# Bovine Tuberculosis Slaughter Surveillance in Albania, Importance of Its Traceback Investigation Based on Singel Cervical Comparative Skin Test

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**Abstract:**

*Bovine tuberculosis is a bacterial contagious disease due by Mycobacterium bovis that primary affects cattle and widest range of mammals. Mycobacterium bovis and Myobacterium caprae are responsible for zoonotic tuberculosis. In Albania, bovine tuberculosis is endemic, however information on the spread of disease is not available and sustainable control measures are not fully enforced. In this study, we present data from a pilot survey for bovine TB after receiving an unofficial information of generalized bovine TB in a slaughtered calf in Dibra region. In total, there were tested 277 cattle by comparative tuberculin skin test and prevalence of bovine tuberculosis was 1.1% at individual level and 1.4% at farm level. There were identify a variety of risk factors that facilitate spreading bovine tuberculosis in animals and interfere with its control programs, such as lack of correct animal identification, animal movement control, age structure etc. Active surveillance in the abattoirs and traced back to their farm of origin and tested all in-contact animals with simultaneous comparative cervical skin test is a rational approach as a first step for control of bovine tuberculosis disease. An active surveillance in large commercial farms must be initiate and when the necessary infrastructure will be available, a national active surveillance must be implement. In addition to field tests, gross examination during meat inspection, histopathological, microbiological and molecular methods must be*

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*employed systematically for bTB diagnosis and epidemiological study. More efforts must be pay in collaboration with human health in the framework of "One Health" philosophy, which will provide the full background of zoonotic tuberculosis in human and animals.*

**Key words:** *Mycobacterium bovis*, bovine tuberculosis, bovine PPD, avian PPD

## INTRODUCTION

Bovine tuberculosis (bTB) due by *Mycobacterium bovis* (*M. bovis*) is a zoonotic disease, spread worldwide, affects the widest range of mammals, including humans [4, 5, 8]. Cattle are target animals for *M. bovis*. Infected cattle serve as main sources of infection, and in different parts of the world there are identify a certain reservoirs of infection [1, 4]. *M. bovis* is slow growing bacteria, able to survive in the environment conditions, which interfere with pathogen isolation and in other hand contaminated environment, including manure may serve as source of infection [6,].

    *M. bovis* transmission occurs in direct and indirect routes, and it is present in aerosol of infected animals, sputum, excretions, secretions and tissues. The inhalation is most common and efficient method of infection, where the infected dose range from 1-10 bacteria [3, 6]. Ingestion is another important method for transmission from infected animal to susceptible animal, but the infected doses is very high compare to the inhalation route. The tubercular lesions could be localised in any tissues or organs, but in general there are known two main forms: pulmonary and extrapulmonary forms. In pulmonary form, which is typically for relatively old animals infected by inhalation, while the extrapulmonary form is related with digestive route of infection and mesenterial lymph nodes often are involve. Despite the above mention forms, in

advanced stages of active bTB, generalised form is likely to occur. Number of mycobacteria have a positive correlation with developing of granulomatous lesions and they are in a large number in lymph nodes, lungs, serous membranes, and *M. bovis* is present in aerosol, faeces, milk, urine etc. Transmission of disease between herds is most likely to occur by introducing infected animals to the free herds, while contaminated vehicles, visitors etc play role in speeding of bTB to the new herds. Applying strict animal movement control and biosecurity measures play important role in reducing the risk of transmission of bTB. Appling the quarantine measures at national and regional level and isolation procedures at farm levels are extremely important for limiting spreading of bTB.

The importance of bTB for animal health and zoonotic potential enforced program control application, which date back since early 1900. Two main factors play significant positive impact on bTB control: milk pasteurisation and identifying of infected animal by using tuberculin skin test. There is estimated that *M. bovis* is responsible for up to 10% of all human cases [3]. Any control program, aim to eliminate bTB, either at regional and/or local level is based on systematic programs of tuberculin skin test, stamping out of positive cattle; active surveillance at abattoirs; strict animal movement control; frequently testing of infected herds; depopulation of wild reservoir animals, heat treatment and milk pasteurisation, farmer training, providing education program etc [3, 5, 6, 7, 8]. In Albania, the first bTB cases were recorded during 1935-1940, but the official reports and control program of bTB started in 1960 [2]. In 1959, the prevalence of bTB was 2.6% at animal level. In 1964, the serious outbreaks occurred in several districts. Interestingly, in 1977 and subsequent years, bTB cases have been reported after ten years in areas that were officially bTB free. The control program was evaluate as effective and the incidence of bovine tuberculosis from 1973 to

1987, decreased 16 to 24 times. The tuberculin test involved almost 50% of the cattle population. In 1988, bTB prevalence at national level was 0.116% [2]. In the 1990, the socio-economic changes have had an impact on farm organisation and veterinary services. Animals were tested on incidental basis and no information was available on disease prevalence. Bovine and avian tuberculin produced at ISUV was used for the field diagnosis. No quality assurance testing was performed during production process. The meat inspection was not done in all cases as many animals are not slaughter under control. In last years, number of tested animals is reduced and laboratory data on bTB confirmation almost do not exists.

In this study, we present results from a pilot study on usefulness of surveillance of bTB based on SICCT by tracing back the farm origin of a calf infected by bTB. In addition, gross lesions and histological changes of affected tissue are described.

## MATERIALS AND METHODS

In this study, we tested all eligible cattle present in 141 holdings in entirely epidemiological unit and tissue samples were collected from slaughtered animals, which were judged as positive in comparative tuberculin skin test. The criteria for including animals were age over 1-month, healthy status and animals that belong to the village that was consider as epidemiological unit. In addition, there was used certified bovine and avian PPD tuberculin, appropriate syringes, clipper and calliper. The details data for each animal were recorded according designated template for the skin test purpose. In total, there were tested 277 animals by single intradermal comparative cervical tuberculin test (SICST). Briefly, the tested animal ear tag was recorded, animal were restrained, the injection sites were prepared, the skin was measured, recorded and both tuberculin were correctly intradermal injected. The

test was applicated according to European Union guideline 64/432/EEC. The injection sites were prepared at the $1/3^d$ of middle of the neck. The doses of the tuberculin was 0.1 ml, which contain 3000UI and 2800 UI for bovine and avian PPD, respectively. The results were read $72 \pm 2h$ and the data were recorded in the same template used at day zero. The criteria for classification of animal health status are described in Table 1. The criteria for classification of animal health status are described in Table 1.

**Table 1 – Criteria used for animal classification according to results obtained from single intradermal comparative tuberculin test (SICTT).**

| Animal status | Skin thickness difference before and 72h ± 2h after avian PPD injection | Skin thickness difference before and 72h ± 2h after bovine PPD injection | Presence of oedema, inflammation and clinical signs | Skin thickness difference between bovine PPD and avian PPD |
|---|---|---|---|---|
| **Positive** | **>4 mm** | **>4 mm** | **+** | **>4 mm** |
| Negative | <2 mm | <2 mm | - | <2 mm |
| Doubtful | 2-4 mm | 2-4 mm | ± | 2-4 mm |

## The tissues

The positive animals were slaughtered, and detailed meat inspection was carried out. The gross lesions were identified, recorded and selected pair samples were collected; fresh samples were used for isolation of suspected mycobacteria (data not show), the tissues fixed in 10% buffer solution of formalin were used for histopathological examination. The samples were collected from the initial case with suspected bTB lesions (liver, spleen and mesenteric lymph nodes) detected during routine meat inspection and from three positive animals (lung lesions, retropharyngeal, mediastinal and mammary lymph nodes) in SICT.

## Gross lesion examination

Examination was done based on knife and eye method and presence of tubercular lesions was judged by naked-eye. From organs with visible tubercular lesions, both fresh and fixed

samples were collected and submitted to the laboratory of animal infectious disease, Faculty of Veterinary Medicine, Tirana.

## Histopathological examination

The fixed samples were stained by using the standard haematoxylin-eosin (H&E) method, and prepared microscopic slides were exanimate under light microscope.

## RESULTS AND DISCUSSION

The single comparative skin test results are present in Table 2. In this study, 277 cattle from 140 holdings were tested with single intradermal cervical comparative skin test (SICCT). The final judgment for classification of individual animals for their health status was based on difference of skin thickness and presence of inflammatory oedema, fluctuation and presence of necrosis etc.

Table 2.  Results of SICT of tested animal

| Parameters | Number | Percent |
| --- | --- | --- |
| Total number of farms | 141 | 100 % |
| Farm tested | 140 | 99.3 %* |
| Positive farms | 2 | 1.4% |
| Farms with doubtful animals | 2 | 1.4% |
| Tested animal | 277 | 100 % |
| Positive animal from simultaneous test | 3 | 1.1% |
| Doubtful animals from simultaneous test | 3 | 1.1% |
| Negative animals (free from bTB) | 271 | 97.8 % |

*one farm refused testing

Based on the study results, disease prevalence of bovine tuberculosis of cattle population at village Blliçe was 1.1% at individual level and 1.4% at farm level. Referring to OIE criteria where disease prevalence should be less than 0.2% in order to classify the unit free from bovine tuberculosis at farm level, the study area could be classify as affected and bTB as enzootic status. Based on available data three main risk factor

that affect the control program and/or can have impact on bTB spreading were identified.

**Herd size:** The average size of farms is two animals/holding, 43% of them own only one cattle, while 1.5% of farmers have over five cattle per farm. Only a very few (0.7%) have 10 animal/holding. This trend of cattle distribution per farm follow the national pattern, where 73% of the farms have a size ranged 1-4 animals and only 5% have a size bigger than 50 animals per farm. Apparently, this size farm does not support bTB transmission, if the close herd management is in place. In the reality, animals managed in one village have close contact between them, they share the routes, water sources, pastures and often the bulls. Those conditions are risk factors that facilitate bTB spreading within and between heads. We strongly suggest the extensions program must consider practical aspects of biosecurity and correct implementation at farm level.

**Age herd structure:** bTB is a typical chronic disease, so it is assume that clinical disease, if appear, could be detect generally in older animals. In our study, the overall age of tested animals was 5.8 years old, ranged from 1 month to 20 years old. Age structure is accepted as a risk factor for developing typical tubercular lesions. At advanced stage of the disease, the number of mycobacteria and severity of lesions obviously increase. It is important to highlight that older animal and animal where the bTB is in advanced case, the cell mediated immunity response is affected negatively and either official skin test and/or INF-$\gamma$ test do not detect all affected animals. To increase sensitivity we recommend using ELISA test as a ̏cleaver ̋ choice aims to detect the infected animals, which may be negative in SICCT and $\gamma$ - INF tests.

**Correct identification of animals:** The first step for disease surveillance is animal identification and strict animal movement control. Animal identification was identified as a poor and risk factor for control of infectious disease in general and bTB in particular. Furthermore, present farm buildings conditions are not adequate for implementing of cleaning and disinfection procedures. As it is shown in Table 3, 121 cattle (43.7%) were unidentified, missing one ear tag was recorded in 301(0, 8%) animals and only 102 of them or 36.8% were correctly identified. Furthermore, 24 animals (8.7%) were not ear tagged, because the farmers refused.

**Tabel 3 – Factors that influence bTB contolle**

| Considered rick factors | The parameter | Comments |
|---|---|---|
| Average farm size (as number of animals) | 2 animals | Farm size ranged from 1 to 10 animals; specifically, 43% with 1 cattle, 32% with 2, 12% with 3, 10% with 4, 1.5% with 5 and only 0.7% with 10 animals. |
| Average age of tested animal | 5.8 years old | 23% of animal tested until 1 years old, 14% 1-3 years old, 21% 4-6 years old, 20% 7-9 years old, 16% 10-13 years old and 6.1% over 14 years old |
| The oldest tested animals | 20 years old | 4 animals or round 1.4 % |
| Unidentified animal (animal without ear tag) at tested time | 121animals | round 44 % |
| Animals with one ear tag at tested time | 30 animals | round 11 % |
| Animals found to be corrected ear tagging at tested time | 102 animals | 36.5 % |
| Number of animals refused to be era tagged by the owners | 24 animals | 8.5 % |

Based on measures made 72±2h, three animals were positive on SICCT. Detailed data for reactors are given in Table 4.

**Table 4 –** Detailed data on positive animal from SICCT

| ID | Breed | Age in years | Skin thickness before and 72±2h after tuberculin injection, their differences and results respectively | | | | | | | | Skin thickness difference Bovine PPD - avian PPD | Comments | Final result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Avian PPD | | | | Bovine PPD | | | | | | |
| 842 | Simmental | 5 | 6.5 | 7.8 | 1.3 | - | 7.3 | 15.0 | 7.7 | + | 6.4 | Oedema | + |
| 018 | Crossed breed | 1.4 | 7.8 | 15 | 7.2 | + | 7 | 34.5 | 27.5 | + | 20.3 | Oedema and necrosis | + |
| 017 | Crossed breed | 6 | 7.2 | 12.5 | 5.2 | + | 6.5 | 17.0 | 10.5 | + | 5.2 | Oedema | + |

**Figure 1-** Positive result from bovine PPD. Animal infected with bTB based on SICCT. The reaction was read 72 hours after injection. The inflammatory response against bovine PPD is clearly visible, while reaction to avian PPD is much less. As the difference of skin thickness between avian PPD and bovine PPD was 20.3 cm and animal was classified as positive.

Positive animals, according to the veterinary low, were slaughtered at regional sanitary abattoir and detailed meat inspection was performed. All affected organs were recorded, filmed and proper samples were collected. The results of close examination of animal carcasses are presented in Table 5 and Figure 2.
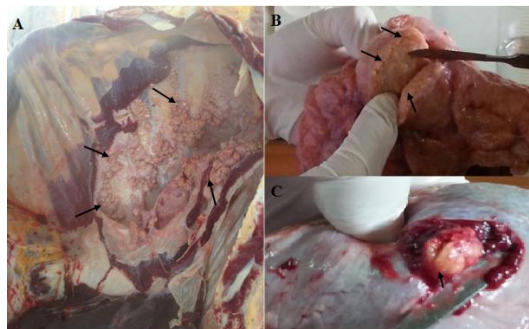


**Figure 2**. Gross lesions in positive cattle. Tubercular lesions **A**. Thoracic cavity, parietal pleura is heavy invaded by miliary

tuberculosis (arrows), thoracic surface of diaphragm is severely affected the fibrous masses of white-pink colour resembling cauliflower. **B**. Mesenteric lymph nodes, arrows indicate the presence of tubercular lesions; they were significantly enlarged and visible from a certain distance. **C**. A typical granulomatous lesion in spleen of SICCT positive animal slaughtered as positive. Encapsulation and calcification were present detected during cutting.

| Organs / lymph nodes | Typical tubercular lesions and their distribution according to organs of each animal | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Calf (index case) | | Animal 1 | | Animal 2 | | Animal 3 | |
| | Yes | No | Yes | No | Yes | No | Yes | No |
| Lungs | | - | + | | + | | + | |
| Liver | | - | + | | | - | NA | NA |
| Spleen | + | | + | | + | | | - |
| Lymph nodes | | | | | | | | |
| Retropharyngeal | | - | + | | + | | | - |
| Mediastinal | | - | + | | + | | + | |
| Mesenteric | + | | + | | | - | | - |
| Mammary | NA | NA | + | | + | | NA | NA |

**Table 5. Distribution of typical tubercular lesions in infected animals included in this study**

Histopathological examination of samples collected from carcases, showed presence of cell alterations and presence of typical structures in response to *M. bovis* infection.

Microscopical examination indicate presence of typical changes in all samples, however the intensity of caseous necrosis, lymphocytic infiltration, presence of giant cells etc varies according to the stage of lesions (Figure 3).
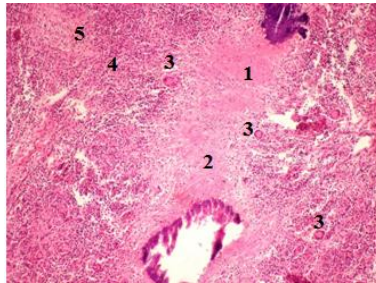
**Figure 3.** Histopathological slight from mediastinal lypnh node showing presence of typical structures stained with Hematoxilin-Eosin (H&E) (**1**-mineralisation, **2**- necrosis, **3**-giant cells, **4**- macrophages, **5**-lymphocytes)

## CONCLUSION

According to our results, incidence of bTB is increased and control of *M. bovis* infection is an important issue of veterinary services, it is becoming a serious challenge. Drafting of a rational and realistic bTB eradication program is necessary; however, it is necessary to consider the risk factors that may have impact on failing the control program. We identify that lack of correct animal identification and strict animal movement control, unavailability of logistic infrastructure for performing skin test and lack of funds for farmer compensation; cleaning and disinfection interfere with the control program [5, 6].

Drafting and implementing a rational strategy based on screening of commercial dairy farms, strict slaughterhouse surveillance and following up the origin of suspected case will be a realistic approach for controlling of bTB. In addition, transferring the new diagnostic tools, smartly using the available diagnostic test and involving the scientific institutions and human health capacities will support positively the bTB control program [7].

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