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SHORT COMMUNICATION

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

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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*

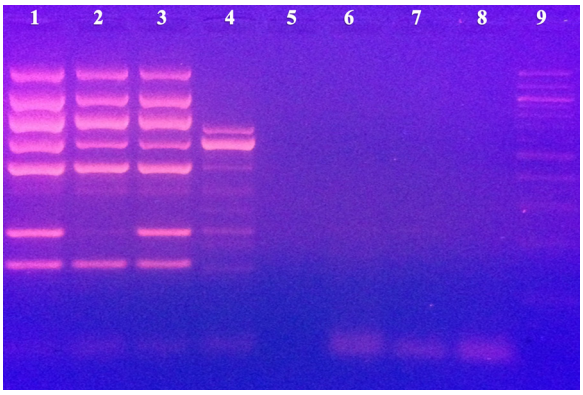

Figure 1 Lane 1: Isolated strain, lane 2: *B. melitensis* 16 M, lane 3: *B. suis* 1330, lane 4—7: test isolates, lane 8: negative control, lane 9: ladder. Lane 1 shows *B. suis* profile, while the rest of the test strains were negative for any known *Brucella* species.

biovar 1 based on conventional biotyping procedures (Table 1).

Discussion

This report is the first to describe a patient with *Brucella* infection due to *B. suis* in Turkey. Detection of *Brucella* species is of critical importance in evaluating pathogen prevalence and disease risk. In countries where domestic animal brucellosis has been eradicated, wild animals may be sources of *B. melitensis*, *B. abortus* or *B. suis* infection in humans and domestic animals [8,9]. Transmission of *B. suis* to domestic animals from wild boars and wild rabbits has been reported in the USA and several European countries [9—11]. Although *B. suis* is among the causes of brucellosis in pigs and humans in Southeast Asia and India, there are no data on *B. suis* infection in animals or humans in Turkey or in neighboring Middle Eastern countries [1,3,12,13]. Studies of the control of animal brucellosis in Turkey have been conducted for many years, and a new period began in 2009 with the involvement of the European Union [13]. Despite claims of an increase in the number of wild boars, there are no current data on many species of wild boars in Turkey. Therefore, data regarding the number of wild boars and the prevalence of *Brucella* infections in the boar population may be beneficial when considering possible transmission between wild and domestic animals.

Accurate diagnosis of brucellosis is generally sufficient to decide the treatment, and subtyping of *Brucella* species is not necessary in daily practice [14]. However, there are many misdiagnosed cases

Table 1 Classical biotyping results of reference and test strains.

	CO_2 requirement	H_2S production	Thionine sensitivity	Basic fuchsin sensitivity	Safranin O sensitivity	Tbilisi phage lysis RTD 10^4	A monospecific Sera aggl.	M Monospecific Sera aggl	R Sera Aggl.
Isolated strain	—	+	+	—	—	+	+	—	—
B. suis 1330	—	+	+	—	—	+	+	—	—
B. melitensis 16 M	—	—	+	+	+	—	+	—	—

of brucellosis. Carrington et al. reported a brucellosis case due to *B. suis* in a pig hunter, which resulted in death because of mistreatment due to misdiagnosis of infection with *Ochrobactrum anthropi* by automated diagnosis methods [15].

Conclusions

B. suis is one of the species responsible for brucellosis in Turkey and should be considered to be a candidate infectious agent, particularly in patients who report outdoor activities, such as wild boar hunting. In addition, *B. suis* infection may be endemic but undiagnosed in wild animals in Turkey; therefore, further epidemiological studies are required to evaluate the need for preventive actions in public health.

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Competing interests

None declared.

Ethical approval

Not required.

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