Case Report

Isolation of *Brucella melitensis* from an abscess on the left foot of a 3-year-old infant

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Received 20 June 2008
Accepted 14 October 2008

An unusual case of a 3-year-old girl with a brucellar foot abscess is reported. Although direct microscopic examination of samples from the lesion did not reveal micro-organisms of any kind, a 7 day culture of caseous material yielded small colonies of Gram-negative coccobacilli in Löwenstein–Jensen medium. These were biochemically and molecularly identified as *Brucella melitensis*. The possibility of foot abscess being caused by *Brucella* should be considered in countries where brucellosis is endemic.

Introduction

The genus *Brucella* consists of alpha-proteobacteria that cause a zoonosis known as brucellosis, acquired mainly through direct contact with infected animals, or the ingestion of raw milk and unpasteurized dairy products. Brucellosis is a major infectious disease distributed throughout the world. *Brucella* is an intracellular facultative pathogen able to persist for long periods of time within the host and establish a chronic disease (Pappas *et al.*, 2005). Ingestion of unpasteurized dairy products is the most common way to acquire *Brucella* in Mexico (López-Merino, *et al.*, 1992; Luna-Martínez & Mejía-Terán, 2002). During the last 5 years a total of 12 447 human cases have been recorded in Mexico (according to the Dirección General de Epidemiología, Secretaría de Salud, Mexico, <http://www.dgepi.salud.gob.mx/>), 14.6 % affecting children <1–14 years old and 41 % affecting people 25–44 years old. In Mexico, 93 % of human brucellosis cases are due to *Brucella melitensis* acquired from goats, and a further 5 % are of bovine origin (Luna-Martínez & Mejía-Terán, 2002). *B. melitensis* has been isolated in a large number of paediatric cases of brucellosis in different countries: reports from Iran, Greece and Saudi Arabia indicate that the ingestion of raw milk or dairy products is the main source of infection (Nabi & Mir, 1992; Feiz *et al.*, 1978; Giannakopoulos *et al.*, 2006; Al-Eissa *et al.*, 1990). In this report we present a case of *B. melitensis* isolated from an abscess of the left foot from a child of 3 years old.

Case report

A 3-year-old girl was admitted to the Hospital Infantil de México 'Federico Gómez' in July 2001 with a painful lesion on the skin of the interior side of her left foot (Fig. 1, Supplementary Fig. S1 available with the online journal). Forty-five days before, without any medical attention, the girl suffered a trauma with an unidentified object that caused the lesion. She lived on a ranch in contact with cattle and had been drinking unpasteurized cows' milk since the age of 1 year. It is likely that the original lesion became contaminated with manure or other animal material. The patient was initially treated in the hospital as an outpatient (18 June 2001). At that time, the lesion exhibited inflammation and erythema, and was causing pain. Hepatomegaly and splenomegaly were not observed. The first diagnosis was pyomyositis, and the patient received 100 mg dicloxacilin kg⁻¹ orally daily for 7 days. On 25 June, the girl was checked again and the lesion showed caseous material as found in an abscess produced by mycobacteria. A sample of the caseous material was cultured on Sabouraud, blood and chocolate agar plates; Löwenstein–Jensen medium (with 2 % malachite green) was also included to look for mycobacteria. A biopsy was taken from the lesion and examined by differential staining. On 29 June, the girl was checked out and results of cultures were analysed. As clinicians were expecting fast-growing bacteria, blood and chocolate plates were discarded after 24 h of incubation. Sabouraud plates were negative after 30 days of incubation at 28 °C. Periodic acid–Schiff, haematoxylin–eosin, Grocott, acid-fast and Gram stains did not reveal any micro-organism in the biopsy specimen. Localized chronic inflammation and

A colour figure of the skin lesion is available as supplementary material with the online version of this paper.

Fig. 1. Skin lesion caused by *B. melitensis*.

ulceration, typical of a fungal infection, were observed at the site of the lesion. The biopsy specimen showed infiltration of lymphocytes, polymorphonuclear leukocytes and eosinophils in the dermis and hypodermis layers. At this time the patient received 100 mg cefalexin kg^{-1} daily for 5 days.

On 2 July, after 7 days of incubation at 35 °C, small colonies of Gram-negative coccobacilli were observed on Löwenstein–Jensen medium (Keness *et al.*, 1993). The colonies were typed following standard bacteriological procedures (Alton *et al.*, 1988) and were identified as *B. melitensis*. PCRs were used to detect the presence of gene *bscp31* (Baily *et al.*, 1992) and insertion sequence IS711 (Bricker & Halling, 1994), specific for *Brucella* spp.; both amplicons, of 231 and 731 bp, respectively, were observed (Fig. 2).

The patient was admitted to the hospital on 10 July, with a diagnosis of brucellosis. A Rose Bengal test was positive,

Fig. 2. PCR products amplified from the clinical isolate of *B. melitensis*. Lane 1, 1 kb Plus DNA ladder (Invitrogen); lane 2, *bscp31* amplification; lane 3, negative control for *bscp31* amplification (water containing no target DNA); lane 4, IS711 amplification; lane 5, negative control for IS711 amplification (water containing no target DNA).

and the standard tube agglutination testing for brucella antibodies was positive at a titre of 1 : 320. A general blood test, C-reactive protein levels and the erythrocyte sedimentation rate were normal. Blood and bone marrow cultures were negative after 30 days of incubation. The patient was treated with 20 mg trimethoprim–sulfamethoxazole kg^{-1} per day and 15 mg rifampicin kg^{-1} per day, for 4 weeks, and 15 mg gentamicin kg^{-1} per day for 2 weeks. The skin lesion resolved 4 weeks after antibiotic treatment had been initiated. Follow up of the patient 1 year later showed normal skin on the left foot and a negative agglutination test.

Discussion

A skin infection caused by *B. melitensis*, most likely traumatically inoculated (as the signs and symptoms normally present in brucellosis caused by *B. melitensis* were absent) has been reported here. Fever has been the predominant presenting feature in most reports of childhood brucellosis; in this context, Benjamin & Annobil (1992) studied 157 children with brucellosis and 100 % of them had a history of fever, while in another report 82.4 % from 114 paediatric brucellosis cases presented with fever (Nabi & Mir, 1992). In our case, the infection was clearly localized to the abscess, and the occurrence of hepatomegaly, splenomegaly or osteoarticular complications was not observed. This case denotes the potential for this pathogen and related bacteria to cause infections that are overlooked. In this case, the unexpected growth on Löwenstein–Jensen medium led to the detection and identification of a pathogen that was not suspected. Although it is known that cattle dealers, butchers, veterinarians and farmers have the occupational risk of acquiring brucellosis through broken skin if in contact with infected animals (Madkour, 1989), skin infections are not contemplated. Metin *et al.* (2001) reported 5.71 % cutaneous manifestation in 103 adults with brucellosis, while Akcali *et al.* (2007) observed 13.59 % from 140 cases of adults with brucellosis. Mantur *et al.* (2004) observed complications by skin lesions in 3 children aged 14 years and younger from a total of 93 brucellosis cases (3.23 %). In areas where brucellosis is endemic, it is important to take account of *Brucella* spp. as a potential causative agent of cutaneous abscesses, when other, more common, aetiologies have been discarded.

Acknowledgements

A.C.R. and A.L.M. were supported by fellowships from COFAA, SIP-EDI and CONACYT. This work was partially supported by SIP-IPN grant no. 20080733 and CONACYT repatriation grant no. 76395.

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