromycin appears to be particularly effective [4,11,33,57,58,60,62–65,71], especially if given for a period of at least 4–6 weeks. In practice, most authors
report the use of several agents given either in combination or serially to achieve cure. Aminoglycosides [33,70], amoxycillin [33], co-trimoxazole [61,64], ceftriaxone [33], quinolones [5,33], chloramphenicol [4,33] and rifampicin and isoniazid [11,57] have all been components of effective regimens. Doxycycline and tetracycline have been variously reported as successful [5,57,58,65,72] and ineffective [4,33].

(d) In a review of 299 cases of infective endocarditis of all causes, Raoult et al attributed 10 cases (3%) to *Bartonella* spp. [74]. Bartonella endocarditis has been reported in at least 34 patients [52,74-83], of whom only five were female. The age of the male patients ranged from 22 to 59 years, while the females tended to be older, ranging from 45 to 81 years. Eighteen patients were homeless and/or alcoholic; 15 were immunocompetent; and one was HIV positive. All cases were of native valve endocarditis, 15 with predisposing valvular factors, such as a bicuspid aortic valve. The aortic valve was by far the most common valve affected; indeed, only three cases were non-aortic, affecting the mitral valve alone. Six cases had dual-valve infection: aortic plus mitral in four; and aortic plus tricuspid in two. *B. quintana* was implicated in 13 cases [74,75,77,78,80,82,83], *B. henselae* in seven [74,76,79,81], and *B. elizabethae* in one [52]. In 13 cases, the species was not determined. Infection with *B. quintana*, in contrast to that with *B. henselae*, occurred predominantly among patients without a predisposing valvular lesion, and in those who were homeless. *B. quintana* is known to be transmitted by an arthropod vector in environments of overcrowding and poor hygiene, conditions similar to those experienced by the homeless. Valvular lesions were visualized in 30 of the cases by echocardiography, and surgery was required in all but four, of whom two died, one was cured by antibiotics alone, and one absconded. Eight patients, of the 29 whose outcomes were known, died.

Raoult et al report an IFAT titre of 1:1600 or greater to have a positive predictive value for endocarditis of 0.884 [74]. Bartonellae were isolated from blood in 12 cases [52,74,75,77-79]. Culture of valve tissue was successful only once [74]. In contrast, PCR on valve tissue looks more promising. In Raoult and colleagues’ report of 22 new cases of bartonella endocarditis, six of 16 valves tested yielded citrate synthase amplicons (see answer to Q6e) [74], fresh tissue proving more ‘fertile’ than paraffin-embedded specimens. Prior to this report, PCR had been valuable in making the diagnosis in nine cases out of 12 [75,76,78,79,81-83].

Experience in the treatment of endocarditis is limited. Overall, a combination of a β-lactam (e.g. aminopenicillin or ceftriaxone) and an aminoglycoside (e.g. gentamicin or netilmicin) for a duration of 4–6 weeks has been used most frequently, commonly with an oral macrolide, doxycycline, rifampicin or 4-quinolone added towards the end of the course to enable continued therapy for up to 3 months. In combination with valve replacement, these regimens are usually effective [74]. It is clear that bartonella endocarditis tends to be very destructive to the valve, and experience suggests that valve replacement is almost inevitable. Suspicion of the diagnosis, therefore, should be raised early in the case of a ‘culture-negative’ endocarditis.

(e) A syndrome of relapsing fever due to persistent bacteremia has been described, and is not confined to the immunocompromised. At least 28 patients have been described with this syndrome of bartonella infection [3-5,33,84-86]. Six patients were previously healthy, including an 8-year-old child; 12 were immunosuppressed (HIV-infected, nine; therapy for organ transplantation, two; splenectomy for thalassemia, one); and ten were homeless, chronic alcoholics. With the exception of one HIV-infected patient, the *B. quintana* isolates were identified only in the homeless group.

HIV-negative individuals, even if pharmacologically immunosuppressed, experience an abrupt onset of fever and rigors which usually responds, without relapse, to short courses of antibiotics. By contrast, HIV-positive patients tend to present with an insidious illness characterized by malaise, weight loss, fatigue, and recurring fevers of gradually increasing magnitude, and require therapy for at least a month [4].

**Question 4**

(a) *Bartonella* spp. are not obligate intracellular pathogens, a key factor in the proposal to remove the genus from the order Rickettsiales [51], although cell-culture systems have been useful in the isolation of these organisms [55,75,79,87].

(b) *B. henselae*, in common with *B. quintana* and *B. elizabethae*, is a fastidious aerobic, slightly curved, Gram-negative bacillus, which is oxidase and catalase negative. Classically, *B. henselae* exhibits ‘twitching’ motility in a wet mount, although it does not possess flagella [88]. *Afipia* spp. differ by being urease and oxidase positive, and by reducing nitrates [88].

(c) The species causing human disease fall into two basic phenotypic groups: *B. bacilliformis* and the non-*bacilliformis* group. The differential features are summarized in Table 3 [23,88].
Table 3

<table>
<thead>
<tr>
<th>Property</th>
<th>B. henselae, B. quintana,</th>
<th>B. bacilliformis</th>
<th>B. henselae</th>
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<tbody>
<tr>
<td>Catalase</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flagella</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Optimal growth + 5% CO₂</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Optimal growth temperature</td>
<td>25–28°C</td>
<td>34–37°C</td>
<td></td>
</tr>
<tr>
<td>Colony size</td>
<td>&lt;1 mm</td>
<td>&gt;1 mm</td>
<td></td>
</tr>
</tbody>
</table>

(b, c) Some standard media, such as chocolate agar and blood agar, will support the growth of Bartonella spp. with extended incubation in the appropriate atmosphere [5,52,75,77,84,85]. However, Bartonella spp. are known to exhibit hemin dependence [91,92] and therefore will not grow on media such as MacConkey agar, tryptic soy agar, yeast extract agar, or brain–heart infusion broth [91], unless supplemented with blood, preferably horse or rabbit rather than sheep [4,55]. Subculture of blood onto solid agar can result in colonies being detected within 4 days of incubation [4], although 1–2 weeks is more usual [52,77,78].

B. henselae has been successfully retrieved by direct plating of lysis-centrifuged blood [3,4,33], lymph node homogenate [21,22], and cutaneous lesion [55] onto blood-containing standard media. B. quintana has also been retrieved from lysis-centrifuged blood by direct plating [55]. Culture of blood, lymph node, cutaneous lesions and even osseous lesions in a cell-line system prior to subculture onto solid agars appears to provide an improved yield [55,77,87]. Attempts at both direct culture and cell-line culture of cardiac valve tissue have proven to be largely unsuccessful.

(d) In cases of endocarditis, relapsing fever and systemic infection in the immunocompromised, bacteremia is relatively common, and Bartonella spp. may be recoverable from blood. Automated blood culture systems (e.g. BACTEC, Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA, and BacT/Alert, Organon Teknika, Durham, NC, USA) have been successful in recovering organisms [52,56,77,80,85,86], but it is generally recommended that detection of growth by CO₂ production is not reliable because these organisms produce insufficient quantities [33,52,78,85]. Instead, subculture from the blood culture bottle onto solid media, usually after 1 week of initial incubation, and repeated at intervals, is the approach described most often. Prolonged incubation for 4–6 weeks or more may be necessary. Some groups report the superiority of direct plating of lysis-centrifuged blood over the BACTEC type method [4,33].

(e) Fresh isolates of B. henselae, B. quintana and B. elizabethae produce small white colonies, often with variable morphology (Figure 1). They are autoadherent to the agar, and corrosive pitting of the surface has been described [3,52,55]. This feature is thought to be due to type IV pili on the exterior of the cell wall [93]. Subculture of the colonies leads to loss of broth and/or solid medium systems [5,55,75,85,86]. There has been no comparative study of these diverse methods to assess their relative sensitivities.

Question 5

(a) The optimal method for isolation of Bartonella spp. has not yet been described. Broadly speaking, two main approaches have been used: first, cell-culture techniques [55,75,79,87], and second, a variety of
autoadherence and more rapid growth. B. haemolitica colonies tend not to be adherent, and are smaller and translucent [23].

Question 6
(a) Histopathologic examination of biopsy specimens has been, and remains, one of the cornerstones of diagnosis of bartonella infection, because of the inherent difficulties of culture. Warthin-Starry silver staining, initially used for the visualization of spirochetes, was instrumental in establishing CSD and BA as infectious diseases due to Bartonella spp. [6,7,9,11,55,57,66]. The stain enables differentiation from other causes of lymphadenopathy and similar skin lesions, particularly KS.

(b) Light microscopy of biopsy specimens demonstrates characteristic changes with both BA and CSD. In the former there is lobular proliferation of blood vessels seen with hematoxylin and eosin staining, and infiltration with neutrophils containing granular aggregates which are clumps of bacteria [23]. Granuloma formation is not a feature of BA. By contrast, the lymph nodes of patients with CSD typically show stellate caseating granulomas, microabsceses, and follicular hyperplasia [34].

(c) The CSD skin test uses heat-inactivated material obtained from the lymph node of a patient already diagnosed. The preparation is injected intradermally, and the reaction read 48–96 h later. Of patients with a clinical diagnosis of CSD, 95–98% are positive for the test [23]. However, because of safety concerns regarding the use of human-derived products, and the lack of general availability, this test is of limited value.

(d) Theoretically, serologic testing is the most practical method of diagnosing bartonella infection. Indirect immunofluorescent antibody tests (IFAT) and enzyme-linked immunosorbent assays (ELISA) have been developed, and have played a pivotal role in identifying the cause of CSD (see answer to Q1b). However, there are drawbacks to serologic testing. Most HIV-infected patients fail to mount a detectable antibody response.
A recent report evaluating the IFAT in a small number of patients diagnosed with CSD by the strictest criteria (n = 91) found a titer of 1:64 or higher in 95% compared with around 4% of controls [94]. However, other reports have found less satisfactory detection rates of around 84–88% [16,17]. In patients for whom paired sera were available, only 66% demonstrated a four-fold rise or fall in titer (n = 132) [94]. A recent report evaluating an ELISA test concluded that the IgM assay was less sensitive than the IFA equivalent, and that the IgG assay was not sensitive enough for diagnosis [15].

Even in cases where a significantly raised antibody titer is present, e.g. in Bartonella endocarditis, the specificity of the antibody tests is frequently poor. Not only are there reported difficulties in differentiating between B. henselae and B. quintana [75,80,81] but significant cross-reactivity with Coxiella burnetii [95] and Chlamydia pneumoniae [75,96] has been reported. Indeed, Raoult et al. [74] found that in nine patients previously diagnosed to have chlamydial endocarditis on the basis of serologic investigation had in fact been infected with Bartonella spp. following antibody adsorption assays.

Answers to the multiple-choice questions
Q1: a. True; b. True; c. False; d. False; e. True
Q2: a. False; b. True; c. False; d. True; e. False
Q3: a. True; b. True; c. True; d. True; e. False
Q4: a. False; b. False; c. True; d. True; e. True
Q5: a. False; b. True; c. True; d. True; e. True
Q6: a. True; b. False; c. True; d. False; e. True

Further reading
Readers are referred to two excellent comprehensive reviews by Anderson and Neuman [23] and Maurin and Raoult [54].

References


Stewart: Human infection with Bartonella species


