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Outbreak of *Brucella melitensis* among Microbiology Laboratory Workers in a Community Hospital

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From May to September 1988, eight employees of a microbiology laboratory developed acute brucellosis (attack rate, 31%). Seven of the eight affected employees had clinical illness ranging from a nonspecific, flulike illness to severe hepatitis. Blood cultures obtained from five of the affected employees (63%) were positive for *Brucella melitensis*, biotype 3. Comparison of cases and controls showed that there were no risk factors besides employment in the laboratory. Based on work locations, assignments, and interviews, it was found that person-to-person, droplet, food-borne, and waterborne spread were unlikely. Our investigation disclosed that 6 weeks before the outbreak began, a frozen brucella isolate from a patient hospitalized 3 years earlier had been thawed and subcultured without the use of a biologic safety cabinet. This clinical isolate was subsequently identified as *B. melitensis*, biotype 3, identical to the employee isolates. It is presumed that transmission occurred via the airborne route. This outbreak reemphasized that all work on *Brucella* species, an established biosafety level 3 organism, must be conducted under a biologic safety hood. Furthermore, it might be prudent to perform all clinical "setups" under a safety hood since aerosolization commonly occurs during the initial processing of specimens and the majority of these specimens are from patients with uncertain diagnoses.

Brucellae are small, nonmotile, gram-negative coccobacilli which cause abortions in a variety of animals including cattle, swine, goats, sheep, and dogs (10). Four species of *Brucella* are associated with systemic disease in humans: *B. melitensis* (found in goats and sheep), *B. abortus* (cattle), *B. suis* (swine), and *B. canis* (dogs). Human brucellosis is rare in the United States; 200 or fewer cases have been reported annually since 1980 (5), and only 8 cases were reported in Michigan from 1983 through 1987. Brucellosis is primarily an occupational disease affecting individuals working with infected animals or their tissues, especially farmers, veterinarians, and abattoir workers (3, 18, 20, 22). Sporadic cases and outbreaks also occur among consumers of unpasteurized milk or dairy products (2, 3, 12, 20, 22), and, on occasion, laboratory workers become inadvertently infected (1, 3, 8, 11, 13, 14, 19, 21, 22). We report here an outbreak of *B. melitensis* involving eight employees of a clinical microbiology laboratory. This outbreak illustrates the protean manifestations and variable incubation periods associated with acute brucellosis which make early diagnosis difficult. This outbreak makes it clear that laboratory personnel must use biologic safety cabinet for handling of materials which are potentially infectious if aerosolized (17).

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MATERIALS AND METHODS

Report of a case. In early October 1988, our Department of Epidemiology was notified of a confirmed case of brucellosis in an employee of the microbiology laboratory. This employee had initial onset of a self-limited, hepatitislike illness in July. A recurrence of fever 10 weeks later led to hospitalization. Serologic studies performed at this time were positive for brucellosis (anti-*Brucella* titer of 1:640 by a standard tube agglutination assay; Febrile Agglutinin Diagnostic—*Brucella abortus*, Fisher Scientific, Orangeburg, N.Y.) (9). Blood cultures were also found to be positive for presumptive *Brucella* species.

Epidemiologic investigation. Because of the possibility of an inadvertent laboratory exposure, serum *Brucella* agglutination titers were obtained for all microbiology personnel. Cases were defined by the presence of a *Brucella* agglutination titer of at least 1:160 and were characterized as clinical cases if the affected personnel had signs or symptoms compatible with brucellosis in the spring, summer, and early fall months of 1988. To determine whether exposure was limited to the microbiology laboratory and to identify potential risk factors for development of brucellosis, workers in adjacent clinical laboratories at the hospital were also evaluated by serologic testing and a questionnaire. All seropositive employees were evaluated clinically by an infectious disease physician, and three sets of blood cultures were obtained. Aerobic specimens were processed by using the Isolator system (Dupont, Wilmington, Del.) and plated on both chocolate and blood agar; anaerobic specimens were inoculated into BACTEC 7D bottles (Becton Dickinson, Diagnostic Instrument Services, Towson, Md.). Blood isolates were sent to the Centers for Disease Control for identification of species and biotyping. In analyzing the data, significance was estimated by using Fisher's exact test and χ^2 analysis with the Yates correction. Two-tailed tests were used for all comparisons.

RESULTS

Eight employees were found to have serologic evidence of infection with *Brucella* species. Seven of these infected employees had developed clinical illnesses consistent with

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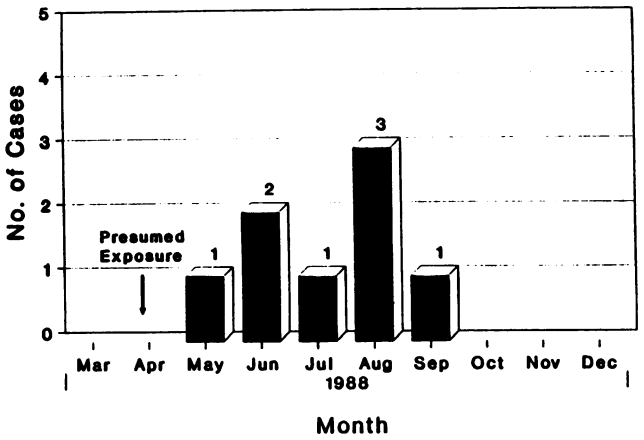

FIG. 1. Cases of brucellosis by month of onset, 1988.

brucellosis. One asymptomatic employee had mildly abnormal liver functions. In the earliest case, symptoms began in mid-May. Additional symptomatic cases occurred over the next four months (Fig. 1).

The clinical features which were observed in affected employees are summarized in Table 1. Myalgia and arthralgia, especially back pain, were reported by all but one of the seropositive employees; three-quarters experienced weight loss. Seventy-five percent also had abnormal liver function studies. Fever, malaise, anorexia, and headaches occurred commonly. These symptoms were initially felt to be due to a nonspecific viral infection when employees were evaluated by their personal physicians. Blood cultures from five of the eight (63%) seropositive employees were positive for *B. melitensis*, biotype 3, even though only one of these employees was acutely ill at the time of culture. Fourfold decreases in serologic titers were observed in all eight employees and confirmed recent infection in the three culture-negative employees. Only persons working in the microbiology laboratory developed brucellosis, where an overall attack rate of 31% was observed (8 of 26 microbiologists). None of 49 employees who worked in adjacent clinical laboratories developed brucellosis ($P < 0.001$).

TABLE 1. Clinical features observed in eight cases of brucellosis

Clinical manifestation	No. (%) of affected personnel
Symptoms	
Myalgia or arthralgia	7 (88)
Fever (temp, ≥38°C)	5 (62)
Anorexia or malaise	5 (62)
Chills	4 (50)
Headaches	4 (50)
Abdominal pain	4 (50)
Sweats	3 (38)
Signs	
Wt loss (≥2.5 kg)	6 (75)
"Hepatitis"[a]	6 (75)
Lymphadenopathy	2 (25)
Skin rash	2 (25)
Pneumonitis	1 (12)
Osteoarticular involvement	1 (12)

[a] Elevation of aminotransferases (serum glutamic oxaloacetic transaminase or serum glutamic pyruvic transaminase) ≥2 times upper limit of normal.

Interviews with affected employees, using case-control analysis, disclosed no risk factors besides employment in the microbiology laboratory. Affected employees had no common source exposures outside the laboratory, denied consuming unpasteurized dairy products, and infrequently processed nonhuman specimens. Affected employees were not more frequently involved in laboratory accidents such as specimen spills, sharp injuries, or mucous membrane exposures to body fluids and, like the controls, rarely used barrier precautions. No one in the microbiology laboratory recalled working with a *Brucella* isolate in the 2 years preceding the outbreak. During our investigation, a frozen *Brucella* isolate was found in the stock freezer with a date of 1 April 1988 on the label. This isolate was from a patient hospitalized in 1985 with brucellosis. Although a written work record on this isolate did not exist, we were able to verify that the isolate had been removed from the freezer, thawed, and replated to test for viability in the last few days of March, approximately 6 weeks before the first case of brucellosis occurred. The isolate was handled on an open workbench and not in a biologic safety cabinet. The original patient isolate and all employee isolates were identified at the Centers for Disease Control as *B. melitensis*, biotype 3.

An examination of work schedules revealed that all eight employees who developed brucellosis had been in the microbiology laboratory on 30 and 31 March, when manipulation of the *Brucella* isolate occurred, as opposed to only 5 of the 18 seronegative microbiology employees ($P < 0.01$). Affected employees worked at different stations in the laboratory (Fig. 2), supporting the hypothesis that airborne transmission occurred. Retrospectively, it was not possible to determine whether a laboratory accident had occurred during the processing of the specimen.

All symptomatic patients were treated either with 100 mg of doxycycline every 12 h for 4 weeks or with the combination of doxycycline plus an aminoglycoside (streptomycin, 1 g intramuscularly per day, or gentamicin, 1.7 mg/kg intravenously per day) for the initial 2 weeks. One patient with brucella spondylitis received 3 months of therapy with high-dose trimethoprim-sulfamethoxazole (480/2,400 mg/day) and rifampin (900 mg/day). One patient developed a clinical relapse requiring retreatment with the combination of trimethoprim-sulfamethoxazole and rifampin. Signs and symptoms resolved in all affected patients. Follow-up blood cultures were negative. The epidemic strain was highly susceptible to a wide spectrum of antibiotics including tetracycline (MIC, 0.5 μg/ml), gentamicin (MIC, 2 μg/ml), and streptomycin (MIC, 8 μg/ml) but only moderately susceptible to trimethoprim-sulfamethoxazole (MIC, 2/38 μg/ml) and rifampin (MIC, 2 μg/ml).

DISCUSSION

Transmission of *Brucella* species to humans occurs via direct contact with infected animals and by ingestion of unpasteurized dairy products (2, 3, 12, 18, 20, 22). Airborne spread via infectious aerosols has also been documented (1, 3, 7, 8, 11, 13, 14, 19, 21, 22). Occupational exposures occur primarily among veterinarians and personnel working at stockyards, dairies, and meat-packing plants. However, 2% of cases occur in research and clinical laboratories and brucellosis remains one of the most common infection risks faced by microbiologists (6, 15, 16).

Sporadic cases and even small clusters can be difficult to identify because of an extremely variable incubation period (weeks to months), a lack of distinctive clinical features, and

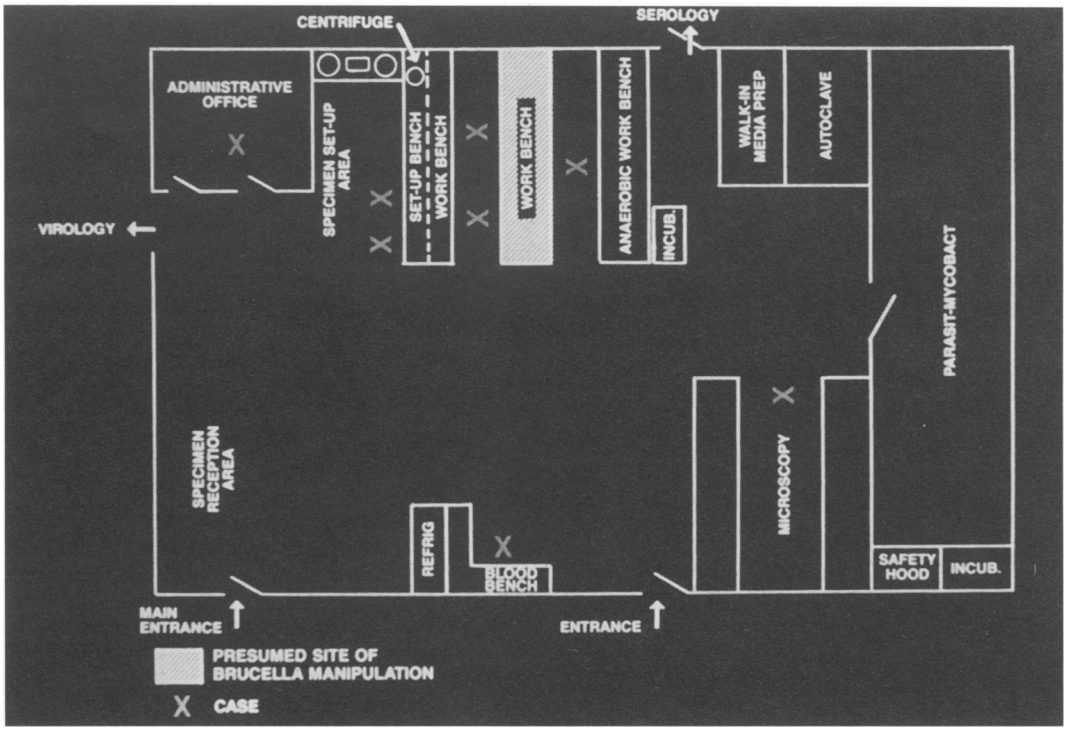

FIG. 2. Schematic of microbiology laboratory with cases (×) by predominant work location.

a varied clinical expression ranging from an abrupt illness in some to an insidious, relapsing, or even subclinical illness in others. In our outbreak, we observed incubation periods ranging from 6 weeks to over 5 months. Symptoms were nonspecific, and clinical expression varied, with some individuals experiencing overwhelming infection while others experienced mild illnesses or even subclinical infection.

Epidemiologically, this outbreak was most consistent with airborne spread. A review of laboratory practices revealed several which may have contributed to the inadvertent spread of *Brucella* species, a biosafety level 3 organism. Because of its relative rarity, many clinical microbiologists, especially those based at community hospitals, will have little, if any, experience with *Brucella* species or other "exotic" agents capable of spread via infectious aerosols. Consequently, knowledge of the degree of transmissibility of the organism may be lacking. As such, we make the following recommendations. (i) Procedures known to produce aerosols should be minimized or conducted under biosafety hoods. (ii) Universal precautions must be completely adopted and regularly monitored. (iii) All work on a presumptive or confirmed biosafety level 3 organism such as brucella must be conducted under biosafety hoods at all times (4, 17). Plates should be sealed for safety when not in use.

Furthermore, we advise that all clinical specimens submitted to a microbiology laboratory from patients with uncertain diagnoses be manipulated under a biosafety hood during initial setup—a time when risk of aerosol production may be highest. Many laboratory-associated infections are acquired from the production of infectious aerosols. With the combination of good microbiologic technique, appropriate safety equipment, and awareness on the part of the laboratory worker, the risk of infection to the laboratory worker can be greatly reduced.

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ADDENDUM

In March 1989, a laboratory worker at the Centers for Disease Control became infected while working with our isolates. A break in technique was not identified. Inspection of the laboratory revealed that the biologic safety cabinet was in working order.

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