A fatal case of brucellosis misdiagnosed in early stages of *Brucella suis* infection in a 46-year-old patient with Marfan syndrome

M Carrington¹, U Choe¹, S Ubillos¹, D Stanek², M Campbell², L Wansbrough³, P Lee³, G Churchwell³, K Rosas³, S R Zaki⁴, C Drew⁴, C D Paddock⁴, M DeLeon-Carnes⁴, M Guerra⁴, A R Hoffmaster⁴, R V Tiller⁴ and **B K De**⁴

¹University of South Florida, Tampa, FL, ²Department of Health, Hillsborough County, FL, ³Department of Health, Bureau of Laboratories, Jacksonville, FL, ⁴Centers for Disease Control and Prevention, Atlanta, GA

**ABSTRACT**

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 of 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, and 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 of 2008, he was admitted with an acute febrile illness. He had similar complaints including daily fever, malaise and fatigue. Extensive workup including...
chest X-ray, repeated blood and urine cultures, HIV (human immunodeficiency virus) and STD (sexually transmitted disease) testing was performed and results were all negative. He then returned to the hospital in January of 2009 with 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 BioMerieux 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 two months. No hunting was reported after the January hospitalization.

In October of 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 zosyn at that time. On evaluation, he was found to have pancytopenia with an absolute neutrophil count of 80 K/UL and platelet count of 63 K/UL, 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 superior to inferior dimension, severe atherosclerosis with stable aneurysmal enlargement of the suprarenal aorta with maximum diameter of 3.3 cm, and generalized edema (anasarca). Analysis of two additional blood cultures revealed a 99% match to *Brucella melitensis* and *Ochrobactrum anthropi* respectively 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 one hour of 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 was performed just prior to his death and were negative for any abnormality in the lymphoid tissue and no visible organisms were detected by standard staining procedures. Formalin-fix, paraffin-embedded heart tissue was tested for *Brucella* antigens by immunohistochemical (IHC) assays using monoclonal anti-*B. melitensis* and *B. abortus* antibodies (12). The *B. suis* blood culture isolate was analyzed by multi-locus sequence typing (MLST) and multi-locus variable-number tandem repeat analysis (MLVA) to compare the genetic profile with that 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 seen 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 α-Proteobacteria (19). Members of the genus Brucella are Gram-negative intracellular facultative pathogens and currently, there are ten 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 B. canis (dogs) (14, 27).

Major routes of Brucella transmission to humans include (i) consumption of unpasteurized dairy products from infected animals, (ii) handling 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 slaughter house or clinical lab (7, 11, 17). Human brucellosis associated with B. suis infection is less prevalent in comparison to 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 US 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 potentially carry not only B. suis but could be chronically infected rarely with field strains of B. abortus including S19 and RB51 vaccines 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 USA 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 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 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 high risk of relapse
with mono-therapy (26). Doxycycline plus streptomycin is first line treatment for brucellosis. Alternatives
include doxycycline plus gentamicin, doxycycline plus rifampin, bactrim plus gentamicin, or fluoroquinolones
plus bactrim as recommended by World Health Organization (WHO)

Routes of transmission of human brucellosis include ingestion of animal products such as
 unpasteurized milk and milk products, undercooked meat products and traditional delicacies; and 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 US, 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 primarily utilized for rapid detection of
 organisms with no prior knowledge of any specific suspected disease. We hope to remind clinicians the
 importance of discussing activities including travel, food-consumption, occupation and recreational activities
 such as feral-swine hunting when obtaining a history on patients with acute febrile illness. We need also to
 emphasis the fact that clinicians should consider brucellosis in such cases where an automated system led to
detect 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

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 despite 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.
REFERENCES


