

## Outbreak of *Brucella melitensis* among Microbiology Laboratory Workers

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**We report on an outbreak of laboratory-acquired brucellosis involving four technicians working at a microbiology laboratory. All cases occurred in a period of 4 months. Blood cultures and the Rose Bengal test were positive for *Brucella* spp. in all cases. Microagglutination was positive for *Brucella* spp. at titers of between 1/40 and 1/160. All patients were cured after treatment.**

Laboratory-acquired infections have occasionally been reported (1, 7-9, 11). However, conclusive information on the actual incidence of this form of disease transmission is not yet available, making it impossible to assess the risk of acquiring infections in the laboratory setting (3). The ratio between the number of infections caused by different microorganisms and the number of hours of exposure of the staff is probably the best indicator of risk (10).

We report on an outbreak of brucellosis that occurred in our laboratory.

Between June and September 1988, four technicians from the microbiology laboratory of the Valme University Hospital, Seville, Spain were diagnosed with acute brucellosis. No evidence of exposure to *Brucella* spp. other than through the laboratory cultures was found for any patient.

The direct microbiological diagnosis was reached by serial blood cultures (three sets per patient), which were processed in the BACTEC NR-730 system (Becton Dickinson Laboratory) by using one aerobic 6A bottle and one anaerobic 7A bottle. The samples were incubated for 30 days and were shaken during the first 48 h of incubation. Each bottle was tested twice on days 1 and 2 of incubation, once a day on days 3 to 7 of incubation, and twice weekly thereafter for the final 3 weeks of incubation. On day 30, samples from all negative aerobic bottles were subcultured on blood agar plates, and the plates were incubated in CO<sub>2</sub> for up to 3 days. For two patients, *Brucella* spp. were isolated in two aerobic bottles on day 4; for another patient, *Brucella* spp. were isolated in two aerobic bottles on day 9, and for the last patient, only one aerobic bottle was positive for *Brucella* spp. on day 11.

Indirect diagnosis was assessed by the Rose Bengal test, microagglutination, and the Coombs test for *Brucella* spp. (bio-Mérieux). One acute-phase serum specimen was obtained from all patients. Two convalescent-phase serum specimens were collected 30 and 60 days after the onset of the disease. The Rose Bengal test was positive for all patients. The results of clinical, biochemical, and serological follow-ups are given in Table 1.

Treatment with doxycycline (100 mg given orally twice daily for 6 weeks) and streptomycin (1.0 g given intramuscularly four times daily for 3 weeks) was given to all patients. They became asymptomatic in a few days, and no complications or relapses were observed during the follow-up.

The Rose Bengal test and microagglutination serological test for *Brucella* spp. performed on serum samples from the remaining members of the laboratory staff were negative.

Members from the Preventive Medicine Department of the hospital carried out an investigation of the possible causes of the outbreak. No laboratory accident was reported during the period of the outbreak as a result of handling either strains of *Brucella* spp. or the blood cultures in which they were isolated. We performed a retrospective study of the 14 cases of *Brucella* bacteremia detected in 1988, with the highest incidence (78.5%) occurring between May and September. All of the strains of *Brucella* spp. isolated from the blood cultures of the affected individuals were identified as *Brucella melitensis* biotype 1 by the National Brucella Reference Centre at Valladolid, Spain.

Transmission of *Brucella* spp. to humans occurs by ingestion of unpasteurized dairy products or by occupational or avocational exposure to animals or laboratory cultures of *Brucella* spp. (2, 3). Transmission by the aerial route because of aerosol formation is widely documented (1, 3, 6, 7). Brucellosis is one of the infections that microbiologists have the highest risk of acquiring (5, 9, 13). Brucellosis is endemic in Spain, and as a consequence, the aerial route of transmission among microbiology laboratory workers could be of importance. However, laboratory-acquired infections are rarely diagnosed or reported.

The laboratory-acquired infections are not due to accidents in more than 80% of the cases (10). The probable source of infection may be apparent in many cases, but the form of transmission is often speculative (10, 12). The inhalation of infective aerosols produced accidentally or unintentionally by numerous microbiological techniques is the most frequent cause of acquisition of infection in the laboratory (10, 13).

The probable source of infection in the patients described here was the handling of blood cultures. All four affected technicians had been working in the room where the blood cultures were handled. No accident occurred in the laboratory at that time, and the blood cultures were handled correctly except that a biosafety hood was not used. Thus, we think that the transmission could have occurred by way of aerosols.

Microbiology laboratory infection hazards must be kept in mind by laboratory workers. Consequently, the need for strict observation of the safety rules when handling infectious material must be emphasized, especially when handling strains of highly infective microorganisms like *Brucella* spp.

All of those facts support the importance of the following universal recommendations. (i) Procedures known to produce

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TABLE 1. Clinical, biochemical, and serological data for four patients with brucellosis<sup>a</sup>

| Clinical feature                      | Patient        |                         |                                   |                   |
|---------------------------------------|----------------|-------------------------|-----------------------------------|-------------------|
|                                       | 1              | 2                       | 3                                 | 4                 |
| Sex/age (yr)                          | Male/32        | Male/37                 | Female/26                         | Male/37           |
| Date of onset of symptoms (mo/day/yr) | 06/01/88       | 07/26/88                | 09/11/88                          | 09/11/88          |
| Date of diagnosis (mo/day/yr)         | 06/08/88       | 08/02/88                | 09/16/88                          | 09/17/88          |
| Symptoms                              | Fever asthenia | Fever, chills, sweating | Fever, sweating, myalgia          | Fever, arthralgia |
| Biochemical changes                   | None           | GOT, 50; GPT, 59        | GOT, 202; GPT, 257;<br>LDH, 1,078 | None              |
| Antibody titer                        |                |                         |                                   |                   |
| Initial agglutinin                    | 1/40           | 1/40                    | 1/160                             | 1/160             |
| Agglutinin at 60 days                 | 1/40           | 1/40                    | 1/10                              | 1/20              |
| Coombs test at 2 mo                   | 1/40           | 1/160                   | 1/10                              | 1/20              |

<sup>a</sup> GOT, aspartate aminotransferase; GPT, alanine aminotransferase; LDH, lactic dehydrogenase.

aerosols should be minimized or should be conducted under a biosafety hood. (ii) Handling of biosafety level 3 organisms, such as *Brucella* spp., must be conducted under biosafety hoods and the plates should be sealed for safety when they are not in use (4, 12).

After the outbreak described here we instituted all of these safety measures, and no new cases have been detected.

We conclude that a combination of good microbiological techniques, appropriate use of biosafety hoods, and awareness of the danger of aerosol spread on the part of the laboratory workers could decrease the risk of laboratory-acquired infections.

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