Fatal Case of Brucellosis Misdiagnosed in Early Stages of *Brucella suis* Infection in a 46-Year-Old Patient with Marfan Syndrome

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We report a fatal case of *Brucella suis* endocarditis initially misdiagnosed by automated identification systems as *Ochrobactrum anthropi* infection in a patient with a history of Marfan syndrome and recreational feral swine hunting. This report emphasizes the need to consider brucellosis as a part of the differential diagnosis of acute febrile illness, particularly in patients with known risk of exposure.

CASE REPORT

In October 2009, a 46-year-old Caucasian male was admitted to a hospital after presenting to the emergency room with fever up to 102°F, extreme fatigue, malaise, and abdominal pain that had progressively worsened over the preceding 3 weeks. He also admitted to occasional shortness of breath with exertion and lower back pain. His past medical history was significant for Marfan syndrome (MFS) with severe scoliosis and multiple aortic aneurysms (21). In his early life, he had several surgeries, including replacement of his aortic valve with a prosthetic metallic valve and four separate aortic graft and spinal fusion surgeries due to complications of MFS. He also was a long-time recreational feral swine hunter in Florida. The patient also admitted to social alcohol use, denied use of tobacco and drugs, and had no recent travel or known contact with infected animals prior to hospitalization.

He had been hospitalized twice prior to this admission. In July 2008, he was admitted with an acute febrile illness. He had similar complaints, including daily fever, malaise, and fatigue. An extensive workup, including a chest X ray, repeated blood and urine cultures, and human immunodeficiency virus (HIV) and sexually transmitted disease (STD) testing, was performed, and results were all negative. He then returned to the hospital in January 2009 with a report of continued fever, fatigue, and malaise, and at this time, he was diagnosed with pneumonia. Analysis of two blood culture samples by both the BioMérieux Vitek 2 system and the Remel RapID NF Plus panel identified *Ochrobactrum anthropi* with a 99% match. He was treated with ciprofloxacin and ertapenem for 2 months. No hunting was reported after the January hospitalization.

In October 2009, he presented to a local hospital with a febrile illness as previously described with extreme fatigue, malaise, and abdominal pain. He was started on empirical intravenous vancomycin and piperacillin-tazobactam (Zosyn) at that time. On evaluation, he was found to have pancytopenia with an absolute neutrophil count of 80,000/μl and a platelet count of 63,000/μl and elevated transaminases with hypoalbuminemia, and the computed tomography (CT) scan of his chest showed cardiomegaly with visualization of his metallic aortic valve and interval resolution of past pneumonia without evidence of pulmonary embolism. Furthermore, the CT scan of his abdomen and pelvis revealed multiple splenic infarcts (data not shown), splenomegaly

with his spleen measuring 16 mm from the superior to the inferior dimension, severe atherosclerosis with stable aneurysmal enlargement of the suprarenal aorta with a maximum diameter of 3.3 cm, and generalized edema (anasarca). Analysis of two additional blood cultures revealed 99% matches to *Brucella melitensis* and *Ochrobactrum anthropi* by an updated Vitek Observa 4.01 instrument. However, the Remel system gave codes reporting a 99% match to *Alcaligenes xylosoxidans* and *Pseudomonas stutzeri*. Both of these blood cultures were later confirmed to be *Brucella suis* biotype 1 by standard microbiological procedures at the state health department laboratory (6).

The patient responded slowly to antimicrobial therapy, and within 1 h of a microbiology report of positive blood cultures for *Brucella*, the patient suffered a fatal cardiac arrest and resuscitative efforts were not successful. Bone marrow aspiration and biopsy were performed just prior to his death and results were negative for any abnormality in the lymphoid tissue. No visible organisms were detected by standard staining procedures. Formalin-fixed, paraffin-embedded heart tissue was tested for *Brucella* antigens by immunohistochemical (IHC) assays using monoclonal anti-*B. melitensis* and anti-*Brucella abortus* antibodies (12). The *B. suis* blood culture isolate was analyzed by multilocus sequence typing (MLST) and multilocus variable-number tandem-repeat analysis (MLVA) to compare the genetic profile with those of other *B. suis* strains previously identified from the southeastern region of the United States (25). Genomic DNA extracted from cardiac tissue was positive for *Brucella* by 16S rRNA gene PCR assay (data not shown), and subsequent *Brucella* antigens were shown to be associated with areas of myocarditis (data not shown). The final diagnosis was fatal brucellosis with myocarditis and endocarditis. We determined the *B. suis* isolate to be sequence type 14 by MLST analysis (data not shown). Comparative genetic profiles by MLVA indicated that this strain was closely related to other *B. suis* strains

associated with recent human brucellosis cases in Florida (3) (data not shown).

Brucellosis is a zoonotic disease caused by the genus *Brucella*, belonging to the family *Brucellaceae* of the class *Alphaproteobacteria* (19). Members of the genus *Brucella* are Gram-negative intracellular facultative pathogens, and currently, there are 10 recognized species based on their phenotypic characteristics, including host preferences and environment (16, 22). Human brucellosis is mostly associated with four *Brucella* spp., which include *B. abortus* (cattle), *B. melitensis* (goat and sheep), *B. suis* (swine), and, rarely, *Brucella canis* (dogs) (14, 27). Major routes of *Brucella* transmission to humans include (i) consumption of unpasteurized dairy products from infected animals, (ii) handling of or exposure to tissues or bodily fluids from infected animals, including feral swine (wild boars), without proper protection, and (iii) inhalation of *Brucella*-contaminated aerosols in a slaughterhouse or clinical lab (7, 11, 17). Human brucellosis associated with *B. suis* infection is less prevalent than *B. abortus* or *B. melitensis* infections worldwide (16). However, despite near-complete eradication of brucellosis in commercial swine through well-managed animal control practices, several cases of *B. suis*-associated infections among hunters and slaughterhouse workers handling infected feral swine/wild boars are reported yearly (1, 4, 9, 15, 18). The U.S. feral swine population has increased dramatically over the past 20 years, and they are recognized as "game species" in 27 states (23). These swine herds not only potentially carry *B. suis* but also may sometimes be chronically infected with field strains of *B. abortus*, including S19 and RB51 vaccine strains, as well as other viruses and parasites (23). Because of the increase in feral swine populations and the lack of serodiagnosis in disease control programs for feral swine herds, control of *B. suis* transmission to recreational hunters in the United States is difficult (8, 18, 23).

The family *Brucellaceae* also includes several genera, of which the most clinically and phylogenetically related to *Brucella* is the genus *Ochrobactrum* (16, 24). *Ochrobactrum anthropi*, a well-studied pathogenic species of this genus, is recognized as a saprophyte and is occasionally associated with endocarditis, bacteremia, and nosocomial infections (2, 24). Because of their similar phenotypic properties, early diagnosis of a suspected brucellosis case is often miscoded as *O. anthropi* infection by rapid automated identification systems in the clinical laboratory (10). Furthermore, widely used serologic assays for early diagnosis of brucellosis, such as standard agglutination testing (SAT) and enzyme-linked immunosorbent assays (ELISA), have demonstrated limited specificity to date due to the cross-reactivity of *Brucella* antigens with other genetically closely related Gram-negative pathogens of the same family (13, 20). Results from these rapid assays for brucellosis should be interpreted with caution due to inaccuracy in differentiation of *Brucella* species from *Ochrobactrum* species and other genetically similar Gram-negative pathogens. Misdiagnosis may result in a delay of appropriate antimicrobial therapy initiation for brucellosis (26). Brucellosis is a treatable disease, and early diagnosis may be critical in patients with underlying conditions, such as in this case. Antimicrobial therapy reduces symptoms, shortens the duration of illness, and decreases the risk of complications and relapse. Furthermore, combination treatment is recommended due to a high risk of relapse with

monotherapy (26). Doxycycline plus streptomycin is the first-line treatment for brucellosis. Alternatives include doxycycline plus gentamicin, doxycycline plus rifampin, co-trimoxazole (Bactrim) plus gentamicin, or fluoroquinolones plus co-trimoxazole, as recommended by the World Health Organization (WHO) (http://www.who.int/csr/resources/publications/Brucellosis.pdf).

Routes of transmission of human brucellosis include ingestion of animal products, such as unpasteurized milk and milk products, undercooked meat products, and traditional delicacies, inhalation of airborne animal manure particles, or mucosal or skin abrasion/wound contact when handling infected animal carcasses, placentas, or animal vaginal secretions (1, 14, 16). In the United States, brucellosis is recognized as one of the most common laboratory-transmitted infections (5). In this specific case, it is clear that clinicians in the local hospital did not suspect brucellosis, therefore prolonging patient treatment and endangering the clinical laboratory staff. The automated identification systems are utilized primarily for rapid detection of organisms with no prior knowledge of any specific suspected disease. We hope to remind clinicians of the importance of discussing activities, including travel, food consumption, occupation, and recreational activities such as feral swine hunting, when obtaining a history of patients with acute febrile illness. We also need to emphasize the fact that clinicians should consider brucellosis in such cases in which an automated system detected *Ochrobactrum* instead of *Brucella* spp. Therefore, it is important for clinicians to alert laboratorians to follow the Sentinel Guidelines for handling suspected brucellosis cases, as recommended by ASM/CDC (http://www.asm.org/index.php/policy/sentinel-level-clinical-microbiology-laboratory-guidelines.html).

Our report emphasizes the need to remind clinicians to consider brucellosis in spite of detection of *Ochrobactrum anthropi* in the differential diagnosis of unexplained febrile illness using results conferred by rapid automated biochemical assay systems and/or serologic testing. We also hope to inspire clinicians to obtain information about patient activities, including travel, food consumption, occupation, and outdoor recreation, such as wild swine hunting. Laboratorians should be aware of the limitations of using the automated identification systems for *Brucella*. Differentiation between *Brucella* species and other genetically related Gram-negative organisms with early diagnosis and treatment can be lifesaving.

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