Original Article

Molecular characterization of Mycobacterium bovis strains isolated from cattle and humans by spoligotyping and 24-locus MIRU-VNTR, and prevalence of positive IGRA in slaughterhouse workers in Southern Turkey

Güven Gökmen, T.1; Yakici, G.2; Kalayci, Y.3; Turut, N.4; Meral Ocal, M.5; Haligür, M.2; Günaydin, E.7 and Köksal, F.5

1Department of Microbiology, Ceyhan Veterinary Faculty, Cukurova University, 01330, Adana, Turkey; 2Tuberculosis Region Laboratory, Tropical Disease and Research Center, Cukurova University, 01330, Adana, Turkey; 3Microbiology Laboratory, Adana City Hospital, 01230, Adana, Turkey; 4MSc, Microbiology Laboratory, Adana Veterinary Control Institute, 01250, Adana, Turkey; 5Department of Microbiology, Medicine Faculty, Cukurova University, 01330, Adana, Turkey; 6Department of Pathology, Ceyhan Veterinary Faculty, Cukurova University, 01330, Adana, Turkey; 7Department of Microbiology, Veterinary Faculty, Kastamonu University, 37150, Kastamonu, Turkey

*Correspondence: T. Gülven Gökmen, Department of Microbiology, Ceyhan Veterinary Faculty, Cukurova University, 01330, Adana, Turkey. E-mail: tulinguven01@hotmail.com

10.22099/IJVR.2022.42580.6186

(Received 22 Dec 2021; revised version 9 Mar 2022; accepted 11 Jun 2022)

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Abstract

Background: Mycobacterium bovis is a zoonotic member of the Mycobacterium tuberculosis complex with a wide range of hosts, mainly cattle. Molecular epidemiological studies should be conducted to determine the transmission route, zoonotic risk factors, and phylogenetic relationships of M. bovis strains. Aims: This study aimed to characterize bovine and human M. bovis isolates by molecular methods. Methods: Molecular characterization and clonal relationship of strains isolated from tissue and organ samples of 76 cattle with positive tuberculin tests were collected from a slaughterhouse, and four M. bovis strains isolated from clinical materials of patients with suspected pulmonary TB isolates were analyzed using 24-locus MIRU-VNTR and spoligotyping methods. QuantiFERON-TB Gold Plus (QFT-Plus; Qiagen) was used to determine the prevalence of latent TB infection among 21 slaughterhouse personnel including 7 veterinarians, 12 butchers, 1 caretaker, and 1 veterinary technician. Results: SB0288/SIT685 type was detected in both cattle and humans by the spoligotyping method. When evaluating MIRU-VNTR, the presence of a 100% compatible pattern between human and bovine isolates was not detected, but some human samples were found to be 91.6% similar to a bovine sample. In addition, 21 slaughterhouse workers were screened with the interferon gamma-released assay (IGRA) and a 23.8% positivity was detected. Conclusion: Clonal similarity was determined between the bovine and human isolates using the MIRU-VNTR and spoligotyping methods and IGRA positivity in the occupational group suggested that M. bovis might be associated with pulmonary tuberculosis in humans.

Key words: IGRA, Molecular epidemiology, Mycobacterium bovis

Introduction

Mycobacterium bovis is the causative agent of bovine tuberculosis (TB), an infectious, zoonotic, and chronic disease that also affects other domestic animals and humans (Carneiro et al., 2020). Transmission occurs mostly through the gastrointestinal tract due to the consumption of contaminated dairy products, or to a lesser extent, contaminated meat. It can be transmitted by the inhalation of aerosols exhaled by infected animals or humans or through direct contact in the presence of an open wound (Djemal et al., 2017). Since the identification of M. bovis is not included in routine tests in pulmonary TB laboratories and clinical and pathological findings are similar to M. tuberculosis complex (MTBC) infection, data on the actual prevalence and incidence in patients with pulmonary tuberculosis is very limited (Jiang et al., 2015). The World Health Organization (WHO) states that there are 7,000 new cases of M. bovis in South America each year, but the incidence is estimated to be eight times higher (O’Reilly and Daborn, 1995). In the south subtropical climate zone, which is close to Turkey, due to a high rate of migration and immigration from countries like Syria, TB constitutes a major risk for such diseases (Ergönül et al., 2020). On the other hand, low income and poor socioeconomic and sociocultural status, close contact with animals, and failure to comply with hygiene rules in food production suggest that in addition to M. tuberculosis, M. bovis may be the cause of pulmonary TB in humans (Djibuti et al., 2014). Programs aiming to eliminate TB all over the world have revealed the
necessity of identifying and monitoring strains isolated from patients with molecular epidemiological methods, such as RFLP-IS6110, MIRU-VNTR and spoligotyping. Thus, the concept of “One Medicine, One Health” has been adopted in the approach to pulmonary TB cases caused by the *M. bovis* agent. Molecular epidemiological studies should be conducted to determine the transmission route of zoonotic TB to humans, to define the dominant genotypes between humans and animals, and to understand the phylogenetic relationships of the strains (Thoen et al., 2016).

The risk of *M. bovis* infection is very high in groups that are in close contact with animals, such as veterinarians and butchers, especially due to occupational exposure. The tuberculin skin test (TST) and the interferon gamma released assay (IGRA) have been available for many years to screen these professional groups (Vayr et al., 2018). However, the sensitivity of TST, which is most commonly used in screening studies, is only 70-85% in latent infection (Menzies et al., 2009). In recent years, The IGRA test has been used to evaluate the cellular immune response (IFN-γ) against ESAT-6 and CFP-10 antigens belonging to MTBC. The sensitivity and specificity of this test are reported to be 98.9 and 98.1%, respectively (Takasaki et al., 2017).

In this study, we aimed to investigate the clonal relationship of strains isolated from animals and humans using the 24-locus MIRU-VNTR and spoligotyping methods and to determine the presence and rates of latent TB in at-risk occupational groups using the QuantiFERON-TB Gold Plus (QFT-Plus) test.

**Materials and Methods**

**Statement of ethics**

Animal experiments and animal management procedures were designed in accordance with the requirements of the animal health and ethics committee of Çukurova University Faculty of Veterinary Medicine and applied after receiving the committee approval. Human sputum samples were included in the study with the permission of the Ethics Committee of Çukurova University Faculty of Medicine.

**Sample recruitment**

In order to determine the epidemiological characteristics of *M. bovis* in our region, the tissue and organ samples of 76 slaughtered cattle with positive tuberculin tests were collected from a slaughterhouse between October 2018 and December 2019. In addition, the study included four *M. bovis* strains isolated from the clinical materials of patients with suspected pulmonary TB, who were presented to Çukurova University Tropical Disease Research and Application Center (TDRAC) and the Turkish Ministry of Health Adana Regional Tuberculosis Laboratory, as well as blood samples collected from 21 slaughterhouse personnel including seven veterinarians, 12 butchers, one caretaker and one veterinary technician. Individuals who agreed to participate were asked to sign a consent form and complete a questionnaire including questions on sociodemographic factors.

**Histopathological and microbiological examinations**

Lung and mediastinal lymph node tissues were fixed in 10% formaldehyde. After the routine tissue follow-up procedure, the tissues were embedded in paraffin. Four micron-thick sections were cut from each paraffin block. The slides were stained with hematoxylin and eosin (HE) and Ehrlich-Ziehl-Neelsen (EZN). The stained sections were examined under a light microscope. EZN staining was performed to examine existing acid-fast stained bacilli in various clinical samples collected from the lung and lymph nodes of cattle with a positive tuberculin test in slaughterhouses and the sputum samples of patients with suspected pulmonary TB.

Tissue samples taken from the lungs and lymph nodes of cattle with a positive tuberculin test and the sputum samples of the patients with suspected pulmonary TB were subjected to the decontamination-homogenization procedure. 3 g of samples taken from the lungs and lymph nodes were dissected in 10 ml 0.85% physiological saline. 1.0 ml of 4% NaOH was added to 1.0 ml of the homogenizer, and the sample was incubated at 37°C for 20 min. It was then centrifuged at 1000 × g for 20 min before PBS was added.

The samples were inoculated in Lowenstein-Jensen and MGIT 960 broth (Becton Dickinson Diagnostic System, Sparks, MD). The isolates grown in the medium from clinical specimens were confirmed to be MTBC using the immunochromatographic MPT64 card test (Bioline, Standard Diagnostics, Seoul, South Korea) following microscopic examinations with EZN staining.

**Genomic DNA isolation**

Mycobacterial DNA was extracted from the cultures grown on Lowenstein-Jensen and BACTEC-MGIT 960 using a Mickle cell disruptor (The Mickle Lab. Engineering Co. Ltd., Gamshall, Surrey, UK) and immediately stored at -20°C to be used in various molecular methods.

**Hsp65-PCR**

TB11 and TB12 primers were used in the PCR method targeting the *hsp65* gene region in which strains belonging to the genus *Mycobacterium* were detected among the isolates grown on the media as previously described (Brunello et al., 2001).

**Spoligotyping**

Spoligotyping was used to identify the genotype of the MTBC strains at the DR locus as previously described (Kamerbeek et al., 1997). All chromosomal DNA was amplified with primary DRA (5′-CCG AGA GGG GAC GGA AAC-3′) and DRb (5′-GTT TTT GTT CTC AGC AC-3′). The amplicons were hybridized after PCR. After hybridization, the products were made visible by adding streptavidin alkaline phosphatase enzyme and
phosphate chemifluorescent substrate (Kremer et al., 1999; Sola et al., 2003). The presence of spacer regions was evaluated considering the blotting resulting from PCR product hybridization. The results were converted into “Octal code” format consisting of 15 digits between 0-7 using the octal coding key. The data was evaluated using various databases (Couvin et al., 2019).

MIRU-VNTR
The 24-locus MIRU-VNTR method was performed to identify and determine clonal relationships between the Mycobacterium bovis isolates. Primers were used for the targeted MIRU loci as described in a previous study (Supply et al., 2006). PCR reactions were performed to determine the VNTR number of the 24 MIRU loci specific to each strain. The number of allele repeats at each MIRU locus was determined based on the band size (Weniger et al., 2010).

Table 1: HGDI values of 24-locus MIRU-VNTR primers

| Locus   | Copy numbers (%) | Allelic diversity |
|---------|------------------|-------------------|
| ETRA    | 4(7), 5(7), 6(8) | 0.69              |
| Qub26   | 3(4), 5(5), 4(13) | 0.59              |
| Muhb21  | 1(9), 3(13)      | 0.50              |
| Qub11b  | 2(9), 4(13)      | 0.50              |
| MIRU27  | 2(9), 3(13)      | 0.50              |
| ETRC    | 3(7), 5(15)      | 0.45              |
| ETRB    | 6(5), 5(17)      | 0.36              |
| MIRU31  | 2(2), 4(2), 3(18) | 0.32              |
| MIRU23  | 3(5), 4(19)      | 0.24              |
| Muhb04  | 1(2), 2(20)      | 0.17              |
| Muhb34  | 2(2), 3(20)      | 0.17              |
| Muhb39  | 3(2), 2(20)      | 0.17              |
| MIRU40  | 3(2), 2(20)      | 0.17              |
| MIRU26  | 6(2), 5(20)      | 0.17              |

PncA-PCR
The pncA-PCR method was applied to the strains analyzed by spoligotyping and MIRU-VNTR methods, as previously described (Sposito et al., 2014). Various primers were used to support the identification of M. bovis. The pncAMTB-1,2F and pncAM primers for M. tuberculosis and the pncAMTB-1,2F and pncAMB primers for M. bovis were used.

QuantIFERON-TB Gold Plus Test
Blood samples were collected from 21 slaughterhouse workers including seven veterinarians, 12 butchers, one caretaker and one veterinary technician, and placed in lithium-heparin tubes. The samples were then transferred to four distinct tubes: Nil tube as a negative control, TB-antigen tube 1 (TB1), TB-antigen tube 2 (TB2), and mitogen tube as a positive control. The QFT-TB Gold Plus tubes were incubated at 37°C for 16-24 h. After incubation, the plasma samples were separated by centrifugation and the amount of IFN-gamma (IU/ml) was measured using the ELISA method according to the instructions of the commercial kit (QFT-Plus; Qiagen, Germantown, MD).

Results
This study included 71 lymph node and 5 lung samples of 76 cattle with positive tuberculin test in farm tuberculosis screening. Also, 7830 suspicious human sputum, pleural and gastric fluid samples were sent to the TDRAC Regional Tuberculosis Laboratory for Diagnosis. The bovine and human isolates were identified using conventional and molecular methods, and M. bovis was further analyzed with the 24-locus MIRU-VNTR, spoligotyping, and PncA-PCR methods. Twenty-five M. bovis were detected by conventional and molecular methods in cattle. In humans, 524 were identified as members of the MTB complex, while four isolates were identified as M. bovis.

MTBC detection in cattle
In the microbiological and histopathological examinations, the presence of acid resistant bacteria was determined in 20 of 76 cattle tissue samples by EZN and HE staining. Macroscopically, granulomas were observed in lung and mediastinal lymph node tissues. When cutting the lesioned area, it felt as if the blade was being pulled through sand. The central part of the cross-sectional surface of the lesions in the lung and lymph nodes was hard and friable. Gray-white mineralization foci were detected in the center of caseification necrosis in some granulomas. Histopathological examination revealed typical lesions in both lungs and mediastinal lymph nodes. The typical lesions were granulomas composed of epithelioid histiocytes, Langhan’s multinucleated giant cells, and mononuclear inflammatory cells, surrounded by fibrous tissue. The central part of the granulomas was observed to have caseification necrosis and some exhibited mineralization. In addition, conglomerated tubercles were found in the lymph nodes. Atelectasis areas were seen in the lungs where the granuloma was compressing on the alveoli. In EZN staining, Mycobacterium bacilli were observed in the alveoli and the cytoplasm of histiocyte and macrophages in inflammatory areas. Free and scattered bacilli were also detected in the connective tissue (Fig. 1). In the Lowenstein-Jensen and MGIT960 broth, growth was observed in 25 of the bovine samples, which was confirmed by EZN staining.

With the MTBC genomic DNA isolated from MGIT broth, using the Mickle extraction method and amplified with the primers of the hsp65 gene region, the strains from the 25 bovine samples were identified as Mycobacterium spp.

MTBC detection in humans
In human sputum samples, EZN staining, inoculation to Lowenstein-Jensen and MGIT960 broth, and post-growth EZN staining processes were applied. DNA extraction from the MGIT960 broth of isolates was performed using the Mickle extraction method and the 524 strains were determined to belong to the MTB complex with the hsp65-PCR.
Fig. 1: Histopathological and microbiological examination of the cattle lymph nodes. (A) Mediastinal lymph node enlarged with granulomas in cattle, (B) Sectional face of the mediastinal lymph node, granuloma (large arrowhead), caseification necrosis (arrows), and gray-white mineralization foci (small arrowheads) in cattle, (C) A granuloma (tubercle) structure, caseification necrosis in the middle (CN), the area around the necrosis consisting of mononuclear inflammatory cells (arrows), and Langhan’s giant cells (arrowheads), (H&E, scale bar, 50 µm), (D) Conglomerated tubercle, capsule (arrows) formed from outer to inner connective tissue, newly formed tubercle (NT), caseification necrosis (CN), and mineralization areas (C), (H&E, scale bar, 100 µm), (E) Acid-resistant bacilli in the cytoplasm of macrophages (arrows), and free-form bacilli in connective tissue (arrowhead), (ZN, scale bar, 10 µm), and (F) Free acid-fast bacilli in the lumen of the alveoli (arrows), (ZN, scale bar, 10 µm)

*M. bovis* detection in cattle and human by spoligotyping, MIRU-VNTR and PncA-PCR

Spoligotyping and 24-loci MIRU-VNTR methods were used to investigate the molecular epidemiological relationship between cattle and human isolates. Eighteen of the bovine isolates and all human samples were evaluated by molecular epidemiological methods. Seven of the bovine isolates were not used because they could not be amplified in the pre-stages of the MIRU-VNTR and spoligotyping methods.

**Spoligotyping**

The clonal group distribution of the 18 bovine and all human isolates included was examined with the spoligotyping method. The spoligopattern of all 18 bovine isolates and the four 524 MTBC isolates included
in the study belonged to M. bovis. In addition, 22 M. bovis isolates were clustered in a total of four spoligotypes.

Spoligotypes determined in cattle were SB0120/SIT482 (40.9%) for nine isolates, SB0288/SIT685 (31.81%) for seven isolates, and SB0140/SIT683 (9.09%) for two isolates. When the spoligotypes of the human isolates were examined, we found that two isolates were SB0989/SIT1118 (9.09%) and the other two isolates were SB0140/SIT683 (9.09%) (Table 2).

Table 2: Spoligotype distribution of the bovine and human samples

| Spoligotypes   | Bovine isolates | Human isolates | Incidence (%) |
|----------------|-----------------|----------------|---------------|
| SB0120/SIT482  | 9 (40.9%)       | -              | 40.9%         |
| SB0288/SIT685  | 7 (31.81%)      | 2 (9.09%)      | 40.9%         |
| SB0989/SIT1118 | -               | 2 (9.09%)      | 9.09%         |
| SB0140/SIT683  | 2 (9.09%)       | -              | 9.09%         |

When we compared the spoligotyping and 24-locus MIRU-VNTR methods in terms of clonal relationship, it was determined that the latter had more discriminatory power than the former, with the number of clusters being determined as 8 and 4, respectively (Table 3). Although the same spoligotypes were determined in the bovine and human isolates, there was no 100% similarity among the total 22 isolates’ patterns according to the 24-locus MIRU-VNTR method.

24-loci MIRU-VNTR

When 18 bovine and all human isolates were evaluated by the MIRU-VNTR method, it was determined that all 18 bovine isolates and four 524 MTBC isolates were M. bovis. According to the 24-locus MIRU-VNTR method, there were eight clusters harboring 20 isolates, and two unique profiles were detected (Fig. 2).

When the number of the repetitions of 24 locus was examined with the MIRU-VNTR method, there was no difference in MIRU02, MIRU04, MIRU10, MIRU16, MIRU20, Mtub29, Mtub30, MIRU24, Qub4156, and MIRU39 locus repeats, but Mtub04, Mtub34, Mtub39, ETRA, ETRB, ETRC, Qub11b, Qub26, MIRU40, MIRU21, MIRU23, MIRU26, MIRU27, and MIRU31 locus repeats showed variability (Table 3). When we calculated the discriminatory powers of the primers used in MIRU-VNTR, we found that the highest values (P≥0.50) belonged to ETRA (0.69), Qub26 (0.59), Qub11b (0.50), Mtub21 (0.50), and Miru27 (0.50) (Table 1).

PncA-PCR

Using the pncA-PCR method used to support M. bovis identification, 185 bp bands were obtained from 25 bovine and 4 human isolates based on amplification with M. bovis-specific primers pncAMTB-1,2F and pncAMB2R.

Table 4: Risk factors in the IGRA test group

| Risk factor       | QFT-positive (n=5) | QFT-negative (n=16) |
|-------------------|--------------------|---------------------|
| Age               | 42.2±9.68          | 37.1±7.44           |
| Gender            |                    |                     |
| Male              | 5                  | 14                  |
| Female            | -                  | 2                   |
| Occupation        |                    |                     |
| Butcher           | 4                  | 8                   |
| Veterinarian      | -                  | 7                   |
| Veterinary technician | 1             | -                   |
| Caretaker         | -                  | 1                   |

QFT-TB Gold-Plus

A total of 21 slaughterhouse workers, including 12 butchers, seven veterinarians, one caretaker, and one veterinary technician, were enrolled for the analysis of the QFT-TB Gold-Plus status. The distribution of the samples according to gender was 19 (90.5%) males and
Fig. 2: N-J tree of \textit{M. bovis} isolates according to the 24-locus MIRU-VNTR method

two (9.5\%) females. The age of the study population ranged from 27 to 51 years (mean age 38.3 ± 8.07 years). According to the results of QFT-TB Gold-Plus, of the 21 individuals, five (23.8\%) tested positive, of whom four butchers and one was a veterinary technician (Table 4).

Discussion

In this study, we used the spoligotyping and MIRU-VNTR methods for the molecular epidemiological determination of \textit{M. bovis} isolated from humans and cattle, and we determined four clusters harboring 22 isolates with the spoligotyping method. The major spoligotype was SB0120/SIT482 with 9/22 (40.9\%) isolates, which was frequently detected in countries such as Iran, Spain, Portugal, Italy, France, Germany, Algeria, Zambia, and Morocco in previous studies (Kubica \textit{et al}., 2003; Duarte \textit{et al}., 2008; Munyeme \textit{et al}., 2009; Sahraoui \textit{et al}., 2009; Rodriguez \textit{et al}., 2010; Yahyaoui-Azami \textit{et al}., 2017; Ghavidel \textit{et al}., 2018). It has been suggested that the reason for the high rate of detection of strains belonging to the SB0120/SIT482 type in Middle Eastern, European and African countries may be the trade of live animals or processed meat products between these countries or homoplasy. In addition, in another spoligotyping study conducted in our region, it was reported that the SB0120/SIT482 pattern was encountered most frequently at a rate of 42.85\% (Tuzcu \textit{et al}., 2020). In our opinion, this spoligotype represents the major \textit{M. bovis} genotypes all over the world.

In our study, SB0288/SIT685 (31.81\%) was the second frequent spoligotype among the 18 (81.8\%) bovine isolates, which is similar to the situation reported in Iran and West Azerbaijan (Tadayon \textit{et al}., 2009; Tadayon \textit{et al}., 2013). This similarity may be related to the widespread cattle breeding and trade carried out in the study area bordering these countries. In Turkey, it was previously reported that six \textit{M. bovis} strains, four of which were isolated from cattle and two from goats, sent to the laboratory for suspected TB from six slaughterhouses in Aydın, Manisa, Muğla, and Izmir between 2010 and 2015, genotypically belonged to the spoligotype SB0288/SIT685 (Avsever \textit{et al}., 2017). The authors noted that this spoligotype also showed homology with the spoligotype patterns of \textit{M. bovis} strains isolated from humans in the Aegean Region. In our study, two (50\%) of the four human isolates belonged to this family, namely the SB0288/SIT685 spoligotype. In a previous study evaluating 482 MTBC strains isolated from clinical samples sent for a routine mycobacteriological examination, the authors determined that 13 isolates were \textit{M. bovis}, of which nine (63.6\%) belonged to the ST685 (SB0288) family (Çavuşoğlu \textit{et al}., 2017).

In the bovine samples, the lowest number of spoligotypes belonged to SB0140/SIT683 detected in two isolates (9.09\%). This clade has been found in studies conducted in many countries such as Iran, Portugal, South Africa, Mozambique, Northern Ireland, Mexico, and Brazil (Cobos-Marín \textit{et al}., 2005; Duarte \textit{et al}., 2010; Skuce \textit{et al}., 2010; Parreiras \textit{et al}., 2012; Çavuşoğlu \textit{et al}., 2017; Machado \textit{et al}., 2018; Sichewo \textit{et al}., 2020). In another study undertaken in Turkey, the rate of the SB0140/SIT683 spoligotype was reported to be 26.19\% (Tuzcu \textit{et al}., 2020). Intense activity in the international livestock trade; i.e., animal imports from many countries, may be the reason for the differences between studies in relation to the percentages of \textit{M. bovis} genotypes. Our minor genotype being the second largest genotype in the previous study, can be attributed to a
periodic change.

We determined that the remaining two (9.09%) M. bovis strains isolated from the human clinical samples belonged to the SB0989/SIT1118 spoligotype. In a study which evaluated only samples isolated from clinical human materials, Çavuşoğlu et al. (2017) reported that 7.7% of the M. bovis isolates belonged to the SB0989/SIT1118 spoligotype. The number of human-derived M. bovis isolates is insufficient for statistical evaluation and proportional calculations. However, the collection of isolates of human origin from two spoligotypes and the isolation of these spoligotypes from human samples in Turkey will guide further studies to be conducted with more isolates obtained from the region.

Considering the diversity of our spoligotyping profile, we found that SB0288/SIT685 was the only common type observed in both the bovine and human groups. However, SB0120/SIT482 and SB0140/SIT683 were detected only in the bovine samples, and SB0989/SIT1118 in two of the four human samples.

In our study, we used the 24-locus MIRU-VNTR method instead of a 12-14-15 locus due to its higher discriminatory power in detecting clonal relationship. We found that the ETRA, Qub26, Qub11b, Mtub21, and Miru27 primers were highly discriminative in identifying the MIRU-VNTR locus. Similarly, many previous studies that used the 24-locus MIRU-VNTR method revealed that the highest discriminatory power was obtained from the ETRA, Qub26, and Qub11b locus (Yang et al., 2015; Carvalho et al., 2016; Elsayed et al., 2019).

In the MIRU-VNTR method, our isolates were divided into eight clusters with two unique profiles. When the similarities between the locus patterns of the human and bovine isolates were evaluated, 100% similarity was not determined. However, the H1 and H2 human samples were found to be 91.6% similar to a bovine sample. In a previous study, 32 bovine and 10 human M. bovis isolates were evaluated with the 12-locus MIRU-VNTR and spoligotyping methods, and the MIRU-VNTR patterns of five human isolates were 100% compatible with the bovine isolates (Skuce et al., 2010). Accordingly, the authors emphasized that bovine isolates could cause infections in humans. Although our data did not provide statistically significant results due to the low number of human strains, it was hypothesized that cattle-originated M. bovis could play a role in active pulmonary TB in humans. Due to this possibility, we also examined slaughterhouse workers as a sampling group that had the most contact with pulmonary TB-suspected animals with a positive tuberculin skin test. However, there were no clinical symptoms in this group. Therefore, we decided to screen for latent TB infection in the blood samples collected from the 21 individuals. MTBC infection was screened using the QFT-TB Gold-Plus assay, and 23.8% positivity (5/21) was detected. In a similar study involving 311 farm and slaughterhouse workers and households, blood samples were taken to screen for latent TB, and tuberculin skin test (TST) and IGRA positivity were found at the rates of 76.2% and 58.5%, respectively (Torres-Gonzalez et al., 2013).

The presence of M. bovis in risk groups was proven by culture and molecular methods, although it could not be determined at the level of Mycobacterium species using TST and IGRA. In a previous study, M. bovis was isolated from the bronchoalveolar lavage of a person who had worked as a butcher for 35 years in Australia (Ingram et al., 2010). In Pakistan, M. bovis was detected from sputum samples in slaughterhouse workers, butchers and veterinarians using the PCR analysis (Khattak et al., 2016). In another case reported from Turkey, M. bovis was isolated from a butcher with a persistent skin lesion and pulmonary infection in Turkey (Mertoğlu et al., 2018). Additionally, M. bovis SB0271 spoligotype was isolated from the sputum sample of a female veterinary nurse in England, while latent TB was also detected in her daughter with the QuantiFERON-TB Gold test. In addition, the same spoligotype was present in the tracheal mucus sample collected from the nurse’s dog. Interestingly, when the spoligotype archives on cattle were screened, the same spoligotype (SB0271) was found in an animal tested for tuberculosis by an animal nurse years ago. This animal was considered as a possible source of infection (Shrikrishna et al., 2009).

In conclusion, although zoonotic TB is not a new disease, it has been neglected for many years. With molecular epidemiological studies, the importance of M. bovis and zoonotic TB has been better understood, and the eradication of this disease globally by 2030 has become one of the United Nations Sustainable Development Goals. Determining the causative agent and breaking the zoonotic chain are essential to the eradication of the disease. Spoligotyping and MIRU-VNTR should always be used as key methods to identify the source of isolates and monitor transmissions in humans and animals. Risk groups should be monitored continuously and the risk of infection of the most exposed group should be eliminated (WHO, 2017).

Acknowledgements

This research was financially supported by Çukurova University Scientific Research Projects (TSA-2018-10909), Adana, Turkey. We would like to thank the staff of “Meat and Dairy Institution Adana Meat Combination Directorate” and “Adana Metropolitan Municipality Slaughterhouse” for whom we collected clinical samples from cattle and blood samples from veterinarians, butchers and technicians in our study.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aseyer, M; Çavuşoğlu, C; Yazoçoğlu, Ö; Eskiüzmirli, S;
Erdal, G; Günen, M; Tunaligil, S; Alparslan, B and Aksoy, A (2017). New spoligotyping pattern of *Mycobacterium bovis* isolates from farm animals in Turkey. Ankara Univ. Vet. Fak. Derg., 64: 37-43.

Brunello, F; Ligozzi, M; Cristelli, E; Bonora, S; Tortoli, E and Fontana, R (2001). Identification of 54 mycobacterial species by PCR-Restriction Fragment Length Polymorphism analysis of the *hsp65* Gene. J. Clin. Microbiol., 39: 2799-2806.

Carneiro, PAM; Pasquatti, TN; Takatani, H; Zumárraga, MJ; Marfil, MJ; Barnard, C; Fitzgerald, SD; Abramovitch, RB; Araujo, FJ and Kaneene, JB (2020). Molecular characterization of *Mycobacterium bovis* infection in cattle and buffalo in Amazon Region, Brazil. Vet. Med. Sci., 6: 133-141.

Carvalho, RCT; Vasconcellos, SEG; Issa, MA; Filho, PMS; Carneiro, PAM; P and Brunello, F (2015). Pulmonary tuberculosis caused by *Mycobacterium bovis* in Brazil. PloS One. 1: 1-16.

Çavuşoğlu, C and Yılmaz, FF (2017). Molecular epidemiology of human *Mycobacterium bovis* infection in Aegean Region, Turkey. Mikrobiyol. Bul., 51: 165-170.

Cobos-Marín, L; Montes-Vargas, J; Zumárraga, M; Cataldi, A; Romano, MI; Estrada-García, I and González-y-Merchand, JA (2005). Spoligotype analysis of *Mycobacterium bovis* isolates from Northern México. Can. J. Microbiol., 51: 996-1000.

Couvin, D; David, A; Zozio, T and Rastogi, N (2019). Macro-geographical specificities of the prevailing tuberculosis epidemic as seen through SITVIT2, an updated version of the *Mycobacterium tuberculosis* genotyping database. Infect. Genet. Evol., 72: 31-43.

Djemaal, SE; Siala, M; Smooui, S; Kammoun, S; Marouane, C; Bezos, J; Messadi-Akrout, F; Romero, B and Gdoura, R (2017). Genetic diversity assessment of Tunisian *Mycobacterium bovis* population isolated from cattle. BMC Vet. Res., 13: 393.

Djibuti, M; Mirvelashvili, E; Makharashvili, N and Magee, JM (2014). Household income and poor treatment outcome among patients with tuberculosis in Georgia: a cohort study. BMC Public Health. 14: 88.

Duarte, EL; Domingos, M; Amado, A and Botelho, A (2008). Spoligotype diversity of *Mycobacterium bovis* and *Mycobacterium caprae* animal isolates. Vet. Microbiol., 130: 415-421.

Duarte, EL; Domingos, M; Amado, A; Cunha, MV and Botelho, A (2010). MIRU-VNTR typing adds discriminatory value to groups of *Mycobacterium bovis* and *Mycobacterium caprae* strains defined by spoligotyping. Vet. Microbiol., 143: 299-306.

Elsayed, MSA (2019). A first insight into the application of high discriminatory MIRU-VNTR typing using QIAXcel technology for genotyping *Mycobacterium bovis* isolated from the Delta area in Egypt. Inf. Gen. Evol., 71: 211-214.

Ergönül, Ö; Tülek, N; Kayi, I; Irmak, I; Erdem, O and Dara, M (2020). Profiling infectious diseases in Turkey after the influx of 3.5 million Syrian refugees. Clin. Micro. Inf. 26: 307-312.

Ghavidel, M; Mansury, D; Nourian, K and Ghazvini, K (2018). The most common spoligotype of *Mycobacterium bovis* isolated in the world and the recommended loci for VNTR typing: A systematic review. Microb. Pathog., 118: 310-315.

Ingram, PR; Bremner, P; Inglis, TJ; Murray, RJ and Cousins, DV (2010). Zoonotic tuberculosis: on the decline Communicable Diseases Intelligence. 34: 339-344.

Jiang, G; Wang, G; Chen, S; Yu, X; Wang, X; Zhao, L; Ma, Y; Dong, L and Huang, H (2015). Pulmonary tuberculosis caused by *Mycobacterium bovis* in China. Sci. Rep., 4: 8536.

Kamerbeek, J; Schouls, L; Kolk, A; van Agterveld, M; van Soolingen, D; Kuijper, S; Bunschoten, A; Molhuizen, H; Shaw, R; Goyal, M and van Embden, J (1997). Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol., 35: 907-914.

Khattak, I; Mushqaq, MH; Ahmad, MU; Khan, MS and Haider, J (2016). Zoonotic tuberculosis in occupationally exposed groups in Pakistan. Occup. Med., 66: 371-376.

Kremer, K; van Soolingen, D; Frothingham, R; Haas, WH; Hermans, PW; Martin, C; Palittapongarnpim, P; Plidhaya, BB; Riley, LW; Yakrus, MA; Mussler, JM and van Embden, JD (1999). Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: inter-laboratory study of discriminatory power and reproducibility. J. Clin. Microbiol., 37: 2607-2618.

Kubica, T; Rusch-Gerdes, S and Niemann, S (2003). *Mycobacterium bovis* subsp. caprae caused one-third of human *M. bovis*-associated tuberculosis cases reported in Germany between 1999 and 2001. J. Clin. Microbiol., 41: 3070-3077.

Machado, A; Rito, T; Ghebremichael, S; Muhate, N; Maxhuza, G; Marranangumbe, AS; Baptista, J; Manguelle, J; Koivula, T; Streicher, EM; Warren, RM; Kallenius, G; van Helden, P and Correia-Neves, M (2018). Genetic diversity and potential routes of transmission of *Mycobacterium bovis* in Mozambique. PLOS Neg. Trop. Dis., 12: e0006147.

Menzies, D; Schwartzman, K and Pai, M (2009). Immune-based tests for tuberculosis. In: Zulma, AS; Grange, JM; Raviglione, MC; Yew, WW; Starke, JR; Pai, M and Donald, PR (Eds.), *Tuberculosis*. (2nd Edn.), Elsevier Health Sciences, W. B. Saunders. PP: 179-196.

Mertoğlu, A; Bİçmen, C; Karsaarslan, S and Buğdayev, MH (2018). Pulmonary tuberculosis due to *Mycobacterium bovis* revealed by skin lesion in slaughterhouse worker. Clin. Respir. J., 12: 317-321.

Munyeme, M; Rigouts, L; Shamputa, IC; Muma, JB; Tryland, M; Skjerve, E and Dønnø, B (2009). Isolation and characterization of *Mycobacterium bovis* strains from indigenous Zambian cattle using Spacer oligonucleotide typing technique. BMC Microbiol., 9: 144.

O’Reilly, LM and Daborn, CJ (1995). The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. Tuber. Lung. Dis., 76: 1-46.

Parreiras, PM; Andrade, G; Nascimento, TV; Oelemann, MC; Gomes, HM; Alencar, AP; Assis, RA; Mota, PMPC; Pereira, MAS; Lobato, FCP; Lage, AP and Suffixys, PN (2012). Spoligotyping and variable number tandem repeat analysis of *Mycobacterium bovis* isolates from cattle in Brazil. Mem. Inst. Oswaldo Cruz., 107: 64-73.

Rodriguez, S; Romero, B; Bezos, J; de Juan, L; Alvarez, J; Castellanos, E; Moya, N; Lozano, F; Gonzales, S; Saez-Lorente, JL; Mateos, A; Dominguez, L and Aranzaz, A (2010). High spoligotype diversity within a *Mycobacterium bovis* population: clues to understanding the demography of the pathogen in Europe. Vet. Microbiol., 141: 89-95.

Sahraoui, N; Muller, B; Guetarni, B; Boulahbal, F; Yala, O; Ouzrouit, R; Berg, S; Smith, NH and Zinsstag, J (2009). Molecular characterization of *Mycobacterium bovis*
strains isolated from cattle slaughtered at two abattoirs in Algeria. BMC Vet. Res., 5: 4.
Shrikrishna, D; de la Rua-Domenech, R; Smith, NH; Colloff, A and Coutts, I (2009). Human and canine pulmonary *Mycobacterium bovis* infection in the same household: re-emergence of an old zoontic threat? Thorax., 64: 89-91.
Sichewo, PR; Hlokwe, TM; Etter, EMC and Michel, AL (2020). Tracing cross species transmission of *Mycobacterium bovis* at the wildlife/livestock interface in South Africa. BMC Microbiol., 20: 49.
Skuce, RA; Mallon, TR; McCormick, CM; McBride, SH; Clarke, G; Thompson, A; Couzens, C; Gordon, AW and McDowell, SWJ (2010). *Mycobacterium bovis* genotypes in Northern Ireland: herd level surveillance (2003 to 2008). Vet. Rec., 167: 684-689.
Sola, C; Filliol, I; Legrand, E; Lesjean, S; Locht, C; Supply, P and Rastogi, N (2003). Genotyping of the *Mycobacterium tuberculosis* complex using MIRUs: association with VNTR and spoligotyping for molecular epidemiology and evolutionary genetics. Inf. Genet. Evol., 3: 125-133.
Sposito, FLE; Campanerut, PAZ; Ghiraldi, LD; Leite, CQF; Hirata, MH; Hirata, RDC; Siqueira, VLD and Fressatti, CR (2014). Multiplex-PCR for differentiation of *Mycobacterium bovis* from *Mycobacterium tuberculosis* complex. Braz. J. Mic., 45: 841-843.
Supply, P; Allix, C; Lesjean, S; Cardoso-Oellemann, M; Rüsch-Gerdes, S; Willery, A; Savine, E; de Haas, P; van Deutkom, H; Roring, S; Bifani, P; Kurepina, N; Kreiswirth, B; Sola, C; Rastogi, N; Vatin, V; Gutierrez, MC; Fauville, M; Niemann, S; Skuce, R; Kremer, K; Locht, C and van Soolingen, D (2006). Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J. Clin. Microbiol., 44: 4498-4510.
Tadayon, K; Mosavari, N and Feizabadi, MM (2013). An epidemiological perspective on bovine tuberculosis spotlighting facts and dilemmas in Iran; a historically zebu-dominant farming country. Iran J. Mic., 5: 1-13.
Tadayon, K; Mosavari, N; Sadeghi, F and Forbes, KJ (2009). *Mycobacterium bovis* infection in Holstein Friesian cattle; Iran. Emerg. Infect. Dis., 14: 1919-1921.
Takasaki, J; Manabe, T; Morino, E; Muto, Y; Hashimoto, Y; Likura, M; Izumi, S; Sugiyama, H and Kudo, K (2017). Sensitivity and specificity of Quantiferon-TB Gold Plus compared with QuantiFERON-TB Gold In-Tube and T-SPOT.TB on active tuberculosis in Japan. J. Inf. Chem., 24: 188-192.
Thoen, CO; Kaplan, B; Thoen, TC; Gilsdorf, MJ and Shere, JA (2016). Zoonotic tuberculosis. A comprehensive one health approach. Medicina (B Aires). 76: 159-165.
Torres-Gonzalez, P; Soberanis-Ramos, O; Martinez-Gamboa, A; Chavez-Mazari, B; Barrios-Herrera, MT; Torres-Rojas, M; Cruz-Hervert, LP; Garcia-Garcia, L; Singh, M; Gonzalez-Aguirre, A; de Leon-Garduño, AP; Sifuentes-Orsorno, J and Bohadilla-Del Valle, M (2013). Prevalence of latent and active tuberculosis among dairy farm workers exposed to cattle infected by *Mycobacterium bovis*. PLOS Neg. Trop. Dis., 7: e2177.
Tuzcu, N and Köksal, F (2020). Genetic evaluation of *Mycobacterium bovis* isolates with MIRU-VNTR and spoligotyping. Turk. J. Med. Sci., 50: 2017-2023.
Vayr, F; Martin-Blondel, G; Savall, F; Soulat, JM; Deffontaines, G and Herin, F (2018). Occupational exposure to human *Mycobacterium bovis* infection: A systematic review. PLoS Negl. Trop. Dis., 12: e0006208.
Weniger, T; Krawczyk, J; Supply, P; Niemann, S and Harmsen, D (2010). MIRU-VNTRplus: a web tool for polyphasic genotyping of *Mycobacterium tuberculosis* complex bacteria. Nucleic Acids Res., 38: 326-331.
WHO (2017). End TB by 2030. Framework for implementing the “End TB Strategy” In the African region 2016-2020. Cited Dec.20.2020. https://apps.who.int/iris/bitstream/handle/10665/259636/TBstrateng.pdf?sequence=1.
Yahyaooni-Azami, H; Aboukhashib, H; Bouslkhane, M; Berrada, J; Rami, S; Reinhard, M; Gagneux, S; Feldmann, J; Borrell, S and Zinsstag, J (2017). Molecular characterization of bovine tuberculosis strains in two slaughterhouses in Morocco. BMC Vet. Res., 13: 272.
Yang, L; Wang, C; Wang, H; Meng, Q and Wang, Q (2015). Evaluation of MIRU-VNTR for typing of *Mycobacterium bovis* isolated from Sika deer in Northeast China. BMC Vet. Res., 11: 93.