JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Microbiology and Pathology

Research Article

Laboratory-Acquired Brucella melitensis Infection in Jiangsu, 2013

Zhongming Tan[1] , Jun Zhang[2] , Weizhong Zhou[1] , Hong Ji[1] ,Changjun Bao[1] , Fenyang Tang1, Yefei Zhu[1*]

[1]*Key Laboratory of Enteric Pathogenic Microbiology, Ministry of Health, Jiangsu Provincial Centre for Disease Control and Prevention, Nanjing, Jiangsu Province, China*

[2]*Yangzhou Center for Disease Control and Prevention, Yangzhou, Jiangsu Province, China*

Zhongming Tan and Jun Zhang contributed equally to this work

**Corresponding author: Dr. Yefei Zhu, Key Lab of Enteric Pathogenic Microbiology, Ministry of Health, Jiangsu Provincial Center for Disease Control and Prevention, 172 Jiangsu Road, Nanjing 210009, China, Tel: 86 +25 +83759404, Fax: 86 +25 +83759409, Email: jszyf@jscdc.cn*

Received: 07-26-2014

Accepted: 07-30-2014

Published: 09-05-2014

Summary

We report a laboratory-acquired *Brucella melitensis* infection from Yangzhou city, Jiangsu Province, China, 2013. Epidemiological investigation found that a laboratory technician (Case 1) and a patient (Case 2) were related. Laboratory confirmation of cases was made by detection of *B. melitensis* genes *bcsp-31* and *IS711* using polymerase chain reaction; source tracking was conducted by multiple loci variable nucleotide tandem repeat (VNTR) analysis (MLVA). The isolates recovered from the two cases were genotypically indistinguishable. Our analyses indicated that Case 1 develop brucellosis most likely during processing of Case 2's isolate; due to inadequate personal protective equipment. Misdiagnosis of Case 2 contributed to subsequent laboratory exposures. Implementing public health measures, such as educating doctors and laboratory technicians about brucellosis, could be used to reduce the risk of laboratory-acquired brucellosis occurring again in Yangzhou.

Keywords: Laboratory-Acquired Brucellosis; *Brucella melitensis*; MLVA

Brucellosis remains an important anthropozoonosis worldwide. Humans may acquire brucellosis through multiple routes, including ingestion, skin and mucosal contact, inhalation of aerosols and percutaneous inoculation. Brucellosis is also the most frequently laboratory-associated bacterial infection in the world [1]. Laboratory workers are at risk when handling specimens containing *Brucella* spp. because of aerosol-generating procedures or accidents that may result in infection of blood or conjunctiva [2].

We report a laboratory-acquired *Brucella melitensis* infection in the microbiology laboratory of a hospital in Jiangsu Province. Epidemiological investigation and MLVA-16 typing confirmed the probable transmission route between a laboratory technician and a fever patient, implicating in adequate personal protection equipment (PPE) for laboratory staff.

Case 1: On 23 June 2013, a 49-year-old female laboratory technician (Case 1) developed fever, characterized as a "heat wave" with temperatures as high as 38.8°C, hyperhidrosis and no other special discomfort. Case 1 applied self-medication with paracetamol and cephalosporins, treatment was not effective and fever continued unabated. On 4 July 2013, blood test and blood smears test were performed, the results revealed reduced white blood cell count of 2.7×10^9 / L, Gram-negative bacilli were found in blood smears, blood culture was subsequently performed. After incubation for 72 h, small Gram-negative bacilli were isolated and identified as *Brucella* spp. using biochemical test; this isolate was designated JS-2013-8. *Brucella* serum agglutination test (SAT) was reactive (1:400). The case was treated as an outpatient with rifampicin and doxycycline for six weeks. Case 1 has not relapsed one year after completing treatment.

Investigation and laboratory detection

To determine the source of the laboratory worker's

infection, an epidemiological investigation was conducted jointly by the hospital laboratory and Yangzhou Center for Disease Control and Prevention. From 29 April to 2 May, an unidentified blood culture isolate from a fever patient was submitted to the microbiology laboratory of hospital, where Case 1 worked, for identification as *Salmonella enterica* serovar Typhi or *Salmonella enterica* serovar Paratyphi. Subculturing and routine laboratory work with cultures of the unidentified isolate was performed outside of a class II biosafety cabinet without PPE except gloves and lab-gown. The results showed the isolate was neither *S*. Typhi nor *S*. Paratyphi, and was subsequently destroyed by the laboratory technician (Fig 1).

Blood tests revealed an elevated white blood cell count of 7.1 × $l0^9$ / L; blood culture was subsequently performed to exclude typhoid and paratyphoid fever. The initial diagnosis of patient was sepsis and viral orchitis. However, treatment with vancomycin and levofloxacin was effective, and the patient was discharge from hospital on 16 May. On 10 July 2013, Case 2 was found by CDC staff, and blood cultures and detection of *Brucella* were then re-performed his preserved blood sample which collected on 24 April 2013 under enough biosafety protection. The isolate was identified as a *Brucella* spp. and designated JS-2013-9 (Fig 1). No risk factor was identified for the source of this infection. He did not get other treatment and has not relapsed after discharged from hospital.

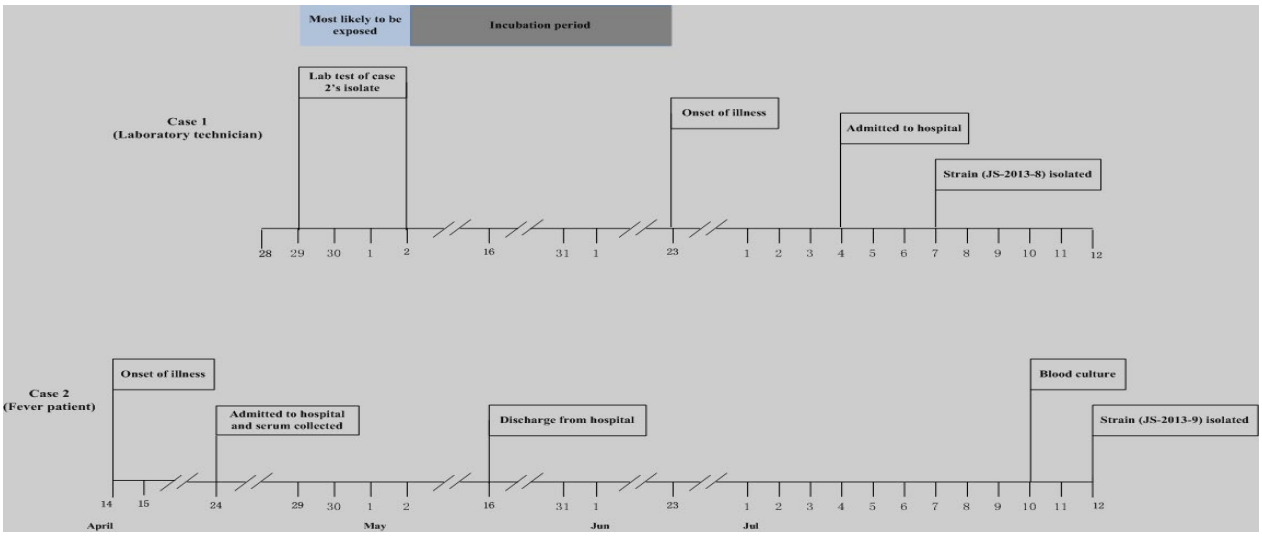

Figure 1. Timeline of laboratory-acquired Brucella melitensis infection from Yangzhou city, Jiangsu Province, 2013. Case 1: a laboratory technician; Case 2: a fever patient.

Case 2: On 14 April 2013, a 27-year-old male who was employed at a local bank reported fever, chills, testicular pain; on 24 April, he presented to a local hospital. Case 2 also reported "heat wave" type of fever, with temperatures as high as 40°C, swelling of the right testis, swollen joints of right knee and right hand, and two lung markings.

To elucidate the origin Case 1's infection, the recovered *Brucella* spp. isolates from both cases were further characterized. Both isolates were identified as *B. melitensis* biovar 1 using agglutination tests and a species-specific polymerase chain reaction (PCR) based on differences in the *bcsp-31* gene [3] of *Brucella* and *IS711* of *B. melitensis* [4].

Table 1. Genotype identified in isolates of *B. melitensis* from both laboratory technician and patient.

	Occupations	Strain NO.	Serotype	Panel 1								Panel 2A			Panel 2B					Panel 1 Type	Panel 2A Type
				bruce06	bruce08	bruce11	bruce12	bruce42	bruce43	bruce45	bruce55	bruce18	bruce19	bruce21	bruce04	bruce07	bruce09	bruce16	bruce30		
Case 1	Laboratory technician	JS-2013-8	Biovar 1	1	5	3	13	2	2	3	2	4	20	8	7	4	3	6	8	42	41
Caes 2	Bank staff	JS-2013-9	Biovar 1	1	5	3	13	2	2	3	2	4	20	8	7	4	3	6	8	42	41

After diagnosis of brucellosis in the laboratory worker, serum samples from her laboratory co-workers were tested by *Brucella* SAT; all samples were nonreactive (<1:50).

Multiple loci variable nucleotide tandem repeat (VNTR) analysis (MLVA) using 16 markers (MLVA-16 typing) was performed. DNA was extracted and amplified at 16 loci by PCR as previously described [5]. PCR products were purified and directly sequenced. Analysis of panel1 (bruce06, bruce08, bruce11, bruce12, bruce42, bruce43, bruce45 and bruce55), 2A (Bruce18, Bruce19 and Bruce21) and 2B (Bruce04, Bruce07, Bruce09, Bruce16 and Bruce30) markers indicated that the isolates were indistinguishable (42, 41, JS2B-6) (Table 1).

Conclusions

Brucellosis is among the most commonly reported laboratory-acquired bacterial infections. Aerosolization is the major source of transmission, but the bacterium can also be transmitted via direct contact. However, in many reported cases, it has not been possible to accurately determine the mechanism for transmission [6-8]. Laboratory-acquired brucellosis has been reported in China as well [9].

Genotyping results provided evidence that the two brucellosis cases described in this report were indistinguishable. The laboratory evidence, combined with the epidemiological investigation, supports the theory that the hospital laboratory technician most likely acquired *Brucella* when processing a sample from Case 2 in the absence of adequate PPE. Our results provide evidence supporting the usefulness of genotyping for epidemiological investigation.

Jiangsu province is a non-endemic area of brucellosis in China, most clinicians have no experience in the diagnosis of *Brucella* infections; in this instance the misdiagnosis of Case 2 led to subsequent laboratory exposures and infection. Some public health measures, such as educating doctors and laboratory technicians about this disease and what level of PPE required, had been used to prevent laboratory-acquired brucellosis occurring again in Yangzhou. We recommended hospital considered all patients' blood and certain body fluids are potentially infectious for some bloodborne pathogens and take universal precautions.

Acknowledgement

The authors thank Dr. John Klena from the US Centers for Disease Control and Prevention for modifying our manuscript. This work was supported by Jiangsu Province Health Development Project with Science and Education (ZX201109 and RC2011085) and Jiangsu Province Science and Technology project of Clinical medicine (BL2014081).

References

1. Weinstein R A, Singh K. Laboratory-acquired infections. Clinical infectious diseases. 2009, 49: 142-147.

2. Olle-Goig J, Canela-Soler J. An outbreak of Brucella melitensis infection by airborne transmission among laboratory workers. American journal of public health. 1987, 77: 335-338.

3. Mukherjee F, Jain J, Patel V, Nair M. Multiple genus-specific markers in PCR assays improve the specificity and sensitivity of diagnosis of brucellosis in field animals. Journal of medical microbiology. 2007, 56: 1309-1316.

4. Bricker B J, Halling S M. Differentiation of Brucella abortus bv. 1, 2, and 4, Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR. Journal of clinical microbiology. 1994, 32: 2660-2666.

5. Le Flèche P, Jacques I, Grayon M, Al Dahouk S, Bouchon P et al. Evaluation and selection of tandem repeat loci for a Brucella MLVA typing assay. BMC microbiology. 2006, 6: 9.

6. Fiori P L, Mastrandrea S, Rappelli P, Cappuccinelli P. Brucella abortus infection acquired in microbiology laboratories. Journal of clinical microbiology. 2000, 38: 2005-2006.

7. Noviello S, Gallo R, Kelly M, Limberger R J, DeAngelis K et al. Laboratory-acquired brucellosis. Emerging infectious diseases. 2004, 10: 1848-1850.

8. Singh K. Laboratory-acquired infections. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009, 49: 142-147.

9. Jiang H, Fan M, Chen J, Mi J, Yu R et al. MLVA genotyping of Chinese human Brucella melitensis biovar 1, 2 and 3 isolates. BMC microbiology. 2011, 11: 256.