

## Spoligotyping of *M. tuberculosis* Strains from Cattle in Turkey

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### Abstract

Although it is generally accepted that *M. bovis* leads to tuberculosis in cattle, there are statements given from the different regions of the world, referring to the fact that *M. tuberculosis*, which is known as the human tuberculosis agent, causes tuberculosis in cattle as well. The material of the study consisted of 13 *M. tuberculosis* isolates which were isolated and identified from the organ pieces of 95 cattle with the culture methods; these organ pieces had been taken from the cattle with granulomatous lesion detection after the slaughtering in slaughterhouses located in Çukurova region and brought to the laboratory under sterile conditions. It was determined in the genotyping conducted by using the Spoligotyping method that 13 of the 55 isolates were *M. tuberculosis* and they belonged to the T1 family (SIT53) by becoming dense in one cluster (100%). Consequently, it was shown with this study that *M. tuberculosis*, which leads to tuberculosis in humans, could be transmitted from humans to animals and from animals to humans again, and researching the human and epidemiological tuberculosis cases by using molecular epidemiology-based methods such as spoligotyping might provide useful information about explaining the ways of transmission of tuberculosis.

**Keywords:** Cattle, *Mycobacterium tuberculosis*, Spoligotyping

## Türkiye’de Sığırlardan İzole Edilen *M. tuberculosis* Suşlarının Spoligotiplendirmesi

### Özet

Tüberküloze sığırlarda, *M. bovis*’in sebep olduğu genel olarak kabul edilmesine rağmen insan tüberküloz etkeni olarak bilinen *M. tuberculosis*’in de sığırlarda tüberküloze neden olduğuna dair dünyanın farklı bölgelerinden yapılmış bildirimler bulunmaktadır. Çalışmanın materyalini Çukurova bölgesinde bulunan mezbahalarda kesim sonrası granülamatöz lezyon tespit edilen sığırlardan alınan ve steril şartlarda laboratuvara ulaştırılan 95 adet sığıra ait organ parçalarından kültür yöntemleri ile izole ve tanımlanmış 13 adet *M. tuberculosis* izolatı oluşturdu. Spoligotyping yöntemi ile yapılan genotiplendirmede 55 izolatın 13 tanesinin *M. tuberculosis* olduğu ve bunların tek bir küme içerisinde yoğunlaşarak (%100) T1 ailesine (SIT53) ait olduğu belirlendi. Sonuç olarak bu çalışma ile insanlarda tüberküloze neden olan *M. tuberculosis*’in insanlardan hayvanlara, hayvanlardan da tekrar insanlara bulaşabileceği, insan ve hayvan kaynaklı tüberküloz vakalarının spoligotyping gibi moleküler epidemiyolojik temelli yöntemlerle araştırılmasının tüberkülozin bulaş yollarının açıklanması konusunda faydalı bilgiler verebileceği gösterilmiştir.

**Anahtar sözcükler:** Sığır, *Mycobacterium tuberculosis*, Spoligotyping

### INTRODUCTION

Tuberculosis is a chronic disease which is comprised of *M. tuberculosis*, *M. bovis*, *M. canettii*, *M. microti*, *M. africanum*, *M. caprae* and *M. pinnipedii* included in the *Mycobacterium tuberculosis* complex (MTC) and has a wide host range including humans, fish, reptiles, birds, wild animals and domestic mammals. *Mycobacteria* are the acid-resistant bacteria known with their abilities to settle in almost all the tissues of the body like lungs, kidneys, bones, liver,

skin and brain and the lymph nodules belonging to these tissues, and with their formation of typical granulomas in the tissues they settle in <sup>[1,2]</sup>.

Although it is generally accepted that *M. bovis* leads to tuberculosis in cattle, there are statements from studies made in different regions, referring to the fact that *M. tuberculosis*, which is known as the human tuberculosis agent, causes tuberculosis in cattle as well. It was reported by many researchers that tuberculosis caused by the *M. tuberculosis* was seen in domestic and wild animals after



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short and long-term contacts with humans and existed especially in cattle [3-5]. Although there are researches in Africa stating that the incidence rate of the *M. tuberculosis* in cattle is under 1% [3,6,7], it was stated that the *M. tuberculosis* dependent tuberculosis rate in cattle is 7.4% in countries like Sudan where the human tuberculosis incidence is high [8]. It was reported in another study conducted in India that the 30.8% of the tuberculosis occurrences identified in cattle were the tuberculosis cases that develop depending on the *M. tuberculosis* [9].

People with tuberculosis spread the agents with their urine, sputum and stools. There are reports stating that the *M. tuberculosis* infection develops in cattle through eating the feeds that have agents or the inhalation of the contaminated respiration air [5,10]. Direct transmission of the *M. tuberculosis* among cattle is doubtful.

The *M. tuberculosis* complex genome includes 36-base-pair direct repeat (DR) loci at different numbers and there are sequences called *spacer* at the length of 35-41-base pairs between these loci. The genetic relation between the strains can be determined by using the spoligotyping method on basis the number of DR copies and the existence or absence of the spacer sequences. The spoligotyping method has been used frequently over the last years to put forth the molecular epidemiology of the tuberculosis in humans and animals. Spoligotyping is a PCR-based reverse dot blot hybridization method and it is fast, simple and repeatable [11].

Simeon Cadmus et al. stated in the spoligotyping they conducted on human and cattle tuberculosis isolates in Nigeria that 51 of the 60 human MTC-member isolates were *M. tuberculosis*, these isolates had 18 different spoligo-patterns and the most observed pattern was the NH1 pattern, which belongs to LAM 10-CAM family including 35 isolates. Same researchers stated that 15 of the 17 MTC members they obtained from cattle were *M. bovis*, one member was *M. tuberculosis*, another member was *M. africanum* and the *M. tuberculosis* isolate belonged to the NH1 (LAM10-CAM) pattern, which is most frequently seen in humans [4].

It was aimed in this study to perform a molecular characterization of the *M. tuberculosis* strains by using the spoligotyping method, which were isolated from the lesions with the suspicion of tuberculosis obtained from the cattle slaughtered in the slaughterhouses located in Çukurova region.

## MATERIAL and METHODS

The material of this study consisted of 13 *M. tuberculosis* isolates which were isolated and identified from the organ pieces of 95 cattle by using the culture methods; these organ pieces had been taken from the

cattle with granulomatous pneumonia detection after the slaughtering in slaughterhouses located in Çukurova region and brought to the laboratory under sterile conditions. These 95 cattle were chosen out of 6.800 cattle slaughtered for meat production.

### Culture

The tissue samples, which were taken from the lesions with the suspicion of tuberculosis observed in the lung and lymph nodes in the macroscopic examination carried out after the slaughtering and brought to the laboratory, were decontaminated in accordance with the protocol specified by Petroff [12].

Samples were inoculated onto the LJ medium with (4 gr/l) and without pyruvate and left for incubation at 37°C [12,13]. EZN-stained preparates were prepared from the bacterial growth. Biochemical tests were applied to the bacterial growth, which were positive in terms of the ARB [14]. The strains which had morphological eugonic growth in the LJ medium, positive niacin accumulation test, nitrate reduction reaction, TCH reaction and negative catalase activity at 68°C were evaluated as *M. tuberculosis* [5,15].

### Spoligotyping

DNA extraction was made by using the Mickle device from the colonies multiplied in the LJ medium of the 13 isolates, which were evaluated as *M. tuberculosis* as a result of the biochemical tests (Mickle tissue disintegrator). The spoligotyping method was applied after the extraction by using the DRa and DRb primer pairs, array of which is given below [16,17].

**DRa:** 5'-GGT TTT GGG TCT GAC GAC-3' (biotin labelled at the 5' end)

**DRb:** 5'-CCG AGA GGG GAC GGA AAC-3'

DRa and DRb primer pairs targeting DR area is synthesized. As DRa primer is labelled with biotin, it is preserved at +4°C. DRb primer is splitted into small amounts and preserved at -20°C. In each process, positive (*M. bovis*, *M. bovis* BCG, *M. tuberculosis* H37Rv, or clinical isolate whose genotype is known) and negative control (dH<sub>2</sub>O) are used. For each strain; dH<sub>2</sub>O 8.5µl, DMSO 1.0 µl, 2x PCR Master Mix (Fermantas) 12.5 µl, DRa (25 pmol/µl) 0.25 µl, DRb (25 pmol/µl) 0.25 µl, template DNA 2.50 µl are used. Tubes containing PCR reaction mixture are placed in Thermal Cycler device (Applied Biosystem AB) and heat cycle as: 95°C 5 min, 40 cycle 94°C 1 min, 55°C 1 min, 72°C 45 sec and then 72°C 10 min 4°C ∞. By using below mentioned octal coding key, results are converted to "Octal code" made of 15 characters between 0 and 7. By using databases, groups and clades are determined by obtained data (<http://www.pasteurguadeloupe.fr:8081/SITVITDemo/outils/Consultation.jsp>, <http://www.miru-vnrplus.org>, <http://www.mbovis.org>).



*M. tuberculosis* in places where the tuberculosis incidence is high in humans and animals.

In another study including the analysis of 768 samples, which were taken from the cattle with the suspicion of tuberculosis after the slaughtering in Northern India, 54 MTC isolates were determined and it was shown in the biochemical tests conducted on the isolated MTC strains that 14 of the isolates were *M. tuberculosis* [5]. The fact that 13 MTC isolates isolated from 95 cattle with the detection of granulomatous lesions were determined as *M. tuberculosis* in this study resembles the results of Srivastava et al. [5]. Besides, this result reveals that the cattle tuberculosis resulting from the *M. tuberculosis* must also be taken into account in the determination of the tuberculosis eradication strategies, which will be applied in our country.

Researchers stated at the end of the study they conducted between 2005-2007 that the cattle-isolates, which were among the 19 isolates isolated from the animals, were included in the T1 family (SIT53) by reporting in the spoligotyping of the 74 tuberculosis agents isolated from humans, who did cattle business with the animals slaughtered in the southwest of Nigeria and had the diagnosis of tuberculosis, that 32 agents were *M. tuberculosis*. The fact that all the *M. tuberculosis* isolates obtained from the cattle with granulomatous lesion detection after the slaughtering belonged to the T1 family in this study matches up with the results of Jenkins et al. [21].

It is stated in the studies related to the molecular epidemiology of the *M. tuberculosis* belonging to the people in our country that the *M. tuberculosis* Beijing strains have started to be observed at growing rates [17,22,23]. With the spoligotyping method, Zozio et al. [24] stated in their study, where they assessed 245 clinical *M. tuberculosis* isolates from Ankara and Malatya regions, that 206 isolates gathered in 33 clusters and 39 isolates were in specific clusters with single members, the LAM7 TUR family constituted the biggest cluster by 21% and the T1 family followed it with the rate of 16.3% and the Haarlem 3 family with 5.3%. Durmaz et al. [23] reported in the spoligotyping they conducted with 145 human MTC isolates in Malatya that LAM7-TUR was the most common pattern (23.96%) and the T1 family (SIT53) was the second most common pattern (22.5%) in Malatya. The fact that the T1 family (SIT53), which was defined as the second density by the researchers in the molecular typing of the *M. tuberculosis* isolates isolated from the tuberculosis cases, included all the *M. tuberculosis* isolates isolated in this study puts forth the necessity to definitely consider the cattle for presenting the transmission dynamics of the tuberculosis seen in humans.

This study has a critical and important role in the control programs that will be applied in our region due to the fact that it is the first molecular epidemiological study

conducted on the *M. tuberculosis* infection in the cattle of our region.

Consequently, the results obtained through this study showed that *M. tuberculosis*, which leads to tuberculosis in humans, could be transmitted from humans to animals and from animals to humans again, animals may have significant roles in the transmission chain of the *M. tuberculosis*, and researching the human and animal-origin *M. tuberculosis* isolates with molecular epidemiology-based methods such as spoligotyping might give useful information about determining the ways of transmission of tuberculosis in the development of struggle strategies against tuberculosis.

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