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Hallucinations, Sensory Neuropathy, and Peripheral Visual Deficits in a Young Woman Infected with *Bartonella koehlerae*▽

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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 *Bartonella koehlerae* seroreactive and bacteremic. Following antibiotic treatment, *B. koehlerae* antibodies and DNA were not detected and all symptoms resolved.

CASE REPORT

An 18-year-old female was sequentially examined by a neurologist, psychiatrist, neuro-ophthalmologist, and infectious disease physician because of a 4-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. In 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 noncontrast magnetic resonance image (MRI) of the brain or an electroencephalogram. The hallucinations were initially infrequent; however, by fall 2008, the hallucinations became frequent, more intense, and at times were accompanied by 1- to 2-min 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 EDTA-anticoagulated blood and serum, inoculated EDTA blood into *Bartonella-Alphaproteobacteria* growth medium (BAPGM), and tested serum for immunofluorescent antibodies using cell culture-grown *Bartonella vinsonii* subsp. *berkhoffii* 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 *Bartonella koehlerae* immunofluorescent-antibody (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* sp. test antigens, and no amplicons were obtained with 16S-23S intergenic spacer (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. The ophthalmologic examination was normal, with 20/20 visual acuity and 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 noncompliant and was not reexamined until 20 months later.

In June 2009, her infectious-disease clinician initiated an 8-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 2 weeks) in hallucination frequency. When the patient was reexamined at the conclusion of the antibiotic course, treatment duration was extended for an additional 6 weeks due to the 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 nondisturbing episodes of hearing her name called, to simply a "white noise." At the patient's request, the dose of oxcarbazepine was reduced.

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TABLE 1. Serological, BAPGM enrichment blood culture, and PCR/DNA sequencing test results for an 18-year-old woman with hallucinations and peripheral visual deficits

Date (mo/day/yr)	*Bartonella* IFA reciprocal titer					PCR/DNA sequencing result[c]			
	B. koehlerae	*B. henselae*	*B. vinsonii* subsp. *berkhoffii* 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	*Bartonella* sp.[a] and *B. koehlerae*[b]
3/10/09	256[b]	<16	64	<16	<16	Neg	Neg	Neg	Neg
5/1/09	256[b]	<16	<16	<16	<16	*B. koehlerae*[b]	Neg	Neg	Neg
11/4/09	256	<16	<16	<16	64	Neg	Neg	*B. koehlerae*	Neg
2/23/10	64	<16	<16	<16	<16	*B. koehlerae*	Neg	Neg	Neg
6/7/10	128	<16	<16	<16	<16	Neg	Neg	Neg	*B. koehlerae*
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	*Bartonella* 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.
[b] 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.
[c] Neg, DNA was not amplified using *B. koehlerae* 16S-23S ITS primers. The identities of all *B. koehlerae* PCR amplicons were confirmed by DNA sequencing.

By November 2009, the Intracellular Pathogens Research Laboratory (IPRL) had developed a *B. koehlerae* immunofluorescent-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 and 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 mixture containing 12.5 μl of Tak-Ex Premix (Fisher Scientific), 0.1 μl of 100 μM 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 PCRs) following the 4-month treatment regimen. During the 9-month posttreatment 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 preinfection 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 bacterium 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 a *B. koehlerae* serological and, subsequently, 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 was 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 cross-reactivity 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 reexposed, 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 and a decrease in *B. koehlerae* antibody titers to a seronegative status, and whole-blood, serum, and BAPGM cultures were PCR negative, with one exception (5 January 2011). It is possible that her infection may not have been eliminated or that the patient was reinfected 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 uninoculated 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* sp. DNA from as many patient samples as possible, the antisense primer used in the genus PCR was designed to amplify pathogenically relevant species, while avoiding nonspecific amplification of *Mesorhizobium* DNA (9). Thus, the design of this primer resulted in greater specificity but decreased sensitivity. The *B. koehlerae* species-specific antisense primer was designed to amplify only *B. koehlerae*, thus sensitivity was improved. As illustrated in the table, direct DNA extraction from blood and serum was repeatedly negative, whereas PCR from 7- or 14-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 subspecies (*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 30 healthy individuals, who were concurrently PCR negative for all components of the BAPGM platform (5). Following suspected needlestick 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 with 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 in 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.

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