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# Diagnosis of Bovine Tuberculosis in Lactating Cattle and Buffaloes by Comparative Intradermal Tuberculin Test and Bovine Gamma-Interferon Immunoassay

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**ABSTRACT**

Two hundred lactating animals (158 cattle and 42 buffaloes) of organized and unorganized farms were investigated for the bovine TB using comparative intradermal tuberculin test (CITT) and IFN-γ assay. CITT was performed using avian and bovine PPD and IFN-γ assay by *Mycobacterium bovis* gamma interferon test kit. Overall, 14.5% and 11.5% animals were found positive by CITT and IFN-γ assay, respectively. However, 22.5% animals were detected TB positive through combination of both the tests. So, both CITT and IFN-γ assay, when used together lead to more accurate screening for bovine TB in dairy herd.

**Keywords:** Bovine tuberculosis, bovine gamma interferon assay, comparative intradermal tuberculin test, cattle, buffalo

Bovine tuberculosis (TB) has been identified as one of the eight worldwide neglected zoonoses which need more attention, especially in developing countries including India (WHO, 2012). Asia is the hardest hit region having the largest numbers of TB cases with the majority in China and India. In bovines, TB is mostly caused by infection with the intracellular acid-fast bacilli *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex. In addition, *M. tuberculosis* has also been reported to cause TB in cattle, although bovines are considered quite resistant to it.

In the early stages of bovine TB, the animals do not show any clinical signs. If not detected early the animal may go undiagnosed and further progress to the chronicity of the disease. Early diagnosis and rapid detection of bovine TB is important for proper management of the disease. Although culture is considered to be the "gold standard" for confirming TB, this procedure is slow and takes several weeks. So, diagnosis of bovine TB in live animals is primarily based on the detection of specific cell-mediated immune responses through the skin test (OIE, 2009) and γ-IFN assay (Gormley *et al*., 2006).

The application of skin test along with IFN-γ 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).

Thus, present study was aimed to diagnose bovine TB in cattle and buffaloes of organized and unorganized farms at Ludhiana and Jalandhar district of Punjab using CITT and γ-IFN assay.

## MATERIALS AND METHODS

### Ethical approval

This study was approved by animal ethics committee of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) [No. VMC/14/2413-43 dated 10/6/14].

### Selection of Area and Animals

Animals under study were cattle (cross bred and HF breeds) and buffaloes (murrah and niliravi breeds) of organized and non-organized farms at Ludhiana and Jalandhar district of Punjab (Table 1).

### Comparative intradermal tuberculin test (CITT)

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 in two sites 12 cm apart in the middle third of the neck of each animal. Skin thickness was measured using calliper on 0 h and 72 h after PPD injections. The result was interpreted as follows:

| Reactors | Difference of the skin thickness |
|---|---|
| Positive reactor | > 4 mm greater |
| Negative reactor | does not exceed 1 mm or no reaction to the bovine antigen |
| Inconclusive reaction | < 4 mm though reaction to both PPD-B and PPD-A exceeded 1mm |

### Bovine gamma-interferon (γ-IFN) enzyme immunoassay (EIA)

Blood samples were collected from jugular veins in commercially available sterile 10 ml heparinized tubes a day prior to CITT (Filia *et al.*, 2016). Briefly, 1.5 ml of heparinized blood from each animals were added in 3 wells of 24-well tissue culture plate and were incubated with 100 µl each of stimulating antigens; PPD-B, PPD-A and PBS (non-stimulating control) respectively in each wells for 16-24 h at 37°C in a humidified atmosphere with 5% $CO_2$. Next day, plasma was collected and assayed for γ-IFN production in duplicate using a commercially available ELISA based *Mycobacterium bovis* gamma interferon test kit (Bovigam®, Prionics, The Netherland). Optical density was measured on an ELISA plate reader (Multiskan, MTX Lab Systems, Inc., USA) at 450 nm.

### Statistical analysis

The proportions of the animals found positive by either CITT or IFN-γ assay were calculated. The proportions for 95 % confidence intervals (95 % CI) were computed as CIs for proportions.

## RESULTS

In the present study, 14.5%, 21% and 64.50% animals were found positive reactors, inconclusive reactors and negative reactors, respectively by CITT (Table 1). Positive reactors in organised and unorganised farms were 5.71% and 35%, respectively. Further in organised farm, out of 140 animals, 8 (5.71%) animals [1 (0.54%) cattle and 7 (5.00%) buffaloes] were positive reactors. While from unorganised farms of total 60 animals, 21 (35%) [19 (31.66%) cattle and 2 (3.33%) buffaloes] were

**Table 1:** Results of comparative intradermal tuberculin test (CITT)

| Farm | Number of Animals | Comparative intradermal tuberculin test (CITT) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive reactors | | | Inconclusive reactors | | | Negative Reactors | | | | |
| | Cattle | Buffaloes | Total | Cattle (%) | Buffaloes (%) | Total (%) | Cattle (%) | Buffaloes (%) | Total (%) | Cattle (%) | Buffaloes (%) | Total (%) |
| 1 | 84 | 56 | 140 | 1 (0.54) | 7 (5.00) | 8 (5.71) | 6 (4.28) | 20 (14.28) | 26 (18.57) | 77 (55.00) | 29 (20.71) | 106 (75.71) |
| 2 | 25 | 3 | 28 | 12 (42.85) | 1 (3.57) | 13 (46.42) | 5 (17.87) | 0 (0.00) | 5 (17.87) | 8 (28.57) | 2 (25.00) | 10 (35.71) |
| 3 | 5 | 3 | 8 | 2 (25) | 0 (0.00) | 2 (25.00) | 2 (25.00) | 1 (12.50) | 3 (37.50) | 1 (12.5) | 2 (25.00) | 3 (37.50) |
| 4 | 10 | 2 | 12 | 2 (16.66) | 1 (8.33) | 3 (25.00) | 3 (25.00) | 0 (0.00) | 3 (25.00) | 5 (41.66) | 1 (8.33) | 6 (50.00) |
| 5 | 12 | 0 | 12 | 3 (25.00) | 0 (0.00) | 3 (25.00) | 5 (41.66) | 0 (0.00) | 5 (41.66) | 4 (3.33) | 0 (0.00) | 4 (33.33) |
| Total | 136 | 64 | 200 | 20 (10.00) | 9 (4.50) | 29 (14.50)* | 21 (10.50) | 21 (10.50) | 42 (21.00) | 95 (47.50) | 34 (17.00) | 129 (64.50) |

* CI values = 9.62% to 19.38%

**Table 2:** Results of the bovine interferon gamma assay (IFN-γ assay)

| Farm | Animals | Interferon gamma assay (IFN-γ assay) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | | | | | Negative | | |
| | Cattle | Buffaloes | Total | Cattle (%) | Buffaloes (%) | Total (%) | Cattle (%) | Buffaloes (%) | Total (%) |
| 1 | 84 | 56 | 140 | 5 (3.50) | 12 (8.57) | 17 (12.14) | 79 (56.42) | 44 (31.42) | 123 (87.85) |
| 2 | 25 | 3 | 28 | 5 (17.8) | 0 (0.00) | 5 (17.8) | 20 (71.42) | 3 (10.71) | 23 (82.14) |
| 3 | 5 | 3 | 8 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 5 (62.5) | 3 (37.5) | 8 (100) |
| 4 | 10 | 2 | 12 | 1 (8.33) | 0 (0.00) | 1 (8.33) | 9 (75) | 2 (16.66) | 11 (91.66) |
| 5 | 12 | 0 | 12 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 12 (100) | 0 (0.00) | 12 (100) |
| Total | 136 | 64 | 200 | 11 (5.55) | 12 (6.00) | 23 (11.50)* | 125 (62.50) | 52 (26.00) | 177 (88.50) |

*CI values =7.08% to 15.92%

positive reactors. By IFN-γ assay, overall 11.5% animals were detected positive for TB (Table 2). In organised and unorganised farms, percentage positivity was 12.14% and 10%. All buffaloes were detected negative by IFN-γ.

## DISCUSSION

In the present study, skin test and IFN-γ test was conducted as a routine bovine TB screening procedure in dairy farms. Positive reactors in organised and unorganised farms were 5.71% and 35%, respectively. Likewise, 14.04% prevalence of bovine tuberculosis using CITT was reported by Filia *et al*. (2016) in sahiwal cattle of an organised farm in Punjab. During present investigation, more positive reactors were found in unorganised farm as compared to organised farm which might be due to the differences in the animal husbandry practice affecting the intensity and distribution of the disease as well as strength of the antigen specific skin reaction (Phaniraja *et al.*, 2010). More numbers of positive reactors were reported in cattle (10%) than buffaloes (4.5%) which is as per findings of Noorrahim *et al*. (2015).

During present investigation, 65.21% animals showed an exclusive positive reaction to INF-γ and a negative response to CITT. The remaining animals showed positive response to both INF-γ and CITT (Table 3). So, the strategic use of the IFN-γ assay provide a means for the early identification of *M. bovis* infected cattle, thus ensuring their removal from an infected herd (Gormley *et al*., 2004). Again, some animals which showed positive reaction in CITT, were detected negative by IFN-γ assay, which corresponds to the findings of Praud *et al.* (2015). In the present study, combination of both the test detected 45 animals positive (Table 3) where as individual test detected only 29 (CITT) and 23 (IFN-γ) animals positive.

**Table 3:** Comparison between CITT and IFN-γ assay

| Test | | Comparative intradermal tuberculin test | | |
|---|---|---|---|---|
| Positive | | Negative | Total | |
| **Interferon Gamma assay** | Positive | 7 | 16 | 23 |
| | Negative | 22 | 0 | 22 |
| | Total | 29 | 16 | **45** |

Gamma-interferon assay is based on the release of IFN-γ from sensitized lymphocytes during a 16 to 24 hrs incubation period with specific antigen. So, the test makes use of the comparison of IFN-γ production following stimulation with avian and bovine PPD (OIE, 2009). Thus CITT alone might resulted false negative and false positive reaction (Lahuerta-Marin *et al.*, 2015) but when IFN-γ assay is used as ancillary test to tuberculin tests, it could detect maximum number of infected animals (Wood and Jones, 2001).

## CONCLUSION

From the present study, it could be concluded that INF-γ assay in supplementary with CITT proved to be a very useful diagnostic method to be incorporated in a control and eradication program for bovine tuberculosis in India. The combination of these two test is highly feasible in the large organised farms and can also be implemented in

unorganized farms through bovine TB control programme looking to the economic importance of disease.

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