An accidental laboratory exposure to *Brucella melitensis*: the prospective post-exposure management and a detailed investigation into the nature of the exposure

C. Wong,* S. Y. Ng and S. H. Tan

Abstract

Our aim was to prospectively manage 22 *Brucella*-exposed individuals and identify the lapses in laboratory practices that lead to the exposure. The exposed individuals were risk-stratified, assessed for post-exposure prophylaxis (PEP), counselled to self-monitor symptoms and followed-up with three serology tests. Staff laboratory practices were recorded. Ten out of 13 high-risk individuals received PEP within 48 h of exposure. Compliance with PEP and serology monitoring was 90 and 96 %, respectively. No brucellosis cases were documented. A single handler manipulated the *Brucella* isolate on the open bench. Movement of the isolate was tracked in detail, highlighting various points of laboratory non-conformance. Early PEP intervention is effective in preventing acquired brucellosis. Our pragmatic post-exposure management achieved high PEP and serology compliance. We experience first-hand how regular staff engagement motivated PEP adherence and interval blood sampling attendance. The enforcement of practical strategies and safety practices was also implemented without compromising our laboratory processing times.

INTRODUCTION

Brucellosis is a zoonotic infection that is usually transmitted by direct contact with infected animal tissues, through consumption of contaminated animal products or via inhalation of aerosolized bacteria. This small Gram-negative coccobacillus has an infective dose of <100 organisms, which, coupled with its ease of aerosolization, makes it highly transmissible, especially in the close confines of the microbiology laboratory [1]. Up to 2 % of all reported cases of human brucellosis are laboratory-acquired and these are predominantly caused by *Brucella melitensis* [2].

Most industrialized countries, including Singapore, have been successful in reducing or eradicating their *Brucella* reservoir in animal livestock by mass animal-based control measures [3]. However, neighbouring countries such as Thailand, Indonesia, Malaysia and Myanmar report a prevalence of bovine and caprine brucellosis that ranges between 1–5 % [4]. Cases of human brucellosis in Singapore are mostly reported in travellers returning from endemic countries. They often present with non-specific symptoms for the unsuspecting clinician. With a low index of clinical suspicion, brucellosis continues to pose an occupational hazard when the organism is unknowingly recovered from clinical samples in the laboratory.

We report our experience of an accidental laboratory exposure of 22 laboratory staff to *B. melitensis* and the post-exposure management.

METHODS

A knee fluid specimen was received from a 66-year-old woman who had been admitted to hospital with a fever of 39.3 °C and a 1-week history of a painful, swollen knee with a recent trauma history for the knee. The index case was a Malaysian resident who was visiting her close relation in Singapore. She is a homemaker in an urban city, with no direct contact with farms or animal livestock, and denied any consumption of unpasteurized dairy products. Her admission temperature was 39.3 °C and a 1-week history of a painful, swollen knee with a recent trauma history for the knee. The index case was a Malaysian resident who was visiting her close relation in Singapore. She is a homemaker in an urban city, with no direct contact with farms or animal livestock, and denied any consumption of unpasteurized dairy products. Her admission serum C-reactive protein and total white cell count were elevated at 88.7 mg l⁻¹ (normal <3 mg l⁻¹) and 11.3 × 10³ µl⁻¹ (normal <10 × 10³ µl⁻¹), respectively. Her liver function tests and renal profile were unremarkable. Concurrent aerobic and anaerobic blood cultures collected on the same day remained negative after 5 days of incubation. Her acute *Brucella* serology titre was 1:80, collected on day 4 of admission. Incidentally, during her admission, a new diagnosis of type 2 diabetes
mellitus was made, with an HbA1c of 7.7%, and her blood sugars were well controlled following the commencement of an oral hypoglycaemic agent.

The clinical information provided in the request form was ‘knee effusion’. Direct microscopy and Gram staining of the specimen did not reveal any organisms, and only white cells were seen (predominantly neutrophils). All slide preparations and the inoculation of specimens onto culture plates on the day of specimen receipt were conducted in the biosafety cabinet class II (BSC-II).

The culture plates did not reveal any growth within the first 48 h. In the absence of growth, our practice is to extend incubation for a further 24 h with the intention of detecting slow-growing organisms and reporting the culture results at 72 h. At 72 h, white pinpoint colonies were visible on the culture plates. Our matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) VitekMS system (BioMerieux, France) failed to identify the organism. Further testing demonstrated the presence of an oxidase-positive Gram-negative coccobacilli. Based on this preliminary result, Brucella spp. was suspected and further manipulations were immediately held off.

Confirmation of identification was performed at the National Public Health Laboratory (NPHL) using the MALDI-TOF Biotyper mass spectrometer (BrukerDaltonics, Germany) followed by whole-genome sequencing (WGS), which identified the organism at the species level as Brucella melitensis.

A single laboratory technician was identified as the main handler of the isolate. Reading of the culture plate up close and manipulation of the isolate with a disposable loop were all performed on the open bench. This included smearing the isolate onto a microscopy slide, transporting the wet microscopy slide across the laboratory onto the slide warmer situated inside the BSC-II, and performing a strip oxidase test directly on the isolate (Fig. 1).

We adapted the revised Centers for Disease Control and Prevention (CDC) risk classification recommendations published by Traxler et al. [5] and identified 21 other potentially exposed individuals in the laboratory (Table 1). Thirteen individuals, including the main handler, were categorized as high risk. Eight of these individuals were within a 5-foot radius of the main handler who performed the open-bench manipulation; three viewed the culture plates placed in the BSC-II wearing gloves and (permeable) laboratory coats; and one staff member could not precisely identify her exact location in the laboratory at the time of the exposure. There were no reported accidental spillages or other specific aerosol-generating procedures performed outside of the BSC-II.

The 13 high-risk individuals were referred to the department of infectious diseases to be counselled for post-exposure prophylaxis (PEP) within 48 h of exposure. Nine individuals were classified as low risk and were not offered PEP. All 22 individuals who were exposed were advised to self-monitor for symptoms daily and to report any febrile illness for a period of 6 months. They were all given a written sheet of information about the symptoms of brucellosis to aid adherence to self-monitoring.

Three Brucella serology samplings were offered to all exposed staff; at baseline, between weeks 10 to 13 and at week 24. Specific Brucella antibody testing against B. abortus and B. melitensis was performed via a serum tube agglutination test. A titre of 1:160 or greater was considered to be clinically significant and a fourfold or greater increase in titre between acute and convalescent phase sera was required to diagnose acute infection.

RESULTS

Ten out of the 13 high-risk individuals agreed to receive the recommended PEP therapy, oral doxycycline 100 mg twice daily and rifampicin 600 mg once daily for 3 weeks. The three individuals who declined PEP elected to be managed as part of the low-risk group. Nine out of the 10 individuals who received the combination therapy completed 3 weeks of antibiotic treatment, achieving a compliance rate of 90% (9/10). Only a few individuals reported mild gastrointestinal discomfort while on the combination therapy. One individual was intolerant of rifampicin due to gastrointestinal side-effects after 1 day of treatment and elected to continue with 3 weeks of monotherapy doxycycline [6].

Similarly, compliance with serology monitoring was high, with 21/22 (96%) completing all three blood samplings. One individual from the low-risk group had only two draws; at baseline and week 24. During the 6-month surveillance period, this person had left for another institution but returned to have week-24 serology performed and did not report any febrile illness during this period.

The baseline serology was normal in 21 individuals, with no evidence of seroconversion. One individual had a baseline titre of 1:80, but did not demonstrate a rise in titres in subsequent samples. No prior history of Brucella exposure was elicited from this person.

During the monitoring period two individuals (who did not receive PEP) reported self-limiting febrile illness in the first 2 weeks of the initial exposure, but the follow-up serology did not demonstrate evidence of seroconversion to support an acute infection.

DISCUSSION

Singapore is considered to be a Brucella-free country, with no documented incidence of animal brucellosis or outbreaks since 2006 [7, 8]. Only a handful of human cases have been reported in the country. The last reported case was in 2009 and concerned a traveller returning from the Middle East who had consumed unpasteurized contaminated camel’s milk [9]. So although endogenous infections are rare, the disease may continue to manifest itself sporadically from returning travellers or transient visitors arriving from neighbouring endemic regions.
Eight years ago, our laboratory had a similar experience of an accidental *Brucella* exposure (not published in the literature). The previous experience had highlighted the importance of safe laboratory practice and heightened awareness for future exposures. Because of the rare encounter with the organism, this state of vigilance gradually waned over time. Even the experienced technicians became less familiar in recognizing the phenotypic characteristics of the organism, resulting in inadvertent manipulation of the cultures on the open bench, as in our present case. The main laboratory handler of this current exposure had been involved in the exposure 8 years previously and had also received PEP at the time. In addition, the clinical history elicited from our index case would not have prompted a suspicion of brucellosis at presentation. To some extent, laboratories do rely on the communication of clinical suspicion from clinical colleagues to adopt additional safety precautions, especially when the disease is uncommon.

*Brucella* is a class 3 pathogen. The minimum recommended safety precautions required for the manipulation of live *Brucella* cultures are within a biosafety level (BSL)-3 facility or a BSL-2 facility complying with BSL-3 safety precautions, as specified by the CDC.

Like most clinical laboratories, we rely on the MALDI-TOF (Vitek-MS system) over traditional phenotypic methods for identification. Our current Vitek-MS library uses the Food and Drug Administration (FDA) approved *in vitro* database.
(IVD) version 3.0, which lacks the ability to identify \textit{Brucella} species. The absence of a reportable \textit{Brucella} result, as in our case, had a significant exposure impact. Additional work on this 'unidentifiable organism', although limited to Gram staining and oxidase testing, contributed to further exposure of other staff. We believe that the inability to alert users to a highly transmissible organism is an important and potentially serious limitation of the Vitek-MS system, particularly in a busy diagnostic laboratory.

There is, however, capacity for the Vitek-MS system, with its Research Use Only (RUO) database, to identify class 3 pathogens, including \textit{Brucella}. This may not be widely adopted by most diagnostic clinical laboratories [10]. We had explored the possibility of incorporating the RUO database into our current Vitek-MS database. To subscribe to this, a technical process of validation and verification has to be met and regularly updated. In addition, staff training and competency checks are required to operate the RUO database. Adopting this database for routine use is highly labour-intensive and not cost-effective. Moreover, our laboratory does not subscribe to a quality assurance and proficiency testing programme for security-sensitive organisms, which would allow for any validation to be performed.

Examination of our laboratory practices has identified gaps and areas for improvement. Practical changes to improve our workflow and safety practices have been adopted into our routine work. The reading of prolonged (48 h or more) incubation plates and the smearing of microscopy slides of unidentified organisms from sterile sites are conducted inside the BSC-II [11]. Additionally, any alerts or communications about suspicious specimens are recorded on a noticeboard at the point of specimen receipt to caution handlers when processing these specimens. Limited access for non-microbiology personnel and the prevention of unnecessary entry into the microbiology laboratory are enforced, especially during peak times of active bench work. Inexperienced and experienced staff are retrained to recognize the characteristics of \textit{Brucella} and are made aware of the specific areas where the breakdown of practices occurred and the corrective measures put in place to address these.

Despite revising our standard operating procedures, we recognize that the risk of accidental laboratory exposure is not negligible, given the high attack rate in a \textit{Brucella} exposure [12]. This risk may be mitigated by the measures taken and reduced to a minimum, but not completely eliminated. A Spanish study has shown how the risk of laboratory-acquired brucellosis correlates with the number of isolates recovered per year [13]. Given the infrequent recovery of this pathogen in our setting, it would seem impractical to categorically apply the strict BSL-3 practices as set out by the CDC. Handling all specimens in the BSC-II, although ideal, is practically challenging and will inevitably compromise specimen processing turnaround times, especially in a busy laboratory with limited staffing resources and safety equipment [14].

Following our post-exposure risk-assessment, 13 individuals were classified as high risk and qualified as candidates for PEP. However, 3 of the 13 individuals perceived their exposure as low risk. They regarded themselves as being reasonably 'well-protected' based on the nature of their exposure, which was limited to viewing or opening of the culture plates inside the BSC-II, while wearing gloves and (permeable) laboratory coats. In a review of 28 case reports by Traxler \textit{et al.}, 85% of the laboratory-acquired brucellosis cases identified aerosolization as the implicated route of exposure during routine identification activities. There was, however, a small number of individuals who acquired brucellosis despite the use of the biosafety cabinet with no recognized lapses in biosafety practices. In view of this, we felt that it was necessary to include the three individuals in the high-risk group to be counselled with the intention of offering PEP, which they subsequently declined.

The timing of symptom onset varies between 1 to 40 weeks. Traxler \textit{et al.} showed that the median time for symptom onset and seroconversion was 8 weeks and 11 weeks, respectively. While early PEP remains the mainstay of averting an infection, Fiori \textit{et al.} [15] demonstrated the rationale behind the importance of serial blood sampling, detecting rising serum titres 2–5 days before symptom onset and therefore pre-emptively instituting treatment. Those started on early treatment based on serum titres exhibited milder symptoms and shorter duration of illness. Meanwhile, Knudsen \textit{et al.} [16] proposed that the emphasis should be placed on

**Table 1. Risk category of the \textit{Brucella} exposure in the laboratory**

<table>
<thead>
<tr>
<th>Risk group</th>
<th>No. of individuals</th>
<th>Exposure</th>
<th>No. of individuals who received PEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>1</td>
<td>Manipulated culture isolate on the open bench</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Working adjacent to the bench where the culture isolate was being manipulated (&lt;5-foot radius)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Working on benches within 5 feet of the microscopy slide travel path</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Viewed the culture plates in the BSC-II, partially complying with BSL-3 safety precautions</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Uncertain of precise location in the laboratory during period of exposure</td>
<td>1</td>
</tr>
<tr>
<td>Low</td>
<td>9</td>
<td>Present in the laboratory during exposure period but was &gt;5 feet away, or in a separate room whilst the culture was being manipulated on the open bench</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

symptom screening and suggested that the number of serology samples be reduced to just three samplings.

Past experiences have demonstrated the challenges faced in attaining high compliance in PEP and serological monitoring [17, 18]. During our 6-month follow-up period, we maintained regular engagement with the individuals exposed. Before commencing PEP, baseline expectations regarding the tolerability treatment were set. Common side-effects were explained and individuals experiencing these were encouraged to report them, however trivial the symptoms might seem. Regular dialogues between individuals concerning their experiences whilst on PEP were encouraged, and this seems to motivate individuals as a group to adhere to treatment. In addition, we also issued periodic reminders to encourage a proactive attitude towards symptom surveillance and attendance for blood samplings. To promote compliance, we decided to offer three serology samplings instead of the recommended [5] five *Brucella* sera. These were performed at baseline, between weeks 10 and 14 and at week 24. Attendance for serology testing was closely monitored, and non-attendees were sent a reminder to reschedule. The three high-risk individuals who declined prophylaxis were prioritized to have their serology performed at week 10, while the others were batch sampled between weeks 12 and 14. This pragmatic approach was adopted to encourage compliance without overtaxing the referring laboratory performing the serology tests.

**Conclusion**

Laboratory-acquired brucellosis is a well-recognized infection. Accidental *Brucella* exposure causes significant anxiety amongst the individuals exposed, and the disease continues to pose a challenge to clinicians and laboratory staff with regard to early recognition, containment and diagnosis. Our experience highlighted areas of laboratory practice non-compliance and the post-exposure impact of the incident on those directly exposed. We undertook a pragmatic post-exposure approach, which resulted in greater compliance in PEP and symptoms and serology surveillance. The overall outcome was favourable, with no acquired brucellosis cases. This may be attributable to the early PEP intervention and the high compliance rate achieved for this exercise. In addition, regular staff engagement provided psychological support and motivated individuals to attend regular blood samplings. Safe laboratory practices were enforced and changes were made in areas where non-conformance was identified. Practical strategies were also implemented to minimize future exposure, all of which were feasible within the limits of our staff capacity and equipment resources.

Funding information

The authors received no specific grant from any funding agency.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**