

Review Article

Neurological Manifestations of Bartonellosis in Immunocompetent Patients: A Composite of Reports from 2005–2012

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Abstract In recent years, an increasing number of *Bartonella* species have been identified as zoonotic pathogens, transmitted by animal bites, scratches or by arthropods. Although historically the term bartonellosis was attributed to infections with *Bartonella bacilliformis*, transmitted by sandflies in the Peruvian Andes, a more inclusive medical use of this term now includes infections caused by any *Bartonella* sp., anywhere in the world. Potentially, because *Bartonella* spp. can infect erythrocytes, endothelial cells, and various macrophage-type cells, including brain-derived dendritic cells in vitro, the clinical and pathological manifestations of bartonellosis appear to be very diverse. The purpose of this review is to focus attention on neurological bartonellosis cases reported in immunocompetent patients since 2005. Among these patients, disease course has varied substantially in length and severity, including one fatal case of encephalitis in a child. Based upon the evolving literature, a high clinical index of suspicion is warranted.

Keywords *Bartonella*; zoonosis; encephalopathy

1 Introduction

The genus *Bartonella* is comprised of a rapidly increasing number of important pathogenic species that induce a spectrum of animal and human diseases, including seemingly diverse neurological abnormalities. These fastidious, hemotropic, Gram-negative bacteria are mainly transmitted by arthropod or insect vectors [25,26]. Most *Bartonella* spp. have evolved to infect one or more mammalian reservoir hosts, within which the bacteria often cause a long lasting intra-erythrocytic bacteremia [11]. During the past decade, there has been a rapid escalation in knowledge relative to those animals that harbor *Bartonella*, the vectors that transmit these bacteria among animals and people, and their pathogenic potential, particularly in pets and people. Infection with one or more *Bartonella* sp. has been associated with a wide range of disease

manifestations; including granulomatous lymphadenopathy, fever of unknown origin, endocarditis, neurological and ophthalmological syndromes, Carrion's disease, trench fever, cat scratch disease (CSD), osteomyelitis, myalgia, peliosis hepatis, splenomegaly and splenic rupture [26] (Table 1), and epithelioid hemangioendothelioma [18,51].

Some of the animals that harbor *Bartonella* include cats, coyotes, cows, dogs, foxes, horses, kangaroos, sheep, numerous rodent species, [35] and bats [3]. There is ample evidence that cats are the primary, but not the sole, reservoirs for transmission of *Bartonella henselae*, *Bartonella clarridgeiae*, and *Bartonella koehlerae* to human beings. Direct cat flea transmission to humans has been suspected [53], whereas epidemiological and experimental transmission studies clearly support cat fleas as the primary vector for transmission of *Bartonella* spp. among cats and likely dogs throughout the world. There are also reports that support bite transmission of bartonellosis from dogs to people [22]. Needle stick transmission has been recently reported in two veterinarians, both of whom developed neurological complications [43,56]. Transmission potentially can occur through cat bites, if infectious flea excrement or cat blood contaminates the bite wound. Other bartonellae reported to cause human infections, their vectors, and primary reservoirs are summarized in Table 1. With the exception of *Bartonella bacilliformis*, for which human beings are the only known reservoir host, all *Bartonella* spp. listed in Table 1 are considered to be zoonotic pathogens. Historically, *Bartonella quintana* was considered a non-zoonotic species because the human body louse was thought to be the sole vector and human beings the sole reservoir host. However, in recent years, *B. quintana* DNA has been PCR amplified and sequenced from cat fleas [63, 66], rodent fleas [66], ticks, [66], dog saliva [31], and from dogs with *B. quintana* endocarditis [39]. In addition, *B. quintana* bacteremia was confirmed in a woman after being

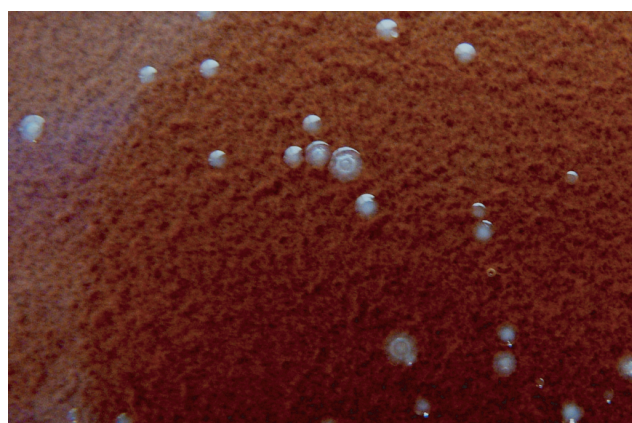
Table 1: Species and subspecies of *Bartonella* that are confirmed or potential human pathogens, primary hosts and vectors, known accidental hosts, and human diseases (adapted from Chomel and Kasten, 2010 [26] and Kaiser [37]).

<i>Bartonella</i>	Primary reservoir	Vector	Accidental host(s) and (human disease)
<i>Bartonella alsatica</i>	(Rabbit <i>Oryctolagus cuniculus</i>)	(Rabbit flea? <i>Spilopsyllus cuniculi</i>)	Human (endocarditis [59])
<i>Bartonella bacilliformis</i>	Human	(Sandfly <i>Lutzomia verrucarum</i>)	None (Carrion's disease, Oroya fever, verruga peruana)
<i>Bartonella clarridgeiae</i>	(Cat <i>Felis catus</i>)	(Cat flea <i>Ctenocephalides felis</i>)	Human, dog (cat scratch disease)
<i>Bartonella elizabethae</i>	(Rat <i>Rattus norvegicus</i>)	(Oriental rat flea <i>Xenopsylla cheopis</i>)	Human, dog (endocarditis, neuroretinitis)
<i>Bartonella grahamii</i>	(Wild mice <i>agrestis</i> , <i>Apodemus Clethrionomys glareolus</i> , <i>Microtus flavicollis</i>)	Rodent fleas	Human (neuroretinitis)
<i>Bartonella henselae</i>	(Cat <i>Felis catus</i>)	(Cat flea <i>Ctenocephalides felis</i>)	Human, dog, horse, marine animals (cat scratch disease, bacillary angiomatosis, endocarditis, neuroretinitis, bacteraemia, epithelioid hemangioendothelioma) [51]
<i>Bartonella koehlerae</i>	Cat	Cat flea	Human, dog (endocarditis) [2]
<i>Bartonella melophagi</i>	(Sheep <i>Ovis aries</i>)	(Sheep ked <i>Melophagus ovinus</i>)	Human [46]
<i>Bartonella quintana</i>	Human	(Body louse <i>Pediculus humanis</i>)	Cat, dog (endocarditis, trench fever, bacillary angiomatosis)
<i>Bartonella rochalimae</i>	Canids	(Fleas? <i>Pulex irritans</i> , <i>Pulex simulans</i>)	Human, dog (bacteraemia, fever)
<i>Bartonella tamiae</i>	(Rat?) unknown	Mites? Ticks?	Humans (bacteraemia, fever [41])
<i>Bartonella vinsonii arupensis</i>	(White-footed mouse <i>Peromyscus leucopus</i>)	(Fleas? ticks?) unknown	Human (bacteraemia, fever, endocarditis)
<i>Bartonella vinsonii berkhoffii</i>	(Coyote <i>Canis latrans</i>) dog (<i>Canis familiaris</i>)	(Ticks?) unknown	Human, cat (endocarditis, epithelioid hemangioendothelioma) [18]
<i>Bartonella washoensis</i>	(Californian ground squirrel <i>Spermophilus beecheyii</i>)	(Fleas <i>Oropsylla montana</i>)	Human, dog (myocarditis, endocarditis)

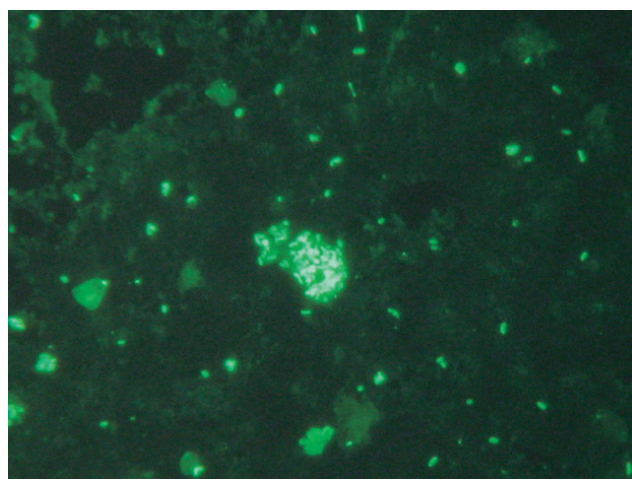
bitten by a feral cat, that months later was shown to be *B. quintana* bacteremic [17]. Thus, there is evolving evidence to support *B. quintana* as a zoonotic pathogen, potentially transmitted among cat and rodent fleas to pets, and thus posing a risk for human infection. Clearly, the zoonotic risk of acquiring bartonellosis is of substantial importance to veterinary professionals, groomers, animal rescue workers, and pet owners, particularly for those individuals who come into frequent contact with feral animals or with privately owned pets for which flea and tick prevention strategies are not used to avoid infestations [47]. Other recent publications have emphasized the ecological complexity of *Bartonella* spp. transmission in nature [23,66]. Tick transmission of a *Bartonella* sp. has been a controversial subject for some years [27,65]. Recently, vector competence for tick (*Ixodes ricinus*) transmission of a *Bartonella* sp. was demonstrated experimentally, thus supporting the possibility that *Ixodes* sp. ticks are transmitting *B. henselae* throughout the northern hemisphere [60]. Previous studies from Europe and North America have documented the presence of *B. henselae* DNA in *Ixodes ricinus* [29], *Ixodes scapularis* [66] and *Ixodes pacificus* [36].

Historically, CSD has represented a prototypical disease process in immunocompetent people, characterized by

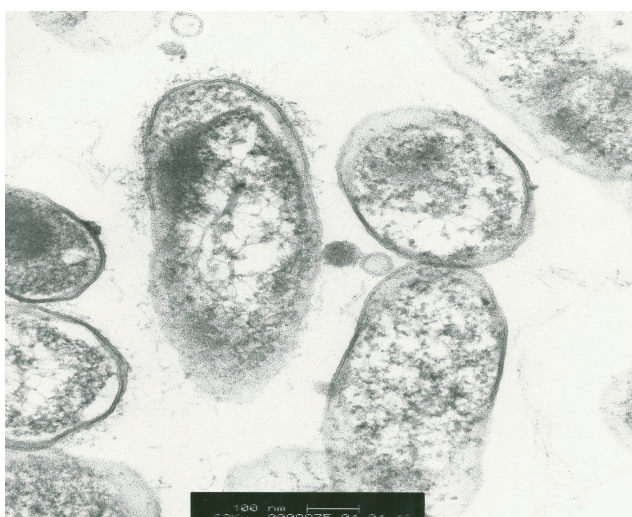
the triad of a cat scratch or bite, fever, and regional lymphadenopathy [9]. Generally, within 7–12 days following transmission of *B. henselae*, the primary if not the sole cause of classical CSD, a papule and then a pustule develop at the inoculation site. One to 3 weeks later, regional lymphadenopathy (usually involving a single lymph node) develops with the persistence of lymphadenomegaly for a few weeks to several months [24,26]. Low-grade fever, malaise, and muscle pain are often reported by CSD patients. Some patients develop severe headaches, anorexia, and splenomegaly. Abscessation of lymph nodes is reported occasionally. Approximately 10% of CSD patients develop what have historically been considered atypical manifestations, including Parinaud's oculo-glandular syndrome, encephalitis, endocarditis, haemolytic anaemia, hepatosplenomegaly, glomerulonephritis, pneumonia, pleural and pericardial effusions, relapsing bacteremia, and osteomyelitis [9,26]. *Bartonella henselae* appears to be the primary, if not the sole, cause of CSD. As discussed in the diagnosis section, isolation, serology, and PCR testing combined with enrichment bacterial culture have facilitated the diagnosis of CSD and neurobartonellosis (Figure 1). With improvements in diagnostic testing modalities, these "atypical" manifestations may be much more prevalent



(a)



(b)



(c)

Figure 1: (a) *Bartonella henselae* colonies on a chocolate agar plate; (b) indirect immunofluorescence microscopy demonstrating cell culture grown *B. henselae*; (c) scanning electron micrograph of *B. henselae* (176, 400X).

(more recent estimates have been revised to 25% of “atypical” cases) in human patient populations than was previously suspected [54]. In the past decade, numerous cases of neuroretinitis or neurobartonellosis optical neuritis have been reported and as these cases have recently been reviewed by Kalogeropoulos et al. [38]; these cases will not be included in this review.

The purpose of this review is to summarize the historical and neurological manifestations reported between 2005 and 2012 in immunocompetent people with bartonellosis. While there are rare exceptions, encephalopathy is a unifying factor in these cases. The additional manifestations are varied; including seizures, cranial neuropathies, aphasia, white matter abnormalities, and peripheral nervous system diseases. Testing limitations, as well as progress in the development of more sensitive and specific diagnostic modalities, will be briefly reviewed. Cerebrospinal fluid findings are often normal or minimally abnormal [49]. Also, because there appears to be a spectrum of disease outcomes ranging from death to self-limiting illness, challenges associated with the selection of an effective treatment regimen and the need for additional research to define the neuropathogenesis of bartonellosis will be discussed.

2 Overview of neurological case reports in immunocompetent patients (2005–2011)

2.1 Encephalitis

Based upon the spectrum of neurological disease manifestations, researchers have hypothesized that *Bartonella* may cause nervous system damage either by producing neural toxins, by direct injury to cells within the CNS, or by triggering an aberrant autoimmune or immune-mediated response. To a great extent none of these hypotheses have been rigorously tested and the remarkable variability among clinical presentations in patients with neurological bartonellosis supports to a varying degree involvement of multiple or individual mechanisms of CNS injury among patients. For example, in 2005, Dyachenko et al. described a 22-year-old patient with fever (38 °C), an epitrochlear mass, and a history of cat scratches, who after a detailed evaluation was treated with a doxycycline for a presumptive diagnosis of CSD [32]. Despite continued administration of oral doxycycline, six days following discharge from the hospital the patient developed generalized tonic-clonic convulsions, followed by a loss of consciousness. CSF analysis revealed only a few leukocytes (3/μL), and normal protein (39 mg/dL), and glucose (80 mg/dL) concentrations. CSF bacterial culture did not result in growth and a herpes simplex virus PCR was negative. Computed tomography of the patient’s brain was normal, but an EEG revealed epileptiform discharges in the temporo-parietal regions. *B. henselae* DNA was amplified from the CSF and following surgical exploration and excision of an encapsulated abscess

in the right epitrochlear lymph node, *B. henselae* DNA was PCR amplified and infection was documented by restriction fragment length polymorphism (RFLP) analysis. Treatment with oral rifampin and intravenous minocycline elicited rapid improvement in consciousness. One year after developing encephalopathy, the patient remained seizure-free without residual neurological deficits. In this patient, PCR amplification of *B. henselae* DNA from the CSF provided a circumstantial support for direct CNS invasion by *Bartonella*. This case also illustrates a potential need to select antibiotics that both penetrate the central nervous system and acquire high intracellular and extracellular antimicrobial concentrations, so as to more rapidly achieve resolution of the neurological symptoms.

In 2008, Nishio et al. described the development of encephalopathy in a 9-year-old girl following admission to the hospital for fever, right cervical lymphadenopathy, and a history of a cat scratch (classical CSD) [55]. Routine laboratory tests were normal, with the exception of slight elevations in erythrocyte sedimentation rate and C-reactive protein (CRP). Initially intravenous ceftriaxone and oral minocycline were administered, with defervescence in the first 24-hours. Due to a minimal decrease in serum CRP over the next 4 days, panipenem/betamiprone was substituted for the initial antibiotics. Once CRP decreased to 1.5 mg/dL, oral clarithromycin was substituted for minocycline. On the 14th day of hospitalization, CRP increased in conjunction with a rise in temperature. On the 16th day of hospitalization, the patient developed generalized tonic convulsions for 5 minutes followed by a comatose state. A CT scan did not reveal any abnormalities, whereas an EEG indicated a generalized slow wave activity, a common but non-specific finding of encephalopathy. CSF analysis was normal with the exception of a slightly increased white blood cell count (12 cells/mm³). The patient was reported to be *B. henselae* seroreactive. After intravenous administration of ceftriaxone and erythromycin, the patient had a rapid neurological recovery. One month after admission, a right cervical lymph node biopsy revealed granulomas with central necrosis. *B. henselae* DNA was amplified from the lymph node, but retrospective attempts to amplify *Bartonella* DNA from CSF were not successful. The patient subsequently remained free of seizures during a six year follow-up period.

In 2007, Fouch and Coventry described a 6-year-old previously healthy Hispanic boy, who was treated with cephalexin after being diagnosed with left axillary lymphadenopathy associated with CSD. During the subsequent 48 hours, the patient developed fever, persistent vomiting, and abdominal pain, indicative of systemic bartonellosis [67] and was hospitalized. During the next two days, the child complained of a worsening headache. By day 4 of hospitalization, mental status deteriorated and the boy

developed seizures. No abnormalities were observed on a brain CT scan. Thirty six hours after the scan he had another seizure, and lumbar puncture revealed 51 WBC/ μ L (94% lymphocytes, 5 % monocytes, 1% segmented neutrophils), glucose (114 mg/dL), and protein (31 mg/dL). Gram stain was negative. Twenty minutes following the lumbar puncture, the boy developed respiratory arrest requiring cardiopulmonary resuscitation. Pupils became fixed and despite medical therapy the boy died. Following autopsy, *B. henselae* DNA (*htr* gene) was amplified from the granulomatous lymph node at the Centers for Disease Control and Prevention; however, *B. henselae* serology results were equivocal (IgM < 16, IgG 1:128) and a Warthin-Starry stain of the lymph node was negative. The brain contained extensive, diffuse perivascular lymphocytic infiltrates with microglial nodules, involving the frontal, parietal, and occipital lobes and pons. *B. henselae* DNA was not amplified from the brain and organisms were not identified by special stains. Tests for other infectious causes of encephalitis were negative (Table 2).

2.2 Aphasia/encephalopathy

In 2007, Fox et al. described a 15-year-old male patient who was sent home from school due to nausea, vomiting, and “an inability to speak” [34]. Expressive aphasia, mild right facial droop, right sided weakness, and an inability to ambulate were found when examined at a local hospital. Laboratory tests and a CT of the head were unrevealing. Subsequently, a diffuse white matter periventricular signal abnormality was interpreted from an MRI of his brain. Aphasia resolved during a one-day hospitalization and as a cerebrovascular insult was suspected, the patient was discharged with instructions for aspirin therapy. Within hours of discharge the boy could not speak and was re-admitted the next day due to agitation. A mildly tender right axillary lymph node was palpated and healing papules and scratches were identified on his chest, which was attributed to being scratched on several occasions by the family kitten. Following review of the previous MRI by a pediatric neuroradiologist, intravenous steroids were administered for acute disseminated encephalomyelitis. The only abnormality identified during an extensive inpatient evaluation was “strongly positive” IgM and IgG *B. henselae* antibody titers. The boy was treated with a 5-day course of azithromycin, after which he remained normal during the 5-month follow-up period (Table 2).

In 2010, Marienfeld et al. reported sudden-onset expressive aphasia, word substitution errors, and impaired repetition in a 59-year-old male with a history of a previous stroke. The patient had recently adopted several stray kittens. Ten days prior to presentation the patient reported a skin infection that progressed to right axillary lymphadenopathy. CSD was diagnosed and treated with

Table 2: A summary of the diagnostic features of cases of neurobartonellosis.

Case	Microbiological blood and CSF examination and CT and MRI examinations of brain	Biochemical assays	References
Recurrent expressive aphasia in a 15-year-old male	Brain MRI suggested an acute disseminated encephalomyelitis. <i>B. henselae</i> titers were positive: IgM > 1:32 and IgG > 1:128. CSF PCR was negative.	Negative for metabolic disorders, hypercoagulability, thromboembolism, toxic ingestions, toxic exposures, autoimmune disorders, encephalitis, and evidence of traumatic injury.	[34]
Encephalitis lethargica in a 16-year-old girl	Increased <i>B. henselae</i> antibody titers. Blood and CSF PCR performed after antibiotic therapy were <i>B. henselae</i> negative. Tests for HHV6, VZV, EBV, HIV, Measles, Enteroviruses, L. monocytogenes, and Rubella were negative. Negative for antibodies against basal ganglia.	Normal WBC count, C reactive protein, antistreptolysin O titer, and all other routine blood analyses. CSF identified lymphocytosis and intrathecal synthesis of oligoclonal bands.	[20]
Encephalopathy in a 9-year-old girl during administration of antibiotics	Elevated erythrocyte sedimentation rate. Sera were negative for EBV, CMV, and <i>T. gondii</i> . <i>B. henselae</i> IgM titer was 1:80 and IgG titer was 1:1024. Lymph node was PCR positive for <i>B. henselae</i> . CSF PCR was negative.	Elevation of C reactive protein. Histology of lymph node biopsy showed granulomatous mass with no malignant cells.	[55]
Fatal encephalopathy in a 6-year-old male	WBC count 9600/mL, CSF contained 51 WBC/mL, CSF was negative for HSV, Eneovirus, WNV, flavivirus, and Eastern equine encephalitis virus. CT was negative. <i>B. henselae</i> IgM titer was 1:16 and IgG titer was 1:128. PCR of lymph nodes was positive.	Toxicology screen was negative.	[33]
Transverse myelitis in a 46-year-old man	Cranial CT was negative. Culture and stain of CSF for bacteria, fungi, and acid fast bacilli were negative. CSF cryptococcal antigens test was negative. Serological testing for coccidioidomycosis, HIV, Lyme disease, and syphilis was negative. <i>B. henselae</i> IgM titer was 1:64 and IgG antibody titer was 1:512.	Chest radiograph, urinalysis, blood chemistry, and ANA were negative; except lactate dehydrogenase which was 266 mU/mL	[4]
Transverse myelitis in a 13-year-old boy	<i>B. henselae</i> and <i>B. quintana</i> IgG antibody titers were 1:512. Warthin-Starry stain was positive.		[4]
Expressive Aphasia in a 59-year-old male	VDRL and CSF culture were negative; PCR for HSV, VZV, and enterovirus was negative and WBCs were absent. <i>Bartonella henselae</i> titers were reported as 1: 1024.	CSF glucose level was 60 mg/dL and protein was 64 mg/dL	[50]
MS-like disease in a 27-year-old-man	Brain and spinal cord MRI were normal. No oligoclonal bands. <i>Borrelia burgdorferi</i> , syphilis, HIV, Epstein Barr virus, cytomegalovirus, HSV 1 and 2, leptospirosis, leishmaniasis, listeriosis, Q fever, tularemia, and <i>M. pneumoniae</i> testing was negative.	ANA, ENA, ANCA, antiphospho lipid antibodies were negative; normal biochemistry profile.	[21]
Psychiatric illness in a 41-year-old male	<i>Bartonella henselae</i> IgM titer was 1:256 and <i>Bartonella henselae</i> PCR was positive.		[64]
Psychiatric illness in a male	<i>Bartonella henselae</i> Ig G titer was 1:128. <i>Bartonella</i> PCR was negative.		[64]
Psychiatric illness in a female	Unusual rash on thigh. No diagnostically supportive data.		[64]
Meningitis in a 47-year-old woman	Cranial and abdominal CT were normal. CSF culture was negative, CSF WBC count was 6 cells/mL. Blood culture after five days of incubation was identified as <i>Bartonella washoensis</i> by FAME analysis and glt A PCR and DNA sequencing.	Coagulation, serum chemistries, and liver function tests were normal.	[57]
Neurological disease in a 50-year-old male	Positive Romberg sign and difficulty with heel-toe walking. Cranial nerves, muscle strength, sensation, and deep tendon reflexes were normal. CSF analyses were normal. By IFA testing, the patient was <i>B. vinsonii berkhoffii</i> genotypes II and III and <i>B. henselae</i> seroreactive. <i>B. vinsonii</i> subsp <i>berkhoffii</i> DNA was amplified and sequenced from blood and CSF using BAPGM enrichment and culture. Periodontal swab sample was <i>B. vinsonii</i> subsp. <i>berkhoffii</i> PCR positive.		[14]
Headaches and insomnia in a 7-year-old female	Seroreactive to <i>B. vinsonii</i> subsp. <i>berkhoffii</i> genotypes II and III and <i>B. henselae</i> , <i>B. vinsonii</i> subsp. <i>berkhoffii</i> DNA was amplified and sequenced from two BAPGM enrichment blood cultures performed 3 weeks apart		[14]
Hallucinations and visual defects in an 18-year-old female	Over a 1-year period, the patient was consistently <i>B. koehlerae</i> seroreactive and <i>B. koehlerae</i> was repeatedly PCR amplified and sequenced from blood or BAPGM enrichment cultures. There were no abnormalities on a noncontrast brain MRI or an electroencephalogram.		[19]

a 5-day course of doxycycline. A head CT identified a low-density lesion in the region of the left insula, with no mass effect. An MRI showed his prior stroke, but no changes consistent with an acute or subacute infarction. EEG demonstrated generalized slowing, but no epileptiform activity. Eighteen hours later, the patient had a generalized seizure and a waning level of consciousness. A lumbar puncture was normal with the exception of a slight increase in protein (64 mg/dL). Viral and bacterial antibody and PCR testing were negative and CSF culture did not result in bacterial growth. Overnight, the patient responded rapidly to intravenous azithromycin, after which he was discharged with instructions to take oral doxycycline and rifampin for 14 days. *B. henselae* antibody titers were positive (IgM 1:20 and IgG 1:1024).

2.3 Encephalitis lethargica (EL)

Encephalitis lethargica is characterized by psychiatric disturbances, lethargy, sleep abnormalities, parkinsonism, and dyskinesia. In March 2005, a 16-year-old girl was referred for evaluation of lethargy, disorientation, and behavioral changes that began 14 days prior to presentation [20]. Six weeks earlier, the girl had complained of swollen lymph nodes in her neck. Physical examination findings were consistent with classical encephalitis lethargica, including lethargy, oral dyskinesias, and dystonia. The girl did not recall exposure to animals and there was no prior history of psychiatric disorders or drug abuse. After hospitalization, seizures progressed from focal to generalized tonic-clonic, accompanied by respiratory arrest requiring mechanical ventilation for over four days. Interictal EEG demonstrated focal slowing over the left temporal lobe. Lumbar puncture revealed a lymphocytosis with intrathecal synthesis of oligoclonal bands. Repeated serological testing identified *B. henselae* antibody titers of 1:64 (day 8 of hospitalization) and 1:256 three weeks later. *B. henselae* PCR from serum and cerebrospinal fluid was negative; however, the samples were obtained after three weeks of antibiotic therapy. Initial and two follow-up cerebral MRIs showed no pathological findings. However, [123]IBZMSPECT revealed a marked decrease of striatal dopamine D2 receptor availability which was more pronounced on the left side corresponding to the predominantly right-sided rigidity. Antibodies reactive against basal ganglia were not found. Symptomatic treatment of parkinsonism with both levodopa (300 mg/tid) and amantadine (200 mg daily IV) did not improve motor disturbances. Treatment with erythromycin (1 g q8h IV) over 14 days in combination with methylprednisolone (initially 1000 mg daily with slow tapering off) also had no effect on lethargy and parkinsonism. Consequently, antibiotic therapy was changed to rifampicin (600 mg daily IV) and doxycycline (100 mg q12h PO) resulting in a significant improvement of her neurological symptoms within one week's time and total

resolution of motor, cognitive, and behavioral disturbances within two months. Encephalitis lethargica is thought to be an immune-mediated disorder due to the presence of intrathecal oligoclonal bands, antibodies reactive against basal ganglia. Steroids are often beneficial. This patient lacked antibodies to basal ganglia and had no discernible beneficial effect from steroids, whereas there was rapid and progressive improvement in neurological status after administration of rifampicin and doxycycline. The authors speculated that *B. henselae* caused encephalitis with the phenomenology of encephalitis lethargica. There is overlap between encephalitis lethargica anti-N-methyl-D-aspartate (NMDA) receptor encephalitis, but this case occurred prior to wide recognition of the latter [28].

2.4 Transverse myelitis, multiple sclerosis (MS) like disease, and Guillain-Barré syndrome (GBS)

Transverse myelitis is associated with spinal inflammation induced by viral infections, syphilis, vaccinations, demyelinating disease, or idiopathic immune reactions that result in extensive damage to nerve fibers of the spinal cord. In 2007, Baylor et al. described two patients with transverse myelitis, both of whom were exposed to young cats and had enlarged lymph nodes (classical CSD). Both patients had positive *Bartonella* serological test results (Table 2). Patient 1 was a 46-year-old man who had a 4-week history of intermittent fever, night sweats, a 5.5 kg weight loss and development of a painful swelling in the right groin. Surgical exploration identified a large lymph node, which was excised. The following day, the patient developed numbness in the right foot and difficulty voiding, and subsequently an inability to ambulate due to flaccid areflexic paraplegia with an incomplete sensory level at T5. Routine laboratory findings were unrevealing, chest radiographs and a cranial CT scan were normal. An MRI identified focal cord changes at C5–C7, consistent with an inflammatory process. Lumbar puncture yielded 47 WBC cells/mm³ (70% lymphocytes, 26% monocytes, and 4% neutrophils) and a protein level of 58 mg/dL. CSF stains and culture were negative, as were a battery of serological tests. The lymph node contained necrotizing granulomatous inflammation. Acid fast and methanamine silver stains were negative, whereas Warthin-Starry staining at the Armed Forces Institute of Pathology identified a myriad of branched bacilli consistent with CSD. Serum *B. henselae* IgM and IgG antibody titers were 1:32 and 1:128 respectively, whereas no antibodies were detected in CSF. Serology, repeated three weeks later, identified a *B. henselae* IgG titer of 1:512, supporting seroconversion. The patient was initially treated with methylprednisone and intravenous immunoglobulin. After diagnosis of bartonellosis, the patient was treated with oral doxycycline and rifampin for two months. Patient 2 was a 13-year-old boy who developed right axillary lymphadenopathy, one

week following a cat scratch. Subsequently fever, anorexia and headaches developed. By day 17 post-scratch, the boy developed dyesthesias, abdominal pain, difficulty in walking followed by paralysis, and urinary retention. CSF contained increased WBC (300 cells/mm^3 with 70% lymphocytes and 30% neutrophils) protein level of 90 mg/dL. Antibiotic treatment with ceftazidime and netilmicin for 10 days and dexamethasone intravenously for 5 days resulted in defervescence, and resolution of neurological symptoms beginning on post-treatment day 7, with complete resolution reported 6 weeks later. A serological diagnosis of myelitis secondary to bartonellosis was made based upon *B. henselae* antibody titers of 1:128 at admission, 1:512 at day 15, and 1:32 at 6 months post-discharge.

In 2010, Brinar and Habek described a patient with multiple sclerosis-like symptoms [21]. The patient had developed encephalopathy, fever, non-tender-pustulous skin lesions, lymphadenopathy, splenomegaly, and relapsing neurological symptoms: slight motor weakness of the left extremities, diminished visual acuity and double vision, paresthesia in the left extremities with demyelinating brain MRI changes. Some symptoms, such as non-tender papules on legs, remittent febrile status, regional lymphadenopathy and splenomegaly pointed to a systemic illness, while the neurological symptoms characterized by remittent encephalopathy, loss of visual acuity, and minor left-sided hemiparesis in conjunction with the MRI findings were consistent with multiple sclerosis. Findings supportive of an infectious process included signs of systemic illness, negative CSF oligoclonal bands, encephalopathy, and an MRI that was more typical of a non-inflammatory/vascular demyelinating disorder. Serological testing for infectious agents was negative (Table 2). Historically, the patient transported wild animal meat across Europe and had exposure to cats. Because of the non-tender pustules, regional lymphadenopathy, splenomegaly, and oscillations in neurological status, neurobartonellosis became a diagnostic consideration. *B. henselae* antibodies were found in serum and CSF. One month after antibiotic therapy, the patient's clinical status normalized and he made a full recovery.

In 2006, Massei et al. described a 10-year-old girl who was hospitalized due to leg weakness [52]. Seven days earlier, the girl had a self-limiting episode of fever and vomiting of 1 day duration. Four days later, she had difficulty walking, became irritable, and complained of severe myalgia in the lower limbs. Laboratory findings were not remarkable. Nerve conduction studies identified decreases in motor conduction velocity and amplitude, consistent with axonal damage. An exhaustive search for known causes of Guillian-Barre syndrome was negative. The patient was treated with intravenous immunoglobulins for 5 days, antibiotics were not administered, and within two weeks her neurological status had normalized. There was no

history of cat scratches, no palpable lymphadenopathy, and no hepatic or splenic lesions on an abdominal ultrasound. Because she lived in a rural area and played with kittens, *B. henselae* serology was requested. She had an IgG titer of 1:1024 and a specific IgM titer (value not reported). CSF PCR was negative. Convalescent serology did not identify IgM antibodies and IgG antibodies were decreased (titer not provided).

In 2007, Mantadakis et al. described a 16-month-old girl who presented to an emergency room for respiratory distress, cough, vomiting, diarrhea, and a decreased level of consciousness during the preceding week. The child lived in a rural area with cat, dog, rabbit, and bird contact. A CT scan of the brain was unremarkable. Lumbar CSF contained 3 cells/mm^3 (all lymphocytes), normal glucose and protein, and negative Gram stain and culture. She was empirically treated with cefotaxime and ampicillin intravenously and was admitted to the pediatric intensive care unit. Thoracic radiographs revealed a right upper lobe infiltrate, requiring intubation and mechanical ventilation. Ampicillin was discontinued and clindamycin and acyclovir were administered intravenously. On the third day of hospitalization, an electroencephalogram showed diffuse slowing and a repeat lumbar puncture identified a marked increase in CSF protein (322 mg/dL) with 5 nucleated cells (80% lymphocytes, 20% neutrophils). While the child remained on mechanical ventilation, there were recurrent episodes of "unexplained" tachycardia and hypertension. During weaning from sedatives, deep tendon reflexes were absent. An MRI scan of the brain and spinal cord with gadolinium showed homogeneous uptake throughout the lower spinal roots. Repeat lumbar puncture identified a protein of 379 mg/dL. GBS was diagnosed and the child was treated with intravenous gamma globulin and a 2-week course of intravenous clarithromycin. Within 3 days, the patient could move her legs. Reduced motor conduction velocity and amplitude supported peripheral axonal damage. The patient was extubated 1 week after receiving immunoglobulin when a repeat MRI identified acute hydrocephalus, necessitating a ventriculoperitoneal shunt. Eight months following discharge, the child had a normal neurological examination. Subsequent efforts to identify an infectious etiology for GBS in this patient resulted in documentation of greater than a four-fold decrease in IgM titer for *B. quintana*. In addition, *B. quintana* DNA was amplified and sequenced from 2 CSF samples. There was no historical evidence of louse infestations or flea bites to the patient's body.

2.5 Meningitis

In 2009, Probert et al. described a 47-year-old previously healthy woman who developed fever, chills, headache, nausea, vomiting, epigastric and lower abdominal pain

and joint pain that had progressed over 24 hours. Examination findings included fever (39.3 °C), photophobia, neck stiffness with mild nuchal rigidity, consistent with meningitis. The patient reported recent handling of a dead mole and dead ground squirrel, but denied flea, tick or other insect bites. Lumbar CSF contained 6 WBC/mm³ (51% neutrophils, 49% monocytes), protein 40 mg/dL and glucose 64 mg/dL. CSF cultures and PCR for enterovirus were negative. When discharged three days later, the platelet count had decreased from 168 to 112 × 10³ cells/μL. The patient was treated empirically with vancomycin and chloramphenicol, followed by replacement of chloramphenicol with aztreonam, and oral moxifloxacin prescribed at discharge. At follow-up 6 days later, urinalysis revealed WBCs, RBCs, and nitrates, and the patient was switched to levofloxacin for 10 days. Eight weeks after hospitalization the patient was clinically improved, but reported persistent body ache and bone pain. Additional patient follow-up was not provided. A blood culture obtained at admission yielded aerobic growth after 5 days of incubation and a subculture isolate was eventually identified as *Bartonella washoensis*. Ground squirrels have been suggested as the major reservoir for *B. washoensis* in California and Nevada. *Oropsylla montana* is the most common flea species to parasitize California ground squirrels. The *gltA* sequence from *O. montana* fleas collected from the patient's putative site of exposure matched the *gltA* sequence from bacteria isolated from the patient's blood. Although the patient did not recall recent arthropod bites, it seems likely that she was bitten by an infected flea while handling the squirrel carcass or while walking near ground squirrel burrows on her property.

2.6 Neurological diseases with psychological illness

In 2007, Schaller et al. described three patients with acute psychiatric disorders (personality changes, agitation, panic attacks, and depression) associated with “*Bartonella*-like” symptoms. Tick transmission was suspected in two patients, and the third was exposed to cat fleas. Symptoms consistent with *Bartonella* infection included lymphadenopathy adjacent to an *Ixodes scapularis* tick bite and a rash in the remaining patients. In order to function normally, all three patients required very high doses of antidepressants, benzodiazepines, or antipsychotics. Medication doses were lowered following antibiotic treatment and all three patients returned to their previously healthy or near-normal baseline mental health status. The authors reported that diagnostic and therapeutic management of these patients did not offer certain proof of *Bartonella* infection, but that patients infected with *Bartonella* might have a variety of mental health symptoms.

In 2011, Breitschwerdt et al. reported *B. koehlerae* bacteremia in a young woman experiencing depression,

anxiety, mood swings, severe headaches, muscle spasms, interphalangeal joint stiffness, decreased peripheral vision, diminished tactile sensation, and hallucinations. When tested against five *Bartonella* spp. antigens, the patient was only *B. koehlerae* seroreactive. Before serological and BAPGM (*Bartonella* alpha Proteobacteria growth medium) enrichment culture/PCR diagnosis, the patient had a slowly progressive history of neurological and neurocognitive abnormalities. Following the initial course of antibiotics (doxycycline and rifampin), there was a decrease in hallucination frequency while receiving a stable dose of antipsychotic medication. Despite an initial treatment failure, total resolution of hallucinations and visual field deficits followed a third course of antibiotics (rifampin and azithromycin). Following treatment, *B. koehlerae* antibodies and *Bartonella* DNA were no longer detectable, all symptoms resolved and antipsychotic drugs were sequentially withdrawn. On the basis of serological and PCR findings (Tables 2 and 3), and the resolution of symptoms following antibiotic treatment; prospective studies are needed to clarify the extent to which *Bartonella* spp. bacteremia might contribute to neurocognitive abnormalities.

2.7 Neurological diseases with *Bartonella* spp. bacteremia

During the past decade, a number of *Bartonella* research laboratories validated the utility of using an insect cell culture medium in conjunction with PCR amplification to enhance the diagnostic sensitivity of confirming *Bartonella* spp. bacteremia in human patients [44,61]. In the Intracellular Pathogens Research Laboratory (IPRL) at the College of Veterinary Medicine, North Carolina State University, *Bartonella* alpha Proteobacteria Growth Medium (BAPGM) was used as an enrichment culture step in conjunction with *Bartonella* sp. PCR. Initially, due to frequent exposure to bacteremic animals and their arthropod vectors, blood or cerebrospinal fluid samples from veterinarians and other animal health professionals with historical animal and arthropod exposures were tested using the BAPGM enrichment culture platform. As a result, it appears that these individuals, a subset of whom report various neurological complaints, are at risk for persistent *Bartonella* spp. bacteremia [12]. Subsequently, the use of the BAPGM platform has resulted in documentation of *Bartonella* spp. bacteremia or cerebrospinal fluid infection in individual patients and small case series summarized below. Clearly there is the need for future case control studies to define the neuropathogenic potential of this genus and to clarify mechanisms of neural injury or dysfunction.

In 2008, Breitschwerdt et al. reported infection with *B. vinsonii* subsp. *berkhoffii* and *B. henselae* in six immunocompetent individuals with symptoms including ataxia, tremors, memory loss, and seizures. In one patient,

Table 3: Serological, BAPGM enrichment blood culture, and PCR/DNA sequencing test results for an 18-year-old woman with hallucinations and peripheral visual deficits [19].

Date (mo/day/yr)	<i>Bartonella</i> IFA reciprocal titer					PCR/DNA sequencing result ^c			
	<i>B. koehlerae</i>	<i>B. henselae</i>	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> genotype			DNA extraction		BAPGM enrichment culture at day:	
			I	II	III	Blood	Serum	7	14
1/13/09	64 ^b	< 16	< 16	< 16	< 16	Neg	Neg	Neg	<i>Bartonella</i> sp. ^a and <i>B. koehlerae</i> ^b
3/10/09	256 ^b	< 16	64	< 16	< 16	Neg	Neg	Neg	Neg
5/1/09	256 ^b	< 16	< 16	< 16	< 16	<i>B. koehlerae</i> ^b	Neg	Neg	Neg
11/4/09	256	< 16	< 16	< 16	64	Neg	Neg	<i>B. koehlerae</i>	Neg
2/23/10	64	< 16	< 16	< 16	< 16	<i>B. koehlerae</i>	Neg	Neg	Neg
6/7/10	128	< 16	< 16	< 16	< 16	Neg	Neg	Neg	<i>B. koehlerae</i>
8/30/10	< 16	< 16	< 16	< 16	< 16	Neg	Neg	Neg	Neg
9/1/10	< 16	< 16	< 16	< 16	< 16	Neg	Neg	Neg	Neg
9/2/10	< 16	< 16	< 16	< 16	< 16	Neg	Neg	Neg	Neg
1/4/11	< 16	< 16	< 16	< 16	< 16	Neg	Neg	Neg	Neg
1/5/11	< 16	< 16	< 16	< 16	< 16	Neg	Neg	<i>Bartonella</i> sp.	Neg
3/28/11	< 16	< 16	< 16	< 16	< 16	Neg	Neg	Neg	Neg
3/29/11	< 16	< 16	< 16	< 16	< 16	Neg	Neg	Neg	Neg
3/30/11	< 16	< 16	< 16	< 16	< 16	Neg	Neg	Neg	Neg

^a*Bartonella* sp., amplicon obtained using *Bartonella* genus ITS primers, but sequencing failed to confirm the species.

^bRetrospective testing was performed using stored frozen serum, blood, or BAPGM enrichment culture samples after the development of a *B. koehlerae* IFA serological assay and a *B. koehlerae*-specific ITS PCR assay.

^cNeg, DNA was not amplified using *B. koehlerae* 16S-23S ITS primers. The identities of all *B. koehlerae* PCR amplicons were confirmed by DNA sequencing.

B. henselae bacteremia was associated with recent onset of migraines with historical support for an acute infection following tick attachment, whereas the remaining five patients were symptomatic for at least one year. Subsequently, the IPRC research group described *B. vinsonii* subsp. *berkhoffii* and *B. henselae* bacteremia in a father and daughter from the same household [14]. The father, a veterinarian, developed progressive weight loss, muscle weakness, and lack of coordination over a one-year time frame. An MRI of the father's brain was notable for an increase in signal intensity throughout the pons and upper medulla lateralizing to left of the midline. Subsequently, the daughter developed headaches, muscle pain, and insomnia. Both patients were being evaluated by a neurologist at the time of initial testing for evidence of *Bartonella* infection. Intravascular infection with *B. vinsonii* subsp. *berkhoffii* genotype II or *B. henselae* (Houston strain 1) was repeatedly identified in father and daughter's blood by enrichment blood culture followed by PCR and DNA sequencing (Tables 2, 5, and 6). *Bartonella vinsonii* subsp. *berkhoffii* DNA was also PCR amplified and sequenced from a BAPGM enrichment CSF culture from the father, with bacteremia concurrently documented. Multiple courses of antibiotics (two courses of doxycycline and rifampin for the father, azithromycin and later doxycycline for the daughter) were administered before both patients' clinical status improved and before there was no longer microbiological, serological and molecular evidence of infection. Four pet cats in the household were *B. vinsonii*

subsp. *berkhoffii* and *B. henselae* seroreactive by IFA testing, whereas antibodies were not detectable in serum samples from the two newly acquired dogs, which were also negative by BAPGM enrichment blood culture/PCR testing. Identical *B. henselae* (ITS Houston 1 strain) DNA sequences (the same ITS strain found in the father and daughter) were found in samples from three of four cats.

3 Diagnosis of neurobartonellosis

In recent years, an increasing number of *Bartonella* spp. have been identified as zoonotic pathogens that are transmitted by animal bites or scratches or by arthropods. Although historically the term bartonellosis was attributed to infections with *B. bacilliformis*, transmitted by sandflies in the Peruvian Andes, a more inclusive medical use of this term now includes infections caused by any *Bartonella* sp., anywhere in the world. Importantly, cat scratch disease represents only a subset of patients with bartonellosis. Potentially, because *Bartonella* spp. can infect erythrocytes, endothelial cells, and various macrophage-type cells, including brain derived dendritic cells in vitro [54], the symptoms and pathological manifestations of bartonellosis appear to be extremely diverse among patients.

The numerous species within the genus *Bartonella*, antigenic and virulence differences among strains and subspecies, the diverse cell tropism, the ability to induce persistent occult infections in both reservoir and non-reservoir hosts, and the extraordinarily low levels of bacteremia

found in accidentally-infected hosts, all contribute to the clinical, microbiological, and pathological complexities associated with the diagnosis of bartonellosis. The use of serology for the diagnosis of *Bartonella* infection is further complicated by the fact that seropositivity is common in some populations. A study of Sicilian blood donors and children demonstrated that 25.1% of children and 11.4% of healthy blood donors had IgG antibodies to *B. henselae* [48]. None of these patients had recent clinical evidence of infection. All were IgM negative. Another project to evaluate *Bartonella* seroprevalence in rural Thailand demonstrated positive IgG antibodies to at least one of four *Bartonella* species in 9.9% of febrile and 19% of afebrile patients [5]. Seroreactivity to *Bartonella* was measured in a population of patients in the Dominican Republic of the Congo, and 4.5% of the samples were found to be positive for *B. henselae*, *B. quintana*, or *B. clarridgeiae* [42]. A recent report of zoonotic infections in employees from Great Smoky Mountains and Rocky Mountain National Parks in 2008 and 2009 found that 26.7% of park employees had serological evidence of past infection with *B. henselae*, and in a 1 year period of observation 5.7% of employees had incident infections [1]. Some of the patients in the above reports may have had mild or asymptomatic infections that went unnoticed, and if detected, the presence of *Bartonella* spp. antibodies can only be used to infer prior exposure. It is important that positive serologic results be interpreted with care and in clinical context. Follow-up titers may also be useful to document infection (seroconversion) or support elimination of the infection following treatment (a decrease in antibody titer, often to undetectable levels).

Culture of blood or other diagnostic specimens (cerebrospinal fluid, joint fluid or cavity effusions) onto blood agar plates has proven to be very insensitive [44,61]. For reasons that remain unclear, *Bartonella* antibodies are not detected in a subset of bacteremic human patients [47]. Therefore, conventional culture approaches and antibody testing are diagnostically insensitive, particularly in persistently-infected immunocompetent patients. Similar to culture onto blood agar plates, unless the patient is immunocompromised, PCR amplification of *Bartonella* spp. DNA from patient blood or CSF samples is also relatively insensitive due to the low level of bacteremia.

3.1 *Bartonella* enrichment culture

Ideally, the diagnosis of *Bartonella* infection should be confirmed by culturing the organism from blood, CSF, lymph node or heart valve (patients with endocarditis) or by amplifying DNA from tissues using PCR. In 2005, a novel, chemically modified, insect-based liquid culture medium (*Bartonella*/alpha-Proteobacteria growth media, BAPGM) that supports the growth of at least seven *Bartonella* species was described [45]. This medium also

supported co-cultures consisting of different *Bartonella* species. Subsequently, a unique approach, which combines enrichment culture in BAPGM, followed by a highly sensitive PCR assay targeting the 16S-23S ITS region, was developed to characterize and quantify *Bartonella* infection in blood and CSF samples. When compared with more traditional methods, this combined approach has facilitated the microbiological diagnosis of bacteremia with *B. henselae*, *B. quintana*, *B. vinsonii* subsp. *berkhoffii*, and a *B. volans*-like organism [13,14,16,23]. Table 2 summarizes the tests performed to diagnose the cases summarized in this review.

It is important that the medical history include questions related to arthropod (lice, fleas, and ticks) and animal exposures. If these questions are not asked, the patient may be subjected to extensive testing prior to consideration of bartonellosis as a differential diagnosis. In neurological cases with arthropod and animal exposure histories, there should be a high index of suspicion for *Bartonella* infection. In patients with suspected autoimmune neuropathy, tests for infectious agents, in addition to an immunological panel, would be indicated. Historically, a serological diagnosis has most often been based upon *B. henselae* and *B. quintana* serology (Table 2). In a case of traverse myelitis [4], a serological diagnosis was obtained using *B. quintana* and *B. henselae* antigens; however, cross reaction between the two antigens did not allow for determination of the infecting species. Mantadakis et al., 2007, also reported the use of two antigens to differentiate *B. henselae* infection from *B. quintana* infection [49]. There was sharp decrease in the *B. quintana* IgM titer after antibiotic treatment, but no decrease in *B. henselae* titer and PCR analysis supported *B. quintana* as the causative agent as *B. quintana* DNA was amplified from CSF.

Breitschwerdt et al., 2011, reported the use of an expanded panel of antigens including *B. henselae*, *B. koehlerae*, and *B. vinsonii* subsp. *berkhoffii* genotypes I, II, and III [19]. Using this panel, a species or subspecies specific serological response was found in some patients infected with *B. koehlerae* or *B. vinsonii* subsp. *berkhoffii* bacteremia, respectively [16,56] (Tables 3 and 4). As the number of recognized zoonotic *Bartonella* sp. that infect human patients expands, the limitations of serology as a sole diagnostic test become much more obvious. Also, as a subset of patients with neurological disease can be co-infected with more than one *Bartonella* sp. [13,14], caution should be exercised when attributing dual seroreactivity (antibodies to more than one antigen) in a patient to non-specific cross reactivity. When used in conjunction with PCR analyses and an enrichment culture approach, serological results can be more accurately interpreted. For example, as illustrated in Tables 5 and 6, a father and daughter tested because of neurological disease had

Table 4: Serological, blood culture, and PCR test results for veterinarian who was infected with *Bartonella vinsonii* subsp. *berkhoffii* [56].

PID	<i>Ehrlichia canis</i>	<i>Bartonella</i> IFA reciprocal titers				PCR/DNA sequencing results		
		<i>Bartonella vinsonii</i> subsp. <i>berkhoffii</i>				Direct extraction*	BAPGM platform enrichment culture**	Subculture isolate***
		Genotype I	Genotype II	Genotype III	<i>B. henselae</i>			
5	<16	16	<16	16	<16	Neg	Neg	Neg
34	<16	16	<16	16	<16	<i>Bvb</i> I	Neg	Neg
81	<16	32	<16	32	<16	Neg	<i>Bvb</i> I	Neg
97	<16	64	<16	64	32	Neg	Neg	Neg
123	<16	64	<16	64	32	Neg	Neg	Neg
144	<16	128	<16	64	32	Neg	Neg	Neg
180	<16	64	<16	64	<16	Neg	Neg	Neg
206	<16	32	<16	<16	<16	Neg	Neg	Neg
234	<16	16	<16	<16	<16	Neg	Neg	Neg

Antibiotic treatment was initiated on PID 138.

^aBoth blood and serum were extracted for PCR.

^bBAPGM enrichment cultures were extracted at 7 and at 14 days for PCR.

^cAgar plate subcultures were generated from the enrichment cultures at 7 and 14 days.

^d16S-23S intergenic spacer PCR results were confirmed by sequencing of the amplicon.

PCR, polymerase chain reaction; BAPGM, *Bartonella* alpha Proteobacteria growth medium; IFA, immunofluorescence antibody assays; Neg, negative; PID, postinoculation day.

B. henselae and *B. vinsonii* subsp. *berkhoffii* genotypes II and III antibodies [14]. Based upon PCR from blood, CSF or enrichment blood cultures, both patients were infected with *B. henselae* and *B. vinsonii* subsp. *berkhoffii*, thus co-infection rather than serological cross reactivity was most likely responsible for the antibodies detected in these patients. Following antibiotic treatment, there was resolution in neurological signs accompanied by a sequential decline in antibodies over a one-year period to undetectable levels. Also, *Bartonella* sp. DNA was no longer amplified from blood or enrichment blood cultures. These two cases illustrate the utility of repeated sampling along with serological and enrichment culturing followed by PCR analyses to enhance patient management decisions. Optimally, serology and enrichment culture/PCR should be performed prior to antibiotics and should be sequentially followed to document therapeutic elimination of the infection. Diagnostic use of enrichment culture is expanding in other laboratories, which should facilitate diagnostic confirmation of neurobartonellosis by physicians throughout the world. Unfortunately, it is rare to obtain subculture isolates following enrichment culture, thus determining antibiotic sensitivity and resistance patterns is often not possible.

4 Treatment challenges

For severe or persistent neurobartonellosis, there is minimal evidence upon which to base treatment decisions. Expert opinion often recommends a combination of doxycycline plus rifampin for 10 to 14 days [8,62]. Based upon in vitro testing, numerous antibiotics appear to be effective for the treatment of *Bartonella* infections [8]. However,

as *Bartonella* spp. induce both intracellular, as well as extracellular infection, in vitro test results can identify antibiotics that are not effective and can also be used to select antibiotics that should be tested in clinical trials of in vivo efficacy. Doxycycline, erythromycin, and rifampin are the most frequently recommended antibiotics for treating *Bartonella* spp. infection in people, but clinical improvement has been reported following the use of penicillin, gentamicin, ceftriaxone, ciprofloxacin, and azithromycin [30,40,58,62]. Treatment for 2 weeks in immunocompetent and 6 weeks in immunocompromised people is generally recommended for treating bartonellosis. However, relapses or ongoing bacteremia have been reported in patients treated with longer courses of antibiotics (three months) [15]. Evolving evidence, although meager, suggests that neurobartonellosis should be treated with two antibiotics that maintain high plasma and high intracellular concentrations for months. It is likely that bartonellosis should be treated like brucellosis, which is caused by a closely related alpha Proteobacteria.

In the cases in this review, third generation cephalosporins, macrolides, and the tetracycline family were often used. Within each antibiotic class, individual antibiotics have different pharmacokinetic characteristics, with some antibiotics within a class eliciting therapeutic improvement in one *Bartonella* patient whereas another antibiotic within the class failing to elicit improvement in another patient. For example, a 6-year-old Hispanic boy died after treatment with a third generation cephalosporin [33], while other patients treated with cephalosporins, often along with other antibiotics such as netilmicin, recovered [4]. Obviously, recovery also depends upon the host immune

Table 5: Serological, culture, and molecular test results for a 50-year-old veterinarian (the father) with chronic weight loss and progressive neurological dysfunction [14].

Date/sample (father)	<i>Bartonella</i> IFA reciprocal titers			PCR/DNA sequencing results		
	<i>B. henselae</i>	<i>Bvb</i> Genotype II	<i>Bvb</i> Genotype III	Direct extraction	BAPGM enrichment culture	Subculture isolate
10-18-07 Blood	512	32	256	<i>Bvb</i> TII	Neg	Neg
11-02-07 Blood	8192	64	128	Neg	<i>Bvb</i> TII	Neg
11-05-07 CSF	NT	NT	NT	Neg	<i>Bvb</i> TII	Neg
12-11-07 Oral swab	NT	NT	NT	<i>Bvb</i> TII	N/A	N/A
1-18-08 Blood	1024	32	128	Neg	<i>Bh</i> H1	Neg
5-27-08 Blood	< 16	16	16	Neg	Neg	Neg
11-04-08 Blood	< 16	< 16	< 16	Neg	Neg	Neg

Table 6: Serological, culture, and molecular test results for a 7.5-year-old girl (the daughter) with progressive neurological dysfunction [14].

Date/sample (daughter)	<i>Bartonella</i> IFA reciprocal titers			PCR/DNA sequencing results		
	<i>B. henselae</i>	<i>Bvb</i> Genotype II	<i>Bvb</i> Genotype III	Direct extraction	BAPGM enrichment culture	Subculture isolate
11-07-07 Blood	256	<16	128	Neg	<i>Bvb</i> TII	Neg
11-28-07 Blood	256	<16	64	Neg	<i>Bvb</i> TII	Neg
1-11-08 Blood	64	32	32	Neg	<i>Bvb</i> TII	<i>Bvb</i> TII
3-20-08 Blood	64	32	32	Neg	Neg	Neg
5-08-08 Blood	64	16	16	Neg	Neg	<i>Ochrobactrum</i> sp.
6-24-08 Blood	64	32	16	Neg	Neg	Neg
7-31-08 Blood	16	32	64	Neg	Neg	Neg
10-13-08 Blood	<16	<16	<16	Neg	<i>Bh</i> H1	Neg
12-01-08 Blood	<16	<16	<16	Neg	Neg	Neg

Neg = DNA was not amplified using *Bartonella* 16S-23S intergenic spacer (ITS) primers.

response and potentially the virulence of the infecting *Bartonella* sp. or strain. However, based upon case data, caution should be used if a cephalosporin is the sole treatment for neurobartonellosis. Another patient had persistent symptoms despite 14 days of erythromycin and corticosteroids, whereas improvement was noted following administration of a combination of doxycycline and rifampin. Macrolides, such as azithromycin, have been used effectively for the treatment of neurobartonellosis patients, but close monitoring is important due to the potential for development of resistance.

In several patients described in this review, combinations of antibiotics were used to eliminate neurological abnormalities, however, for many patients the antibiotic combination was changed when the patient's clinical status deteriorated. Biswas et al., 2010, have reported that resistance to azithromycin evolves more rapidly than drugs from floxacillin family [6]. It has also been demonstrated that cat and human *B. henselae* isolates are more susceptible to pradofloxacin than azithromycin and enrofloxacin [7]. Similar in vitro studies are needed to characterize the resistance of doxycycline and rifampin antibiotics when used in combination and alone. There is a paucity of data regarding the appropriate length of treatment for patients with neurobartonellosis, and further study is needed to identify optimal treatments to effectively treat these infections.

5 Future perspective

It is now well known that *B. quintana* and *B. henselae* can cause vasoproliferative lesions such as bacillary angiomatosis or peliosis hepatis in immunosuppressed individuals and that *B. henselae* is the primary, if not the sole, cause of CSD. In immunocompetent patients, including those with neurological disease, angiomatosis is rarely reported. It seems likely that expression of those factors responsible for vasoproliferation and neoangiogenesis is immunologically blocked or suppressed by the immunocompetent host, at least at the gross anatomic level. The extent to which chronic intravascular or endotheliotropic infection contributes to immune-mediated or autoimmune (molecular mimicry) neurological phenomena in neurobartonellosis patients has not been investigated to any degree. The mechanisms by which some *B. henselae* strains cause aphasia, encephalopathy, neuropathy, seizures or transverse myelitis should be studied. Based upon limited case data, *Bartonella* sp. can be isolated from the CSF of patients with minimal pathological evidence (i.e., increased protein and cell counts) to support inflammation or infection [10, 14, 15].

Finally, it is critical for the physician to consider each patient's travel history, exposures to domestic animals and wildlife, exposure to arthropod or insect vectors, and potentially whether there is a history of blood product

administration. For future cases, diagnosis can be enhanced by using a panel of serological assays in conjunction with enrichment culture/PCR from blood, serum, tissues or cerebrospinal fluid [15,47]. Efforts to standardize antibiotic dose and duration treatment regimens, based upon both in vitro antibiotic susceptibility testing and patient outcome assessments are critically needed to effectively manage patients with neurobartonellosis. There is also a critical need for research to elucidate the mechanism(s) by which chronic interplay between the host and bacteria ultimately leads to neurological manifestations. In addition, research is needed to determine whether antibiotic resistance or other factors contribute to treatment failures in some patients with neurobartonellosis [10].

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