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SHORT COMMUNICATION

The first report of *Brucella suis* biovar 1 isolation in human in Turkey

Murat Kutlu[a,*], **Nural Cevahir**[b], **Sevil Erdenliğ-Gürbilek**[c], **Şerife Akalın**[a], **Mehmet Uçar**[a], **Selda Sayın-Kutlu**[a]

[a] *Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey*
[b] *Department of Medical Microbiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey*
[c] *Department of Microbiology, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey*

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Summary
Background: *Brucella melitensis* and *B. abortus* are the species generally isolated from human samples in Turkey. Several studies have also demonstrated the presence of antibodies against *B. canis*.
Case report and study: *Brucella* spp. was isolated from blood culture from a 35-year-old male with clinical signs and symptoms of acute meningitis, including fever lasting for 1 week. Multiplex PCR demonstrated *B. suis*, and biochemical features indicated biovar 1.
Conclusions: This report is the first emphasizing that *B. suis* should be considered among the causes of brucellosis in Turkey.

Introduction

Brucellosis remains an endemic zoonosis in Middle Eastern and Mediterranean countries, including Turkey. *Brucella melitensis*, *B. abortus* and *B. suis* are responsible for a substantial proportion of infections in humans. Although observed less frequently, *B. canis* and *Brucella* species from aquatic mammals can cause brucellosis in humans [1].

* Corresponding author at: Department of Infectious Diseases and Clinical Microbiology, Pamukkale University, Faculty of Medicine, 20070 Denizli, Turkey. Tel.: +90 0258 2118585 5767; fax: +90 0258 2134922.
E-mail address: muratkutlu72@yahoo.com (M. Kutlu).

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The relative abundance of *Brucella* species vary among geographic regions. *B. melitensis* is the most common species isolated in the Middle East, whereas *B. abortus* and *B. suis* are more frequently isolated in regions of South America, the USA and many European countries [1]. In Balkan countries, such as Greece, *B. melitensis* is a more common cause of infection in humans than is other *Brucella* species [2]. Similarly, in Turkey, *B. melitensis* accounts for the majority of isolated species, while *B. abortus* is isolated less frequently [3,4]. Moreover, the presence of antibodies against *B. canis* was demonstrated in humans previously [5].

However, there have been no reports from Turkey demonstrating *B. suis* infection in animals or humans. In this study, we report the first case of acute *Brucella* meningitis from Turkey in which the infectious agent was *B. suis*.

Case report and study

Case

A 35-year-old man was admitted to the hospital with a 7-day history of fever, chills and shivering, fatigue, low back pain, and headache. He was a sheep and cattle breeder and reported hunting wild boars several times in the last 6 months and feeding the boar meat to his dogs. Upon physical examination, his fever was 39.2 °C, and he exhibited confusion. He had neck stiffness and positive Kernig's sign. Laboratory results were hemoglobin 15.5 g/dl, hematocrit 46.8%, white blood cell count 7290/mm^3, platelet count 257,000/mm^3, and C-reactive protein (CRP) 10.1 mg/dl; other biochemical tests were within the normal ranges. The contrast-enhanced cranial and lumbar magnetic resonance imaging results were normal. CSF leukocyte count was 10/mm^3, and the CSF glucose level was 69 mg/dl (simultaneous serum glucose was 121 mg/dl) and protein level was 59 mg/dl. He was started on empirical antibiotic treatment with ceftriaxone 2× 2 g, doxycycline 2× 100 mg and rifampicin 1× 900 mg for *Brucella* meningitis because of his occupation and hunting activities, as well as clinical presentation associated with Rose Bengal positivity. Serum *Brucella* tube agglutination test (SAT) was positive at 1/80 titer, and SAT with Coombs antiserum was positive at 1/160 titer, whereas a CSF *Brucella* tube agglutination test was negative. On the third day of blood culture, growth was detected. Gram staining revealed Gram-negative coccobacilli. Samples from blood culture were plated on blood agar, eosin-methylene blue (EMB) agar and chocolate agar. Inoculated plates were incubated at 37 °C in normal atmospheric conditions and with the addition of 10% CO_2 for 4–5 days. At the end of the incubation, small, convex, non-hemolytic colonies were observed in the blood and chocolate agars. EMB agar was negative for growth.

Identification and biotyping

The culture was identified in a three-stage procedure [6]. In stage 1, the isolate was checked for colony morphology by stereomicroscope and for agglutination with neutral acriflavin (0.1%, w/v) (Sigma A8126). In stage 2, for species determination, the following tests were performed on all isolates: serum requirement for growth, oxidase and urease production and lysis with Tbilisi phage at routine test dilution (RTD) and $10^4 \times$ RTD and R/C phage at RTD. In stage 3, for biotyping, the production of H_2S, CO_2 requirement for growth, growth in media containing thionine (T3387, Sigma) (20 μg/ml), basic fuchsine (115937, Merck) (20 μg/ml), and safranin O (S2255, Sigma) (100 μg/ml) dyes, agglutination with A and M monospecific antisera, and R antiserum were investigated.

Molecular typing

Molecular typing of *Brucella* species by multiplex PCR (Bruce-ladder) was undertaken using the method described by Mayer Scholl et al. [7]. For extraction of bacterial genomic DNA, a loopful of bacterial culture was taken from the petri plate and re-suspended in 200 μl sterile distilled water, which was mixed and incubated at 99 °C for 10 min and finally centrifuged at 12,000 × *g* for 20 s. The resulting supernatants were used as the DNA template for Bruce-ladder. The assay was carried out in a 25 μl reaction mixture containing 2× Qiagen Multiplex Master Mix (Qiagen, Germany), 0.2 μM of each primer in cocktail of nine primer sets and 1 μl template DNA. Amplifications were initiated by denaturing the sample for 15 min at 95 °C followed by template denaturation at 94 °C for 30 s, primer annealing at 58 °C for 90 s, and primer extension at 72 °C for 180 s for a total of 30 cycles. After the last cycle, samples were incubated for an additional 10 min at 72 °C. Amplification products were separated on 1.5% agarose gels.

Results

The isolated strain showed a profile matching *B. suis* (Fig. 1), and the isolate was identified as *B. suis*