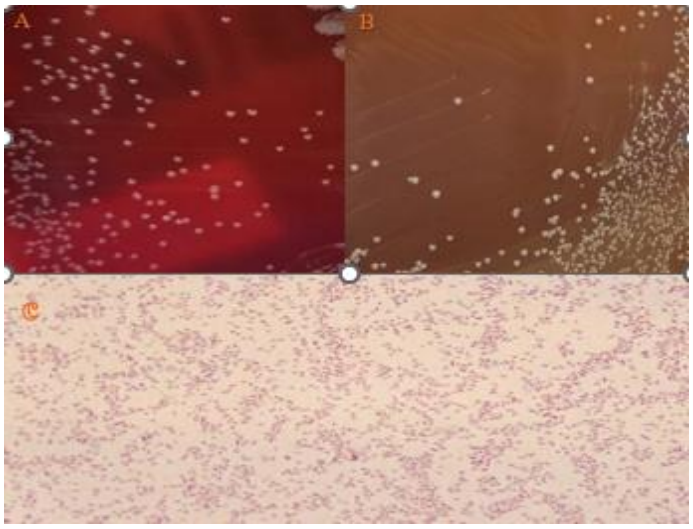
The Uninvited Guest: Bacterial spondylodiscitis

An elderly male presented with a three-month history of worsening lumbar pain. He endorsed a one-month history of chills and night sweats. While plain radiographs were unremarkable, an outpatient MRI revealed spondylodiscitis at L3-L4 with adjacent psoas abscess, prompting hospital admission. Computed tomography (CT) of the abdomen and pelvis revealed a right psoas abscess adjacent to the L3 inferior endplate, further suggesting an infectious process extending from the lumbar spine. The patient is originally from Central America, with recent travel to the continent within the last year. *M. tuberculosis* screening was negative.

An IR-guided spinal aspiration of the accumulated serosanguinous fluid from the disc space yielded murky fluid which was sent for culture. Gram stain of the aspirate revealed moderate neutrophils, but no organisms were observed. Blood cultures were also obtained. Both blood and aspirate cultures turned positive after 48-72 hours of incubation and empiric therapy with ceftriaxone and vancomycin was initiated. Blood culture Gram stains and Gram stains from the punctate colonies recovered from the aspirate revealed small Gram-negative coccobacilli. Importantly, growth was observed on Sheep blood agar (BAP) and chocolate agar, but growth was noticeably absent from the MacConkey agar suggestive of a fastidious organism. Biochemical testing revealed positive oxidase, catalase, urease, and β-lactamase results. Due to a concern for a possible laboratory biological hazard, further manipulation of cultures containing suspect organisms were performed using enhanced biosafety precautions

MALDI-TOF mass spectrometry was performed for identification without success using FDA-cleared and RUO databases. Per laboratory protocol, a Security Relevant database for the identification of Select Agents was attempted, identifying this organism as *Brucella* sp. The isolate was subsequently referred to the Maryland Department of Health (MDH) laboratory, which confirmed as *B. abortus* by a *Brucella*-specific PCR assay. Antimicrobial therapy was adjusted to a combination of PO doxycycline and rifampin to be continued for three months. Following initiation of appropriate therapy, the patient's symptoms significantly improved and they were discharged after one week of hospitalization.

Representative images of small, punctate *Brucella* colonies recovered from blood culture on BAP (A) and CHOC (B). Gram stain of *Brucella* spp. from a positive blood culture bottle (C, 100X, oil immersion)



Which of the following is/are false?

A. Brucella species are classified as BSL-3 organisms, requiring strict biosafety precautions.
B. Brucella species are fastidious and require X or V factors or both for growth.
C. Gram stain may appear faint because the organisms are small intracellular coccobacilli.
D. Brucella species exhibit positive results for oxidase, catalase, and urease tests.

Answer: B

Brucella spp. and *Haemophilus* spp. both appear as small coccobacilli by Gram stain. However, many *Haemophilus* spp. With fastidious growth requirements do not grow on routine blood agar plates containing sheep blood, as opposed to *Brucella* sp. which are often recovered after extended incubation. Fastidious *Haemophilus* sp. typically exhibit satellite growth around *S. aureus* on sheep blood-containing BAP, reflecting their species-specific requirements for X (hemin) and/or V (NAD) factors. Sheep blood contains high levels of endogenous NADases, which rapidly degrade V factor, rendering routine blood agar plates unsuitable for cultivation of most fastidious *Haemophilus* sp.

Discussion:

Brucellosis is one of the most widespread zoonotic infections with an estimated 1.6-2 million new human cases annually. *Brucella* sp. cause infections in a variety of mammals, with the severity of disease ranging from asymptomatic carriage to fulminant infections impacting the reproductive, osteoarticular, and central nervous systems. Clinical brucellosis is primarily caused by four *Brucella* species: *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis* and is acquired through contact with fluids from infected animals or by consuming contaminated animal products, such as unpasteurized milk and cheese, and exposure to infectious aerosols. The disease is primarily endemic in developing and tropical regions, with an estimated 500,000 cases reported worldwide each year. In the United States, brucellosis is rare, with only 100 to 200 cases occurring annually.

Brucella species are small, non-motile, Gram-negative coccobacilli which are often faintly stained with conventional Gram counterstaining using safranin. Because of this, cells are sometimes overlooked in direct Gram stains. Staining of *Brucella* can be enhanced using carbolfuchsin as a Gram counterstain in lieu of safranin. Colonies grow slowly on blood and chocolate agar but do not grow on MacConkey, like other fastidious Gram-negative species. Colonies typically appear after 2–4 days as small (0.5–1 mm), convex, nonpigmented, nonhemolytic colonies. They are oxidase-, catalase-, and urease-positive, with some species exhibiting rapid urease activity that supports survival in acidic environments.

Brucella are facultative intracellular pathogens that replicate primarily within monocytes and macrophages. Most possess two circular chromosomes with relatively high GC content. They do not produce classical toxins; instead, their virulence is mainly associated with lipopolysaccharide (LPS) and the Type IV secretion system. Smooth LPS strains exhibit full virulence, whereas rough strains are less virulent. Within host cells, brucellae survive in a specialized compartment known as the *Brucella*-containing vacuole (BCV), which prevents fusion with lysosomes facilitating persistence and survival. The BCV later interacts with endoplasmic reticulum membranes, creating a compartment that supports bacterial replication. Because *Brucella* reside intracellularly, effective treatment requires antimicrobials with intracellular activity. Commonly used drugs include tetracyclines, aminoglycosides, and fluoroquinolones. To minimize the risk of relapse, combination regimens are recommended, such as doxycycline with rifampicin, doxycycline with gentamicin, or TMP-SMX with rifampin.

Symptoms of brucellosis typically appear two to four weeks postexposure to the bacteria, often with subclinical or intermittent, mild symptomology. Because of this, it is not uncommon for protracted courses of disease to be reported, sometimes spanning months or years. Brucellosis is endemic in Central America, where exposure to unpasteurized dairy products or animal contact is frequent. These fastidious organisms enter the bloodstream and can subsequently seed distal sites including regional lymph nodes, bone marrow, bones, joints, and other organs. Clinical manifestations range from fever, night sweats, and fatigue to musculoskeletal complaints. Osteoarticular involvement, such as that seen in this case, occurs in 10-85% of cases with spondylodiscitis representing one of the most serious manifestations. This patient's presentation, lumbar spondylodiscitis with psoas abscesses, night sweats, and a subacute three-month course, is characteristic of brucellar infection.

Despite recent removal from the Federal Select Agent Program in the United States, *Brucella* sp. are classified as BSL-3 organisms requiring strict biosafety precautions because of their high infectivity and potential for aerosol transmission. All manipulations involving *Brucella* should be conducted in a BSL-3 laboratory using appropriate personal protective equipment (PPE), including gloves, gowns, and face and eye protection. In the clinical laboratory, appropriate biosafety precautions are essential as laboratory-acquired infections are well documented.

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