VETERINARSKI ARHIV 82 (3), 303-310, 2012

Molecular epidemiology of *Mycobacterium tuberculosis* transmission between cattle and man - a case report

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ŠPIČIĆ, S., M. PATE, S. DUVNJAK, V. KATALINIĆ-JANKOVIĆ, M. OBROVAC, D. DEŽĐEK, G. KOMPES, B. HABRUN, M. OCEPEK, Ž. CVETNIĆ: Molecular epidemiology of *Mycobacterium tuberculosis* transmission between cattle and man - a case report. Vet. arhiv 82, 303-310, 2012.

ABSTRACT

We describe a case of transmission of *Mycobacterium (M.) tuberculosis* infection from a man to cattle. *M. tuberculosis* was isolated from the bronchial lymph nodes of a heifer that reacted positively to bovine tuberculin but showed no gross pathological changes at slaughter. The cattle owner died of tuberculosis the same year the heifer was diagnosed with *M. tuberculosis* infection. *M. tuberculosis* strains isolated from the heifer and its owner were genotyped by mycobacterial interspersed repetitive units - variable-number tandem repeat (MIRU-VNTR) typing, which revealed identical MIRU profiles for both isolates. This is the first described case of *M. tuberculosis* infection in cattle and the first case of human-to-animal transmission of *M. tuberculosis* in Croatia.

Key words: *Mycobacterium tuberculosis*, cattle, Croatia

Introduction

Among the diseases that have threatened the health and lives of people and animals in the past century, tuberculosis (TB) has played a significant role. In Croatia, bovine TB occurrence has decreased as a result of an ongoing control program against the disease in the past decades. Despite the effort, it has not yet been eradicated completely.

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ISSN 0372-5480
Printed in Croatia

Bovine TB represents a major problem in terms of epidemiology, especially in countries where certain wild animal species, e.g. badgers (*Meles meles*), possums (*Trichosurus vulpecura*) and wild boars (*Sus scrofa*), represent environmental reservoirs and a source of infection for domestic animals and humans (NUGENT and WHITFORD, 2000; DELAHAY et al., 2001; NARANJO et al., 2008). In Europe, the infection in cattle is most frequently caused by *Mycobacterium (M.) bovis* and *M. caprae* (PRODINGER et al., 2002; KUBICA et al., 2003; CVETNIC et al., 2006). Apart from cattle, both species may also affect other mammals. In humans, they are causative agents of zoonotic TB, i.e. TB transmitted from animals to humans. Zoonotic TB is most frequently caused by *M. bovis*, although *M. caprae* infection has also been recorded frequently in recent years (KUBICA et al., 2003; PRODINGER et al., 2005; BONIOTTI et al., 2009). In Croatia, *M. caprae* infection in humans was first described in 2006 (CVETNIĆ et al., 2007).

Albeit rare, *M. tuberculosis* infection may occur in cattle. *M. tuberculosis* does not appear to have an indigenous animal host or reservoir and the infected animals most probably represent accidental hosts. Humans suffering from active TB are strongly believed to represent the main source of *M. tuberculosis* in animals, including cattle (STEELE, 1980; THOEN et al., 1981).

In this report we describe a case of transmission of *M. tuberculosis* infection from a human to cattle, confirmed by a modern epidemiological method, i.e. mycobacterial interspersed repetitive units - variable-number tandem repeat (MIRU-VNTR) typing, a technique that analyzes the number of tandem repeats at loci distributed within the *M. tuberculosis* genome.

Materials and methods

Case report. During routine tuberculin skin testing in cattle in March 2008, a six-month-old heifer from a small cattle farm (5 animals) reacted inconclusively to bovine tuberculin. In July 2008, the heifer was retested using a comparative tuberculin skin test with bovine (≥20.000 IU per mL, Veterina d.d., Croatia) and avian (≥20.000 IU per mL, Veterina d.d., Croatia) tuberculin. All skin tests were performed and interpreted according to the manufacturer's instructions. Following the positive reaction to bovine tuberculin, the heifer was slaughtered. No gross pathological changes were visible in the lymph nodes and tissue specimens inspected at slaughter. The submandibular, tracheobronchial, retropharyngeal, bronchial, mediastinal, portal and mesenteric lymph nodes were collected for bacteriological investigation. *M. tuberculosis* was isolated from the bronchial lymph nodes. The farm owner (born in 1911) died of TB in April 2008. After *M. tuberculosis* infection was detected in the heifer, the family members underwent Mantoux skin testing. The testing was performed using TUBERKULIN PPD RT 23 SSI 2 T.U. (Statens Serum Institute, Denmark) recommended by WHO. The injection was given intradermally in

the middle third forearm. The diameter of induration was evaluated 72 hours after the injection (positive reaction >5 mm), yielding negative results for all four persons. All animals on the farm tested negative to bovine tuberculin in two consecutive skin tests performed in 2009.

Laboratory investigations. Cultivation. Bacteriological investigation of the collected lymph nodes was performed according to the protocol described previously (KENT and KUBICA, 1985). Following homogenization, decontamination and concentration, the material 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.

Identification of the colonies. A PCR test targeting the *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-GAG-GAT-CC-3') and TB2 (5'-AGC-TGC-AGC-CCA-AAG-GTG-TT-3') and the protocol described previously (HANCE et al., 1989) were used. A GenoType® MTBC culture identification kit (Hain Lifescience, Germany) was used for further identification according to the manufacturer's instructions. As this version of the kit does not discriminate between *M. tuberculosis*, *M. africanum* II and *M. canettii*, biochemical tests, including niacin accumulation, nitrate reduction, thiophene-2-carboxylic acid hydrazide susceptibility, pyrazinamide susceptibility and Lebek test for oxygen preference, were performed with the purpose of further identification to the species level.

MIRU-VNTR typing. Genotyping of the *M. tuberculosis* isolates was performed using an in-house MIRU-VNTR method. The 15-locus MIRU-VNTR set (Table 1) with the primers described previously (SUPPLY et al., 2006) was used. The tandem repeat copy numbers were determined by agarose gel electrophoresis, i.e. by comparison of the PCR product using molecular weight markers (Invitrogen, USA).

Results

Colony growth was observed on the Stonebrink slant inoculated with the processed bronchial lymph node material after 42 days of incubation. The suspected colonies were identified as members of the genus *Mycobacterium* by PCR and as the members of *M. tuberculosis*/*M. africanum* II/*M. canettii* group using the GenoType® MTBC kit. Based on the results of biochemical tests, summarized in Table 2, the isolate was identified as *M. tuberculosis*. The MIRU profile of the heifer isolate was compared with the MIRU profile of the isolate obtained from the heifer owner present in the database of the Croatian National Institute of Public Health. Both isolates were shown to share the same MIRU profile (Table 1).

Table 1. Loci designations and results of MIRU-VNTR typing

Locus	580	960	1644	2996	3192	802	424	577	1955	2163	2165	2401	3690	4052	4156
Alias	MIRU 04	MIRU 10	MIRU 16	MIRU 26	MIRU 31	MIRU 40	VNTR 42	VNTR 43	VNTR 1955	QUB 2163b	ETR A	VNTR 47	VNTR 3690	QUB 26	VNTR 53
Human isolate	3	3	3	5	2	2	3	4	1	5	3	2	3	8	2
Heifer isolate	3	3	3	5	2	2	3	4	1	5	3	2	3	8	2

Table 2. Results of the biochemical tests performed to identify the isolate to the species level

Test	Result
Niacin accumulation	+
Nitrate reduction	+
Thiophene-2-carboxylic acid hydrazide susceptibility	-
Pyrazinamide susceptibility	+
Lebek test for oxygen preference	aerobic

Discussion

Although *M. tuberculosis* is considered to be primarily a human pathogen, it has also been reported to be present in domestic and wild animals, most frequently living in close contact with humans (ALFONSO et al., 2004; ARANAZ et al., 1999; MONTALI et al., 2001; OH et al., 2002; STERNBERG et al., 2002). Among domestic animals, infection with *M. tuberculosis* has most frequently been identified in cattle(STEELE, 1980; THOEN et al., 1981; CHANDRASEKHARAN and RAMAKRISHNAN, 1969). A number of *M. tuberculosis* infections in cattle have been identified as a result of the routine tuberculin testing of cattle herds (STEELE, 1980; LESSLIE, 1960; SMITH, 1984). Even though *M. tuberculosis* in cattle usually produces a quickly vanishing infection, the infected animals react positively when challenged with tuberculin (KRISHNASWAMI and MANI, 1983). The sensitization to tuberculin is usually short and the reactivity disappears when the infection source is removed (LESSLIE, 1960). Thus, when a tuberculin-positive animal is recognized during routine tuberculin testing for the first time in a previously tuberculin-negative herd, andparticularly,whenthe tuberculin-positive animal is young, the possibility of human TB infection among farm workers should be considered (LESSLIE, 1960). In this reported case, a young animal was affected, no TB-characteristic pathological changes were detected and subsequent skin TB testing revealed no inconclusive or positive reactors among the other animals on the farm. The fact that the farm owner was infected with TB strongly suggested the possibility of an anthropozoonotic transmission of *M. tuberculosis* infection.

Supporting data suggesting that humans suffering from active TB are the most probable source of *M. tuberculosis* in cattle have been described in the past, but the first unequivocal evidence of human-to-cattle transmission of *M. tuberculosis* confirmed by IS*6110* restriction fragment length polymorphism (RFLP) analysis was not reported until 2005 (OCEPEK et al., 2005). In recent years, novel PCR-based typing methods have been developed, e.g. MIRU-VNTR typing, that enable faster and more detailed strain differentiation. The *M. tuberculosis* MIRU-VNTR typing system based on 15 loci, used in this study, was proposed as the new standard for routine epidemiological discrimination of *M. tuberculosis* isolates in 2006 (SUPPLY et al., 2006). Although MIRU-VNTR typing has been used widely for genotyping *M. tuberculosis* and *M. bovis* isolates, it has been, to the best of our knowledge, employed only twice to characterize *M. tuberculosis* isolates from cattle (CHEN et al., 2009; DU et al., 2011). CHEN et al. (2009) reported a common MIRU profile revealed for both human and cattle *M. tuberculosis* isolates, suggesting a common source of infection in the cow and an epidemiological link between the cow and human *M. tuberculosis* infections. In a recent investigation of throat swabs from cattle that reacted positively to bovine tuberculin, *M. tuberculosis* strains were isolated in an even larger number than *M. bovis* strains (DU et al., 2011). *M. tuberculosis* strains isolated in cattle mostly belonged to the Beijing and Beijing-like family (CHEN et al., 2009; DU et al., 2011). This can be explained by the fact that China is a highly-burdened TB country (incidence rate 96 per 100,000 population in 2009) (ANONYM., 2010: Global tuberculosis control. World Health Organization. http://www.who.int/tb/publications/global_report/2010/gtbr10_a2.pdf) where humans and animals still live in close contact.

In this paper, we present the first case of *M. tuberculosis* infection in cattle in Croatia. The MIRU profile of the heifer isolate matched with MIRU profiles of two *M. tuberculosis* isolates detected in humans in 2008: namely, of the heifer owner and of another epidemiologically unlinked person residing in another Croatian county. Apart from these three cases, this particular MIRU profile has not been reported in Croatia in the period from 2007 until now. The lower incidence rate of human TB (25/100.000 in 2009) (ANONYM., 2010) and a largely urban population most likely contribute to the very low risk of *M. tuberculosis* transmission from humans to animals in Croatia.

Conclusions

MIRU-VNTR typing proved to be useful tool for the epidemiological investigation of the first documented case of *M. tuberculosis* infection in cattle in Croatia. Though rare, the possibility of an anthropozoonotic transmission of *M. tuberculosis* infection should not be overlooked. In regions where bovine TB and human TB still coexist, a detailed microbiological investigation of specimens of tuberculin-positive animals should always be performed in order to discriminate between *M. tuberculosis* and *M. bovis* infections.

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Received: 6 April 2011
Accepted: 4 November 2011

ŠPIČIĆ, S., M. PATE, S. DUVNJAK, V. KATALINIĆ-JANKOVIĆ, M. OBROVAC, D. DEŽĐEK, G. KOMPES, B. HABRUN, M. OCEPEK, Ž. CVETNIĆ: Molekularna epidemiologija infekcije vrstom *Mycobacterium tuberculosis* u goveda i ljudi - prikaz slučaja. Vet. arhiv 82, 303-310, 2012.

SAŽETAK

Opisan je slučaj prijenosa zaraze vrstom *Mycobacterium* (*M.*) *tuberculosis* s čovjeka na govedo. *M. tuberculosis* je izdvojen iz bronhalnih limfnih čvorova junice koja je pozitivno reagirala na tuberkulin, a prilikom klanja nisu utvrđene patomorfološke promjene karakteristične za tuberkulozu. Iste godine vlasnik goveda je preminuo od posljedica tuberkuloze. Izolati *M. tuberculosis* iz goveda i čovjeka bili su genotipizirani pomoću metode određivanja promjenjivog broja opetovanih sljedova nukleotida (engl. mycobacterial interspersed repetitive units - variable-number tandem repeat [MIRU-VNTR]) i u oba je slučaja bio utvrđen identičan rezultat genotipizacije. Ovo je prvi opisani slučaj zaraze vrstom *M. tuberculosis* u goveda i prvi slučaj prijenosa ove bolesti s čovjeka na govedo u Hrvatskoj.

Ključne riječi: *Mycobacterium tuberculosis*, govedo, Hrvatska