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Research article
Ερευνητικό άρθρο

First isolation of *Mycobacterium bovis* SIT 482 BOV from beef cattle in Turkey

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ABSTRACT: Bovine tuberculosis is a zoonotic disease which should be emphasized in our country as in many countries. A large number of genotypes have been revealed by spoligotyping method of *M. bovis* in the world. *M. bovis* SIT 482. BOV is one of these genotypes and it is also *M. bovis* genotype in which BCG vaccine is administered in humans. The BCG vaccine is obtained through multiple passages of this genotype. However, this genotype, like other genotypes, can cause serious infections in humans and animals. There are data on the isolation of *M. bovis* SIT 482 BOV from animal and human tuberculosis cases in the world. In our country, the isolation of this genotype has been reported only in humans and no data have been found in the animals. In this study, *M. bovis* SIT 482. BOV was isolated from the samples of six cattle with internal organ samples obtained from licensed slaughterhouses in Aksaray. While isolation is carried out with BACTEC MGIT 960 liquid media, spoligotyping was carried out according to the manufacturer's with kit (Isogen LifeScience, The Netherlands). This study aims to report *M. bovis* SIT 482 BOV from cattle in Turkey for the first time, to draw attention a very limited number reported in *M. bovis* cases in Turkey, highlight the importance of genotyping of tuberculosis factors and contribute to epidemiological studies. These and similar studies will contribute to the creation of genetic maps for eradication of *M. bovis* from cattle in our country. The study was also conducted to investigate whether *M. bovis* SIT 482. BOV isolated from cattle in Aksaray province is BCG strain or another strain giving the same pattern.

Keywords: Cattle; *Mycobacterium bovis* SIT 482. BOV; Turkey

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INTRODUCTION

Mycobacterium bovis is a pathogen within the *Mycobacterium Tuberculosis* Complex (MTC) that causes tuberculosis in animals and humans (Rodwell et al., 2008). *M. bovis*, the causative agent of bovine tuberculosis, has the widest host range among the MTC members (Wei et al., 2004), and infects animals, especially cattle, and sometimes humans (Mignard et al., 2006).

Among the cattle, *M. bovis* is mostly transmitted by the airway, but by ingestion of the contaminated material, the infection may also occur. When the bacterium enters the lungs it begins to multiply and usually spreads to the lymph nodes of the lungs (Menzies and Neill, 2000). The transmission of *M. bovis* from the cattle to the people is the most contaminated, non-pasteurized milk consumption, but it is observed by direct contact with infected animals (Lari et al., 2006) or by inhalation of infectious aerosols extracted from the sick animals (Mignard et al., 2006).

On the other hand, in many cases, the clinical and pathological features of *M. bovis* cannot be distinguished from *M. tuberculosis* infection (Rodwell et al., 2008; Lari et al., 2006) and the incidence of *M. bovis* in human infections is unknown, as many laboratories provide general diagnoses under the name MTC. Therefore, identification and genotyping of the mycobacterial agents are important in diagnosing humans. Again, considering the presence of multi-drug resistant *M. bovis* strains and *M. bovis* co-infection with HIV, *M. bovis* studies are also gaining value (Rodwell et al., 2008). The American General Health Organization and World Health Organization state that in South America every year, 7,000 new cases of human tuberculosis caused by *M. bovis* have been reported and the actual incidence is estimated to be eight times higher (Wei et al., 2004).

M. bovis BCG was obtained by passaging *M. bovis* 230 times over 13 years and is used as a vaccine strain throughout the world (Keating et al., 2005). *M. bovis* SIT 482. BOV has the same spoligotype patterns as the BCG vaccine strain and can cause infections in humans and animals. *M. bovis* SIT 482 BOV was isolated from humans (Lari et al., 2016; Mokrousov et al., 2010) and cattle (Sahraoui et al., 2009; Munyeme et al., 2009; Parreiras et al., 2012) in the World. *M. bovis* SIT 482 BOV has been reported in humans in our country (Çavusoğlu et al., 2007; Aslan et al., 2009) but data from this genotype was not detected from animals in Yurkey. Knowing which genotypes are available to combat *M. bovis* in our country will be of great benefit.

In the diagnosis *of M. bovis*, isolation is the gold method and identification is usually made by geneticall-ly based methods. Although the Restriction Fragment Lenth Polymorphism (RFLP) method is commonly used in genotyping the agents, Spoligotyping (Spacer Oligonucleotide Type Analysis) method based on PCR amplification of the Direct Repeat (DR) region of MTC has been preferred in recent years for identification (Kamerbeek et al. 1997; Schlossberg, 2017). Spoligotyping is a method based on the polymorphism of DR regions in mycobacterial DNA. The DR regions were formed by the spacing of non-repetitive spacers between a number of repetitive sequences specific to mycobacteria belonging to the member of the MTC. In this method, all DR regions are amplified by PCR following DNA extraction. The resulting amplicons are hybridized with membrane-bound separator oligonucleotides and made visible by streptavidin-peroxidase autoradiography (Marchetti et al., 1997). Spoligotyping results are in the form of numbers and are converted into number series that define the hybridization model.

This study aims to report *M. Bovis* SIT 482 BOV from the slaughterhouse samples of cattle in Turkey for the first time, to draw attention to a very limited number reported in *M. bovis* cases, highlight the importance of genotyping of tuberculosis factors and contribute to epidemiological studies. This and similar studies will contribute to the creation of genetic maps necessary for eradication of *M. bovis* from cattle in the World.

MATERİALS AND METHODS

Samples

This study performed during wintger months (January and February), after the permission received from the Ministry of Food, Agriculture and Livestock. In this research, a sample of the internal organs (lung and related lymph nodes) of six cattle obtained from the licensed slaughterhouses in Aksaray province was used. These samples were transported in hazardous material transport containers and in the cold chain to the Mycobacteria laboratory of Ege University Medicine Faculty. The laboratory work was done.

Bacterial examination

Isolation

Sterile samples (weight 5 to 10 g) were homogenized using the stomacher and the homogenate was taken to the decontamination process. For this purpose, tissue homogenate in 0.375-0.75% hexade-

cylpyridiniumchloride (HPC, detergent) was shaken at room temperature for 10-15 minutes. Sieves were thawed from BACTEC MGIT 960 liquid media which were thawed for primary isolation and the cultivated media were incubated at 37 ° C for 8 weeks. (Arda et al., 1997, Songer and Post, 2011). The BACTEC MGIT 960 automatic mycobacteria detection system was detected by stimulation in the liquid media.

S*poligotyping*

Spoligotyping was performed as described by the manufacturer of the kit. In summary; DNA was obtained from the isolates and *M. tuberculosis* H37Rv and *M. bovis* BCG strains used as positive control and DNA was obtained by ultrasonic bath method. 5% of the DNA obtained was used as template DNA in PCR process. The PCR procedure was determined by DRa 5'-GGTTTTGGGTCTGACGAC-3 and biotin labeled DRb 5AC- CCGAGAGGGGACGGAAAC-3 'primers used by Kamerbeek et al. (1997). These primers were used in the amplification of the DR region to be spoligotyped. DH_2O was used as the negative control in PCR. PCR mix with a total volume of 50 ml were 3 U Hot Star Taq DNA polymerase (0.6 µl), 10X buffer (5 µl), 25 mM $MgCl_2$ (5 µl), 20 pM DRa primer (4 µl), 20 pM DRb primer (4 µl), molded DNA (5 µl), a mixture of 2.5 mM dNTP (4 µl), and nuclease purified dH_2O (22.4 µl). Amplification conditions was in the form of after 15 min pre-denaturation at 95º C, 30 cycles of amplification at 95º C for 1 min denaturation, connecting for 1 min at 55ºC and elongation for 45 sec at 72ºC and 10 min final elongation at 72ºC. The amplified PCR product was hybridized with the nitrocellulose membrane attached to the oligonucleotide with the help of the mini blotter. Hybridized DNA was detected by chemiluminescence. Following incubation with streptavidin-peroxidase followed by detection with ECL (Enhanced Chemo-luminescence), the hybridized regions were visualized as black squares on the film. Spoligotypes were entered into the SpolDB4 spoligotyping database at http://www.pasteur-guadeloupe.fr in the form of a binary format and converted to a 15-digit octal code and compared with the strains recorded in the database

RESULTS AND DISCUSSION

In this study, *Mycobacterium bovis* SIT 482 BOV was isolated from the specimens (lungs and related lymph nodes) of the six cattle obtained from licensed slaughterhouses in Aksaray province BACTEC MGIT 960 automatic mycobacteria detection system showed positive signal İn three weeks ago after planting in BACTEC MGIT 960 fluid medium. Macroscopic image samples of organ are given in **Figure 1.** The study was also conducted to research whether *M. bovis* SIT 482 BOV isolated from cattle in Aksaray province was BCG strain or another strain giving the same pattern.

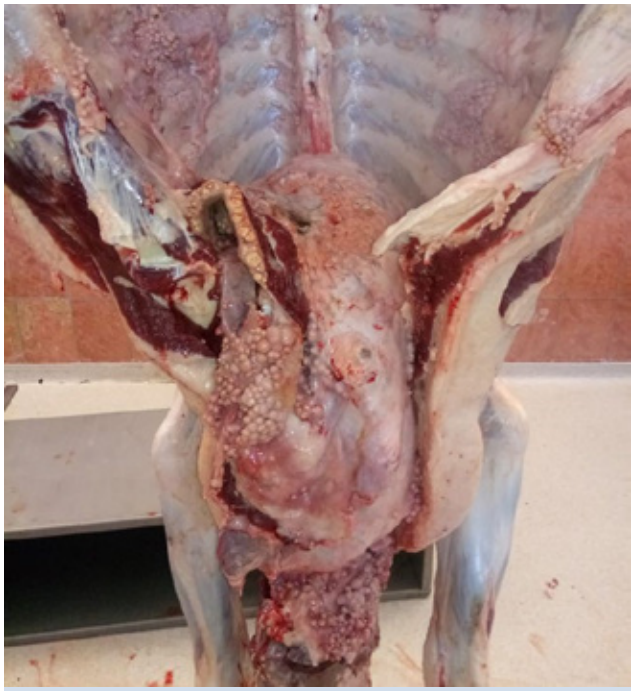

Figure 1. Macroscopic image of organs with tuberculosis nodules

Mycobacterium bovis SIT 482 BOV was isolated from humans (Lari et al., 20016; Mokrousov et al., 2010) and cattle (Sahraoui et al., 2009; Munyeme et al., 2009; Parreiras et al., 2012) in the world. In our country, although it was reported by Çavusoğlu et al. (2007) and Aslan et al. (2009). *M. bovis* SIT 482 BOV isolation data were not found from the animals. The reason of the fact that the agent is not bitten in animals in our country is that *M. bovis* is one of the least studied pathogens in our country due to the difficulties such as zoonosis, isolation and identification difficulties and a level three laboraztories required to work.

Cavusoglu and Yilmaz (2017) spoligotyped in the Aegean region of 13 *M. bovis* isolates 9 (63.6%) *M. bovis* SIT 685 BOV, 1 (7.7%) *M. bovis* SIT 1118 BOV, 1 (7.7%) *M. bovis* SIT 820 was determined and found no strains registered in the database for two isolates. Avsever et al. (2017) isolated *M. bovis* SIT 685 BOV from the goats in the same region. Again, Çavusoğlu et al. (2007) and Aslan et al. (2009) isolated *M. bovis* SIT 482. BOV from humans.

In this study, *M. bovis* SIT 482. BOV was isolated

from cattle. These data indicate that at least *M. bovis* SIT 482. BOV and SIT 685 BOV in our country can be widespread from animals to humans. This and similar studies are important in terms of understanding which genotypes are a more important problem in humans and animals in our country and which are more contagious.

In the study, BACTEC MGIT 960 fluid media and BACTEC MGIT 960 automated mycobacteria detection system for the isolation of bacteria has multiple advantages over the conventional method using Löwenstein-Jensen (LJ) solid media (Saini et al. 2017) The first advantage is that fewer bacteria can be produced in a faster time, the other advantage is that the system gives a warning and that the first growth is understood in the fastest time and that the human eye is not mistaken.

Tuberculosis disease is a holistic problem that does not distinguish between human or animal in our country. In order to solve this problem, veterinary and medical microbiologists should work together and support multidisciplinary studies. Neither the solution of tuberculosis alone or human tuberculosis alone will provide an effective remedy.

As a result, Mycobacterium of the slaughterhouse samples of cattle in Turkey in this study *M. bovis* SIT 482 BOV reported for the first time, the country has drawn attention to the very limited number of reported *M. bovis* cases, it highlighted the importance of genotyping of tuberculosis agent and has tried to contribute to epidemiological studies. This and similar studies will contribute to the creation of genetic maps for the eradication of *M. bovis* from cattle in our country. Finally, this is mulutdisciplinary study among veterinarians and human doctors.

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest**.**

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