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## Detection of tuberculosis in goats using anti-mortem techniques

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#### Abstract

A total of 50 goats of smallholders and different farms from in and around Nagpur were investigated for tuberculosis using comparative intradermal tuberculin test (CITT) and IFN- $\gamma$  assay. CITT was performed using avian and bovine PPD and IFN- $\gamma$  assay by *Mycobacterium bovis* gamma interferon test kit. Overall, 4% (2/50) goats were found inconclusive by CITT and 4% positive by IFN- $\gamma$  assay. However, in goats which found inconclusive in CITT, only those turned positive in IFN- $\gamma$  assay. Hence, when CITT and IFN- $\gamma$  used together lead to more accurate screening for tuberculosis in animals.

**Keywords:** tuberculosis, bovine gamma interferon assay, comparative intradermal tuberculin test, goat

#### Introduction

Bovine tuberculosis (TB) has been identified as one of eight globally neglected zoonoses that require more attention, particularly in developing nations such as India (WHO, 2011) [1]. Asia is the most severely affected region, with the largest numbers of TB cases occurring in China and India. TB in small ruminants is caused by members of *Mycobacterium tuberculosis* complex predominantly by *Mycobacterium bovis* and *Mycobacterium caprae* (OIE 2009) [2] and few caused by *Mycobacterium tuberculosis* (Cadmus *et al.* 2009) [3]. Epidemiological studies indicated that tuberculosis in goat has wide global distribution and has been reported in various countries of the world including India. In India, bovine TB has been known to be endemic in cattle; however, the status of TB in goats has not been well studied in spite of their close contact with cattle. This zoonotic disease continues to have considerable economic and public health implications. Since both the species of *Mycobacterium* (MTB and *M. bovis*) pose a threat to health of animals and thereby capable of infecting humans and viz. (reverse zoonosis), detection of the bacteria in the early stage is needed.

Intradermal tuberculin test is recognized by the World Organization for Animal Health (OIE) as the primary screening test for detection of bovine TB in large and small ruminants (OIE, 2009) [2]. The application of this test, supplemented with interferon gamma assay, help in the detection of the disease in infected or suspected animals. The animals show no clinical indications in the early stages of bovine tuberculosis. If the condition is not recognised early, the animal may go undetected, leading to the disease becoming chronic. For successful therapy of bovine tuberculosis, early diagnosis and prompt detection are critical.

Although culture is regarded the "gold standard" for diagnosing tuberculosis, it is a time-consuming operation that can take several weeks. So, in live animals, diagnosis of bovine tuberculosis is essentially reliant on the detection of specific cell-mediated immune responses by the skin test (OIE, 2009) [2] and IFN- $\gamma$  assay (Gormley *et al.* 2006) [4].

The purchase of infected animals and the interaction with infected cattle or goats at common pastures could be the external sources of bovine TB (Okafor *et al.* 2011) [5]. The application of skin test along with IFN- $\gamma$  assay may increase sensitivity and specificity and help in the better diagnosis of the disease in infected or suspected animals either in early stage or in latent stage of TB (Praud *et al.* 2015) [6].

Taking into account all these, the current study aimed to look into diagnosis of *tuberculosis* in goats in and around Nagpur region.

#### Materials and Methods

##### Study animals and collection of samples

In present study, goat population suspected of tuberculosis having the symptoms like poor

body condition, emaciated, hacking cough, swollen lymph node and loss of weight were selected. About 50 goats were screened by comparative intradermal tuberculin test and blood samples from respective goats were collected for Interferon gamma assay in heparin vacutainer. All the samples were collected from different farms and smallholder farmers from in and around Nagpur, Maharashtra. No previous screening of tuberculosis was done in the selected farms.

#### Comparative intradermal tuberculin test (CITT)

This test compares immune responses to *M. bovis* (bovine) and *M. avium* (avian) tuberculin in the cervical region. All the animals were subjected to CITT as per the guidelines of OIE (2009). Briefly, 0.1 ml, each of avian tuberculin PPD-2500 (PPD-A) (Prionics) and bovine tuberculin PPD-3000 antigens (PPD-B) (Prionics) were injected intradermally on each side of the neck at identical sites in the centre of the middle third of the neck of goats (Fig.1). Skin thickness was measured using digital vernier calliper on 0 hrs and 72 hrs after PPD injections. The result was interpreted as follows:

Reactors	Difference of the skin thickness
Positive	>4mm
Negative	< 1mm or no reaction to antigens
Inconclusive	Between 1 to 4 mm

Fig 1: Tuberculin test in goat

#### Bovine Interferon Gamma (IFN- $\gamma$ ) Assay

Interferon gamma assay was performed by employing BOVIGAM<sup>TM</sup> (Thermo Fischer, Switzerland) commercially available kit. Blood samples were collected from jugular veins in sterile 10 ml heparinized tubes before CITT was conducted. The immunoassay was performed according to the manufacturer's instructions. Briefly, 1.5 ml aliquots of heparinized blood was dispensed into individual wells of 24-well tissue culture plates and incubated with 100  $\mu$ l each of stimulating antigens (PPD-B and PPD-A) and PBS (non-stimulating control) for 16-24 h at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub>. The plasma was then collected and assayed for IFN- $\gamma$  production in duplicate using a commercially available EIA kit (Bovigam), and optical densities were measured on an ELISA plate reader (Multiskan Go, Thermo scientific, Finland) at 450 nm.

#### Results and Discussion

In present investigation, comparative intradermal skin test and Interferon gamma assay was performed in 50 goats suspected of tuberculosis. Out of 50, two (4%) were found in-conclusive and rests of other were non-reactors in CITT whereas in IFN- $\gamma$  assay two (4%) were found positive. The details of the results are given in Table 1.

Table 1: Results for CITT and IFN- $\gamma$  in goats

Species	No. of animals	Tuberculin test		IFN- $\gamma$ (Positive)
		Positive	In-conclusive	
Goat	50	0	2 (4%)	2 (4%)

Detection of tuberculosis in goats by skin test is not much popular in India. The screening for tuberculosis is not routinely performed in goats in India. Further, there are few reports available pertaining to tuberculosis diagnosis in small ruminants of Maharashtra. Hence the data for comparison in goats is lacking. Even though bovine TB is known to be endemic in Indian cattle and buffalo, very little information on TB in small ruminants is available. Best of our knowledge, this is the first study performed in goat population in given study area for tuberculosis. Due to lack of detailed sources on the topic, the observations could not be compared extensively. However, a few publications pertaining to the diagnosis of tuberculosis in small ruminants are available.

Various researchers reported the prevalence of tuberculosis in goats by employing intradermal tuberculin test worldwide. The results of present findings are in accordance with Kassa *et al.*, (2012) [7] who reported 3.8% of in-conclusive reactors. Also, Noorrahim *et al.*, (2015) [8] reported zero reactors from goats and Tschopp *et al.*, (2011) [9] reported positivity of 0.4% in small ruminants. Many other researchers also reported the low prevalence by CITT in small ruminants but the fact that they also get infected with tuberculosis cannot be denied. In contrast to the of present findings, other studies by Ashenafi *et al.*, (2013) [10] reported 5.29% of prevalence, Rahman *et al.*, (2013) [11] reported 1.29% overall positive reactors in goats from Bangladesh. Also, Vidal *et al.*, (2018) [12] reported 10% in goats, Tafess *et al.*, (2011) [13] reported 3.1% individual animal prevalence in goats and 26.18% prevalence when doubtful reaction was considered positive. Bezos *et al.*, (2015) [14] screened goat flock for tuberculosis with Single intradermal test and comparative intradermal test, 22.6% were reactive for CITT and 27.1% for SID.

Interferon gamma assay is a quick, laboratory assay of a cell-mediated immune response that can be used to diagnose tuberculosis (TB) infection in animals. It is based on the detection of gamma interferon (IFN- $\gamma$ ).

The IFN- $\gamma$  test has been used successfully to diagnose bovine tuberculosis worldwide. It has also been used in goats to detect the tuberculosis in early progression of the disease. But very few reports are available about use of IFN- $\gamma$  used in small ruminants as most of the researchers performed combination of the intradermal tuberculin test and other test like isolation of the organism for TB diagnosis. The findings of present study could not be compared sufficiently for want of the numbers of references for study in goats.

The finding of the present study could be compared with Shanahan *et al.*, (2011) [15] who reported 18% positivity in goats which were at high risk of tuberculosis and 0% in lowrisk goats from the different farms at Ireland by employing IFN- $\gamma$ .

The finding of present study is at lower side compared with Bezos *et al.*, (2015) [16] who reported positivity with the prevalence of 60.5%.

The advantage to use IFN- $\gamma$  assay is that the, IFN- $\gamma$  could detect the infection in early progression of disease, considering this point the positivity found in present study is low. Blood collection and the start of the IFN- $\gamma$  assay at the laboratory in present study, a minimum of 8 hours of holding time was required. But the samples were collected from the distant regions and minimum holding time of blood samples was 16-24 hrs, this could have possibly lowered the sensitivity of the assay.

Delaying antigenic stimulation of samples for more than 8 hours reduced test sensitivity, putting the ability of assay to detect sick animals in danger exhibiting role of those in IFN- $\gamma$  assay Bezos *et al.*, (2011) [14]. The other factors such as set-up delays and initial high sample temperatures, diminish IFN- $\gamma$  responses in infected cow cells, increasing the probability of false negatives Waters *et al.*, (2007) [17].

The gamma-interferon assay can be used to diagnose and treat tuberculosis in goats. The test can detect *M. bovis* infection in its early stages. Avian reactors with enhanced reactivity to bovine PPD in the gamma-interferon assay should be considered positive for *M. bovis*. It is feasible to obtain a group of animal, test negative for TB from a herd of goats with high immunoreactivity to this infection by serial testing with the gamma-interferon and single intradermal tuberculin tests, as well as a policy of segregation of kids at birth (Liebana *et al.*, 1998) [18].

The disparity in results could be explained by the fact that investigations are conducted in different geographical areas, and the epidemiology of the disease may differ in different areas. The proportion of positive reactors was much greater in these studies, which could be associated to the different husbandry practices, favoring a potential transmission of mycobacterial species between goats (Kassa *et al.*, 2012) [7]. The other possible reason for such variations in result could be that some researches performed single intradermal tuberculin test rather than comparative intradermal test, as CIDT can differentiate the animals infected from *M. bovis* and other environmental Mycobacteria. As in SIT, only bovine tuberculin PPD was used and it can react to other Mycobacteria also including *M. bovis*.

According to Gormley *et al.*, (2006) [4], a tuberculin test combined with an IFN- $\gamma$  assay was able to identify 95.2% of infected cattle. Ganesan (2012) [19] found that IFN- $\gamma$  assay detected 77.7% of bTB positives compared to 49.2% using SID in the same animals, implying that IFN- $\gamma$  assay is more sensitive than SID, which supports the findings of the present study.

The IFN- $\gamma$  assay proved to be a valuable tool for TB diagnosis in goats. It has a higher sensitivity than the CITT test and allowed us to detect the infection at an early stage. Goats may operate as a reservoir for tuberculosis, hence caprine TB should be considered a hurdle block to the success of specific TB eradication projects, particularly in areas where goat rearing is a tradition.

Intradermal tuberculin test is based on the CMI response to mycobacterial antigen, which can result in a false negative if the animal is immunocompromised or has severe disease. These diseased animals could be a continuous source of infection for other healthy animals, as well as posing major health risks to humans.

## Conclusion

The strategic use of the gamma-interferon (IFN- $\gamma$ ) assay can help in early identification of *M. bovis* infected animal, allowing them to be separated from healthy herd. When used in conjunction with a tuberculin test, it can detect sick animals that would otherwise go undetected for months, if at all. The earlier these animals are identified and removed, the less likely they are to become a source of illness for other animals and humans. Thorough surveillance studies like the present one will add into animal TB control programs.

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