



NEW YORK CITY DEPARTMENT OF
HEALTH AND MENTAL HYGIENE
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Commissioner

2015 Alert #15: Imported Brucellosis: Recent Laboratory Exposures Requiring Prophylaxis and Long-term follow up

Please distribute to Departments of Emergency Medicine, Family Practice, Geriatrics, Pediatrics, Internal Medicine, Obstetrics/Gynecology, Infectious Disease, Infection Control, Pulmonary Medicine, Critical Care, Pharmacy, and Laboratory Medicine

- **Since April, two separate incidents in New York City exposed 51 healthcare workers in two hospital clinical laboratories to *Brucella melitensis* from patients infected overseas**
- **Clinicians are reminded to elicit travel histories and risk exposures from patients presenting with fever and infectious disease symptoms**
- **If brucellosis or infection from another highly infectious agent is suspected, inform the clinical laboratory so that laboratorians can take special precautions to prevent exposure**
- **Clinical laboratories should insure that appropriate protocols are followed to recognize, rule out and refer potential biological threat agents**
- **Please notify the NYC Health Department promptly of any suspected brucellosis cases**

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Dear Colleagues,

Since 2010, there have been six incidents in which NYC hospital laboratories isolated a *Brucella* species unexpectedly, requiring that steps be taken to address possible laboratory exposures. In April and May 2015, two laboratory-confirmed cases of *Brucella melitensis* were reported to the NYC Health Department. Both patients reported symptoms consistent with brucellosis, traveled to a country where the disease is endemic, and consumed unpasteurized milk while there. The diagnosis was not suspected, and the laboratories were not notified to consider brucellosis and take appropriate precautions when handling clinical specimens and isolates. No laboratory workers developed brucellosis. We urge clinicians and laboratorians to take specific steps to prevent transmission of brucellosis and other highly infectious diseases.

Background

Brucellosis is a common zoonotic disease throughout Africa, Asia, the Middle East, Mediterranean Basin, Eastern Europe, the Caribbean, Central America and South America. In the United States, only 100 – 140 brucellosis cases are reported annually, including 2 – 3 cases from NYC.

Brucellae are small, slow-growing Gram negative coccobacilli that typically infect livestock (goats, sheep, cattle, swine). Most exposures occur in countries where brucellosis is endemic, with consumption of unpasteurized milk or milk products being the most common risk factor. Infection in NYC and elsewhere also has occurred following consumption of cheese that originated from another country (e.g., soft cheese from Mexico). Less commonly, brucellae are inhaled in farm settings or inoculated into cuts and other skin breaks after direct contact with blood or body fluids when an infected animal is slaughtered (e.g., feral pig). The primary human pathogens are *B. melitensis*, *B. abortus* and *B. suis*.

After an incubation period that ranges from weeks to months, predominant symptoms are fever (intermittent and/or undulating), profuse sweating (often described as malodorous), fatigue, headache, weight loss and

arthralgia. Cytopenias, hepatitis, lymphadenopathy, hepatomegaly and splenomegaly are common. Combination antibiotic therapy for weeks to months is required, and infection is prone to relapse.

Brucellosis is the most commonly reported laboratory-acquired bacterial infection, which is likely due to the low infectious dose of *Brucella* spp. and the ease by which brucellae can be aerosolized in laboratory settings and inhaled or ingested (e.g., mouth pipetting or sprays to the face) by unprotected personnel. CDC recommends that laboratories use procedures that minimize splashes or aerosols of unidentified isolates, that culture plate sniffing should be prohibited, and that slow-growing Gram negative and Gram variable organisms should be handled in a biological safety cabinet. (See <http://www.cdc.gov/brucellosis/laboratories/index.html>)

Bacterial isolation is the most reliable way to diagnose brucellosis. In addition to blood, *Brucella* spp. can be isolated from bone marrow, joint fluid, CSF, splenic/hepatic abscesses, and purulent discharge. Of note, more than 72 hours of growth is typically needed to detect *Brucella* in automated blood culture systems. Clinicians must notify their microbiology laboratory that brucellosis is suspected so that cultures can be incubated for at least 10 days and biosafety measures taken to prevent laboratory exposures.

Incident 1

A male patient in his 40s presented to a NYC hospital with debilitating neck pain. He had been living in Africa for 6 – 9 months. On presentation, he was afebrile, though he developed fever, chills and sweats after he arrived at the emergency department (ED). His CBC was normal, and transaminases were not tested. Two days after admission, a consultant elicited a history of recurrent fever, weight loss, and anorexia while he was in Africa. He ultimately was diagnosed with extensive cervical prevertebral and retropharyngeal phlegmons.

After 4 days of incubation, 2 sets of blood cultures were flagged as positive by the instrument. Blood culture bottles were vented and Gram stained in a biological safety cabinet, and small, Gram negative coccobacilli were observed. Colony growth on subculture was apparent after roughly 24 hours of incubation. The following laboratory work was done on an open bench: subculturing; catalase test; a qualitative method for identifying *Neisseria* and *Haemophilus* spp. that requires inoculation with standardized suspensions of bacteria; and spotting bacterial growth on a steel plate for analysis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

MALDI-TOF MS could not identify the organism, because *Brucella* is not included in the instrument's reference database. Approximately 1 week after the blood cultures initially demonstrated growth, the isolate was sent to an outside laboratory for additional testing which identified a presumptive *Brucella* sp. pathogen of voles and sea mammals, and the NYC Health Department was notified. The patient was re-interviewed when this information was received, and he reported weekly consumption of unpasteurized cow, goat, and camel milk in Africa. The clinical isolate was sent to CDC and confirmed as *B. melitensis*.

A laboratory risk assessment was conducted. (For information about assessing laboratory risk after exposure to *Brucella* spp., see <http://www.cdc.gov/brucellosis/laboratories/risk-level.html>) A total of 35 persons were classified as having had either high or low risk exposures in the microbiology laboratory during the week that laboratorians worked with the unidentified isolate on an open bench.

Incident 2

A patient in his 20s presented to a NYC hospital with a 2-month history of recurrent fever, dry cough, night sweats, weight loss, myalgia, arthralgia, and fatigue. He had returned recently from a stay of 2-3 months in the Middle East. On evaluation, he had fever (T 102 °F), anemia, and elevated transaminases. Chest x-ray was negative; chest CT showed hepatosplenomegaly.

The initial concern was that the patient might have tuberculosis, but this was ruled out. After 5 days, 2 sets of blood cultures that were collected on admission were flagged as positive for microbial growth. All

microbiology laboratory work, including blood culture bottle venting, Gram staining, and vortexing, was conducted on an open laboratory bench. The blood culture Gram stains were read as tiny, Gram positive cocci in pairs and chains. After subculture and incubation for 2 days, colony growth of tiny, Gram negative cocci was observed. An automated microbial identification system identified a presumptive *B. melitensis* the following day, and the NYC Health Department was contacted. A MALDI-TOF MS instrument undergoing validation at the facility also failed to identify the isolate, because *Brucella* is not in the instrument's reference database.

The patient was re-interviewed by clinical staff, and he reported drinking unpasteurized sheep milk during his time in the Middle East. The NYC Public Health Laboratory confirmed the isolate as *B. melitensis*.

A laboratory risk assessment was conducted by the hospital's infectious disease department and infection control program. Because multiple potential aerosol-generating procedures and other manipulations of *Brucella* cultures were performed on an open laboratory bench, 18 people were classified as having had either high or low risk exposures.

Prevention of laboratory exposures to *Brucella* and other potentially hazardous organisms

Incidents involving unrecognized biological hazards can be prevented. These two incidents highlight aspects of clinical and laboratory practice requiring greater attention.

First, brucellosis was not suspected or considered as a potential diagnosis until blood culture isolates were presumptively identified by the laboratory. Both incidents involved patients with recurrent fever, travel to countries where zoonoses are common, and with histories and findings consistent with brucellosis. If the diagnosis had been considered, risk exposures for the disease could have been readily identified and the microbiology laboratories alerted that brucellosis was a suspected diagnosis. **Clinicians should always obtain travel histories and query for risk exposures when patients present with fever and infectious disease symptoms and should share the information with their microbiology laboratory when it may contribute to laboratory diagnosis. If brucellosis or infection from another highly infectious agent is suspected, inform the clinical laboratory so that laboratorians can take special precautions to prevent laboratory exposures.**

Second, work with clinical specimens and slow-growing, small Gram negative organisms occurred on an open bench, including procedures that could aerosolize brucellae. **Microbiology laboratories are advised to work with clinical specimens (body fluids, blood, tissues, etc.) and suspect clinical isolates in a biological safety cabinet until highly infectious agents have been ruled out. Laboratories are advised to incorporate controls that prevent work with slow-growing Gram negative isolates from being done on open benches unless they have been identified as organisms that can be handled safely in a BSL-2 environment.** Similarly, if meningococcus is suspected after Gram stain, all further manipulations of the isolate should be conducted in a biological safety cabinet.

In both incidents, blood cultures required at least 4 days of incubation before growth was detected. This was an important clue that a potentially hazardous isolate (e.g., *Francisella* or *Brucella*) was present; that it needed to be handled safely within a biological safety cabinet rather than on an open bench; that appropriate personal protective equipment was indicated for laboratorians working with the unknown isolate; and that the patient's history, travel and risk exposures should be reviewed with the clinical team. *Brucella* spp. are commonly mistaken for small cocci and may retain crystal violet stain in blood culture smears, appearing Gram positive. **If slow-growing blood culture smears suggest the presence of small, Gram positive cocci or Gram variable or Gram negative organisms, laboratorians are asked to continue all subsequent work in a biological safety cabinet and to contact the NYC Health Department for referral when *Brucella* spp. and other potential biological threat agents (BTAs) cannot be ruled out.**

Finally, automated identification systems and mass spectrometry were used when referral to the NYC Public Health Laboratory was indicated. As above, diagnosis would have been more rapid and laboratory exposures prevented if the microbiology laboratories had contacted the NYC Health Department and referred the unknown isolates to the NYC Public Health Laboratory instead of attempting identification with automated systems or mass spectrometry. Laboratories are asked to review the American Society of Microbiology (ASM) protocols for ruling out and referring potential BTAs including *Bacillus anthracis*, *Brucella* spp. and *Francisella tularensis*. Detailed sentinel level laboratory protocols are available on the ASM website (<https://www.asm.org/index.php/guidelines/sentinel-guidelines>). BTA bench cards for sentinel laboratories that summarize steps to recognize, rule out and refer potential BTAs also are available through the Association of Public Health Laboratories. (<http://www.aphl.org/aphlprograms/preparedness-and-response/documents/aphl-sentinel-laboratory-biothreat-bench-cards.pdf>)

Microbiology laboratories are cautioned that automated systems commonly misidentify brucellae as *Moraxella* spp., *Micrococcus* spp., *Corynebacterium* spp., “slow growing” *Staphylococcus* spp., *Oligella ureolytica*, *Bordetella bronchiseptica*, *Haemophilus* spp., or *Pasteurella* spp.

MALDI-TOF MS was used unsuccessfully in both institutions to identify the isolate. This technology is emerging as a promising rapid diagnostic tool in microbiology laboratories. However, the instruments used by both hospital laboratories did not include organisms that are considered select agents, including *Brucella*, in the reference database. When this technology is being utilized, it is essential for laboratories to understand the database and software limitations and to employ the above ASM protocols to recognize and refer potential BTAs to the NYC Public Health Laboratory.

These two recent incidents exposed a large number of laboratorians to *B. melitensis*, requiring many of them to take weeks of antibiotic post-exposure prophylaxis to prevent brucellosis and months of serologic surveillance and fever/symptom checks. We ask clinicians and laboratories implement processes and procedures that address potential gaps in clinical assessment and laboratory biosafety.

Sincerely,

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