The first case of *Mycobacterium terrae* infection in cattle in Bosnia and Herzegovina

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Abstract

We here describe a case of *Mycobacterium terrae* infection in a cow in a small dairy household. At necropsy of the cow otherwise positive on routine annual tuberculin skin testing, severe multifocal granulomatous dermatitis and panniculitis, and moderate granulomatous (parasitic) colitis were observed. Histopathology revealed granulomatous dermatitis. Bacteria isolated from regional and thoracic lymph nodes, and skin lesions were identified by colony morphology, biochemical testing and molecular methods as *M. terrae*. Our findings confirm the difficulties that non-tuberculous mycobacteria can cause *in vivo* diagnosis of mycobacterial infections, especially in conjunction with parasitic infestations.

Keywords

Cattle — *Mycobacterium terrae* — Granulomatous dermatitis — Bosnia and Herzegovina

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Introduction

Non-tuberculous mycobacteria (NTM) are group of “atypical” mycobacteria not belonging to those classified in the *Mycobacterium tuberculosis* complex. Their potential pathogenicity for humans and animals has long been recognized, especially in individuals with immunodeficiencies (1). Unlike in humans, the distribution and epidemiology of NTM in animals is poorly understood (7). However, many of NTM species were isolated from different animal hosts (1). It is assumed that natural infections with these mycobacteria may influence the development of tuberculosis in different animal hosts (3). Also, immune responses induced by NTM may interfere with current diagnostic tests (skin test and γ-interferon test) for bovine tuberculosis (12, 13), and with efficacy of the *M. bovis* BCG vaccine against tuberculosis (3). *Mycobacterium terrae* is a slow-growing, non-photochromogenic species of *Mycobacterium* (10). Although commonly regarded as non-pathogenic in humans, *M. terrae* isolates can cause serious skin infections (hand infections) as well as the infections of the joints, tendons, lung, gastrointestinal and genitourinary systems. These infections are regarded as relatively resistant to antibiotic therapy (4, 9). In animals, *M. terrae* was isolated in two out of 570 nasal and pharyngeal swab samples from cattle and buffaloes in South Africa, and represents one of four most frequently isolated NTM (7). It was isolated from tuberculosis-like skin lesions on the udder of a cow, but its pathologic role is still unknown (17). Little is known about cattle tuberculosis in Bosnia and Herzegovina. It was considered to be eradicated from the former Yugoslav Federation in 1973, with sporadic outbreaks recorded until 1990. No information is present about the war period (1992–1996), and only two outbreaks were documented between 1996 and 1999 (14). Recently, several outbreaks of bovine tuberculosis caused by *M. caprae* were recorded in three different municipalities (Livno, Tomislavgrad and Vitez) of Bosnia and Herzegovina (2). To the authors’ knowledge, there is no data about animal NTM in Bosnia and Herzegovina. Thus, the aim of the present report is to describe a case of *M. terrae* infection in a cow on a small dairy household in the municipality Vitez.

Material and Methods

A cow in a small household herd in the municipality Vitez in Central Bosnia and Herzegovina was tested positive on routine annual tuberculin skin test (TST). Later, comparative tuberculin skin test confirmed the previous result. Prior to euthanasia the cow was clinically examined. Later, complete necropsy of the cow was carried out near the farm. Samples collected for histopathology (subcutaneous granulomatous lesions, regional and thoracic lymph nodes, and colon) were fixed in 10% buffered neutral formalin for 24 hours, routinely processed and embedded in paraffin blocks. Multiple semithin sections (4-5 micrometers) were cut, stained with hematoxylin, eosin and Ziehl-Neelsen (Z-N) for microscopic evaluation. Following homogenization, decontamination and concentration, the material (subcutaneous granulomatous lesions and lymph nodes) was inoculated onto four Löwenstein-Jensen slants (two of them supplemented with glycerol) and one Stonebrink slant, which were checked for growth once a week for eight weeks (18). A PCR test targeting the heat shock protein 65 (*hsp65*) gene was employed in order to identify the colonies as the members of the genus *Mycobacterium*. Primer set TB1 (5'-GAG-ATC-GAG-CTG-GAT-CC-3') and TB2 (5'-AGC-TGC-AGCCCA-AAG-GTG-TT-3') and the protocol described previously (8). Later, isolates were characterised to the species level. The identification
of isolated NTM was performed by means of DNA hybridization test (GenoType® CM/AS; Hain Lifescience) and by phenotypical methods. GenoType® CM/AS was performed according to manufacturer’s instructions (18). Test procedures include DNA isolation from cultivated mycobacteria, amplification using biotin-labeled primers and reverse hybridisation. The final identification of these mycobacterial isolate at species level was done by niacin production, growth rate on different temperatures (25°C, 31°C, 37°C and 42°C), morphology of colonies, pigment production in the dark and after exposure to light, and standard biochemical tests like catalase, nitrate reduction, Tween 80 hydrolysis at 5 and 10 days, arylsulphatase at 3 and 21 days, growth on Lowenstein Jenson (L-J) medium containing 5% NaCl and utilization of carbon sources (citrate, mannitol, inositol) (11).

Results

The cow was healthy and in good body condition. No clinical signs of chronic illness were noted. No reactions were observed on auscultation and percussion of the chest cavity. At necropsy, severe multifocal granulomatous dermatitis and panniculitis were observed. Observed granulomatous foci were up to 2 cm in diameter and filled with granular, yellow caseous material (Fig. 1). Foci were surrounded with thin haemorrhagic rim. Furthermore, moderate to severe granulomatous (parasitic) colitis characterised by numerous up to 0.5 cm nodular foci protruding on the colonic serosal surface, was also noted. Histopathology revealed granulomatous dermatitis characterised with caseous necrosis, mineralization and thick inflammatory infiltrate (lymphocytes, macrophages and neutrophils) surrounding the foci (Fig. 2). Z-N staining was negative for acid fast bacteria. In colon, multiple granulomatous foci with centrally located cross sections of nematode parasites (not identified, presumably Bunostomum sp.) were observed.

After 32 days of incubation at 37°C on Löwenstein-Jensen slants supplemented with glycerol suspicious colonies were found from the thoracic lymph nodes and granulomatous foci from the skin material. Molecular identification by conventional PCR targeting the hsp65 gene was positive to Mycobacterium sp. Isolate was classified in NTM by positive result by specific hybridization with GenoType® CM/AS. The growth of the strain was examined on Löwenstein-Jensen (L-J) egg medium and 7H10 medium incubated at 25, 31, 37 and 42°C. The strain was growing at 25°C and 37°C but not at 42°C. According to the rate of growth and pigment production, it was classified as non-chromogenic mycobacteria. Due to the positive test for Tween 80 hydrolysis, 68°C and SQ catalase, nitrate reduction, β-galactosidase, and negative test for niacin production, growth in the presence of 5% NaCl, pyrazinamidase production, 3d arylsulphatase, and tellurite reduction it was further characterised as M. terrae.

Discussion and conclusion

Reports on M. terrae in animals are very scarce, and there is a lack of information on lesions and pathogenesis of infection in animals and humans (6). Also, the ecology and physiology of the NTM are significantly different from those of M. tuberculosis (11, 16). Our finding confirms that the M. terrae like other NTM infection in cattle can interfere with intradermal tuberculin test, especially in conjunction with parasitic infestations, and hamper the in vivo diagnosis of bovine tuberculosis resulting in significant economic losses due to unnecessary restrictions and culling of reactor animals. However, the majority of false positive reactions in this manner have been overcome by single intradermal comparative cutaneous test (SICCT) (5). Having in mind
the presence of HIV positive persons in Bosnia and Herzegovina and other parts of population at risk, the infections with NTM deserve more attention. The pathologies in humans reported to be caused by *M. terrae* contribute to this assumption (4, 9).

Most countries in the European Union (EU) achieved bovine tuberculosis free status, while in some EU and non-EU member states tuberculosis remains non-eradicat-ed, but with ongoing eradication programs. In developing countries tuberculosis still remains a public health concern (15). In Bosnia and Herzegovina, the epidemiological status of bovine tuberculosis is still unknown in animals and humans.

Our finding represents the first report on isolation of NTM in cattle in Bosnia and Herzegovina. Absence of strict control measures such as annual TST testing, animal movement control, and trade control cause the continued presence of mycobacteria in animal populations. In addition, in most cases, tubercle-like and other granulomatous lesions found in animals at necropsies by local veterinarians and at abattoir inspections are not subjected to histopathology and bacterial culture to exclude tuberculosis or other infectious agents. Furthermore, there is obvious lack of communication between veterinary sector and human health sector on the prevalence of tuberculosis and non-tuberculous infections in animals and humans. Due to the economic problems, the poverty is particularly present in rural population that further contribute to spread of tuberculosis. For a better understanding of the epidemiology, better planning of control measures and eradication of bovine tuberculosis and NTM all TST positive animals should be submitted for necropsy, histopathology and microbiology for cultivation, identification and typisation of precise mycobacterial species involved. These measures will give a better insight into the epidemiological situation of bovine tuberculosis in Bosnia and Herzegovina and hopefully prevent further spread of the disease to the human population.

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References


**NOVO!!** Dijagnostika herpes virusne infekcije pasi!

Canine herpesvirus je uzročnik fatalne neonatalne hemoragije štenadi i jedan od najvažnijih uzročnika nesplodnosti i pobačaja kuja. Dokaz uzročnika je najpouzdaniji dijagnostički metod i može se obaviti u mikrobiološkom laboratoriju Veterinarskog fakulteta metodom qPCR.

Informacije na telefon 033 617 370
Prvi slučaj Mycobacterium terrae infekcije krave u Bosni i Hercegovini

Sažetak

Uvod i ciljevi

Materijal i metode
Krava s malog posjeda u Općini Vitez u centralnoj Bosni je pozitivno reagirala na tuberkulozni kožni test (TKT) u sklopu godišnje akcije tuberkulinizacije goveda. Nakon kliničkog pregleda, životinja je eutanazirana i podvrgnuta obdukciji. Uzorci organa (torakalni regionalni limfni grla, subkutani granuloma i koloni) i ustanovljene lezije (subkutani granuloma, multipli presjeci nematodifikovanih, s multiplim mineraliziranim, s multiplim mineraliziranim fokusima, a periferno ograničena obilnim infiltratom limfocita, makrofaga i neutrofila. U središnima granuloma u zidu kolona ustanovljeni su obilnim infiltratom limfocita, makrofaga i neutrofila. U središnima granuloma u zidu kolona ustanovljeni su obilnim infiltratom limfocita, makrofaga i neutrofila. U središnima granuloma u zidu kolona ustanovljeni su obilnim infiltratom limfocita, makrofaga i neutrofila.

Rezultati i interpretacija
Na obdukciji krave pozitivne na TKT ustanovljen je multiokokalni teški granulomatozni dermatitis i panikulitis, i umjereni do teški granulomatozni (parazitarni) kolitis. Ustanovljena granulomatozna žarišta su bila ispunjena granulatiranom kazenom sadder, sa siferom mineraliziranim fokusima, a periferno ograničena obilnim infiltratom limfocita, makrofaga i neutrofila. U središnima granuloma u zidu kolona ustanovljeni su multipli presjeci nematodifikovanih fokusa. Identifikacijom hsp65 gena utvrđeno je da izolirane bakterije pripadaju rodu Mycobacterium. Specifičnom hibridizacijom pomoću GenoType CM/AS testa (Hain Lifescience) i fenotipskim i biohemijskim testovima, identificirana je vrsta M. terrae.

Glavni zaključci
Naši rezultati predstavljaju prvi izvještaj o izolaciji NTM iz tkiva goveda u Bosni i Hercegovini. Oni potvrđuju da M. terrae, kao i ostale NTM infekcije kod goveda, mogu utjecati na zahtjeve intradermalnog tuberkulinog testa, izolacije u slučaju parazitarnih koinfekcija, time otežavajući in vivo dijagnostiku tuberkuloze goveda, što rezultira značajnim ekonomskim gubicima, zbog nepotrebnih restrikcija i uklanjanja životinja reaktora. U cilju boljeg razumijevanja epidemiologije, planiranja kontrolnih mjera i eradikacije tuberkuloze goveda i drugih NTM infekcija, sva TKT pozitivna grla trebalo bi podvrgnuti obdukciji, histopatološkoj i mikrobiološkoj pretrazi u svrhu izolacije, identifikacije i tipizacije prisutnih vrsta mikobakterija.