

## Laboratory-acquired Brucellosis

Dear Editor,

Brucellosis is a serious disease seen worldwide and has been historically known as undulant fever, Bang's disease, Gibraltar fever, Mediterranean fever, and Malta fever. Brucellosis has a limited geographic distribution but remains a major problem in Mediterranean and Middle Eastern countries.<sup>1</sup> In 2001, a total of 15,510 brucellosis cases were reported in Turkey.<sup>2</sup> In our centre there are approximately 20 to 25 new cases each year. *Brucella* is most commonly transmitted by the consumption of contaminated raw or unpasteurised milk and cheese. Laboratory-acquired brucellosis has also been documented and is considered the most important laboratory-acquired bacterial infection. All *Brucella* spp. have been implicated in laboratory-associated infections, and they may account for up to 2% of all laboratory-associated infections.<sup>3</sup> In this paper, we report 3 laboratory-acquired brucellosis.

Case 1 was a 26-year-old female laboratory worker, who works as microbiology technologist. She presented with joint pain and fever of 1 week's duration. There was no history of trauma. The patient had given birth 2 weeks prior. Physical examination and haematological parameters were normal. However, the levels of aminotransferases were elevated [aspartate transferase (AST), 45 U/L; alanine transferase (ALT), 55 U/L]. The past history of the patient did not reveal any raw cheese consumption. She had been working on the determination of subgroups of *Brucella* positive cultures. She was tested for brucella using the Rose Bengal microagglutination test (Tulip Diagnostics Ltd. Goa, India) as well as serological titre of anti-*Brucella abortus* antibodies were evaluated by using a standard tube agglutination test (Seromed Laboratory Products, Turkey). The Rose Bengal microagglutination test was positive. The *Brucella* serum agglutination test was reactive (1/640). Two sets of blood samples were obtained for culture. The blood cultures showed bacterial growth (Bactec 9050, Becton Dickinson, USA) following 72 hours of incubation. Bacteria were isolated in 5% sheep blood agar. Grams' stain revealed small gram-negative coccobacilli. The organism was confirmed to be *B. melitensis* by standard biochemical reactions (production of urease, catalase-positive, oxidase-positive, H<sub>2</sub>S and indole negative, the dyes basic fuchsin, thionine, thionine blue are positive). In addition, biochemical identification using an API 20 NE (BioMerieux, France) was done. Treatment was initiated with a combination of 2 g of ceftriaxone plus 600 mg of rifampin every day for 6 weeks. The patient had a full recovery without any coexisting problem.

Case 2 was a 28-year-old female laboratory worker who presented with non-specific symptoms of malaise, vomiting and fever. She had been working in the same microbiology laboratory as Case 1 and Case 3. She had worked with the same *Brucella* samples as the patient in Case 1 two weeks prior. She did not have any risk factors for brucellosis exposure such as ingestion of unpasteurised milk products. The results of her physical examination and haematological and biochemical studies were normal. *Brucella* serology was positive at 1/160. The blood culture revealed *B. melitensis* growth after 3 days. She received doxycycline at a dosage of 100 mg po twice daily and rifampin at a dosage of 600 mg po qd for 6 weeks. She recovered completely.

Case 3 was a 24-year-old female microbiology technologist who was suffering from fever and pain of the lower extremities. Her physical examination and routine laboratory tests were normal. *Brucella* serology was positive at 1/640. *B. melitensis* was isolated from the blood culture. She received the same treatment as the second case and recovered fully. Like the preceding 2 patients, she denied other exposure to brucellosis.

Brucellosis has been considered the most important laboratory-acquired bacterial infection. Aerosol transmission generated accidentally or during microbiologic techniques from contaminated materials are the proposed routes of transmission. Our present report includes 3 patients with an exposure history of working *Brucella* bacteria in a microbiology laboratory. No accident occurred in the laboratory during the time they were exposed. All 3 cases were found to be working on the specimen of the index case patient. The index case was a 45-year-old man who had a *Brucella* serology of 1/640 and blood culture that grew *B. melitensis*. Only 3 of the microbiology staff work on the specimen. The other personnel in the laboratory who did not work with the specimen were also examined with Rose-Bengal test, brucella antibody and brucella-related symptoms. The results were negative for brucellosis.

Brucellosis is an endemic disease in our country. The 3 patients presented did not have any suspicious history of unpasteurised milk consumption or animal contact. The absence of this kind of contamination led us to believe that the transmission to our patients was through the laboratory route. The patients were thought to have been infected during subculturing for collecting bacteria. These laboratory workers were unaware of the hazards of aerosol transmission of *Brucella* spp. They handled the biosafety cabinet, and used gloves and masks. Sniffing culture plates is another

risk factor, and is a common practice in bacteriology laboratories in Turkey, as in other countries.<sup>4</sup> *Brucella* spp. are highly infectious because the infectious dose by an aerosol is only 10 to 100 organisms.<sup>5</sup> Laboratory-acquired brucella infections are very important in developing countries and in countries with endemic disease, such as Turkey. In our country, biosafety cabinets do not exist in most hospital laboratories. Therefore, using gloves, masks and goggles and also continuous education on biosafety where brucellosis is endemic is very important. The transmission in our cases was probably due to aerosol contamination because of the current practice of sniffing culture plates. Additionally, catalase test used for bacteria identification might have produced aerosol.

In 1986, the World Health Organization recommended the use of doxycycline in combination with rifampin for 6 weeks as the preferred treatment for adult acute brucellosis. The second and third cases received doxycycline and rifampin for 6 weeks. However, the first case received rifampin + ceftriaxone for 6 weeks. It is known that tetracyclines accumulate in fetal bones and teeth, and furthermore, pass into breast milk.<sup>1</sup> For this reason, the combination of rifampin + ceftriaxone was preferred instead of the combination of rifampin + doxycycline for Case 1, who had given birth 2 weeks prior and was breastfeeding.

Laboratories should be aware of laboratory-associated hazards and take adequate safety precautions even after the use of biosafety cabinet, gloves and masks. In addition, all laboratory workers should be educated periodically for occupational risks in the laboratory. In our hospital, all microbiology laboratory staff were educated concerning laboratory-acquired bacterial infections. The laboratory

staff are discouraged from smelling the culture plaques. In order to prevent future infections, close collaboration between clinicians and laboratory staff was initiated. Biosafety level 3 has to be advocated and used when working with micro-organisms such as *Brucella* spp. Clinicians should alert laboratory personnel if they suspect Brucellosis in patients so they can take extra precautions.

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