Brucellae growing on Thayer–Martin medium: a source of inadvertent exposure for laboratory personnel in endemic areas

Brucellosis is a zoonotic disease that is endemic in many developing countries. It can be transmitted to humans, usually via the consumption of dairy products from infected animals (Pappas et al., 2005). The low infecting dose of Brucella species and the fact that the organism can enter the body by several routes relevant to laboratory practices, including the gastrointestinal and respiratory tracts, conjunctiva and abraded skin, make brucellosis the most common laboratory-acquired bacterial disease (Noviello et al., 2004). The bacteraemic character of the infection, and frequent seeding of the organism to bones and joints, mean that processing cultures of blood and skeletal system exudates poses the highest risk of transmission of brucellae to laboratory workers. However, human brucellosis can also affect other body sites, including the reproductive, urinary and central nervous systems and, therefore, a variety of other clinical specimens can contain viable Brucella organisms. The purpose of this communication is to report on the inadvertent exposure of laboratory technicians to Brucella melitensis during the processing of obstetric and gynaecological specimens. The identity of the organism was missed because of its growth on selective media used to isolate gonococci from genital cultures.

The Soroka University Medical Center (SUMC) is a 1200-bed facility serving the urban and rural inhabitants of southern Israel. The population of the region includes a large Bedouin minority in transition from a nomadic lifestyle to permanent settlements. Brucellosis is endemic in the Bedouin community, which maintains herds of unvaccinated sheep and goats, and an annual incidence of 152 laboratory-confirmed cases per 100 000 population was calculated for this community in 2012 (unpublished data). The Clinical Microbiology Laboratory of the SUMC provides diagnostic bacteriologic services to the inpatient population, as well as to 191 community clinics spread across the region. Blood cultures detected as positive by a Bactec 9240 instrument (Becton Dickinson) and all normally sterile body fluids (NSBFs) are dealt with in a class II biological safety cabinet. Genital and obstetric specimens are processed on an open bench and are routinely plated on trypticase soy agar with 5 % added sheep haemoglobin (blood agar), chocolate agar, and modified Thayer–Martin, MacConkey and Sabouraud media, and a thioglycolate tube is inoculated. Seeded media are incubated at 35 °C in a 5 % CO₂-enriched atmosphere. To increase laboratory safety and awareness, the identification details of all patients with recent positive Brucella serology are conveyed to the blood culture laboratories. In the three exposure events, the organism was missed because of its growth on the selective media used to isolate gonococci from genital cultures.

Between 2004 and 2013, three separate incidents of exposure to Brucella organisms occurred among laboratory technicians working at the genital cultures station of the SUMC Clinical Microbiology Laboratory. In each event, the organisms were recovered from female Bedouin patients living in the area. In the first patient, a diagnosis of brucellosis was reached only after the organism was isolated from a Bartholin’s gland exudate and identified. Clinical details of this patient have been published elsewhere (Peled et al., 2004). In the other two cases, the patients suffered from a febrile non-specific disease characterized by vague skeletal system complaints. By the time the organisms were detected in the genital cultures, the diagnosis had already been made based on the basis of a positive serological test and/or a positive blood culture, but this information was unknown to the personnel working at the genital cultures station.

In the three exposure events, the organism grew after 2–3 days on blood, chocolate and modified Thayer–Martin media as small, white, non-haemolytic colonies, and a Gram stain revealed small Gram-negative coccobacilli. Because of the growth on the presumptively selective Thayer–Martin agar, the true identity of the bacterium was not suspected for 3–4 days, and isolates were subjected to repeat subcultures and biochemical assays, including the dangerous aerosol-generating catalase test, on an open bench. The organisms were non-motile, exhibited positive oxidase, catalase and urease reactions, and did not ferment sugars. At this point, the possibility of a Brucella species was considered and all further work-up with the isolates was performed in a safety cabinet. The suspicion was confirmed by a positive agglutination test with specific antiserum (Wellcome Diagnostics) and the isolates were finally identified as B. melitensis by speciation with phages TB and Iz at the Kinner Veterinary Institute at Bet Dagan, Israel.

Post-exposure prophylactic therapy with 100 mg oral doxycycline twice daily for 14 days was administered to all three potentially exposed individuals. None of these individuals developed clinical disease and their serological tests for brucellosis remained negative on follow-up. The concentration of Brucella organisms in blood and human exudates is usually low, with a median of 88 c.f.u. ml⁻¹, and clinical specimens therefore probably pose a low risk for transmission (Yagupsky et al., 1997). The danger of contagion, however, increases exponentially after incubation on media, and routine bacteriological practices, such as preparing bacterial suspensions, centrifugation, vortexing and performing the catalase test, may create dangerous aerosols that can infect through the conjunctival and respiratory mucosal surfaces. In the three events described here, the technologists were unaware of the fact that Brucella organisms may grow on the modified Thayer–Martin medium used to facilitate detection of Neisseria gonorrhoeae.
(Paolicchi et al., 1991), resulting in extensive and unsafe manipulation of cultures and potential exposure of laboratory personnel to Brucella. While brucellar involvement of the female genitourinary tract is common in ruminants, among which the disease is commonly transmitted by the venereal route and causes septic abortions, infection of human female reproductive organs and miscarriages attributable to the organism are deemed rare (Porreco & Haverkamp, 1974). The present report suggests that the diagnostic laboratory could easily miss many of these infections.

Because of the substantial risk posed by brucellae, the Centers for Disease Control and Prevention (CDC, 1998) has explicitly recommended that all procedures with live cultures of the organism should be confined to a class II biological safety cabinet. However, by the time the identity of the isolate is definitely established, contamination of the laboratory environment and transmission to personnel may have already occurred. In addition, this recommendation is difficult to implement in poor countries because of a lack of expensive safety cabinets. It therefore seems prudent to advise that, in areas endemic for brucellosis, all positive obstetric and gynaecological cultures, including those exhibiting growth on Thayer–Martin media, should be processed in a safety cabinet, where available, pending final identification of the isolate. Even where this safety equipment does not exist, simple and inexpensive measures may help in reducing the risk of contagion.

Communication with attending physicians should be improved and the clinical microbiology laboratory should be informed in advance when clinical specimens have been obtained from patients with risk factors for brucellosis or presenting with a clinical picture suggestive of the disease. Better communication within the laboratory should also be encouraged, particularly with personnel working at the serology station. Familiarity of laboratory technicians with the characteristics of Brucella, the differences in colony morphology between brucellae and gonococci, and the safe handling of cultures should be strengthened and maintained through periodic education.

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