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Prevalence of tuberculosis in cattle in Turkey*)

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Summary

The aim of the study was to determine the prevalence of bovine tuberculosis in the Kayseri province in Turkey and to compare histopathology and direct microscope examinations, culture and BACTEC radiometric methods.

The lungs and bronchial and mediastinal lymph nodes, collected from 3216 cattle slaughtered at 5 slaughterhouses belonging to both the public and private sector, were examined from September 2003 to August 2004.

In macroscopic examinations lesions were noted in 248 (7.7%) of the cattle. In histopathology examinations caseous tuberculosis was observed in 57% of the lungs, in 57% of the bronchial and in 53% of the mediastinal lymph nodes. In direct microscopy, acid-fast bacilli were seen in 33% of the lungs, in 33% of the bronchial and in 28% of the mediastinal lymph nodes. In culture, Mycobacterium bovis was isolated in 19% of the lungs, 19% of the bronchial and in 17% of the mediastinal lymph nodes. In the BACTEC method, Mycobacterium spp. was isolated in 21% of the lungs, in 21% of the bronchial and in 17% of the mediastinal lymph nodes.

In conclusion, the prevalence of bovine tuberculosis in slaughtered cattle in Kayseri province is 1.49%, and the BACTEC radiometric method is a rapid and sensitive method for diagnosing M. bovis.

Keywords: Tuberculosis, cattle

Bovine tuberculosis (BTB) is a chronic disease of cattle which is characterized by granulomatous lesions mainly seen in the respiratory tract and associated lymph nodes. The aetiological agent of bovine tuberculosis is *Mycobacterium bovis* (16).

M. bovis infection in cattle is now mostly confined to the respiratory system, which reflects transmission and establishment to infection mainly by this route. A single bacillus transported within a droplet nucleus is probably sufficient to establish infection within the bovine lungs. Once the bacteria enter the lungs, they begin to multiply and generally spread to the lymph nodes near the lungs (14). At this primary site of infection, the lesions can remain quietly inactive or develop further depending on the ability of the cow to fight off the infection (9).

Bovine tuberculosis can not be diagnosed by clinical examination because of its sub-clinical course. Definitive diagnosis of infection relies on skin test, evaluation of macroscopical and microscopical findings as well as isolation procedures (7, 16). At present, direct microscopic examinations of smears stained by Ziehl-Neelsen (ZN) stain is the most rapid and inexpensive method. However, this method gives false negative results in some cases. The growth of the agent in the culture takes between 4 and 8 weeks (19). Considering the zoonotic characteristics of the disease, rapid, less cumbersome, accurate and inexpensive methods are needed for the diagnosis of animal tuberculosis. Currently, the half automated radiometric BACTEC 460 TB system is one of the techniques widely used among commercial systems (1). The bacilli of TB can be determined between 7 and 18 days (mean 13 days) regardless of species of Mycobacterium and samples (19). Most of the previous studies with regard to BTB conducted in Turkey focused on the clinic and pathological aspects of the disease (2, 5, 18).

In the present study, determination of the prevalence of BTB in cattle slaughtered in the slaughterhouses located in Kayseri province, and the comparisons of histopathological and direct microscopical examinations, culture and BACTEC radiometric methods for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic value (DV) were aimed.

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Material and methods

A total of 3216 cattle slaughtered at 5 slaughterhouses belonging to public and private sector were examined for BTB from September 2003 to August 2004. Lungs and bronchial and mediastinal lymph nodes were macroscopically examined.

Histopathology. Tissue samples were fixed in 10% neutral-buffered formalin. The pieces of preserved organs were embedded in paraffin, sectioned (5-6 µm), and mounted on glass slides, stained with haematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN), and were examined microscopically (12).

Bacteriology. Tissue samples with lesions were homogenised and decontaminated (10), and the sediments were used for direct microscopical examination, culture and BACTEC method.

Direct microscopy. The smears prepared from sediments were stained with ZN stain and examined for acid-fast bacilli (13).

Culture. The sediments were inoculated onto Lowenstein-Jensen (LJ) slants (Merck, Darmstasdt, Germany) with or without glycerol and LJ slants were incubated at 37°C for 4-8 weeks. Identification of isolates of mycobacteria was based on growth on LJ slants, colony morphology and biochemical tests such as catalase, urease, niacin accumulation, nitrate reduction, pyrazinamidase and thiophene-2--carboxylic acid hydrazide (13).

BACTEC radiometric method. The sediment were inoculated into the Middlebrook 7H12 (Bactec 12B medium, Becton Dickinson, Maryland, USA). Inoculated vials were placed in BACTEC 460 (Becton Dickinson Diagnostic Instruments, Towson, Maryland) instrument, and they were incubated at 37°C and read daily. Growth index (GI) points for the specimens were recorded until a GI of 999 was obtained or until 108 days post inoculation. Ziehl Neelsen staining was performed on BACTEC cultures when a GI of 999 was obtained (21).

Statistical analysis. Data were analysed by SPSS for Windows release 13.0 packet programme. McNemar Chi-Square test was done for the sensitivity, specificity, PPV, NPV, and DV were calculated.

Results and discussion

Macroscopical findings. Diffuse fibrinous adhesions were observed between the visceral surface of the lungs and pleura. Lesions were changed from irregular caseous bronchopneumonia to conglomera caseous lobar pneumonia especially in the caudo-dorsal lobs depending on the rapidity and extent of spread. The diameters of the firm, dark yellow nodules were ranged from 0.4-3 cm. Furthermore, dense consolidation areas ranged from dark red to purple were observed almost in all the lung tissue. Bronchial and mediastinal lymph nodes were enlarged 2-3 fold in diameter and caseous yellowish content was present in the cut surface.

Histopathology. Caseous tuberculosis was observed in 142 (57%) of the lungs, in 142 (57%) of the bronchial and in 132 (53%) of the mediastinal lymph nodes. Classical tuberculosis granulomas were found in nearly all of the cuts of lungs and lymph nodes. Granulomas had centrally or peripherally mineralized caseous necrosis and they were surrounded by inflammatory cells and a thick connective tissue (fig. 1). Inflammatory cells were consisting of too many neutrophil leucocytes lymphocytes, plasma cells, macrophages, epithelioid cells, Langhans' giant cells. The interlobar and intralobular septa were thickened due to inflammatory cell infiltration and connective tissue proliferation. A lot of acid-fast bacilli were observed in caseous necrotic area, and Langhans' giant cells and macrophages (fig. 2).

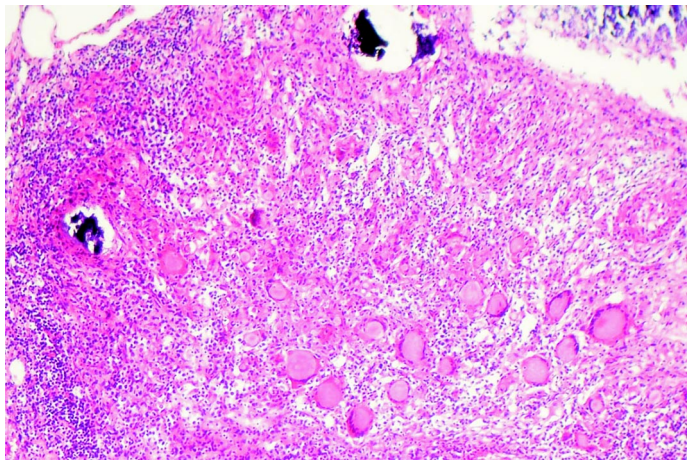

Fig. 1. Lungs, mineralized small necrotic areas surrounded by Langhans' giant cells and inflammatory cells. H&E stain, × 200

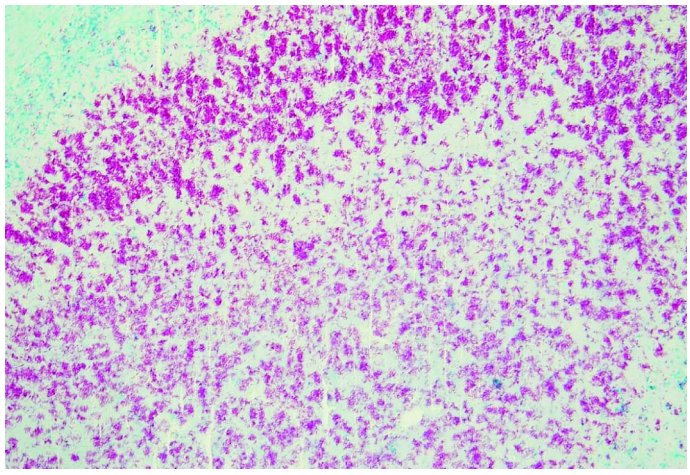

Fig. 2. Caseous necrotic areas with acid fast bacilli in granulomas. ZN stain, × 1000

Bacteriology. In direct microscopical examination, acid-fast bacilli were seen in 81 (33%) of the lungs, and bronchial lymph nodes and in 70 (28%) of the mediastinal lymph nodes. *Mycobacterium bovis* isolated and identified from 48 (19%) of the lungs, and bronchial lymph nodes and from 41 (17%) of the mediastinal lymph nodes by LJ slants without glycerol. The first growth on LJ slants was observed on 24th day. Agent was isolated in 49 (21%) of the lungs, and bronchial lymph nodes and in 41 (17%) of the mediastinal

lymph nodes by BACTEC radiometric method. The first isolation by BACTEC 12B was made on 8th day. In microscopical examination of the smears prepared from both LJ and BACTEC cultures, acid-fast bacilli were seen. No growth was observed on LJ slants with glycerol. The prevalence of BTB in cattle slaughtered in Kayseri was found as 1.49%.

The sensitivity, specificity, PPV, NPV and DV were 100, 53, 33.8, 100 and 62% respectively for histopathological examinations; 97.9, 83, 58, 99.4 and 85.8% respectively for direct microscopic examinations and 100, 99.5, 98, 100 and 99.5% respectively for the BACTEC radiometric technique, compare to culture as gold standard used for the examinations of lungs and bronchial lymph nodes. The sensitivity, specificity, PPV, NPV and DV were 100, 56, 31.1, 100 and 63.3% respectively for histopathological examinations; 97.6, 85.5, 57.1, 99.4 and 87.5% respectively for direct microscopic examinations and 100, 100, 100, 100 and 100% respectively for the BACTEC radiometric technique used for the examinations of mediastinal lymph nodes. In this study the results of histopathology, direct microscopy, culture and BACTEC radiometry were consistent ($p < 0.001$, $p > 0.05$).

Bovine tuberculosis represents a potential health hazard to both animals and humans. Economical losses in animals as well as psycho-sociological losses in human arising from the tuberculosis are of importance in Turkey. Cattle are considered the primary and most well-known reservoirs of tuberculosis (1). In recent years, increases in the prevalence of BTB have been reported (1, 4, 7). The primary factor causing the increase of the prevalence of BTB is political instability, and politico-economic factors also causes failure in enforcement of control and eradication programmes (1). Furthermore, the increase of the prevalence might be attributed to environmental factors such as management. The prevalence studies conducted in Turkey (2, 18) and in many other countries (4, 7, 8, 15, 23) based on the detection of the diseased cattle at slaughterhouses by tuberculin testing and macroscopic inspection of the carcases as well as laboratory diagnostic tools. In the present study, the prevalence of BTB was determined as 1.49% based on the culture. The prevalence of tuberculosis in cattle is accepted to be low when it is less than 5%, and at this point, the test and slaughter control method might be considered an economical option for the control and eradication of the disease (4). The low prevalence determined in the present study may be due to the modern cattle breeding and appropriate application of the control and eradication programmes. In suspicious cases, accurate and rapid diagnosis of *M. bovis* should be done by histopathological examination and culture following the macroscopic observation of the typical lesions.

There have been limited studies investigating BTB in Turkey (2, 5, 18). In the present study, caseous tuberculi were observed in 142 (57%) of the lungs, in 142 (57%) of the bronchial and in 132 (53%) of the mediastinal lymph nodes consistent with the findings of Ortatatlı et al. (18) who found granulomas with mineralized necrotic areas. Whipple et al. (24) isolated *M. bovis* from 3 cattle that had no gross lesion of tuberculosis. This finding suggests that tuberculosis could be missed by inspection of carcases at slaughter (24). Furthermore, some suspicious samples collected in the present study gave false-positive results with direct microscopic histopathological examination which were found negative with culture that is accepted as reference method (13). Therefore, culture should be performed when BTB is suspected. Acid-fastness is not restricted to the mycobacteria; other acid-fast organisms include *Corynebacterium* spp., *Nocardia* spp., and *Rhodococus* spp. (1, 11). The false-positive results obtained in this study by direct microscopy and histopathological examination may results from other acido-resistant microorganisms.

Currently, the solely definitive diagnostic method is the isolation of the *M. bovis* from the tissue samples of internal organs (11). In the present study, in culture, *Mycobacterium bovis* was isolated in 19% of the lungs, in 19% of the bronchial and in 17% of the mediastinal lymph nodes. Pavlik et al. (20) found BTB in 1.4% of the lungs and bronchial lymph nodes of slaughtered animals in the Chech Republic which is similar to the findings of the presented study. However, in the studies conducted in South East Brazil, the isolation rate of BTB from mediastinal and retrophrangeal lymph nodes of cattle was reported as 64.6% (26) and 42.6% (25). The differences between the results of the present study and the studies of Zanini et al. (25, 26) may be due to the variation of geographical location as well as the differences between the decontamination methods used in the mentioned studies. Currently, a combination of conventional solid media with broth-based method such as BACTEC TB system is accepted as a reference standard for the diagnosis of mycobacterial infection. Previous studies have shown that BACTEC system has higher isolation rate than solid media (6, 17, 22) as in the present study. Simultaneous use of conventional solid media and broth based methods such as BACTEC is suggested for efficient isolation of Mycobacterium species (3, 6, 19).

In conclusion, determination of a 1.49% prevalence has shown that the diagnosis of the BTB in slaughtered cattle is of importance. In diagnosis of BTB, the BACTEC radiometric method which is found as rapid and sensitive method can be used along with the conventional methods.

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