Case Report

Isolation of *Brucella melitensis* biotype 3 from epidural empyema in a Bosnian immigrant in Germany

Dennis Tappe,¹ Falk Melzer,² Gernot Schmook², Mandy Elschner², Thiên-Trí Lâm¹, Marianne Abele-Horn¹ and Christian Stetter³

¹Institute of Hygiene and Microbiology, University of Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany
²Friedrich-Loeffler-Institute, Naumburger Str. 96a, 07743 Jena, Germany
³Department of Neurosurgery, University Hospital of Würzburg, Josef-Schneider-Str. 11, 97080 Würzburg, Germany

Brucellosis is a regionally emerging infectious disease in Mediterranean countries with an increasing number of human cases and high morbidity rates. Here, we describe a case of severe *B. melitensis* biotype 3 infection in an immigrant who had contact with ruminants during a short-term stay in Bosnia before he returned to Germany. The patient developed thoracic spondylodiscitis accompanied by a large epidural empyema and neurological deficits. The isolated strain was characterized and compared to other strains from the Mediterranean region by multiple locus variable number of tandem repeat analysis (MLVA).

Case report

In September 2010, a 37-year-old Bosnian immigrant experienced severe pain in the thoracic spine. Despite physiotherapy and analgesic treatment for 3 weeks, increasing pain led to hospitalization. The patient developed progressive numbness in both legs and urinary retention and was transferred to our neurosurgical department at the end of the month. Physical examination showed severe local pain in the mid-thoracic spine, lower limb paraparesis with a sensory level T9, ataxia, increased deep tendon reflexes on the left side and a positive Babinski sign. There was no fever or meningismus. White blood count was normal and C-reactive protein was slightly increased (3.50 mg dl⁻¹, normal, 0.5 mg dl⁻¹). T2-weighted magnetic resonance imaging (MRI) of the spine showed a hyperintense lesion in vertebra T6 and T7 and an epidural mass extending from vertebra T5 to T8. Prior to surgery, the patient received levofloxacin and ampicillin/sulbactam for suspected epidural empyema. Upon further questioning, the patient reported two short-term stays near Tuzla, Bosnia, in April and August, where he had been in close contact with sheep and goats at the home of his mother. The animals had

Introduction

Brucellosis is an important zoonosis caused by Gram-negative bacteria of the genus *Brucella*. The disease is endemic to many Mediterranean countries and *Brucella melitensis* infection is a regionally emerging infectious disease in the Federation of Bosnia and Herzegovina, with an increasing number of human cases (>770 in 2008; Obradovic & Velic, 2010) and high morbidity rates. *B. melitensis* is found most frequently in sheep and goats and brucellosis has become an important health problem linked to direct occupational exposure to livestock in rural areas as well as consumption of unpasteurized dairy products in urban environments (Obradovic & Velic, 2010).

Here, we describe a severe infection in an immigrant returning to Germany after visiting relatives in Bosnia. The patient developed thoracic spondylodiscitis with epidural empyema and neurological deficits. The *B. melitensis* strain recovered was characterized as biotype 3 and compared to other isolates by multiple locus variable number of tandem repeat analysis (MLVA).

Abbreviations: MLVA, multiple locus variable number of tandem repeat analysis; MRI, magnetic resonance imaging.
been kept in a barn directly attached to the family’s residence and the patient had been around them on multiple occasions. He could not recall if there had been any sick animals. Husbandry tasks, such as milking or delivering new-born animals had not been performed by him. The patient denied having had fever or nausea.

After 7 days of culture, growth of catalase- and oxidase-positive coccoid Gram-negative rods was noted on blood agar. Serology for brucellosis was highly positive with a complement fixation test result of 1:16. At that time, the patient reported residual lower limb ataxia.

Discussion
Since 2001, the number of human *B. melitensis* infections has rapidly increased in Bosnia and Herzegovina and mass vaccinations of livestock were started in 2009 (Obradović & Velić, 2010). The country was free of brucellosis from 1980 until 2000. Since then, infections have been recorded in almost the entire territory (Obradović & Velić, 2010). Animal farms and human habitats are closely related (Tandir et al., 2008) and the number of reported outbreaks of brucellosis in ruminants was paralleled by an increase in human infections (Šeric-Haracic et al., 2008). Brucellosis inflicts high costs to the economy as a result of animal reproductive failure, medical treatment for humans and employee absenteeism (Obradović & Velić, 2010). Brucellosis causes systemic symptoms, most often fever and malaise, and can involve many organs. Osteoarticular manifestations are frequent focal complications (Al Dahouk et al., 2007a). As observed in other Balkan countries, the male population accounted for most cases of brucellosis reported in Bosnia and Herzegovina (71%) and infection resulted from occupational exposure (Obradović & Velić, 2010). Inhalation of contaminated dried birth products and secretions or skin cuts and abrasions after contact with infected livestock are known occupational risks and constitute a major route of human infection, also in Bosnia and Herzegovina. The incidence rate of human brucellosis sharply increased from 3.83 per 100 000 inhabitants in 2004 to 33.4 per 100 000 inhabitants in 2008. The age group of 35–45 years accounted for 48% of these cases (Obradović & Velić, 2010). In Germany, a country which was declared brucellosis-free in 2000, the epidemiology changed from a former occupational endemic infection to a travel-associated food-borne zoonosis, mainly affecting Turkish immigrants (Al Dahouk et al., 2007a). Since 2001, between 19 and 37 cases of brucellosis per year have been diagnosed in Germany. Accordingly, *B. melitensis* strains isolated in Germany are most often of the East Mediterranean genotype (Al Dahouk et al., 2007b). As was the case here, onset of symptoms often occurs after the end of the German summer holiday season (August/September) and coincides with the return of immigrants from disease-endemic countries where the peak of human brucellosis cases is in June and July (Al Dahouk et al., 2007a).

All *Brucella* species are highly related at the genetic level. They exhibit identical 16S rRNA gene sequences and share highly similar genomes, suggesting a monospecific genus.
with *B. melitensis* as the sole true species with several biovars. However, the old taxonomy describing several nomenspecies and biovars was kept (Scholz et al., 2010). The MLVA genotyping database for *Brucella* species (http://mlva.upsud.fr/brucella/) has registered 96 isolates of *B. melitensis* with the same combination of MLVA-8 (genotype 43) and MLVA-11 (genotype 125) results. Most of these isolates are strains from the Eastern Mediterranean region and differences between our strain compared to these isolates were visible in the more variable loci Bruce 04, Bruce 09, Bruce 16, Bruce 30 and Bruce 06 only. These data show that the isolated strain of *B. melitensis* differs from, but is closely related to, emerging strains from the Balkan region. The closest related strain was isolated from a human in Bosnia in 2003 and is different in locus 16 (7 repeats) only. It is a current topic of discussion, at an expert level, that isolates showing differences in only one locus might be considered as the same strain. Based on this assumption, it seems possible that both Bosnian patients were infected by the same *B. melitensis* strain. Most of the above-mentioned closely related isolates from the Eastern Mediterranean region are of human origin. They were mainly isolated in Turkey and Lebanon. Only a few isolates are of animal origin (cattle from Greece and sheep from Syria). In a recent study (Gwida et al., 2012), isolates from people with a Turkish background revealed epidemiological concordance with 20 sheep isolates originating from Eastern Anatolia, Turkey.

This report underlines the proposal that immigrants from areas where brucellosis is endemic who present with infections of the skeletal system should undergo rapid diagnosis for brucellosis, a process which is often delayed (Al Dahouk et al., 2007a).

**References**


