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ORIGINAL ARTICLE **OPEN ACCESS**

Screening of cattle population for Bovine Tuberculosis using Single Intradermal Tuberculin Test

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ABSTRACT
Bovine tuberculosis (BTB) is a chronic bacterial disease of cattle, caused by Mycobacterium bovis (M. bovis). The OIE (World Organization for Animal Health) recommended test for screening of bovine tuberculosis is Single Intradermal Tuberculin Test (SIDT). In the current study, a total of 82 randomly selected cattle from four gaushalas of Haryana were screened by SIDT. Out of these, 6/82 (7.31%) animals were found to be doubtful/ inconclusive by SIDT using bovine tuberculin. From this study, it could be concluded that bovine tuberculosis is prevalent in Haryana and need to be further investigated by other modern diagnostic tools like interferon gamma assay (IFN-γ assay) and DNA based techniques.
Keywords: *Economic, Mycobacterium bovis, Screening, Tuberculin, Zoonotic.*

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INTRODUCTION

Bovine tuberculosis (BTB) is an OIE (World Organization for Animal Health) listed chronic, debilitating, bacterial disease of livestock, wildlife and humans, caused by *Mycobacterium bovis* (*M. bovis*) and is economically significant because of trade restriction and its zoonotic perspective [1]. It has been estimated that *M. bovis* might infected over 50 million cattle worldwide with resulting economic losses of approximately $3 billion [2]. A wide range of animals is susceptible to *M. bovis* infection making its eradication difficult [3].

There are many available diagnostic tests for bovine tuberculosis but tuberculin skin test (TST) is the OIE recommended test for screening against TB [4]. Limitations in the sensitivity and specificity of tuberculin testing result in a failure to detect all *M. bovis* infected animals and contribute significantly to disease persistence [5]. The tuberculin used for in vivo tuberculin test in cattle contains a crude mixture of mycobacterial secreted proteins prepared by precipitation of heat killed cultures. Despite of limitations of tuberculin skin test, it is widely used for screening purpose. Traditional test and slaughter/segregation policies based on tuberculin skin testing have not been fully successful so that additional more sensitive and specific diagnostic tests are required [6]. The current study was performed on randomly selected 82 cattle of four different gaushalas of Haryana to screen for bovine tuberculosis by SIDT.

MATERIAL AND METHODS

The study was approved by the institutional animal ethics committee. The present study was conducted on randomly selected 82 cattle from four different gaushalas of Haryana. These animals were screened by single intradermal tuberculin testing (SIDT) using bovine origin purified protein derivative (PPD-B) (IVRI, Izatnagar, U.P.) using standard protocol [4] with the exception of calves of less than 6 months age, animals of more than 8 months pregnancy and one month post-partum animals [7]. The animals were categorized on the basis of breed, age, gender and location (Table 1). Briefly, before PPD-B injection, the injection site of neck region was shaved with sterile razor and cleaned properly. Skin thickness of the neck region of each cattle was measured prior to tuberculin injection with Vernier caliper. A single dose of 0.1 ml (2000 IU) PPD-B (1 mg/ml) was administered intradermally using tuberculin syringe having a

short needle. Skin thickness at the injection site was measured with Vernier Caliper after 72 hrs. of PPD administration. The SIDT was considered positive if the increase in skin thickness at the site of injection was 4 mm or more than 4 mm, inconclusive if the increase in skin thickness was more than 2 mm and less than 4 mm and negative if the increase in skin thickness at site of injection was less than 2 mm.

Table 1: Categorization of animals under study

Variables	No. of cattle screened
Breed	
Cross-bred	39
Hariana	26
Rathi	2
Sahiwal	15
Location	
A	25
B	19
C	19
D	19
Gender	
Male	19
Female	63
Age	
Heifer	0
Adult	82
Total cattle screened by SIDT	**82**

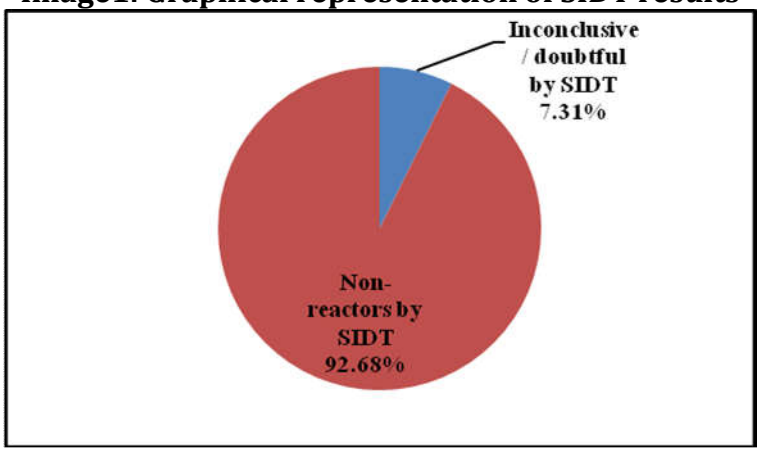
RESULTS

Out of 82 cattle, 6 (7.31%) were found doubtful/inconclusive as the difference in skin thickness before and after PPD-B administration was more than 2 mm but less than 4 mm. Out of these six cattle, four cattle (4.87%) were from gaushala 'A', one cattle (1.21%) from gaushala 'B' and one cattle (1.21%) from gaushala 'C'. None of the tested cattle from gaushala 'D' was found positive by SIDT. A total of five female cattle (6.09%) and one male (1.21%) animal (cow bull) were found positive by SIDT. Out of these six cattle, five (6.09%) were crossbreds and one (1.21%) was Hariana cattle. All the six doubtful/ inconclusive cattle by SIDT were adult animals (Table 2, Image 1).

Table 2: Categorization of animals in relation to positivity (%) to SIDT

Variables	No. of cattle found reactor/doubtful by SIDT (%)
Breed	
Cross-bred	5 (6.09%)
Hariana	1 (1.21%)
Rathi	0
Sahiwal	0
Location	
A	4 (4.87%)
B	1 (1.21%)
C	1 (1.21%)
D	0
Gender	
Male	1 (1.21%)
Female	5 (6.09%)
Age	
Heifer	0
Adult	6 (7.31%)
Total cattle found inconclusive/doubtful by SIDT	**6 (7.31%)**
Total cattle found non-reactor by SIDT	**76 (92.68%)**

Image1: Graphical representation of SIDT results



DISCUSSION

Similar to the present study, it was reported that 2.8% animals reacted to the tuberculin test (8) and low prevalence (1.93%) was also reported in cattle from Maharashtra (9). Similarly, in Morogoro, the prevalence was 1.3% by single intra-dermal comparative cervical tuberculin (SICCT) test (10) and in another study, an individual prevalence of 3.7% was estimated using SIDT test in Morogoro (11). The estimated prevalence of TB in animal population of Charsadda (Pakistan) by tuberculin skin testing (TST) found was 4.33% (12).

However, in Tamil Nadu and Karnataka, the prevalence rate was 34.58% and 30-35%, respectively (13). In Himachal Pradesh, BTB prevalence in cattle found was 14.31% by SIDT testing and 20% prevalence by comparative cervical tuberculin (CCT) test (14) and in U. P., 13.12% animals were found positive by SIDT testing (15). In a study of West Bengal, a total of 25.4 % animals from organized and 3.2% from backyard farming sector were found positive by tuberculin testing (16).

In the current study, the prevalence of BTB by SIDT testing was very low. The possible explanation for low prevalence of BTB by SIDT test could be early infection, dormant infection, presence of other environmental mycobacteria, immune response of the host, environmental stress factors, poor nutritional status *etc.* It can be speculated that animals with dormant infections fail to respond to PPD stimulation or repeated testing of animals with PPD may increase the number of animals failing to respond (17).

The current findings could also be supported with the findings that the infection with *M. avium* subsp. *paratuberculosis* interferes in the diagnosis of BTB by SIDT test (18). It has been reported that infection with either *Fasciola* spp. or *Stronglus* spp. significantly reduces the skin indurations in response to PPD-B in *M. bovis*-infected heifers (19).

CONCLUSIONS

The complex immune response of cattle to infection with *M. bovis* predisposes difficulties in diagnosis. These can best be dealt with by identifying the herd rather than the individual animal, and using combinational approach *i.e.* gamma interferon gamma assay, nucleic acid based techniques for diagnosis, to quantify the disease burden. The strategic use of IFN-γ assay, as an adjunct to the tuberculin skin test, can facilitate the early removal of the infected animals that are otherwise negative to the tuberculin skin test.

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