



Short report

Staggered enforcement of infection control and prevention measures following four consecutive potential laboratory exposures to imported *Brucella melitensis*

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ARTICLE INFO

Article history:

Received 28 October 2020

Accepted 12 February 2021

Available online 18 February 2021

Keywords:

Brucellosis

Laboratory workers

Laboratory exposure

Risk assessment

Biosafety

Infection control and prevention practices

SUMMARY

From 2015 until 2020, *Brucella melitensis* was isolated four times in our microbiology laboratory. All patients had travelled in endemic-areas. Immediately after the first occurrence, all laboratory staff were risk-stratified and preventive and protective measures were applied according to CDC guidelines. Nineteen workers were exposed and needed chemoprophylaxis and follow-up. At each subsequent occurrence, risk analysis was performed, and additional measures were implemented accordingly, leading to a progressive reduction of exposed staff members to none the fourth time. We describe here the additional measures that permitted this important exposure reduction.

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Abbreviations: Biovar, (bv); Center of Disease Control, (CDC); Centre Hospitalier Universitaire, (CHU); Cliniques Universitaires de Bruxelles, (CUB); Emergency Room, (ER); Laboratoire Hospitalier Universitaire de Bruxelles – Universitair Laboratorium Brussel, (LHUB-ULB); National Reference Centre, (NRC); Laboratory Acquired Infection, (LAI); Post-Exposure Prophylaxis, (PEP).

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<https://doi.org/10.1016/j.inpp.2021.100128>

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Introduction

Acquired human infection with *Brucella* spp. is rare in Belgium (less than 3 cases/year) and the majority of confirmed cases are imported [1]. Conversely, exposure to *Brucella* spp. is the most common Laboratory Acquired Infection (LAI) reported over the world [2]. Indeed, a low dose of bacteria is enough for infection by aerosol transmission. Spilling blood-culture bottles, sniffing plates, mucocutaneous exposure to spray, and centrifugation steps are the most common sources of laboratory acquisition. Infection risk is enhanced by routine work outside of a biosafety cabinet. Imported *Brucella* infection has been increasingly reported in the recent years in Europe as a consequence of migrations flows, especially from Syria and Eritrea [3–5].

The LHUB-ULB (Laboratoire Hospitalier Universitaire de Bruxelles – Universitair Laboratorium Brussel) is a clinical laboratory (founded in 2015), serving five University Hospitals located in the Region of Bruxelles-Capitale (CHU Saint-Pierre, Institut Jules Bordet, Hôpital Universitaire des Enfants Reine Fabiola, CHU Brugmann and CUB Hôpital Erasme). Preserving hygiene, security and health of our 400 laboratory workers is therefore one of the main goals of the management team. That implies iterative biosafety risk analysis to point out lapses in laboratory practices followed by implementation of measures to reinforce safety and avoid LAI. From 2015 to 2020, *Brucella melitensis* was isolated four times in our clinical microbiology laboratory. We describe here the risk analysis performed and additional measures implemented leading to a progressive and significant reduction of the number of exposed staff members at each occurrence.

Materials and methods

We prospectively followed over a five year period, all the *Brucella* spp. isolation cases in our microbiology laboratory and analysed the measures subsequently taken to prevent LAI and their respective impact.

Routine identification of suspected *Brucella* spp. includes, in our laboratory, MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and Vitek-2 identification (BioMérieux, Marcy L’Etoile, France). For each isolation case, speciation of the *Brucella* isolate, biochemical and molecular typing were conducted at the *Brucella* National Reference Centre (NRC) as previously described [1].

For every *Brucella* spp. isolation, potential occupational exposure incidents, such as manipulating a positive blood

culture or opening a positive culture plate without using Biosecurity Level-3 (BSL-3) precautions were actively searched for and recorded. All members of the laboratory staff were risk-stratified and managed according to the CDC guidelines [6]. “High risk” level workers were those who performed potentially exposing handling and all those present in the room within a 5 feet radius (1.52m) at the time such manipulation occurred. “Low risk” level workers were those present in the room at the time but at a greater distance than 5 feet.

Both high and low risk workers were followed with six consecutive *Brucella* serology tests (baseline, week 3, week 6, week 12, week18 and week 24).

Additionally, a PCR test in whole blood (not advocated by CDC) in parallel to those serology tests was performed according to the recommendation of the *Brucella*’s NRC.

Post-exposure prophylaxis (PEP) treatment was given to all the workers identified as high risk (doxycycline 100 mg twice daily, and rifampicin 600 mg once daily, for three weeks).

All the exposed workers were counselled to self-monitor symptoms including daily temperature taking for 24 weeks.

Results and discussion

From 2015 to June 2020, *Brucella* spp has been isolated from four different patients. The cases are summarised in Table I.

Clinical data of patients

In October 2017, a *Brucella melitensis* bv1 strain was fortuitously isolated from blood culture of a female Syrian refugee. The patient had a history of travel in various Middle-East and Maghreb countries, as well as in Spanish areas known to be endemic for Brucellosis. She was admitted at CHU Saint-Pierre’s emergency room (ER) with abdominal pain in the context of an advanced stage cervical cancer complicated by bilateral ureteral invasion with suspicion of urinary tract infection.

In August 2018, a *Brucella melitensis* bv3 strain was isolated from blood culture of a Lebanese woman who came to Brussels for a few days. The infection was diagnosed when she had already flown back to Lebanon.

In October 2018, a *Brucella melitensis* bv1 was isolated from an osteitis sample of a homeless man who had immigrated to Brussels a few days before. Native Eritrean, he left his country four years earlier and had been living in Italy since then.

Finally, in June 2020, a *Brucella melitensis* bv1 was isolated from a blood culture of a 36 year old, Eritrean asylum-seeking

Table I

Description of potential exposure incidents and risk-stratification of the laboratory workers over the study period

Case #	Date (Month and year)	Origin of lab exposure	# Workers in the lab	# High risk	# Low risk	# Lab acquired infection
				Lab workers	Lab workers	
1	October 2017	Positive blood culture	26	7	12	0 ^a
2	August 2018	Positive blood culture	20	3	5	0
3	October 2018	Osteitis sample (in blood culture bottle)	21	1	0	0
4	June 2020	Positive blood culture	4	0	0	0

^a One case of LAI was suspected, but not proved.

patient, who had arrived in Belgium four months earlier. He was admitted to CHU Brugmann's ER with a two week history of persistent abdominal pain. Clinical investigations revealed disseminated infective endocarditis.

Brucella identification and typing

For the first three cases, *Brucella melitensis* was isolated in our laboratory and further confirmed and identified at the NRC. For the final case, after visualisation of Gram-negative coccobacillus in the Gram stain, the positive blood culture was immediately sent to the NRC (since the patient's serology was known to be positive). *B. melitensis* was subsequently identified.

Molecular typing (Multiple Locus Variable Number of Tandem Repeat Analysis), performed by the NRC, demonstrated that all strains were of foreign origin.

Risk analysis and biosafety management

After recognition of the first case, and in association with the NRC, a risk analysis was performed to assess staff exposure in the laboratory. Personnel categories exposed included laboratory technicians, microbiologists, students and administrative employees in charge of encoding the request forms.

The immediate actions included: (1) tracking all the movements of positive samples and potential exposure incidents, (2) identification of ongoing patient samples for safe handling, (3) establishment of a list of the activities of all workers which were in the laboratory the day(s) the potential exposure incidents occurred, (4) contact all the hospital employer's partners involved in the laboratory exposure follow-up, (5) various information communications tailored to the receiver (disease declaration organism, laboratory workers, occupational medicine, hospitals management) and (6) establishment of the follow-up procedures (see Materials and Methods section) for high and low risk workers.

For this first exposure case, risk assessment following CDC criteria revealed seven "high risk" and 12 "low risk" workers among the 26 present in the laboratory during the three days when exposure incidents occurred. No relationship could be found between the classification of risk compared to the category of personnel.

The high risk workers received rifampicin and doxycycline Post-Exposure Prophylaxis (PEP) treatment during three weeks, except a pregnant woman who received sulfamethoxazole and trimethoprim. Potential toxicity was monitored by liver enzyme level measurement at day 21.

One high risk worker, from this first case, developed fever around three weeks after exposure, with weight loss and fatigue. At the same time the PCR test taken at third week of follow-up came back positive, serology was negative. Close monitoring was undertaken, using blood culture specimens, PCR tests and serology everyday over the course of a week. Symptoms resolved after one week. All tests (serology as well as the PCRs performed up to 6 months after exposure) remained negative. This first positive PCR could be due to an aspecific reaction or an aborted infection following prompt and full compliance to PEP.

At the end of the follow up of this first case, 24 weeks serology remained negative for all the other (low and high risk)

staff. All the PCR tests were also negative (with the exception of the one described above).

Compliance to PEP and serology monitoring was 100% and 93% respectively (eight of the 114 programmed serology tests were missed). No other adverse event was recorded.

Risk-assessment identified several potential areas for improvement, all linked to a lack of experience or preparedness in diagnosing *Brucella spp.* This inexperience led to a multitude of *Brucella spp.* isolate manipulations, a long identification period and consequently a greater number of workers exposed to the organism: inadequate behaviour such as handling positive blood culture on the open bench without using safety cabinet, or sniffing bacterial cultures, but also absence of *Brucella spp.* in the MALDI-TOF IVD database and misinterpretation of the serology test (Rose Bengal test) that led to a false negative report. Indeed, the identification using MALDI-TOF was delayed due to the necessity of using a security database as described by others [7].

Accordingly, several corrective and preventive actions have been taken. Regarding the workflow, (1) a re-organisation of blood culture bench was implemented (moving the blood culture just in front of the dedicated safety cabinet so that the technicians can easily turn from side to side using a swivel chair) (2) *Brucella spp.* was added in the MALDI-TOF IVD data base and (3) the staff were reminded of the quality system procedures relating to health and safety, as well as Good Laboratory Practices through lecture sessions. (4) The serology team were sent for additional training at the NRC. During this training, the need for more performant material (agglutination test plates) was pointed out and implemented accordingly. (5) Clinicians were asked to warn the Microbiology laboratory of any suspected case of highly contagious disease, including brucellosis and prion disease. (6) Additionally, open lectures have been given by Infectious Diseases specialists to clinicians to raise their awareness regarding imported brucellosis.

As soon as the second *Brucella spp.* case was detected (10 months later), the risk-stratification and follow-up were identical but easier to manage: PEP treatment was initiated within 48 hours of exposure for the three high risk workers identified. Implementation of six serology & PCRs tests and self-monitoring of symptoms over a 24 week period for all eight exposed workers identified began (5 low risk and 3 high risk).

The same strategy was maintained for the last two cases and according to the CDC specific criteria, a single high risk exposure was recorded for one laboratory worker handling the third case's specimens. Despite an extension to the MALDI-TOF IVD database, we encountered a practical problem for this case, as the technicians used the RUO database for rapid MALDI identification on the blood culture pellet. The lesson learned was the mandatory use of the IVD database.

The fourth case did not cause any occupational exposure. Fortunately (or thanks to the awareness-raising seminars organized), the clinician in charge of the patient at admission asked for an initial battery of serological tests, among which *Brucella* serology, that was positive. We were thus able to warn all laboratory workers (day and night shifts) to carefully handle all the patient's samples in the safety cabinet and send directly the positive blood-culture bottles to the Brucella NRC without sub-culturing them.

For all three cases described above, no Brucellosis case was documented among both high and low risk workers, as the 24 week follow up serology and PCR testing remained negative.

One worker of the low risk group was not compliant with follow-up (only one serology and PCR performed). Nevertheless, he did not declare any symptom during the six month follow up period.

Some cases of *Brucella* spp developing after post-exposure management have been described [8–10]. But, to our knowledge, this is the first description of follow-up procedures and prevention practices implementation over time in the same laboratory.

In summary, we highlight the positive effect of a temporal and thorough risk analysis and risk management approach applied within a clinical laboratory to reduce exposure to the most common LAI, in a non-endemic area.

The improvement in laboratory biosafety and increased awareness are evidenced by the significant decrease in the number of exposed workers. The medical management was fully achieved, including laboratory testing, PEP and clinical follow up of exposed workers.

This successful approach implies a close and multi-disciplinary collaboration between the Infectious Diseases team, Infection Control units, the *Brucella* NRC, Emergency units, Occupational Medicine and clinical microbiology laboratory management team.

Authors contributions

Miendje Deyi Véronique Y: Conceptualization, Methodology, Investigation, Writing-original Draft, Writing-Reviewing and Editing.

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Acknowledgements

We acknowledge all the partners of all the laboratories who were involved in these issues, particularly, pharmacists, infectious diseases specialists, occupational medicine departments, Infection control units, hospitals management and laboratory technicians.

Conflicts of interest

None declared.

Funding sources

The NRC activity is supported by the Belgian Ministry of Social Affairs (Health Insurance System), Belgium through a fund of the Health Insurance System.

Ethics statement

None required.

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