

CASE REPORTS

Human Coinfection with *Bartonella henselae* and Two Hemotropic Mycoplasma Variants Resembling *Mycoplasma ovis*[∇]

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Two variants of an organism resembling the ovine hemoplasma, *Mycoplasma ovis*, were detected by PCR in blood samples from a veterinarian in Texas. Coinfection with similar variants has been described in sheep. This represents the first report of human infection with this organism. The veterinarian was coinfecting with *Bartonella henselae*.

CASE REPORT

A 49-year-old man with a 15-year history of intermittent numbness of the left leg and left hand subsequently developed an acute febrile illness (39.2°C) with nausea and malaise that abated over a 1-week period. In the subsequent months, progressively severe neurological symptoms developed, which included stumbling during jogging, muscle weakness, and fatigue, which were thought to be associated with a viral neuropathy. During the next year, running became impossible, the patient could not walk unaided, he developed leg myoclonus and numbness of the hands, and resting two to three times a day was required. Routine laboratory testing was unremarkable. Magnetic resonance imaging (MRI), performed 6 years before and 6 months after the onset of febrile illness, showed progressive multifocal paraventricular lesions of the white matter in the brain and spinal cord suggestive of a demyelinating disease. Tibial nerve conduction velocities were slightly slowed, consistent with a mild to moderate demyelinating peripheral neuropathy. Cerebrospinal fluid analysis, which had been performed before the onset of febrile illness, revealed the presence of oligoclonal bands in the absence of corresponding serum bands. Ultimately, on the basis of these findings, multiple sclerosis (MS) was diagnosed. Methylprednisolone sodium succinate (1 g) was administered intravenously once daily for 5 days in early 2005, prior to the diagnosis of MS, and again in 2006, followed by oral prednisolone at 15 mg once daily for 1 year, the dose of which was subsequently tapered slowly and discontinued in late 2008. Treatment with subcutaneous beta 1a interferon three times weekly was initiated in April 2005 and continued until March 2008. Low serum concentrations of IgM and IgG were detected in May 2006. Subsequently, intravenous immunoglobulin was administered monthly for 6 months. The man was a veterinarian and was “case 5” in a

publication describing *Bartonella* bacteremia in patients with neurological dysfunction (4). He reported frequent bites or scratches from cats, dogs, rodent pocket pets, and an assortment of wild and zoo animals (4). He had also worked with sheep, goats, llamas, and camels and had frequent deer contact during his career. He had traveled as a veterinary student to Central America and Colombia and to Mexico on numerous occasions before that time.

Commencing 6 months after the onset of febrile illness, serial blood and serum samples were submitted over a 3.5-year period (15 June 2005 to 11 February 2009) to the Intracellular Pathogens Research Laboratory at North Carolina State University. Serology using indirect immunofluorescence for evidence of *Bartonella* exposure was performed as reported previously (4). A PCR assay targeting the *Bartonella* 16S-to-23S intergenic spacer region was performed following direct extraction of nucleic acid from blood and following preenrichment culture of blood samples in *Bartonella* *Alphaproteobacteria* growth medium (BAPGM), as previously described (4, 10). Amplicons were sequenced to confirm their identity and determine the infecting *Bartonella* species. To assess for laboratory contamination, an uninoculated culture flask was processed simultaneously and in an identical manner with each batch of patient samples tested. All PCR and culture controls were negative throughout the study.

In June 2005, serology and PCR for detection of *Bartonella* spp. were negative (Table 1). In July 2005, the patient was treated with doxycycline for 5 weeks. Initial PCR documentation of *Bartonella* infection occurred in August 2005 (Table 1). The sequence of this initial PCR product could not be ascertained. Repeat testing in November 2005 was negative. In July 2006, due to continued deterioration in neurological status, treatment with azithromycin was instituted, followed by levofloxacin, with slight clinical improvement. In March 2007, *B. henselae* DNA was amplified from enrichment culture and found to have a sequence matching that of the SA2 strain (7). Doxycycline and rifampin were administered continuously from August 2007 until August 2008. During this time period, there was significant improvement in the patient's muscle

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TABLE 1. *Bartonella* IFA serology, PCR, BAPGM culture, and *Mycoplasma* PCR results for a 49-year-old male veterinarian with progressive neurological dysfunction

Date of sampling	<i>Bartonella</i> IFA reciprocal titer			<i>Bartonella</i> PCR result		Hemoplasma PCR result(s) (Genbank accession no.)	Treatment date(s) and antimicrobial(s) used ^a
	<i>B. henselae</i>	<i>B. quintana</i>	<i>B. vinsonii</i> subsp. <i>berkhoffii</i>	Blood extraction	BAPGM Enrichment Culture		
15 June 2005	<16	<16	<16	–	–	–	July 2005; doxycycline, (100 mg q24h p.o. for 35 days)
16 August 2005	<16	<16	<16	–	<i>Bartonella</i> spp.	<i>M. ovis</i> -like 16S rRNA (GU383115)	
1 November 2005	<16	<16	<16	–	–	–	July 2006; azithromycin (250 mg q24h for 49 days)
							October 2006; levofloxacin (500 mg q24h p.o. for 84 days)
21 March 2007	NA ^b	NA	NA	–	<i>B. henselae</i> (SA2) ^c	<i>M. ovis</i> -like 16S rRNA (GU230141–GU230144, GU383116); <i>M. ovis</i> -like RNase P (HM059715, HM159553)	August 2007 to August 2008; doxycycline (100 mg q24h p.o.) and rifampin (300 mg q12h p.o.)
15 November 2007	<16	NT ^d	<16	–	–	–	
15 September 2008	<16	NT	<16	–	–	–	
17 November 2008	<16	NT	<16	–	–	–	
11 February 2009	<16	NT	<16	<i>B. henselae</i> (SA2) ^c	<i>B. henselae</i>	–	May 2009 to March 2010; doxycycline (100 mg q24h p.o.) and rifampin (300 mg q12h p.o.)
21 September 2009	<16	NT	<16	–	–	NT	
2 March 2010	<16	NT	<16	<i>B. henselae</i> (Houston 1)	–	NT	March 2010 to the present; azithromycin (500 mg q24h p.o.) and rifampin (300 mg q12h p.o.)

^a q24h, every 24 h; p.o., *per os*.

^b NA, serum not available for testing.

^c Denotes 16S-to-23S ITS DNA sequence result, identical to San Antonio 2-like strain.

^d NT, not tested.

strength and coordination and reduction of myoclonus. Relapses, which occurred frequently prior to treatment with doxycycline and rifampin, did not occur. PCR testing and enrichment culture results were negative in September and November 2008. In December 2008, symptoms of fatigue returned, and in February 2009, *B. henselae* DNA was amplified and sequenced directly from blood, the sequence again matching that of the SA2 strain. A 30-bp insertion at the 3' end of the internal transcribed spacer (ITS) region distinguishes the SA2 strain from other *Bartonella henselae* strains such as Houston-1 (22). A sequence generated from the enrichment culture aligned with that of *B. henselae*; however, overlapping sequences at the 3' end of the ITS region did not permit identification of an ITS strain type and suggested concurrent infection with two *Bartonella* species or strains. Subsequently, doxycycline and rifampin were administered continuously until March 2010, again with improvement of the patient's symp-

oms. Testing for *Bartonella* infection in September 2009 was negative. In March 2010, *B. henselae* DNA was amplified directly from blood, the sequence of which matched that of the Houston-1 isolate (22), but growth, as detecting using PCR, was not obtained in BAPGM enrichment culture. Azithromycin was then substituted for doxycycline. The patient never seroreacted to *Bartonella* antigens. Additional MRI scans showed no changes to previously documented lesions and no evidence of active plaques.

As part of a larger study designed to determine the prevalence of hemoplasma DNA in blood samples from veterinarians, extracted DNA from the sample collected in August 2005 was sent to the School of Veterinary Medicine at the University of California, Davis. A partial hemoplasma 16S rRNA gene sequence was amplified from the sample using conventional PCR primers for detection of hemotropic mycoplasma species (13). Each 50- μ l reaction volume contained 1 \times reac-

tion buffer, 2.5 U AmpliTaq Gold polymerase (Invitrogen, Carlsbad, CA), 1.5 mM MgCl₂, 1 μM each primer, 200 μM each deoxynucleoside triphosphate (dNTP), and 5 μl DNA template. The cycling conditions were 95°C for 5 min, followed by 45 cycles of amplification (1 min at 95°C, 1 min at 60°C, and 30 s at 72°C) in a Dyad thermocycler (MJ Research Inc., Waltham, MA). The DNA of “*Candidatus Mycoplasma haemominutum*” was used as a positive control. Ultrapure water was used as a negative thermocycling control and a negative extraction control. Samples were analyzed on a 2.5% agarose gel containing GelStar nucleic acid gel stain (Lonza, Rockland, ME). DNA sequencing was performed using automated methods (¹³C-DNA Sequencing Facility, Davis, CA). The PCR assay yielded a 159-bp PCR fragment with 100% sequence homology to a partial 16S rRNA gene sequence of *M. ovis*.

Testing of additional archived blood samples from the patient resulted in detection of DNA from a *Mycoplasma ovis*-like agent in one additional sample from March 2007 (Table 1). Subsequently, a 16S rRNA PCR fragment approximately 1,450 bp in length was amplified from this sample using primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), as described previously (25). Sequencing of this product revealed a mixed sequence containing two PCR products, one possessing a 17-bp deletion. The PCR product was ligated into a vector (pGEM-T Easy; Promega, Madison, WI), and the plasmids were transformed into competent cells (XL1 Blue; Stratagene, La Jolla, CA). Plasmids were then purified from cell lysates using a plasmid purification kit (UltraClean; MO BIO Laboratories, Carlsbad, CA), and inserts were sequenced using automated methods. Three of five cloned sequences showed 99 to 100% homology with *M. ovis* (GenBank accession no. AF338268 and EU165510), and two showed 98% similarity to a novel variant detected in Hungarian sheep, containing the 17-bp deletion (EU165513). Subsequently, a 111-bp *mpB* gene PCR product was amplified from the March 2007 blood sample as described previously (19) but instead using 400 nM each primers *ovirnpB-F* (5'-GTTGATGGCGGAGAACTTTT-3') and *ovirnpB-R* (5'-GGTTTGCTCGTTTCACTCT-3') and an annealing temperature of 55°C. Direct sequencing of the PCR product yielded a 78-bp sequence with 100% identity only to the *mpB* gene product of *M. ovis* in the GenBank database (EU078612). The *mpB* gene PCR product was then cloned and sequenced. Five clones were selected for sequencing, each of which yielded 112-bp sequences. Four of five cloned sequences had 100% homology to the *mpB* gene product of *M. ovis*, and one had 99% homology (1 nucleotide substitution). GenBank accession numbers for sequences that were identified in this report are shown in Table 1.

Hemotropic mycoplasmas (hemoplasmas) are unculturable, epierythrocytic mycoplasmas that cause disease in a variety of animal species, which is generally manifested as hemolytic anemia, of variable chronicity and severity. The development of PCR assays, primarily targeting the 16S rRNA gene, to detect these organisms has resulted in recognition of several novel animal hemoplasmas, and for some pathogens, the host range has been extended.

Prior to the advent of PCR, hemotropic organisms other than *Bartonella* spp. had been occasionally documented, using cytologic examination of blood smears from humans, including anemic patients with AIDS and systemic lupus erythematosus (SLE) (1, 9, 14, 21). Recently, *Mycoplasma haemofelis* was detected using PCR in a Brazilian man who was coinfecting with human immunodeficiency virus and *Bartonella henselae* (8). The existence of a genetically similar organism was documented in a splenectomized patient with SLE (15). *Mycoplasma suis* has been identified using PCR among humans in contact with infected pigs in Shanghai (26).

Mycoplasma ovis is a recognized pathogen of sheep flocks worldwide. Previously known as “*Eperythrozoon ovis*,” the organism was reclassified as *M. ovis* based upon 16S rRNA gene sequence analysis (17). The organism attaches to erythrocytes and causes “ill thrift,” hemolytic anemia, and occasionally icterus, hemoglobinuria, and mortality in lambs (20, 24). Decreased growth rate and wool production have also been reported in flocks, as well as immunosuppression with predisposition to pneumonia and abscessation (20), although the mechanisms of immunosuppression have not been elucidated. In adult sheep, chronic, subclinical infections are typical, unless immunosuppression, including the stress of parturition, triggers the development of hemolytic anemia. Lymphoid depletion within the spleen and lymph nodes was also described in one affected sheep (20). Coinfection of sheep with two distinct strains, differing with respect to a 17-bp fragment, was recently reported in sheep from Hungary (11). It was suggested that the difference in homology between these strains may be sufficient to justify renaming the deletion variant as a separate species, “*Candidatus Mycoplasma haemovis*” (11). Similar variants also coinfecting the veterinarian in this report. This is the first detection of an organism resembling *M. ovis* in our laboratory.

The detection of multiple different 16S rRNA gene sequences in this patient and the Hungarian sheep flock might also be explained by the existence of multiple copies of the 16S rRNA gene within a single strain of *M. ovis*, each having a variable genetic composition, as reported for other bacteria (16). The only hemotropic mycoplasma species for which 16S rRNA gene copy number has been ascertained is *Mycoplasma haemofelis*, which has a single copy of the 16S rRNA gene (2). Furthermore, a similar large deletion in the 16S rRNA gene helps discriminate *Mycoplasma haemofelis* from “*Candidatus Mycoplasma haemominutum*.”

The reported host range of *M. ovis* includes goats, in which it causes severe disease, and possibly deer (17, 23, 24). Transmission among sheep is thought to occur by blood-feeding arthropods, and reuse of needles and blood-contaminated instruments during procedures such as flock immunization and ear-tagging (6, 17, 20, 24). Oral transmission has been documented experimentally (18). How the veterinarian in this report became infected is unknown. The *Bartonella* coinfection may be a clue to vector-borne transmission. Coinfection with *B. henselae* was not associated with seropositivity in this veterinarian, possibly because of underlying drug-induced immunosuppression, which may also have contributed to impaired clearance of both *B. henselae* and the *M. ovis*-like agent.

The full-length 16S rRNA gene sequence homologies between the organisms detected in this report and those of ovine

M. ovis variants do not prove their identity to one another. The 16S rRNA gene sequences of *Mycoplasma haemocanis* and *M. haemofelis* are identical, yet they differ in their host tropisms and morphologies and are better differentiated based on their *mpB* gene sequence (3, 19, 25). Amplification of a short fragment of the *mpB* gene sequence from the *M. ovis*-like organisms showed 100% identity to that of the *mpB* gene amplified from an ovine *M. ovis* strain. Repeated attempts to amplify a longer segment of the *mpB* gene were unrewarding, possibly as a result of sequence variation.

Bartonella DNA was first detected in the veterinarian in August 2005. Retrospective efforts to define the *Bartonella* species and strain by DNA sequencing for that sample were unsuccessful. *B. henselae* DNA with a sequence identical to that of the SA2 strain was detected in March 2007 and again in February 2009, following prolonged doxycycline and rifampin therapy. Therapeutic elimination of *Bartonella* sp. may be difficult (5). Also, some individuals remain persistently infected without developing a specific humoral immune response as detected by immunofluorescent antibody (IFA) testing (4). Similarly, *M. ovis* disappears from blood smears following treatment with tetracyclines, but infection may persist with disease recrudescence following discontinuation of therapy (11). Infection appeared to persist in the human patient reported here despite prolonged therapy with azithromycin and then levofloxacin. Immunocompromise may have contributed to persistent infection, as a result of the immunomodulatory drug therapy used to treat MS. Immunosuppressive effects associated with chronic *Bartonella* infection, as suggested previously (4), and possibly chronic hemoplasma infection may also have contributed to bacterial persistence. The only laboratory evidence for immunosuppression in the patient was hypoglobulinemia. White cell counts were within reference ranges, although lymphocyte subset counts were not performed.

Reinfection remains another possible explanation for repeated detection of *B. henselae* and *M. ovis*, as the patient continued to work as a veterinarian throughout his illness, and the detection of *B. henselae* Houston-1 in March 2010 suggested that ongoing infection with two *B. henselae* strains might have been a possibility. The prevalence of *B. henselae* bacteremia in cats and dogs within the patient's practice was unknown, but *Bartonella* seroprevalence in cats in the region exceeds 50% (12).

The extent to which the infections with *B. henselae* or *M. ovis* contributed to or initiated disease symptoms in this patient remains unanswered, although the dramatic clinical improvement following antimicrobial therapy suggested they may have played a role. Hemoplasma infection was not associated with laboratory evidence of hemolysis in this patient. Given the increasing number of reports of hemoplasma infection in humans in contact with animals, especially the immunosuppressed, continued investigations into the prevalence and clinical significance of hemoplasma infections in this group of individuals seem warranted.

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