Brucella Species Staining as Gram-Positive Rod and Gram-Positive Cocci in Chains

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ABSTRACT
Two cases of Brucellosis were identified at a hospital in Rhode Island. In both cases, the organisms were isolated from the blood cultures. The bacteria did not appear as the classical textbook description of Brucella spp. as short, Gram-negative rods; instead, Gram-positive rods and Gram-positive cocci in chains were observed. Due to the atypical Gram stain morphology, Brucella spp. were not initially considered as a possible pathogen. Antimicrobial prophylaxes were offered to the technologists who were exposed to the organisms.

KEYWORDS: Brucellosis, Brucella, Gram stain, Gram-positive rod, Gram-positive cocci

INTRODUCTION
Brucellosis is a zoonotic disease caused by Brucella spp. According to the Centers for Disease Control and Prevention (CDC), seventy-three cases were reported in the United States in 2021. Brucella spp. are short, Gram-negative, non-encapsulated, non-motile, non-spore-forming, slow-growing, facultatively intracellular coccobacilli that are pathogenic to different domestic and wild animals. Brucellosis can be acquired through ingestion of contaminated food such as unpasteurized dairy products, through skin wounds or mucous membranes after contact with infected animals, or inhalation of the bacteria. Although rare, transmissions from human to human have been reported through different routes. Here, we report two cases of Brucellosis that presented to the same emergency department in Rhode Island five days apart with the organisms showing atypical morphology on Gram stain.

CASE REPORT
Case 1
A 59-year-old male with a past medical history of diabetes mellitus type 2, hyperlipidemia hypertension, atrial fibrillation and transient ischemic attack presented to an emergency department in Rhode Island during March 2022 with subjective fever, malaise, headache, diaphoresis, nausea, left lower quadrant pain and back pain for five days duration. He recently traveled to Aruba for a fishing trip with friends three weeks prior to becoming symptomatic. An extensive infectious disease work-up was performed and the patient’s test results came back negative for most bacterial, viral, and parasitic infections such as Borrelia burgdorferi, Human Immunodeficiency Virus and Anaplasma phagocytophilum. Blood cultures were collected on the day of his presentation to rule out sepsis. Gram-positive rods were seen after two days of incubation (Figure 1). The patient’s primary care team prescribed an empiric treatment of doxycycline, vancomycin and cefepime (for empiric treatment of Gram-positive rod bacteremia) after receiving the initial Gram stain result. Upon subculture, the positive blood culture grew short Gram variable rods (Figure 2). Due to the suspicious nature of the colony Gram stain and colony growth on culture, biochemical tests were performed to rule out Brucella spp.

Figure 1. Gram stain, from blood culture bottle (BACT/ALERT FA plus).

Figure 2. Case 1, Gram stain from colony after subculture.
The organism was oxidase, catalase, and urease positive which matched the expected results for Brucella spp. The laboratory was unable to rule out Brucella spp., so the isolate was sent to Rhode Island Department of Health (RIDOH) for confirmatory testing. The isolate was identified as Brucella spp. by the RIDOH and was sent to CDC for further testing. The isolate was later speciated as Brucella abortus by CDC. Serologic testing was positive for Brucella IgM and IgG. He was treated with doxycycline 100 mg twice a day and rifampin 600 mg once a day (rifampin was chosen due to its availability, ease of administration and lower toxicity). Upon further questioning, the patient admitted consuming unpasteurized milk/cheese while in Aruba. Eight days after presentation, the patient’s symptoms began to improve, and he was discharged on doxycycline and rifampin. He finished the treatment, remained afebrile and was at his baseline health during the six weeks follow-up visit.

Case 2
A 21-year-old female with a past medical history of migraines presented to the same emergency department in Rhode Island with high fever (up to 104.7°F), myalgia, headache, diaphoresis, rhinorrhea, and otalgia for twelve days duration that started just prior to a trip to Puerto Rico. Three months before the illness, she traveled to Sudan for one month and stayed with family and friends. She admitted that she did not take any malaria prophylaxis or follow strict food and water precautions. Blood cultures were drawn on the day the patient presented to the emergency department. She was discharged from the hospital the next day after clinically improving. After approximately fifty hours of incubation, both sets of blood cultures were positive and Gram stain showed Gram-positive cocci in chains. The patient was called back to the emergency department for further work-up and was started on ceftriaxone for bacteremia that was thought to be caused by Streptococcus spp. based on the original gram stain. The organism was identified as Brucella spp. by MALDI-TOF (Vitek MS). The identification was confirmed by RIDOH and was sent to CDC for speciation. The organism was identified as Brucella melitensis by CDC. After diagnosis, the patient was treated with doxycycline 100 mg twice a day for six weeks and gentamicin 240 mg intramuscularly for nine days (she weighed 56.0 kg). The gentamicin was not given on Day 10 since she felt lightheaded; a symptom that might indicate early vestibular toxicity. The patient was discharged from the hospital one week after the initial presentation with follow-up.

**DISCUSSION**
In a single emergency department in Rhode Island, two cases of Brucellosis presented five days apart. In both cases, the organism did not present with its typical morphology on Gram stain. The organisms appeared as Gram-positive rods or Gram-positive cocci in chains on the original Gram stains. Brucella spp. are known to stain Gram variable or be faintly staining, which can have significant impact on the patient care and safety of laboratory staff. In both cases, the organism presented atypically on Gram stain leading to routine handling of the organism by the medical technologists. In the first case, the organism stained as short Gram-positive rods on the original gram stain (Figure 1) and stained as short Gram variable rods on colony Gram stain (Figure 2). In the second case, the organism stained as Gram-positive cocci in chains (Figure 3). As a part of our laboratory procedure, identification by MALDI-TOF is performed on all organisms that are suspicious for Streptococcus spp. The identification of Brucella spp. was unexpected based on the original gram stain. In both cases, the organisms appeared more consistent with the typical morphology of Brucella spp. after subculture. Due to the atypical Gram stain appearance, Brucella spp. was not initially considered as a possible pathogen in these cultures. Some of the biochemical testing methods were not performed in a Class II biosafety cabinet, which caused some medical technologists in the laboratory to be exposed to the organism. This is significant because Brucella spp. have been known to cause laboratory-acquired infections. Retrospectively, Gram-stain slides from both cases were reviewed by the senior technologists and laboratory director, and the initial results were confirmed. The organisms did stain as Gram-positive. These Gram-stain results were most likely due to the intrinsic nature of the organism rather than a technical error in the Gram-staining procedure. Variable Gram-staining morphology were also identified in cases reported in New York. Antimicrobial prophylaxis was offered to the technologists, and they were monitored by the Employee Health. Lessons learned from these two cases: slow growing organisms from positive blood cultures, regardless of the Gram staining result, should be handled in a Class II biosafety cabinet until Brucella spp. and other highly infectious organisms are ruled out. This is not a standard laboratory practice in most clinical microbiology laboratories, however, laboratories with experience...
of *Brucella* spp. exposure advocate that this should become a routine laboratory procedure.\(^6\)\(^7\)

Although the incidence of Brucellosis is rare in the northeastern part of the United States, it is still one of the most common zoonotic diseases in developing countries.\(^8\)\(^9\)\(^10\) The rarity of Brucellosis in the United States can easily lead to a misdiagnosis and potentially cause laboratory-acquired Brucellosis.\(^8\)\(^11\)\(^12\) Brucellosis is one of the most common laboratory-acquired infections and laboratory staff should be aware of the potential for this organism to be recovered in culture despite the low incidence of Brucellosis in the developed countries.\(^6\) Even though Brucellosis is rare in the United States, the amount of immigration and travel in the United States increases the likelihood that *Brucella* spp. could be recovered from patients.\(^8\) In addition to the rarity of Brucellosis, its non-specific symptoms and varying presentations can delay diagnosis and appropriate treatment.\(^13\) Taking a detailed travel and occupational history as well as questioning consumption of raw or unpasteurized dairy products is vital in the diagnosis of Brucellosis.\(^8\)\(^9\) A thorough investigation is required after *Brucella* spp. are isolated in the laboratory to identify, notify, and follow up with laboratory staff and healthcare personnel at risk. This includes offering prophylactic treatment to employees who are exposed and monitoring the health of these employees.\(^14\)

References


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