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BUREAU OF PUBLIC HEALTH LABORATORIES

The Florida Lab Link

"A Biological and Chemical Preparedness Newsletter"

Proficiency Testing Event Involving *Brucella abortus* RB51 Exposures

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On February 14, 2017, the American Association of Bioanalysts (AAB) sent out a bacteriology program proficiency test (PT) panel to 138 participating laboratories nationwide. This PT panel consisted of five samples containing various bacterial organisms for identification.

The microbiology section at the Bureau of Public Health Laboratories (BPHL) in Jacksonville received the AAB PT on February 16, and a senior microbiology technologist began to work on the panel. Instructions included general safety precautions, but did not state that the samples should be handled in a biological safety cabinet (BSC). The microbiologist worked with the samples on the open bench for several days and isolated two organisms from sample number 5; one identified as *Listeria monocytogenes*, and the other described as a Gram negative, fastidious organism that was hard to separate in pure culture.

On February 24, 2019, 16S rDNA Sanger sequencing was performed on the Gram-negative organism. Results indicated a 99% homology to *Brucella suis.* Although 16S rRNA gene sequencing can produce accurate genus-level identification for *Brucella*,[1] it does not accurately identify to the species level. Due to the sequencing result, the organism was referred to the biodefense section for analysis using Laboratory Response Network (LRN)

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protocols. The LRN real-time PCR was performed and reported that same day and corroborated the sequencing results as a *Brucella* species. A list of potential organisms that could be identified from this PT sample was provided by AAB with their PT instructions; however, *Brucella* was not on this list and was an unexpected finding.

Due to the potential biosafety risk with handling *Brucella* species on the open bench, AAB was immediately contacted and asked to provide the identification of the Gram negative organism: it was *Brucella abortus* strain RB51.

Brucella abortus RB51 is an attenuated strain most commonly used as a live vaccine in veterinary medicine. Since this strain is less potent than a wild-type *B. abortus* it is not considered a severe threat to public health and safety and it is exempt from the legal reporting requirements of the Federal Select Agent Program. However, it is not avirulent and therefore does pose an exposure risk when not handled appropriately using biosafety level 3 (BSL-3) practices in a certified biological safety cabinet (BSC).

Immediately following the identification of the *B. abortus* RB51 isolate in the AAB PT, the Florida Department of Health (FDOH) Bureau of Epidemiology, Centers for Disease Control and Prevention (CDC) Bacterial Special Pathogens Branch, and the Association of Public Health Laboratories' (APHL) Public Health Preparedness and Response Program were contacted by our BPHL CLIA laboratory director to coordinate a national response. Actions taken on February 24, 2019, were as follows:

1. AAB provided a complete list of laboratories that had received the PT to APHL and CDC;
2. CDC notified and distributed an exposure risk assessment and guidance to state epidemiologists and state public health veterinarians;
3. APHL informed state and local public health laboratory directors which lab in their states had received the PT to enable them to provide appropriate guidance;
4. APHL shared the information with the Council of State and Territorial Epidemiologists, the Association of State and Territorial Health Officials, and the National Association of County and City Health Officials.

CDC, APHL and AAB instructed all laboratories to destroy the PT samples and subcultures. Standard laboratory disinfection with 10% bleach was also recommended.

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Proficiency Testing Event Involving *Brucella abortus* RB51 Exposures (continued)

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The CDC risk assessment guidance for determining potential *Brucella* exposure was provided to all laboratories that received the PT. The guidance can be found at https://www.cdc.gov/brucellosis/laboratories/risk-level.html. Symptom monitoring for both high-risk and low-risk exposures was recommended for 24 weeks' post-exposure. Symptom monitoring is especially important for *B. abortus* RB51 due to the lack of serological tests available to identify seroconversion. CDC provided recommendations regarding a 21-day course of antibiotic post-exposure prophylaxis (PEP). For a wild-type *Brucella* exposure, doxycycline and rifampin are recommended for PEP. However, *B. abortus* RB51 is resistant to rifampin and therefore another suitable antimicrobial must be used in addition to the doxycycline.

According to AAB, 15 laboratories in Florida were scheduled to receive the PT. Ten had not received the PT yet or had not started working with the samples. Of the five laboratories that had worked on the sample and had exposures, BPHL-Jacksonville had the most extensive number of exposures with 3 high risk and 11 low risk and the remaining four laboratories had low risk exposures only; two in BPHL-Miami, one each in three sentinel clinical laboratories, and two in a single sentinel clinical laboratory. The Bureau of Epidemiology worked closely with each laboratory to ensure PEP was available based on the CDC risk assessment. PEP is recommended for all personnel with a high-risk exposure, and should be considered for pregnant or immunocompromised personnel with a low-risk exposure.

To date, no known human brucellosis has been reported in association with this AAB PT. However, brucellosis caused by vaccine strains, such as *B. abortus* strain 19*, B. abortus* strain RB51 *and B. melitensis* Rev-1, has been reported as an occupational disease for people who work with livestock. In 1997, a farmer, four veterinary clinicians, and four veterinary students were exposed to infected tissue from a stillborn calf whose mother had been vaccinated with *B. abortus* RB51. All received PEP and none developed brucellosis.[2]

A report in 2004 describes 26 individuals with accidental exposure to the RB51 vaccine.[3] At least one systemic symptom was reported by 19 of the 26 individuals and *B. abortus* strain RB51 was isolated from the wound of an individual who required surgery. In 2007, numerous laboratory exposures to *B. abortus* strain RB51 occurred due to inadequate biosafety precautions when manipulating specimens included in a College of American Pathologists Laboratory Preparedness Survey.[4]

The survey was used to assess bioterrorism preparedness capabilities of sentinel clinical laboratories. A total of 916 exposed personnel was identified in 254 laboratories nationwide. Following medical intervention, no cases of brucellosis related to these exposures has been reported. In 2017, a patient in Texas was

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diagnosed with brucellosis caused by *B. abortus* strain RB51. This was the first reported case of infection acquired through the consumption of raw, unpasteurized cow's milk from an animal inoculated with the RB51 vaccine.[5]

In the most recent reports from January 2019, the New York State Department of Health and Pennsylvania Department of Health describe human cases of brucellosis caused by consuming raw milk from a farm in Pennsylvania.[6] This represented the third known case of *B. abortus* RB51 brucellosis due to raw milk acquired in the United States since August 2017. The epidemiological link was confirmed when milk samples from the farm tested positive for *B. abortus* RB51. A cow that also tested positive for RB51 was removed from the milking herd. Potential exposures associated with this single Pennsylvania farm have now been identified in persons from 19 states.


Third Case of Rifampin/Penicillin-Resistant Strain of RB51 *Brucella* from Consuming Raw Milk

HAN HEALTH ALERT NETWORK

Distributed via the CDC Health Alert Network
January 23, 2019 1430 ET (2:30 PM ET)
CDCHAN-00417

This is an official CDC HEALTH ADVISORY

In Florida, *Brucella suis* is endemic in the feral pig population, and thus the organism is periodically isolated from hunters. The potential for exposure to *Brucella* in the clinical laboratory is now exacerbated due to these vaccine-related strains from the consumption of raw milk from vaccinated cattle or the inadvertent inclusion in PT samples. Therefore, microbiologists need to remain more vigilant when working on specimens. *Brucella* species are most often isolated from whole blood and bone marrow. However, the organism may also be isolated from a wide variety of body tissues, such as spleen, liver, placenta, joint fluid, cerebrospinal fluid, abscess exudate, semen, pulmonary excretions, and occasionally urine.

Clinical diagnostic laboratories should follow the American Society for Microbiology Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases.[7] *Brucella spp.* cannot be ruled out if an isolate exhibits the following characteristics: dysgonic (slow-growing) aerobic growth; nonmotile Gram-negative coccobacillus, 0.5 to 0.7 µm by 0.6 to 1.5 µm; 48 to 72 hours to the appearance of individual colonies, particularly from clinical specimens; growth on most laboratory media but no growth on MacConkey or eosin methylene blue agar (may be pinpoint at 7 days); small, convex, glistening colonies with an entire edge; non-hemolytic and non-pigmented; catalase positive; does not satellite around *Staphylococcus*

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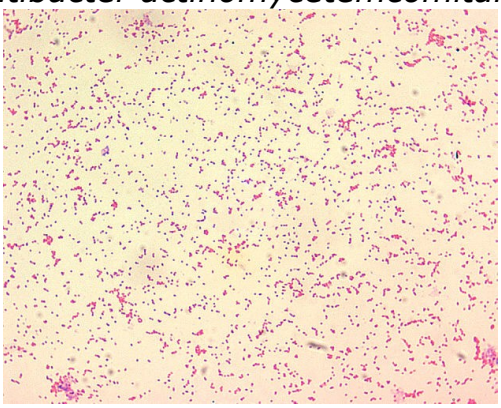
aureus; oxidase positive; and urease positive.
The isolate should be referred directly to an LRN reference laboratory for rapid real-time PCR analysis and species confirmation testing. *Brucella* spp. are LRN priority organisms, and all LRN reference laboratories have the capability of rapidly identifying *Brucella* spp. to the genus level by real-time PCR within a few hours of receiving an isolate.

Other urea-positive, oxidase-positive, Gram-negative coccobacilli that are likely to be encountered in clinical laboratories include:

- ·Oxidase-producing strains of *Aggregatibacter actinomycetemcomitans*
- ·*Bordetella bronchiseptica*
- ·*Bordetella hinzii*
- ·*Cupriavidus pauculus*
- ·*Haemophilus influenzae*
- ·*Oligella ureolytica*
- ·*Paracoccus yeei*
- ·*Psychrobacter immobilis*
- ·*Psychrobacter phenylpyruvicus*.



Brucella spp.

Automated identification systems often do not accurately identify *Brucella* species and also have the potential to generate aerosols of the infectious organism. Many automated systems use phenotypic methods to generate biochemical profiles and slow-growing organisms like *Brucella* tend to produce low-confidence results or are misidentified because insufficient enzymatic activity occurs during the limited incubation times, and the databases for these systems may contain few isolates for comparison and identification.[8]

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-ToF) mass spectrometry, can also misidentify *Brucella* spp. and present an exposure potential during sample preparation.[9] In addition, *Brucella* species are occasionally misidentified since they do not always decolorize on Gram stain and can appear as Gram positive or Gram variable.

Creating a culture of safety in clinical and public health laboratories is essential. Biosafety improvements require leadership support and recognition of biosafety as a priority. At a minimum, laboratories should maintain a current biosafety manual,

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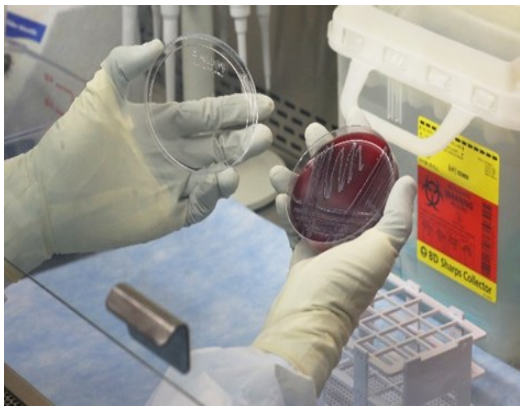
review the safety program and provide biosafety training annually, and perform risk assessments on all new procedures or amendments to existing protocols.

CDC recommendations for safe laboratory practices when working with *Brucella* spp. to prevent exposures and laboratory-associated infections[10]



- ⇒ when brucellosis is suspected, clinicians should note "suspect (or "rule out") brucellosis" on the laboratory submission form.
- ⇒ Review laboratory containment methods and microbiologic procedures to ensure compliance with recommendations of the 5th edition of the CDC's Biosafety in Microbiological and Biomedical Laboratories.[11]
- ⇒ Use primary barriers (safety centrifuge cups, PPE, and class II or higher BSCs) for procedures with a high likelihood of producing droplet splashes or aerosols.
- ⇒ Use secondary barriers; restrict access to the lab when work is being performed.
- ⇒ Maintain the integrity of the laboratory's air-handling system by keeping external doors and windows closed.
- ⇒ Perform all procedures on unidentified isolates carefully to minimize the creation of splashes or aerosols.
- ⇒ Prohibit sniffing of opened culture plates to assist in the identification of isolates.

This article is a summary of our recent publication. For the full details see:

Rowlinson MC, Lee PA. 2019. *Brucella abortus* RB51 strain sent in a proficiency testing panel to clinical and public health laboratories. Clin Microbiol Newsl 41 (3):23-32.

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Sentinel Laboratory Guidelines Updated—Note New Link

The American Society for Microbiology (ASM) website containing the Sentinel Level Clinical Protocols has moved. The updated protocols designed to offer Laboratory Response Network (LRN) Sentinel Level Clinical Laboratories standardized, practical methods and techniques to rule out microorganisms suspected as agents of bioterrorism, or to refer specimens to public health laboratories for confirmation can be found at: https://www.asm.org/Articles/Policy/Laboratory-Response-Network-LRN-Sentinel-Level-C.

(Please note that the ASM website has been recently redesigned. If you keep a link to the Sentinel Guidelines on your personal or lab computer, you will need to update your bookmark to the link above.)

A Change of Hats for Biosafety Outreach Officer

After three and half years, the grant funding that BPHL (the Bureau of Public Health Laboratories) received from CDC through the Epidemiology Laboratory Capacity grant for biosafety outreach has ended, and the BOO (biosafety outreach officer), Ed Kopp, has moved to a different role in the BPHL Tampa lab. While there will not be money available for travel to provide biosafety training at a clinical lab's site, you can still expect to get a healthy dose of biosafety in BPHL's Biological Defense LRN (Laboratory Response Network) Training provided at clinical lab sites (see page 9 for more information). In addition, while Ed's primary duties have shifted, you are welcome to reach out to him via his existing contact information, and he will try to answer any laboratory biosafety questions you may have and provide you with resources and assistance with biosafety risk assessment. You can find his contact information at http://www.floridahealth.gov/programs-and-services/public-health-laboratories/biosafety/ along with BPHL's biosafety risk assessment SOP and related tools as well as a selection of especially useful biosafety resources.

BT Coordinators representing each of Florida's BPHL branch locations accept the Excellence in Public Health Response award at the 2018 LRN National Meeting in Atlanta. Pictured with plaques, left to right: Justin Hubsmith (BPHL-Tampa), Stephen White (BPHL-Miami), and Phil Lee (BPHL-Jacksonville). Florida's public health labs were selected to receive the award due to their coordinated response to a series of white powder letters as well as Zika virus testing.

Chemical Threat (CT) Preparedness Training

The CT laboratory coordinators continue to reach out to the health and medical community by offering training for CT preparedness at hospitals and county health departments (CHDs). This training covers chemical terrorism awareness and the collection of clinical specimens after a chemical terrorism event. Hospital and CHD staff play an important role in the response to a chemical exposure event when clinical specimens are collected for analysis. For your convenience and to increase participation, this training can be presented at your facility. Each course lasts approximately one hour with one 15-minute break between courses. Training manuals, "hands-on" exercise materials, and CT preparedness kits will be provided. This training is recommended for physicians, nurses, epidemiologists, emergency department personnel, phlebotomists, hospital and health department laboratory personnel and others who may collect clinical specimens. Contact the CT laboratory coordinators in your region for more information (see the Bureau of Public Health Laboratories Directory for contact information).

Laboratory Response Network (LRN) Training—Biological Defense

The Bureau of Public Health Laboratories is currently offering an LRN sentinel laboratory training course at no cost to your facility. This training follows the American Society for Microbiology (ASM) Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases. Scheduling the training at your facility is a relatively easy process. Determine when you would like to have the training and how many people will be attending. A time will be set up that is convenient for all. The training materials are provided as well as the biodefense reference manuals for your laboratory.

The training syllabus includes: an overview of the LRN; biosafety risk assessment and biosafety for the clinical laboratory; the ASM protocols for ruling out potential bioterrorism agents and how to refer a sample to the state LRN Public Health Reference Laboratory when a bioterrorism agent cannot be ruled out; and an introduction to the CDC Select Agent Program.

Please contact Betty Wheeler at 904-791-1568 (Betty.Wheeler@FLhealth.gov) to schedule a class for your facility.

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