

## *Brucella abortus* Infection Acquired in Microbiology Laboratories

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**We report an outbreak of laboratory-acquired *Brucella abortus* infection originating in the accidental breakage of a centrifuge tube. A total of 12 laboratory workers were infected (attack rate of 31%), with an incubation time ranging from 6 weeks to 5 months. Antibody titers were evaluated weekly in all personnel exposed, allowing the diagnosis of the infection in most cases before the onset of clinical symptoms, so that specific therapy could be administered.**

Brucellosis is a disease typically affecting individuals that work in contact with infected farm animals or with animal-derived tissues (17, 19, 20); the incidence of this disease has fallen in the countries that have attempted to eradicate the infection in animals. The food-borne transmission of brucella is well known and is especially common through the consumption of contaminated raw milk and cheese (2), while person-to-person transmission has been reported but remains extremely rare (15). Transmission occurs frequently via aerosol inhalation of infected fluid, allowing entry of brucella through the respiratory mucosa (8). A number of cases of laboratory-acquired infections have been reported (9–12, 15, 16, 18). Laboratory-associated infections represent 2% of reported cases of brucellosis (4, 5, 13), demonstrating the high risk of acquiring brucella infection in clinical microbiology laboratories where these highly infective bacteria are handled. The attack rate in cases of accidental laboratory exposure ranges from 30 to 100%, depending on the location of workers and the quantity of bacteria involved (1, 6, 7, 11, 22). The recommended treatment for acute infection is based on the combination of tetracycline or doxycycline with streptomycin or rifampicin for a period of 4 to 6 weeks.

We report on an outbreak of brucellosis in the Experimental Microbiology Laboratory of the Institute of Microbiology and Virology of the University of Sassari, Italy, after an accidental exposure to a laboratory strain of *Brucella abortus*. Between November 1990 and March 1991, a total of 12 people working at different locations in the laboratory developed acute brucellosis, with an attack rate of 31%. The outbreak originated from the accidental rupture of a polystyrene centrifuge tube containing live microorganisms during transfer of the tube from one room to another. The source of infection was a *B. abortus* biotype 1 atypical strain previously isolated from a camel. Immediately after the tube rupture, the person that caused the accident (patient 1) used directly applied 3% phenol solution and paper towels soaked with the same germicide to immediately decontaminate the area, wearing a single-use mask and rubber gloves. The laboratory was evacuated within 45 min, and the germicide was removed after 60 min by the same operator.

The accident occurred in the first week of October 1990, and despite the immediate application of all recommended safety

guidelines (14, 16), 6 weeks later three laboratory employees (including the one that provoked the accident) suffered from fever, chills, sweats, weight loss, malaise, headaches, myalgia, and arthralgia. Diagnosis of brucellosis was made by the Rose Bengal microagglutination test, and the serologic titer of anti-*B. abortus* antibodies was evaluated by using a standard tube agglutination test (21). The original *B. abortus* biotype 1 strain was obtained from blood samples of all the three infected persons after 5 to 10 days of cultivation by using the BACTEC NR-730 system (Becton Dickinson Laboratories); bacteria were isolated in 5% sheep blood agar and were then identified by using standard biochemical techniques. At the time of the first diagnosis, agglutination titers were 1:640 for patients 1 and 2 and 1:320 for patient 3 (Table 1).

All laboratory workers, including those working in other laboratories located on the same floor, the administrative office personnel, and people that visited the institute in the first week of October were then enrolled in a prospective study. Blood samples were taken weekly for the first 3 months following the accident, then monthly until December 1991, and sera were tested to detect specific anti-*B. abortus* antibodies by using the standard tube agglutination test.

Nine weeks after the centrifuge tube rupture, four additional employees (patients 4 to 7) (including a woman who worked in the administrative office), tested positive by the anti-*Brucella* agglutination test, with a titer ranging from 1:340 to 1:1,280. Symptoms began 2 (patient 7) to 5 (patients 4 to 6) days after the detection of antibodies and included fever, myalgia, and

TABLE 1. Antibody titers of patients with brucellosis

Patient no.	Titer on:					
	15 Nov. 1990	8 Dec. 1990	7 Jan. 1991	15 Mar. 1991	15 Apr. 1991	15 Dec. 1991
1	1:640	1:1,280	1:640	1:320	1:80	1:20
2	1:640	1:640	1:1,280	1:320	1:80	1:20
3	1:320	1:640	1:640	1:320	1:40	0
4	1:20	1:640	1:320	1:160	1:40	0
5	1:20	1:320	1:320	1:320	1:40	0
6	0	1:640	1:320	1:320	1:40	0
7	0	1:1,280	1:1,280	1:320	1:160	1:80
8	0	1:80 <sup>a</sup>	1:160	1:40	0	0
9	0	0	1:160	1:40	1:40	1:20
10	0	0	1:640	1:640	1:40	1:20
11	0	0	1:640	1:320	1:20	0
12	0	0	0	1:640	1:320	1:20

<sup>a</sup> Serological test performed on 16 December 1990.

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malaise. One week later, patient 8 showed an antibody titer of 1:80, and on 7 January 1991, three more workers tested positive by agglutination (patients 9 to 11), with antibody titers ranging from 1:160 to 1:640, as shown in Table 1. All seropositive patients were immediately treated with a combined antibiotic therapy (see below), in most cases before the appearance of symptoms. The last patient (patient 12) seroconverted after an incubation period of more than 5 months, presenting an antibody titer of 1:640 (Table 1). Also in this case, the antimicrobial therapy was administered before the onset of symptoms. In December 1991, 1 year after the outbreak, only patient 7 still tested positive for specific anti-*Brucella* antibodies, albeit at a low titer (1:80).

The incubation time of laboratory-acquired brucellosis ranged from 6 weeks to 5 months, in accordance with other reports (1, 18); there was no correlation between incubation time and the location of workers in the laboratory when the accident occurred, with the exception of patient 1, who perpetrated the accident and was the first to show signs of infection.

All symptomatic (patients 1 to 3) and asymptomatic but seropositive (patients 4 to 12) patients were treated immediately after seroconversion with a combination of 200 mg of doxycycline plus 600 mg of rifampicin every day for 6 weeks. Since the first group of patients (patients 1 to 3) experienced notable side effects at the beginning of therapy (including body temperature raised to as high as 40°C after every antibiotic administration and hallucinations), the workers who were treated next (patients 4 to 12) were administered low doses of cortisone during the first week of antibiotic therapy in order to avoid the toxic effects of endotoxin release.

Symptoms were milder in patients who received specific therapy before clinical manifestations of infection; fever, headaches, and chills were resolved in these patients in a few days, in contrast to the 2- to 3-week duration of these symptoms in patients 1 to 3. Anorexia, malaise, and myalgia or arthralgia generally lasted for 2 or 3 additional weeks in most patients.

The milder course of disease in patients who received antimicrobial therapy immediately after seroconversion and before clinical onset (patients 4 to 12) indicates the importance of a prompt diagnosis based on serological tests when an outbreak occurs in a laboratory. In this work, we show that seroconversion occurs in most cases before the onset of symptoms, thus allowing the administration of early treatment. These data emphasize the importance of monitoring the antibody titers in patients exposed to brucella.

The antibody titers of all 12 patients were monitored for more than 1 year after the laboratory outbreak; results shown in Table 1 clearly demonstrate that the antibody titer rises rapidly and decreases very slowly after antibiotic therapy, falling to undetectable levels only after 1 year. The decrease of antibody titer was faster in patients treated before the appearance of clinical symptoms.

No incomplete recovery, relapse, or complications occurred in any patient during the following 8 years. Ariza et al. reported a relapse rate of 38.8% in brucellosis patients treated with rifampicin and doxycycline (3). The absence of relapse or complications in all patients involved in the described laboratory outbreak could be related either to a low virulence for humans of the *B. abortus* strain that was isolated from a camel or to the prompt and effective therapy administered, especially in asymptomatic but seropositive patients.

These data highlight the importance of monitoring antibody titers of all individuals exposed to brucella, in particular in

large outbreaks of infection, when preventive treatment described (22) for small epidemics is not practicable.

The number of personnel affected by brucellosis confirms the high risk of transmission of the infection after a laboratory accident, despite the immediate applications of safety measures, and demonstrate the very fast aerosol spread of bacteria. The reported case of laboratory outbreak underlines the fact that *Brucella* species should be handled according to the most stringent safety measures, including the use of safety boxes for biohazard transport within laboratories, not only in clinical microbiology but also in research departments.

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