Answer to Photo Quiz: Brucellosis Caused by *Brucella melitensis*

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KEYWORDS *Brucella*, Gram stain, MALDI-TOF MS, bioterrorism, laboratory safety

The organism was identified as *Brucella* species using 16S rRNA gene sequencing at the reference laboratory and subsequently confirmed as *Brucella melitensis* with Bruce-ladder multiplex PCR at the Centers for Disease Control and Prevention (CDC) (1). Brucellosis is widely known as a zoonotic disease and one of the most common laboratory-acquired infections (2). Three *Brucella* species, *B. melitensis*, *Brucella abortus*, and *Brucella suis*, are classified as category B bioterrorism (BT) agents by the CDC for their low infectious dose and ease of spread through aerosols. The manifestation of the disease is often characterized by intermittent or remittent fever, malaise, anorexia, joint pain, back pain, and prostration. The incubation period ranges from 5 days to 5 months, with an average of 2 to 4 weeks (3). Without timely diagnosis and appropriate treatment, cardiovascular complications of brucellosis may occur, accompanied by a high mortality rate (4).

Preliminary identification of *Brucella* relies on its small, Gram-negative appearance, slow growth on media, positive oxidase and catalase reactions, and strong urease activity. While *Brucella* species are exclusively described as Gram-negative coccobacilli in textbooks and guidelines, a few studies have reported Gram-positive staining of the organism (3, 5–7). In the current case, the *B. melitensis* isolate stained Gram positive in all positive blood culture bottles as well as on the medium plates, regardless of the day of isolation and the technician. The Gram-variable pattern and the resistance to decolorization in *Brucella* were acknowledged by the CDC (personal communication). This rare Gram-positive presentation of *Brucella*, resembling that of *Corynebacterium* or *Streptococcus*, inevitably misled the workup and identification process; the oxidase test was not performed, and the MacConkey medium plate was not set up. Presumed to be a slow-growing Gram-positive organism, the culture was worked up on an open bench. The first sign of discrepancy was revealed when the BD Phoenix system (Becton, Dickinson and Company, Franklin Lakes, NJ) identified the organism as *Ochrobactrum anthropi*, a Gram-negative bacillus closely related to *Brucella*, which contradicted the original Gram stain result. Simultaneously, identification of the organism was attempted using MALDI-TOF MS with the IVD database, but no reliable identification was obtained. Multiple studies have evaluated the potential of MALDI-TOF MS for identification of BT agents with specialized libraries (8, 9), but Rudrik et al. recently showed that even using the security-relevant (SR) library cannot fully identify all the *Brucella* species and other BT agents (10).

Aerosol exposure due to manipulation of unidentified *Brucella* isolates outside a biological safety cabinet remains the major cause of laboratory-acquired brucellosis. Since the introduction of the CDC assessment and prophylaxis guideline in 2008 (11), the percentage of disease development from reported potential exposures in the United States has remained low (3). Seven of our laboratory staff were categorized as having a high risk of potential exposure, while another six were at low risk. All 13 staff members were monitored by symptom watch and the *Brucella* species serology offered

Citation West T, Warren RA, Chow S-K. 2019. Answer to Photo Quiz: Brucellosis caused by *Brucella melitensis*. J Clin Microbiol 57:e00382-18. <https://doi.org/10.1128/JCM.00382-18>.

Editor Paul Bourbeau

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See page <https://doi.org/10.1128/JCM.00381-18> in this issue for photo quiz case presentation.

Published 27 February 2019

by the CDC. All seven staff at high risk received doxycycline (100 mg BID) and rifampin (600 mg daily) as prophylaxis for 3 weeks. None of the staff seroconverted. After further investigation, it was found that the patient is a farmer in India. He raises cows but denies drinking unpasteurized milk. It was unclear if his cows suffered from abortion, still birth, weight loss, or unusual sickness resembling brucellosis. He was treated with doxycycline (100 mg BID) and rifampin (600 mg daily) for 6 weeks, and subsequently his back pain resolved.

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