A large exposure to \textit{Brucella melitensis} in a diagnostic laboratory

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\textbf{SUMMARY}

\textbf{Background:} \textit{Brucella} species are easily transmitted by aerosols and can be acquired in the laboratory.

\textbf{Aim:} To report the management of a large exposure to \textit{Brucella melitensis} that occurred over six days in a hospital diagnostic laboratory.

\textbf{Methods:} Fifty-one exposed staff were managed according to Centers for Disease Control and Prevention guidelines. A further 96 non-exposed laboratory staff were tested for seroprevalence. Testing was carried out using the \textit{Brucella} sp. serum agglutination test.

\textbf{Findings:} Twenty-seven people had high-risk exposure and 24 had low-risk exposure. High-risk staff were offered post-exposure prophylaxis. Twelve (44.4\%) agreed to this, of whom eight (66.7\%) completed the course. Overall compliance with serological follow-up at baseline, 2, 4, 6 weeks and 8 months was 45.9\%. Despite this poor compliance there were no clinical brucellosis cases and no seroconversion in the 47.1\% of staff tested at 8 months. \textit{Brucella} sp. seroprevalence among all staff tested was 3/147 (2.0\%).

\textbf{Conclusion:} Lack of experience with \textit{Brucella} spp. and lack of policies for handling potentially hazardous organisms contributed to this prolonged exposure. As compliance with current recommendations may be poor, the optimum frequency of serological follow-up and target groups for prophylaxis should be reassessed. Laboratories in low- or non-endemic areas must prepare for potential isolation of \textit{Brucella} spp. The impact of human brucellosis in Malaysia requires further study.

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\textbf{Introduction}

The most common worldwide bacterial zoonosis is brucellosis.\textsuperscript{1} Human brucellosis is mainly caused by \textit{Brucella melitensis}, \textit{Brucella abortus}, and \textit{Brucella suis} in people exposed to infected animals such as cows, goats, sheep and pigs. Disease in humans produces a variety of clinical manifestations in all organs.\textsuperscript{2} Although mortality is low, brucellosis may result in debilitating chronic infection.

Microbiology laboratory workers are at increased risk of brucellosis through unsuspected exposure to cultures from clinical specimens. Transmission occurs mainly via inhalation of organisms aerosolized through laboratory procedures such as
making bacterial suspensions and pipetting. As the infectious dose is low, reported attack rates from laboratory exposures are high, ranging from 30% to 100%. Brucella spp. are among the most frequently reported laboratory-acquired infections, and if these organisms are suspected they should be handled under biosafety level 3 conditions.

Centers of Disease Control and Prevention (CDC) guidelines on management of laboratory exposures to Brucella spp. advise serological follow-up and antibiotic prophylaxis, but these are based more on expert opinion than evidence. Reports of managing incidents using these guidelines are limited but important for judging their effectiveness. This paper reports our experience and difficulties in managing a large exposure to B. melitensis in a busy hospital laboratory in tropical Malaysia. Since there are few data on human brucellosis in Malaysia, a secondary aim was to determine seroprevalence in laboratory workers.

**Methods**

The University of Malaya Medical Centre is a 1000-bed teaching hospital in Kuala Lumpur. The diagnostic microbiology laboratory is a biosafety level 2 facility which processes >250,000 specimens annually. A 15-year-old female was admitted in February 2009 with fever. There was no indication on the microbiology request forms that brucellosis was suspected. Aerobic bottles from two sets of blood cultures and a bone marrow aspirate flagged positive between 25 February and 3 March, after 4 days of incubation each. No organisms were seen in direct Gram stains. Standard laboratory procedures such as inoculating plates, antimicrobial susceptibility testing and biochemical testing were carried out on an open bench in the bacteriology laboratory. Colonies were visible after 2 days of incubation and found to be Gram-negative coccobacilli. Brucella sp. was first suspected based on preliminary results on 3 March, following which all processing of the cultures was stopped. All remaining plates, bacterial suspensions and inoculated biochemical tests were sealed and autoclaved. The clinicians and public health authorities were informed, and further history was obtained from the patient that she had consumed unpasteurized cow’s milk.

16S rDNA sequencing was performed, confirming Brucella sp. (accession number JN571438) on 5 March. Species identification was carried out later using a previously published polymerase chain reaction assay, which confirmed Brucella melitensis.

All potentially exposed microbiology staff were identified for further management following CDC guidelines. Staff were divided into high- and low-risk exposure groups. Staff at high risk had handled cultures on the open bench or were within 1.5 m of such activities. Staff at low risk were present in the laboratory at the time the processing was done, but further than 1.5 m away. Post-exposure prophylaxis (PEP), comprising doxycycline 100 mg twice daily and rifampicin 600 mg daily for 3 weeks, was recommended to high-risk staff and offered to the rest.

Serological testing by serum tube agglutination test (SAT) using B. abortus and B. melitensis antigens (Plasmatec, Bridport, UK) was performed at baseline, 2, 4, 6 weeks and 8 months (instead of the CDC’s recommended 6 months). The baseline sample was taken between 4 and 9 days after potential exposure. Samples with titres ≥1:160 from patients with symptoms consistent with brucellosis were considered positive. Samples from exposed but asymptomatic staff with titres of ≥1:80 were considered suspicious and were sent to the Veterinary Research Institute in Ipoh for confirmation using Rose Bengal, Coomb’s, and complement fixation tests. The latter is the most useful test during the incubation period. Staff were briefed to report symptoms of brucellosis developing in the following 6 months. For the seroprevalence study, serum was also collected from staff from other areas of the microbiology department (N = 28) and from the chemical pathology laboratory (N = 68). All interventions were optional for the staff and informed consent was obtained.

**Results**

The main potential exposure occurred in the bacteriology laboratory between 25 February and 3 March, a 6-day period that included a weekend when there were different shifts of staff. As many as 20 staff work during the day in this laboratory, with many others entering from other parts of the department. The cultures had been handled openly on the blood culture bench, located on the right-hand side of the laboratory (Figure 1). The class II biosafety cabinet was not used and there is no directional airflow system in this laboratory. Centrally cooled air is circulated in the room via ceiling vents and there are three exhaust fans. The laboratory windows are always kept shut. There were no reported spills, episodes of catalase testing which may generate aerosols, or smelling of cultures. However, this bench is close to, although >1.5 m away from, the common sink where Gram staining is performed, and close to one of the two doors to the laboratory. The doors open directly into a common corridor and there is no interlocking door system, so this was a particularly busy part of the laboratory. The adjacent genitourinary bench was within 1.5 m. In this laboratory alone, 17 people had high-risk exposures (Table I).

There were potential exposures at several other sites. The 16S rDNA sequencing was performed in another laboratory on 3 March. However, the bacterial suspension was made in a biosafety cabinet which had not been switched on, and the suspension was boiled in a closed tube on an open bench before DNA extraction. This resulted in high-risk exposure of a further six people. Another three people in the autoclave room handled bottles containing inoculated biochemical tests prior to 3 March. Finally, a technician in the mycology laboratory had performed a Gram stain from a positive fungal blood culture bottle from the patient.

A total of 51 people were considered exposed. There were 27 people with high-risk exposure of whom 12 (44.4%) decided to take PEP (Table I). Of these, 8/12 (66.7%) completed the course. Three people who did not finish PEP had gastrointestinal side-effects, while the fourth had abnormal liver function tests. The 24 other people who worked in the bacteriology laboratory had low-risk exposures and none elected to take PEP.

Compliance with testing at baseline, 2, 4, 6 weeks and 8 months was 88/135 (65.2%) of all planned tests for the high-risk group, and 29/120 (24.1%) for the low-risk group. Only 7/51 (13.7%) were tested at 4 weeks, with many deferring testing to 6 weeks. Seven staff (13.7%), all from the low-risk group, declined any testing.
Brucella sp. SAT titres of $\geq$1:80 were seen in baseline serum of four high-risk staff. Serial samples were sent for confirmatory testing using other assays but none was diagnostic of recent Brucella sp. infection (Table II), suggesting that these were pre-existing antibodies or non-specific reactivity. For patient B, there was an apparent seroconversion in SAT titres at week 2, so PEP antibiotics were continued for a further three weeks as a full treatment course. Other test results suggested that this SAT result represented non-specific reactivity. Patient D had a baseline titre of 1:160, had a history of drinking raw milk as a child, and declined prophylaxis. Seroconversion did not occur and he remained well.

Two years after the exposure, none of the 51 exposed staff had had clinical disease consistent with brucellosis, and none of the 24 who were tested at 8 months had seroconverted. Using SAT titres of $\geq$1:160 there was no significant difference between the Brucella sp. seroprevalences of 2/79 (2.5%) among microbiology staff and 1/68 (1.5%) among chemical pathology staff ($P = 1.0$).

Discussion

Brucella sp. infection is reported in animals throughout Malaysia, but there are few data on human disease (World Organization for Animal Health. Annual animal health report on the notification of the absence or presence of all diseases, Malaysia, Jan–Dec 2010; http://web.oie.int/wahis/public.php?page=report_ann_sem&country=MYS&year=2010&semester=0&aquatic=2&WAHID=1). A recent study of 184 suspected brucellosis cases found that 10 (5.4%) had detectable IgM and/or IgG, mostly in animal workers, whereas none of 100 blood donors was seropositive. Total seroprevalence for all staff tested in our study was 3/147 (2.0%) using SAT titres of $\geq$1:160. The cutoff for a given population depends on the background endemicity, which is not known in Malaysia, but a range from 1:80 to 1:320 may be used. Seroprevalences of 4.0–5.4% are reported in endemic Brazil and Turkey. Although seroprevalence studies are not easily compared due to non-standardized assays and cut-off levels, the prevalence of human brucellosis in Malaysia requires further study, particularly in at-risk occupations such as laboratory workers.

As human brucellosis appears to be rarely encountered in cities in Malaysia, there may be a low level of suspicion among clinicians, who then do not alert the laboratory. Nor was it initially suspected based on the characteristics of the isolate: Brucella sp. was last isolated more than 20 years ago in our laboratory and there were also no standard operating procedures addressing the potential isolation of Brucella spp. This

### Table I

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Exposure</th>
<th>No. of staff</th>
<th>No. taking PEP (finished course)</th>
<th>Total compliance with testing at baseline, 2, 4, 6 weeks and 8 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Handled open cultures</td>
<td>12</td>
<td>10$^a$ (7)</td>
<td>44/60 (73.3%)</td>
</tr>
<tr>
<td></td>
<td>Within 1.5 m of open cultures</td>
<td>15</td>
<td>2$^a$ (1)</td>
<td>44/75 (58.7%)</td>
</tr>
<tr>
<td>Low</td>
<td>Worked in same laboratory &gt;1.5 m from cultures</td>
<td>24</td>
<td>0</td>
<td>29/120 (24.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td>12 (8)</td>
<td>117/255 (45.9%)</td>
</tr>
</tbody>
</table>

PEP, post-exposure prophylaxis.

$^a$ One person from each high-risk group took trimethoprim-sulfamethoxazole 160 mg/800 mg twice daily instead of doxycycline.

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Figure 1. Floor plan of the bacteriology laboratory. The Brucella spp. cultures were handled on the blood cultures bench, which is located near one of the main doors and the common sink used for Gram staining, and next to the genitourinary bench. B, blood culture analyser; BSC, class II biosafety cabinet; E, exhaust fan.
frequency and duration of follow-up needs to be determined.15
is widespread, particularly with large groups, the optimal
contention that PEP is discussed, rather than recommended
supports the use of PEP in those at greatest risk, since 10/12 of
background explains the extended period of potential exposure
as staff worked unsuspectingly on the cultures, as well as
further exposures in other parts of the department as the
cultures were not adequately contained. Most reported cases
of laboratory-acquired Brucella sp. are due to aerosols from
handling cultures on an open bench.13–17 Variability in Gram
staining or misidentification by commercial biochemical assays
may further delay recognition of Brucella spp.16,17
As a result of this incident, new SOPs were introduced.
Inoculation of all positive blood cultures for Gram stains or on
to media is now performed in a class II biosafety cabinet. All
specimens suspected of Brucella from clinical history or lab-
atory findings (such as slow-growing organisms) are now
processed in a biosafety cabinet with personal protective
equipment including gloves and coat. Safe laboratory practices
become particularly important in resource-limited settings
where biosafety level 3 infrastructure is not available.
During medical surveillance of the staff there was poor
overall compliance with the testing schedule. The rationale for
serological follow-up is to identify and treat asymptomatic
infected patients, although the benefit is not proven. In one
exposure incident, treating seroconverted asymptomatic
workers led to milder symptoms compared with those treated
for clinically apparent disease.17 Prolonged follow-up of exposed
workers is required, since the incubation period varies from 1
week to 5 months.17,18 As poor compliance with frequent testing
is widespread, particularly with large groups, the optimal
frequency and duration of follow-up needs to be determined.15
As trials cannot be carried out, support for the use of PEP
is merely anecdotal and the regime commonly causes side-
effects.15,19,20 In this report, all 51 exposed microbiology staff
were clinically well after 2 years. Experience at this institution
supports the use of PEP in those at greatest risk, since 10/12 of
the staff who handled cultures had PEP. We also support the
contention that PEP is discussed, rather than recommended
outright, for those at low risk, since none of the 24 low-risk staff
received PEP and none developed evidence of infection. No
evidence was found to support the recommendation that all
staff within 1.5 m of open Brucella sp. cultures should be
designated as having high-risk exposure, since only 2/15 in
this group received PEP and none became ill or seroconverted.
Nevertheless, with many reports of personnel being infected
despite not handling cultures, we continue to support the
recommendation that PEP should be advised for these staff.
The main limitation of this study was that only 35/51 (68.6%)
and 24/51 (47.1%) exposed staff were tested at 6 weeks and 8
months, respectively. Hence seroconversions due to mild or
asymptomatic infections, which occur occasionally, could not
be excluded.21 It was perhaps surprising that no clinical cases
resulted from this prolonged exposure of so many people to
aerosol-generating procedures previously associated with
laboratory transmission, particularly since the laboratory does
not have directional airflow, the underlying population sero-
positivity was low, and since 39/51 (76.5%) of the exposed staff
did not take prophylaxis. The reasons for the lack of trans-
mision in this study are unclear but may include a less virulent
or transmissible bacterial strain, and/or low genetic suscepti-
bility of the population. Alternatively the high rates of trans-
mision in other studies may reflect publication bias towards
confirmed outbreaks. It is also possible that the early prophy-
laxis given, particularly to the highest risk group that handled
cultures, may have averted infections. If so, it is not known
whether effective prophylaxis also blunts or aborts the anti-ody response since no seroconversion was seen in the high-risk
staff tested.
As brucellosis cases increase worldwide due to globaliza-
tion, and with the threat of deliberate release, laboratoried in
low- or non-endemic areas must prepare for the possibility of
isolating Brucella spp.14 This Brucella exposure spurred our
laboratory to improve practices and procedures for potential
isolation of hazardous organisms. In the event of laboratory
exposure to such organisms, there should be effective

<table>
<thead>
<tr>
<th>Patient</th>
<th>PEP</th>
<th>Sample date</th>
<th>Serum agglutination test</th>
<th>Rose Bengal test</th>
<th>Coomb’s test</th>
<th>Complement fixation test</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. abortus</td>
<td>B. melitensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Incomplete (2 weeks)</td>
<td>Baseline</td>
<td>1:80</td>
<td>1:80</td>
<td>Negative</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 2</td>
<td>–</td>
<td>1:80</td>
<td>Negative</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 6</td>
<td>1:40</td>
<td>1:80</td>
<td>Negative</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Month 8</td>
<td>1:40</td>
<td>&lt;1:40</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>Completed</td>
<td>Baseline</td>
<td>&lt;1:40</td>
<td>&lt;1:40</td>
<td>Negative</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 2</td>
<td>1:80</td>
<td>–</td>
<td>Negative</td>
<td>1:10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 6</td>
<td>&lt;1:40</td>
<td>&lt;1:40</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>Incomplete (1 week)</td>
<td>Baseline</td>
<td>1:80</td>
<td>1:80</td>
<td>Negative</td>
<td>1:40</td>
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<td>1:80</td>
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<td>Negative</td>
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<td>Week 4</td>
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<td>Week 6</td>
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<td>1:160</td>
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<td>–</td>
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<tr>
<td></td>
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<td>Month 8</td>
<td>1:160</td>
<td>1:160</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D</td>
<td>Declined</td>
<td>Baseline</td>
<td>1:160</td>
<td>1:160</td>
<td>Negative</td>
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<tr>
<td></td>
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<td>Week 2</td>
<td>1:160</td>
<td>1:160</td>
<td>Negative</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 4</td>
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<tr>
<td></td>
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<td>Week 6</td>
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<td>Month 8</td>
<td>1:160</td>
<td>1:80</td>
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</tbody>
</table>

PEP, post-exposure prophylaxis.
communication of risks and benefits to staff to ensure maximum compliance with PEP and testing.

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Conflict of interest statement
None declared.

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