Hallucinations, sensory neuropathy and peripheral visual deficits in a young woman infected with *Bartonella koehlerae*

*Bartonella koehlerae* and neurological abnormalities

Edward B. Breitschwerdt, D.V.M.¹*, Patricia E. Mascarelli, Ph.D.¹, Lori A. Schweickert, M.D.², Ricardo G. Maggi, Ph.D.¹, Barbara C. Hegarty, B.A.¹, B., Julie M. Bradley, B.S.¹, Christopher W. Woods, M.D., M.P.H.³

¹Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

²3C Family Services, Cary, NC

³Duke University School of Medicine, Durham, NC

*Corresponding Author: Dr. Edward B. Breitschwerdt:

North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Street Raleigh, NC 27606; Phone: 919-513-8277; Fax: 919-513-6336 ; E-mail:

ed_breitschwerdt@ncsu.edu

Key Words: *Bartonella*, hallucinations, peripheral visual loss, sensory neuropathy

Word Counts: Abstract- 45 words, Text- 1887 words
ABSTRACT

A young woman experiencing depression, anxiety, mood swings, severe headaches, muscle spasms, interphalangeal joint stiffness, decreased peripheral vision, diminished tactile sensation and hallucinations was persistently *B. koehlerae* seroreactive and bacteremic. Following antibiotic treatment, *B. koehlerae* antibodies and DNA were not detected and all symptoms resolved.

**Clinical Relevance:** *Bartonella koehlerae* bacteremia may be associated with hallucinations, sensory neuropathy and peripheral visual deficits.
CASE REPORT

An eighteen-year-old female, was sequentially examined by a neurologist, psychiatrist, neuro-ophthalmologist and infectious disease physician because of a four-year history of slowly progressive neurological and neurocognitive abnormalities. Biopsy-proven Celiac disease was diagnosed in December 2004, following complaints of frequent stomach cramps. Despite dietary control of gastrointestinal symptoms, she developed intermittent joint pain, primarily involving the ankles. During 2005, the patient reported reduced tactile sensation in her hands and by 2007 frequent severe headaches, back pain, generalized muscle spasms and an inability to extend her fingers due to stiffness in her proximal and distal interphalangeal joints. During 2008, she was referred to a neurologist for evaluation of depression, anxiety, mood swings, dizziness, auditory and visual hallucinations and a progressive decrease in peripheral vision. No abnormalities were observed on a non-contrast MRI of the brain or an electroencephalogram. The hallucinations were initially infrequent; however, by the fall 2008, hallucinations became frequent, more intense, and at times were accompanied by 1-2 minute dissociative episodes. The patient’s psychiatrist addressed her anxiety with cognitive behavioral therapy and prescribed oxcarbazepine (300 mg twice daily) and quetiapine fumarate (300 mg in the evening), which reduced hallucination frequency to less than once daily.

In January 2009, at the request of the patient’s parents, we performed PCR on aseptically obtained ethylenediaminetetraacetic acid (EDTA)-anti-coagulated blood and serum, inoculated EDTA blood into Bartonella Alpha Proteobacteria Growth medium (BAPGM), and tested serum for immunofluorescent antibodies using cell culture grown Bartonella vinsonii subsp. berkholffii genotypes I, II, and III and Bartonella henselae antigens, as previously described (2,5,6,11). At
the time of her original testing, our research laboratory did not perform *B. koehlerae* IFA testing and we had not yet developed a *B. koehlerae*-specific PCR assay. Therefore, her first samples were not seroreactive (IFA titer ≥ 1:64) to any of the *Bartonella* spp. test antigens and no amplicons were obtained with 16S-23S ITS *Bartonella* genus primers from blood, serum, or the 7-day BAPGM enrichment blood culture. (Table 1) However, a PCR amplicon was obtained from the 14-day BAPGM enrichment culture, suggesting *Bartonella* sp. growth following incubation for 14 days. Efforts to determine the *Bartonella* species by sequencing the amplicon were unsuccessful; therefore, serology and BAPGM enrichment blood cultures were repeated in March and May 2009. Repeat testing generated no additional serological or molecular evidence to support previous or current infection with a *Bartonella* sp.

In March 2009, the patient was referred to a neuro-ophthalmologist because of decreased peripheral vision. Ophthalmologic examination was normal with 20/20 visual acuity, no eye pain or redness. By formal perimetry, peripheral vision was reduced to the central 5 degrees bilaterally. The patient was advised to avoid driving a car and to repeat perimetry testing in a few weeks; however, the patient was non-compliant and was not re-examined until twenty months later.

In June 2009, her infectious diseases clinician initiated an eight week course of doxycycline (100 mg twice daily) and rifampin (300 mg twice daily), due to ongoing symptoms and the January 2009 *Bartonella* genus PCR result in the 14 day enrichment culture. After starting antibiotics and while continuing cognitive behavioral therapy, oxcarbazepine and quetiapine fumarate, the patient reported a further decrease (from daily to one episode every two weeks) in hallucination frequency. When re-examined at the conclusion of the antibiotic course, treatment duration was extended for an additional six weeks due to patient’s maladherence.
Following completion of antibiotics, the patient regained the ability to extend her fingers and described improved tactile sensation. Her psychiatrist documented a further improvement, with her hallucinations changing from disturbing visual and auditory sensations, to non-disturbing episodes of hearing her name called, to simply a “white noise”. At the patient’s request, the dose of oxcarbazepine was reduced.

By November 2009, the Intracellular Pathogens Research Laboratory (IPRL) had developed a *B. koehlerae* indirect fluorescent antibody assay, using an isolate derived from a sick cat (2,5). Retrospective IFA testing of frozen serum samples (January, March and May 2009) and prospective testing of serum obtained in November 2009, February and June, 2010 consistently detected *B. koehlerae* antibodies. (Table 1) During the same time period, *B. koehlerae* species-specific 16S-23S ITS PCR primers repeatedly amplified organism-specific DNA sequences from the blood or BAPGM enrichment cultures. Specifically, amplification of the *B. koehlerae* ITS region was performed using oligonucleotides Bkoehl-1s: 5’ CTT CTA AAA TAT CGC TTC TAA AAA TTG GCA TGC 3’ and Bkoehl1125as: 5’ GCC TTT TTT GGT GAC AAG CAC TTT TCT TAA G 3’ as forward and reverse primers, respectively.

Amplification was performed in a 25-µl final volume reaction containing 12.5 µl of Tak-Ex® Premix (Fisher Scientific), 0.1 µl of 100 µM of each forward and reverse primer (IDT® DNA Technology), 7.3 µl of molecular grade water, and 5 µl of DNA from each sample tested.

With repeated PCR documentation of *B. koehlerae* bacteremia, the patient was treated with rifampin (300 mg twice daily) and azithromycin (250 mg/day) for an additional 4 months. Following this treatment regimen, her hallucinations stopped completely and normal peripheral vision was confirmed by visual field perimetry in November 2010. The quetiapine fumarate dosage was gradually decreased and hallucinations and dissociative episodes have not returned.
B. koehlerae antibodies were not detected in August and September 2010 and January and March 2011. (Table 1) With the exception of a single 7-day BAPGM enrichment culture PCR result (January 2011), for which the amplicon could not be successfully sequenced, Bartonella sp. DNA was not amplified from blood, serum or enrichment blood cultures (n=31 independent PCR reactions) following the 4-month treatment regimen. During the nine-month post-treatment follow-up period, the patient has experienced no hallucinations, peripheral vision has remained normal and tactile sensation has improved substantially. Family members reported that the patient was much improved and had returned to her pre-infection baseline, both neurologically and psychiatrically.

DISCUSSION

Bartonella koehlerae was initially characterized from blood culture isolates obtained from two flea-infested healthy cats from northern California in the early 1990s (7,8). In 2004, the bacteria was first reported as a human pathogen in a patient from Israel with aortic valve endocarditis (1). Investigators subsequently isolated B. koehlerae from flea infested (Ctenocephalides felis) stray cats in Israel (1). In 2010, we reported B. koehlerae bacteremia in eight patients with variable symptoms (5). The diagnostic evaluation of the patient in this case report benefitted from development of B. koehlerae serological and subsequently, a species-specific PCR assay (5). When first tested using Bartonella genus ITS primers in January 2009, only the 14-day BAPGM enrichment blood culture was PCR positive. As multiple attempts to sequence this amplicon were not successful, Bartonella species bacteremia was suspected, but not confirmed. Subsequent serological and Bartonella genus ITS PCR (genus primers) testing of BAPGM enrichment blood cultures spanning a 17 month time period were consistently negative,
whereas retrospective and prospective IFA testing consistently identified *B. koehlerae* antibodies, until after the third course of antibiotics when the patient also became seronegative. Of serodiagnostic importance, there was no crossreactivity to *B. henselae* or *B. vinsonii* subsp. *berkhoffii* antigens. Similarly, *B. koehlerae* DNA was repeatedly amplified and sequenced using species-specific primers until after the third antibiotic course. Assuming that the patient was not re-exposed, treatment with doxycycline and rifampin for 14 weeks in 2009, did not eliminate *B. koehlerae* bacteremia. Lack of patient compliance may have contributed to the initial treatment failure, but was not a factor during administration of the subsequent treatment regimens.

Following the third course of antibiotics, the patient experienced clinical resolution of symptoms, a decrease in *B. koehlerae* antibody titers to a seronegative status and whole blood, serum, and BAPGM cultures were PCR negative, with one exception (January 5, 2011). It is possible that her infection may not have been eliminated or that the patient was re-infected by animal or arthropod contact. Her pet cat and dog were seronegative and BAPGM PCR negative. Follow-up serological and BAPGM enrichment culture testing in March 2011 was negative.

In this study, all patient samples were processed in a biosafety cabinet with Hepa filtration, located in a limited access laboratory. To avoid DNA carryover, PCR sample preparation, DNA extraction, and PCR amplification and analysis were performed in three separate rooms with a unidirectional work flow. For PCR, *Bartonella* sp. negative controls were prepared using 5 µl of DNA from the blood of a healthy dog. As newly designed *B. koehlerae* species-specific PCR primers were used in this study, we purposely did not use a *B. koehlerae* positive control. To assess for potential contamination during processing of enrichment blood cultures, an un-inoculated BAPGM culture flask was processed simultaneously and in an identical manner with each batch of patient blood and serum samples tested. For all components
of the BAPGM blood culture enrichment platform (PCR from blood, serum, enrichment cultures and subcultures) PCR negative controls remained negative throughout the course of the study. Based upon the results of this study, the *B. koehlerae* ITS species-specific primers were more sensitive than the *Bartonella* genus ITS primers. In order to successfully amplify *Bartonella* spp. DNA from as many patient samples as possible, the anti-sense primer used in the genus PCR was designed to amplify pathogenically relevant species, while avoiding non-specific amplification of *Mesorhizobium* DNA. (9) Thus the design of this primer resulted in greater specificity, but decreased sensitivity. The *B. koehlerae* species-specific anti-sense primer was designed to only amplify *B. koehlerae*, thus sensitivity was improved. As illustrated in the table, direct DNA extraction from blood and serum was repeatedly negative, whereas PCR from seven or fourteen day BAPGM enrichment cultures contained adequate bacterial numbers (ITS targets) to allow for successful amplification and DNA sequencing. Despite growth in the liquid enrichment culture flasks, we were never able to obtain a *B. koehlerae* subculture isolate from this patient, which remains a technical problem with this genus of highly fastidious bacteria. Interestingly, there was no cross reactivity to antigens of other *Bartonella* sp. (*B. henselae*) or subsp. *B. vinsonii* subsp. *berkhoffii* genotypes I, II and III).

Fatigue, insomnia, joint pain, headache, memory loss, and muscle pain were among frequent symptoms in eight patients with *B. koehlerae* bacteremia, of which four patients reported decreased tactile sensation or numbness and three reported blurred vision (5). *B. koehlerae* antibodies were not detected in thirty healthy individuals, who were concurrently PCR negative for all components of the BAPGM platform (5). Following suspected needle stick transmission of *Bartonella vinsonii* subsp. *berkhoffii*, a veterinarian reported frequent headaches, fatigue, and intermittent paresthesias in the left arm in focal, nondermatomal areas (11).
Bartonella henselae infection was reported in a patient distal axonal sensomotor polyneuropathy and Raynaud’s phenomenon (13). Brachial plexus neuropathy was diagnosed in three patients with neuralgic amyotrophy (12,13). Prospective studies are needed to evaluate B. koehlerae bacteremia patients with unexplained fatigue, joint pain, sensory neuropathies, visual deficits and hallucinations.

With the advent of B. henselae serology, PCR and improved enrichment culture techniques, case studies are suggesting an important and expanding role for Bartonella sp. infection in patients with memory loss, expressive aphasia, word substitution errors, and impaired repetition (3,4,10). In our patient, there was a decrease in hallucination frequency following the initial course of antibiotics and total resolution of the hallucinations and visual field deficits following the third course of antibiotics, while on a stable dose of antipsychotic medication. We conclude that this constellation of symptoms should raise the index of clinical suspicion for Bartonella infection.

ACKNOWLEDGEMENTS

This research was supported in part by a grant from the American College of Veterinary Internal Medicine Foundation, the Kindy French Foundation, the State of North Carolina, and Bayer Corporation. The authors are grateful to Tonya Lee for editorial assistance.

COMPETING INTERESTS

In conjunction with Dr. Sushama Sontakke and North Carolina State University, Dr. Breitschwerdt holds U.S. Patent No. 7,115,385; Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is the chief scientific officer for Galaxy
Diagnostics, a newly formed company that provides diagnostic testing for the detection of Bartonella species infection in animals and in human patient samples. Dr. Ricardo Maggi has lead efforts to optimize the BAPGM platform and is the Scientific Technical Advisor and Laboratory Director for Galaxy Diagnostics. The remaining authors have no potential conflicts of interest to declare.
REFERENCES


Table 1: Serological, BAPGM enrichment blood culture and PCR/DNA sequencing test results for an eighteen year-old woman with hallucinations and peripheral visual deficits.

<table>
<thead>
<tr>
<th>Date</th>
<th>Bartonella koehlerae</th>
<th>Bartonella henselae</th>
<th>Bartonella vinsonii subsp. berkoffii</th>
<th>DNA Extraction</th>
<th>Days</th>
<th>BAPGM Enrichment</th>
<th>Culture</th>
<th>PCR/DNA Sequencing Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-13-09</td>
<td>64</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>&lt;16</td>
</tr>
<tr>
<td>3-10-09</td>
<td>256</td>
<td>&lt;16</td>
<td>64</td>
<td>&lt;16</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>&lt;16</td>
</tr>
<tr>
<td>5-1-09</td>
<td>256</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>&lt;16</td>
</tr>
<tr>
<td>11-4-09</td>
<td>256</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>Neg</td>
<td>Neg</td>
<td>B. koehlerae*</td>
<td>Neg</td>
</tr>
<tr>
<td>2-23-10</td>
<td>64</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>Neg</td>
<td>Neg</td>
<td>B. koehlerae*</td>
<td>Neg</td>
</tr>
<tr>
<td>6-7-10</td>
<td>128</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>Neg</td>
<td>Neg</td>
<td>B. koehlerae*</td>
<td>Neg</td>
</tr>
</tbody>
</table>
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 Neg
3-28-11 <16 <16 <16 <16 <16 Neg Neg Neg
3-29-11 <16 <16 <16 <16 <16 Neg Neg Neg
3-30-11 <16 <16 <16 <16 <16 Neg Neg Neg

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

**= retrospective 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.

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

The identity of all B. koehlerae PCR amplicons was confirmed by DNA sequencing.