



## Short Communication

## A Case-Study of Implementation of Improved Strategies for Prevention of Laboratory-acquired Brucellosis



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## ABSTRACT

**Background:** In 2012, the Alaska Section of Epidemiology investigated personnel potentially exposed to a *Brucella suis* isolate as it transited through three laboratories.

**Methods:** We summarize the first implementation of the United States Centers for Disease Control and Prevention 2013 revised recommendations for monitoring such exposures: (1) risk classification; (2) antimicrobial postexposure prophylaxis; (3) serologic monitoring; and (4) symptom surveillance.

**Results:** Over 30 people were assessed for exposure and subsequently monitored for development of illness. No cases of laboratory-associated brucellosis occurred. Changes were made to gaps in laboratory biosafety practices that had been identified in the investigation.

**Conclusion:** Achieving full compliance for the precise schedule of serologic monitoring was challenging and resource intensive for the laboratory performing testing. More refined exposure assessments could inform decision making for follow-up to maximize likelihood of detecting persons at risk while not overtaxing resources.

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## 1. Introduction

Laboratory-acquired brucellosis (LAB) cases have been previously reported in the United States. Anecdotally, clinical labs periodically misidentify or fail to anticipate the presence of *Brucella* spp., which creates a risk of episodic exposure at both clinical and reference laboratories. *Brucellae* are Gram-negative intracellular coccobacilli. There are several different species of *Brucella*, which have different host specificities and clinical manifestations. *Brucellae* are facultative and grow slowly, which sometimes contributes to their misidentification. Brucellosis can be a very debilitating and serious disease with symptoms that range in severity from fevers and body aches to arthritis and endocarditis. Because symptoms can be vague, brucellosis may not be immediately diagnosed and people can progress toward chronic brucellosis.

In 2012, an isolate collected from a blood culture taken on February 8, 2012, was presumptively identified as *Haemophilus* spp. at Laboratory A (an Alaska hospital laboratory) and was forwarded to Laboratory B (Arctic Investigations Program, a referral laboratory) for confirmation. Laboratory A uses a Siemens Microscan (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA) and Laboratory B uses culture-based methods for bacterial identification. On March 6, 2012, the Alaska Section of Epidemiology (SOE) received a call from Laboratory B that the isolate was suspected as *Brucella* spp. Subsequently, *Brucella suis* was confirmed by Laboratory C [Alaska State Public Health Laboratory (ASPHL)] on March 9, 2012. Alaska records an average of one case of brucellosis biennially. Locally-acquired cases are *B. suis* biovar 4 and historically have been associated with contact to infected reindeer or caribou carcasses [1].

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The blood specimen was drawn from an adult male patient in rural Alaska who had presented several times to a regional hospital emergency department (ED) for non-specific systemic illness of 6–8 weeks' duration. History and specific exposures elicited during ED visits were unremarkable. Following diagnosis and more extensive interviews, no further discrete exposures were revealed. Brucellosis typically results from contact with infected animals or animal products contaminated with the bacteria, with the most commonly infected animals including sheep, cattle, goats, pigs, and dogs [2]. Although patient occupation did not put him in close contact with animals, he lived in a rural area where hunting and a subsistence lifestyle are common. Therefore, patient exposure was presumed to be zoonotic.

Secondary exposures to, and ultimately disease from, *Brucella* spp. in a laboratory setting have been published [3–9], although no exposures were specific to *B. suis* biovar 4. In 2012, the United States Centers for Disease Control and Prevention (CDC) had begun revising recommendations for monitoring such exposures, including a change to the schedule for serial serologies, and published updated prevention strategies to prevent LAB, including: (1) risk classification; (2) antimicrobial postexposure prophylaxis (PEP); (3) serologic monitoring; and (4) symptom surveillance [10]. In this brief report, we describe implementation of these strategies to manage a laboratory exposure to an isolate of *B. suis* in Alaska.

## 2. Materials and methods

We implemented steps published in Traxler et al [10] as follows.

### 2.1. Risk classification

Alaska SOE staff consulted with the CDC *Brucella* spp. subject matter experts to develop a plan for assessing exposures of any affected laboratory workers and implementing a health monitoring schedule. Staff at each of the three laboratories were administered a questionnaire to assess specific practices performed with the specimen/isolate and the presence of appropriate personal protective equipment (PPE) or engineering controls in place during execution of those practices. Examples of risk classification criteria included assessing whether isolates were manipulated on an open bench without using the appropriate level of biosafety precautions and whether other staff were within a 1.5 meters radius of someone who was performing those activities [10]. Staff that had been present in the laboratories were then classified as either “High” or

“Low” risk based on their participation in the work-up of the specimen/isolate or proximity to the work [10]. If specimen handling occurred with adequate PPE in the biological safety cabinet (BSC) as required [11], exposure was classified as “None”. Additionally, laboratory practices at each facility were evaluated to ensure that future exposures could be minimized.

### 2.2. Antimicrobial PEP

Persons identified as being at high risk were recommended to receive the standard dosages of doxycycline (100 mg) twice daily and rifampin (600 mg) once daily for 3 weeks [10].

### 2.3. Serologic monitoring

Baseline serum samples as well as follow-up serological testing from blood collected at 6 weeks, 12 weeks, 18 weeks, and 24 weeks after last known exposure was recommended. ASPHL evaluated serum samples using the *Brucella* spp. micro-agglutination test (BMAT). In general, testing was batched for efficiency, although time for a single test may take a minimum of 1–2 days.

### 2.4. Symptom surveillance

Laboratory personnel identified through the investigation process were monitored for development of symptoms and referred to care and/or testing as appropriate.

## 3. Results

### 3.1. Risk classification

During the > 4 weeks from specimen collection to final confirmation of the isolate, a total of 32 people were identified who had either worked with the specimen or isolate or had been in the vicinity of the work, with most being associated with Laboratory B (Table 1).

### 3.2. Antimicrobial PEP

Six microbiologists who directly participated in the testing of the specimen or were in the immediate vicinity were identified as “High” risk and were recommended to receive the standard prophylaxis. Because of the delay in final confirmation and the

**Table 1**  
Summary of serologic testing and postexposure monitoring for persons exposed to a *Brucella* spp. isolate at three laboratories, stratified by risk

Laboratory	No. of persons assessed	Risk categories, n (%)	Testing* intervals from exposure (wk)	Postexposure monitoring
A	4	High: 2 (50) Low: 2 (50)	2 staff: B <sup>†</sup> , 8, 12, 20, 26 1 staff: B <sup>†</sup> , 12, 26 1 staff: B <sup>†</sup> , 8, 12	Doxycycline/rifampin postexposure antibiotics Fever watch Fever watch
B	27	High: 4 (15) Low: 23 (85)	1 staff: O <sup>‡</sup> , 2, 6, 12, 20 3 staff: O <sup>‡</sup> , 6, 12, 20 13 staff: O <sup>‡</sup> , 6, 12, 20 9 staff: O <sup>‡</sup> , 6, 12 1 staff: O <sup>‡</sup>	Doxycycline/rifampin postexposure antibiotics Fever watch Fever watch
C	1	None <sup>§</sup>	—	Fever watch

\* Testing via the BMAT (*Brucella* spp. micro-agglutination test).

† The first follow-up blood draw for persons working in Laboratory A was in early March, ~1 month after the specimen had been manipulated by microbiologists. Therefore, the baseline or Day 0 interval listed here as 1 is actually 4 weeks from exposure. The other intervals were calculated based on the exact interval from exposure.

‡ Because the specimen had originally arrived in Laboratory B ~3–4 weeks prior to suspicion of *Brucella* spp. identification, the timing intervals for some of the exposed people may actually indicate an additional 3–4 weeks from exposure.

§ At Laboratory C, the isolate had been handled in a biological safety cabinet from the outset.  
B, baseline.

potential for repeated exposures at Laboratory B, the exact time from exposure to initiation of prophylaxis could not be calculated for every person, but ranged from 3 days to 31 days. Aside from the microbiologist mentioned below, the remaining three high-risk persons completed their courses of prophylaxis.

### 3.3. Serologic monitoring

Of the total number of exposed staff members from Laboratories A and B, only 10% (3/31) completed five serial serologic tests, although the specified timing of the blood draws was not followed. Those three staff represented 100% (2/2) of people with high-risk exposures at Laboratory A and 25% (1/4) of high-risk exposures at Laboratory B. In the low-risk groups, 52% of staff (13/25) completed four tests and 40% (10/25) completed three. Overall, 65% (20/31) of staff had serology drawn more than 12 weeks' postexposure.

### 3.4. Symptom surveillance

At Laboratory B, one high-risk microbiologist reported a fever 3 weeks after exposure and was immediately referred for medical evaluation. Baseline titers had been negative. Polymerase chain reaction (PCR) testing of a blood specimen at the time of symptoms was also negative for brucellosis. This person was taking antibiotic prophylaxis at the time of fever onset, however, these antibiotics were discontinued shortly thereafter due to side effects and because additional broad-spectrum antibiotics were prescribed from the ED.

Following the incident, several changes were implemented at both laboratories. At Laboratory A, Gram-negative specimens with uncertain identification were recommended for work-up in a BSC. At Laboratory B, all *Haemophilus influenzae* surveillance cultures were recommended for work-up inside a BSC until confirmation was complete. Additionally, a room previously designated for storage was repurposed into a central receiving area for specimens in order to eliminate unnecessary traffic in the laboratory. A sign-in log was instituted for maintenance workers in order to track entrance into the laboratory area.

## 4. Discussion

The initial identification of the *B. suis* isolate as *H. influenzae* has been previously documented because of similarities between the two genera [11–13]. Similarities include the fact that both bacteria are slow-growing, tiny Gram-negative organisms, however, *Brucellae* grow on blood agar and chocolate agar, while *H. influenzae*, only grow on chocolate agar. Other instances where laboratory exposure occurred have involved misidentification of *Brucellae* as *Psychrobacter* spp. or *Moraxella* spp. [4,7–10]. This potential for misidentification was incorporated into the revised practices implemented at both Laboratories A and B. As mentioned, Laboratory A used a Microscan machine (Siemens, Tarrytown, NY, USA) for bacterial identification and Laboratory B used culture-based methods, specifically growth on blood agar plates, Gram stain consistent with *Brucellae*, and positive reactions to both urease and oxidase. Laboratory C used a combination of PCR and biochemical testing consistent with Laboratory Response Network procedures at the time of testing.

In this incident, none of the 31 potentially exposed persons had a detectable titer at the start of the monitoring period and no seroconversion was documented among those who had serial serologic testing. For Laboratory A staff, the baseline serum draw done in March was actually 3–4 weeks after the isolate had been referred to Laboratory B. Half of the high-risk people had the recommended five serial serologic tests, although the precise schedule

was not followed. Most of the people in the low-risk group had at least four tests. Only 65% had serology drawn after 12 weeks' postexposure.

Factors influencing compliance with the timing of serial draws were not formally assessed. Anecdotally, people reported a perception of low risk for the exposure and that the timing of serial draws was inconvenient. The guidelines and schedules were communicated to affected persons at the first blood draw, however, no specific constraints or conditions of continued work were made to compel completion.

Compliance with PEP appeared to be good. Other studies have noted that poor compliance is often related to occurrence of side effects [5] or that people may refuse PEP based on low perception of risk when they are healthy [12,14].

The CDC schedule for serial serological testing of samples was revised for a few reasons. The new schedule is more evenly spread over 24 weeks to enable prompt identification of infections that occur after 6-weeks postexposure (the previous schedule was 0 weeks, 2 weeks, 4 weeks, 6 weeks, and 24 weeks) [6]. Also, the spread may improve mindfulness of laboratory workers and physicians that brucellosis has a long incubation period, which might reduce the number of additional exposures associated with a laboratory-acquired case. Traxler et al [8] found that 21% of laboratory-acquired brucellosis had onset > 12 weeks after exposure [8]. Finally, one laboratory worker developed brucellosis after completing a full course of antimicrobial PEP [10]. Although the reason for PEP failure is unclear, a multipronged approach for disease prevention is indicated.

The premise behind the serial blood draws is to presage symptoms of acute infection to allow for rapid institution of treatment. One study demonstrated detection of a positive titer 2–5 days before symptom onset [3]. Additionally, persons starting therapy based on titers before the start of symptoms had milder symptoms and a shorter illness duration [3]. The timing of symptom onset has varied widely from 1–40 weeks' postexposure [8], therefore, developing the optimal schedule to maximize likelihood of early detection of infection without draining resources is challenging. Further challenges may occur if testing is batched at a state or commercial laboratory because the short lead time from seroconversion to symptom onset may be negated. Knudsen et al [12] suggested revising the schedule to eliminate draws at 6 weeks and 24 weeks and focusing on symptom screening [12]. In Alaska, the adherence to a fixed schedule was somewhat artificial because the exact nature and/or timing of the exposure was unclear and the proscribed schedule did not necessarily capture true intervals after exposure.

In previous studies, although high-risk persons not taking PEP were over nine times more likely than low-risk persons to develop brucellosis, a proportion of low-risk persons still became ill [8]. In that report, exposure specifics about the low-risk persons were not available. Therefore, one suggestion to refine future exposure assessments would be to incorporate more quantitative measures. For example, the criteria for low-risk assessment could be narrowed to include a time component (e.g., present for > 30 minutes after known aerosol-generating procedures) or a larger concentric ring (e.g., distance > 3 meters from the specimen) to reduce the overall number of persons classified as "exposed". The 2013 criteria for low-risk assessment consider those persons in the laboratory > 1.5 meters from manipulation who did not directly participate in procedures that would constitute a higher risk, such as any aerosol-generating procedures. However, limited data exist from which to draw such quantitative measures and exposure to few organisms can lead to disease [15]. In the Alaska example, sufficient detail was not collected about individual exposures to assess whether quantitative measures could have reduced the number of low-risk

persons. Additionally, the species of *Brucellae* might also be considered in the decision-making for follow-up in order to maximize the likelihood of detecting persons at risk while not overtaxing resources. Admittedly relevant only to a small number of geographic locations, the lower virulence of *B. suis* biovar 4 could be factored into the aggressiveness of postexposure response in the future. Similarly, there are not enough current data to stratify LAB risk by species of *Brucellae*.

### Conflicts of interest

All authors have no conflicts of interest to declare.

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