JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2016. Vol. **10**(2), p. 1527-1532

Screening of Bovine Tuberculosis Cattle using the Tuberculin Skin Test in Barsana

S.K. Shukla[1], A Chauhan[1], S. Shukla[2], M. Panigrahi[1], B. Bhushan[1], S. Maurya[1], Sarvjeet[1], R. Maurya[1], S. Kumar[1], C. Prakash[3], V.K. Saxena[1] and R.V. Singh[1]

[1]Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly - 243122, India.
[2]Department of Biosciences, Integral University, Lucknow, U.P., India.
[3]CADRAD, Indian Veterinary Research Institute, Izatnagar, Bareilly, India.

(Received: 23 December 2015; accepted: 12 March 2016)

The intradermal tuberculin tests used worldwide for the diagnosis of bovine tuberculosis. The tuberculin skin test can provide a means for the early identification of *Mycobacterium bovis* infected cattle, thus ensuring their removal from an infected herd. We have done the skin test in three hundred numbers of cattle and results of skin testing after 72 hours revealed a pattern as depicted; positive cattle comprised of fifty six bovine tuberculosis infected animals from tuberculosis-infected herds, one hundred fifty one non-infected animals from tuberculosis-free herds and ninety three doubtful animals from tuberculosis-free herds. The aim of this study is, identification of bovine tuberculosis animal based on tuberculin skin test; which is considered as the primary method of bTB detection in India. The skin test is based on a differential delayed type hypersensitivity (DTH) response to intradermal injections of purified protein derivative (PPD) from *M. bovis* (PPD-B) and *M. avium* (PPD-A).

Keywords: Bovine tuberculosis, Btb, Cattle, *M. bovis*.

The skin test is the international standard for the diagnosis of bTB, and accordingly the cervical Single Intra-dermal Tuberculin test (SIT) is primarily used to screen both individual cattle and herds in continental Europe[1,2,3]. Examples of such tests include the single intradermal comparative tuberculin test (SICTT). Numerous novel diagnostic tests have been developed specifically to detect *M. bovis* infection in non-bovine animals including the multi-antigen print immunoassay (MAPIA), rapid lateral-flow antibody tests (RLFATs), PCR, and IFN- assays[4,5,6]. The MAPIA assay is a laboratory-based, multi-step method that employs a cocktail of antigens to detect antibody responses to mycobacterial infections[7], while RLFATs have been used to detect antibodies to both *M. bovis* and *M. tuberculosis* infection in elephants, badgers and non-human primates[8].

For TB control, the tuberculin skin-test, based on the inoculation of purified protein derivatives (PPDs), has been employed worldwide as the standard diagnostic method designated by the World Organization for Animal Health[9]. This test, based on the measurement of the increase in the skin fold thickness induced by the inoculation of PPD of *M. bovis* (bovine PPD) or *M. avium* (avian PPD), is the basis of tuberculosis diagnosis in cattle. However, PPDs consist of a complex mixture of proteins, lipids, sugars, nucleic acids and include a great variety of antigens, many of which are shared with other mycobacterial species and close related bacteria[10, 11], by help of the Adjunctive biomarkers for improving diagnosis of tuberculosis and monitoring therapeutic effects[12]. A number of factors such as age, sex, season, body condition and management type can affect the responses detected in the skin tests in deer

* To whom all correspondence should be addressed.
E-mail: sanjeevcloning@gmail.com
Mob.: +91-9457273980;

compromising both sensitivity and specificity[13]. The identification and early disposal of such animals form the basis of national bovine tuberculosis eradication programmes world-wide. Success is dependent upon the removal of the infected animal before it becomes a source of infection of *M. bovis* for other animals. PPD bovis contains immunogenic proteins that are also present in non-tuberculous mycobacteria[14] which would compromise the specificity of the assay. Particularly, this specificity has been discussed when the most strict criterion (OD cut-off point PPD bovis e" negative control + 0.05) has been used to classify animals as positive[4].

The aim of this study is, identified bovine tuberculosis animal based on tuberculin skin test; outcome of a recent intradermal infection of PPD on the sensitivity of the SICTT, in a group of cattle naturally infected with bovine tuberculosis.

MATERIALS AND METHODS

Experimental animals

Three hundred randomly selected animals, Kosi (46), Gir (54), Sahiwal (44), Deoni (56), Tharparkar (43) and Rathi (57) from dairy farm of Barsana, Mathura, Uttar Pradesh were selected for this study. All animal procedures were approved by animal ethics committee of Institute.

Mycobacterial antigens

Both avian and bovine Purified protein derivative (PPD) supplied by Prionics and the Tuberculin PPDs of Prionics Lelystad are already applied in over 50 million skin tests annually in Europe.

The single intradermal comparative tuberculin test (SICTT)

The most widely employed diagnostic method is based on intradermal tests but they have limitations in both sensitivity and specificity, detailed information about the SICTT and diagnosis of tuberculosis in cattle is discussed before[15]. Briefly, the test is read 72 h later, by comparing the relative millimetre increase in skin fold thickness (*in-vivo* cell mediated response to each tuberculin) at each injection site. The preparation, potency testing and labelling of each batch of tuberculin PPD must conform to the provisions of the standards laid down in the European Pharmacopoeia monographs for tuberculin PPDs[16], the OIE manual for diagnostic tests and vaccines for terrestrial animals, WHO[17] requirements and the standards for the manufacture and use of bovine tuberculin as laid down in European Commission Directive 64/432/EEC[18]. According to WHO Technical Report Series No. 384 (World Health Organization, 1987), and as referenced in the OIE Terrestrial manual[19], potency testing should be performed in the animal species, and under the conditions, in which the tuberculin will be used in practice. It goes on to say that periodic testing in tuberculous cattle is necessary however, this is not mandatory under any of the above.

Test interpretation

In accordance with directive 64/432/EEC, as amended[18], the reaction at an individual injection site (either bovine or avian) is determined and considered negative 'if only limited swelling is observed, with an increase of not more than 2 mm without clinical signs such as diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes, inconclusive 'if no clinical signs as mentioned (previously) are observed and if the increase in skin-fold thickness is more than 2 mm and less than 4 mm'; or positive (Table.1).

The comparative intradermal tuberculin test was conducted in all cattle using both avian and bovine purified protein derivatives (PPD). Intradermal injections of 0.1 mL (2500 IU/ mL) bovine PPD and 0.1 mL (2500 IU/ mL) avian PPD were administered in two shaved areas, 12 cm apart from each other in the middle of the neck, after having measured and recorded skin thickness with a Vernier Caliper. Skin thickness was measured again at both injection sites after 72 h. The reaction at each site was derived by measuring the difference of the skin thickness before and 72 h after the injection. An animal was considered positive if the bovine minus the avian reaction was greater than 4 mm. (Fig.1)

RESULTS

Descriptive results of (SICTT)

Our team member experts in rural practice ranging participated generally in bovine TB testing. A mean delay of 72 h post-injection is generally respected before the reading of the test result

(range 36–78 h). Regarding the type of reading of the response, the majority of veterinarians include the qualitative reading, which consists of the observation of inflammatory clinical signs such as oedema, exudation, necrosis, pain or inflammatory reaction of the vessels and local lymph nodes. The simple palpation of the site of injection is also included in this category. Visual observation takes as well part of the reading procedure.

The quantitative reading by the measurement of the skin fold thickness with Vernier Caliper is practiced by a minority of veterinarians, not always systematically applied. In field conditions, many practitioners first rely on a visual observation and the palpation of the site of injection; only in case of a suspect reaction, they measure the swelling with a Vernier Caliper.

Increase in skin thickness recorded for the tuberculosis infected cattle in response to the intradermal injection of avian PPD was 6.0 mm three days after the first test and 9.1 mm three days after the second. At the same time the mean increase in skin thickness recorded in response to the intradermal injection of bovine PPD were 6.4 mm and 9.3 mm.

Breed-wise response to Intradermal Tuberculin Test

Breed-wise detail of results of skin testing after 72 hours revealed a pattern as depicted through (Fig. 2). Among the three hundred animals in six different breed of cattle, positive on skin testing fifty six, doubtful ninety three and negative one hundred fifty one were found

Table 1. Criteria for interpretation of reactions of the intradermal tests used in this study according to European and Spanish legislation (EU Council Directive 64/432/EEC and R.D. 2611/1996)

Experiment Name	Severe interpretation[a]	Standard interpretation[b]
Single Intradermal Tuberculin Test **(SIT)**[c]	Negative reaction: Limited swelling with an increase 2 mm in the skin-fold thicknesses without clinical signs	Negative reaction: Limited swelling with an increase 2 mm in the skin-fold thicknesses without clinical signs. Inconclusive reaction: Increase in skin-fold thickness > 2 mm and < 4 mm and no clinical signs observed
	Positive reaction: Increase > 2 mm in the skin-fold thickness and/or clinical signs are observed	Positive reaction: Increase > 2 mm in the skin-fold thickness and/or clinical signs are observed
Single Intradermal Comparative Cervical Tuberculin Test **(SICCT)**[e]	Negative reaction: Bovine difference minus avian difference is < 1 mm and no clinical signs are observed	Negative reaction: Bovine difference minus avian difference is < 1 mm and no clinical signs are observed Inconclusive reaction: Bovine difference minus avian difference is 1 and 4 mm and no clinical signs are observed
	Positive reaction: Bovine difference minus avian difference is 1 mm and/or clinical signs are present.	Positive reactione: Bovine difference minus avian difference is > 4 mm and/or clinical signs are present.

[a]Severe interpretation: used in geographical areas with high prevalence and in herds with confirmed Mycobacterium bovis infection.

[b]Standard interpretation: used in geographical areas with low tuberculosis prevalence and in herds with absence of M. bovis infection (unless they are situated in geographical areas with high prevalence).

[c]SIT, single intradermal tuberculin test. Results are recorded increase(s) in skin-fold thickness at the sites of injection 72 h after injection of tuberculin (s) and observation of clinical signs.

[d]Clinical signs: diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes.

[e]SICCT, single intradermal comparative cervical tuberculin test. Results are recorded increase(s) in skin-fold thickness at the sites of injection 72 h after

Fig. 1. Measuring skin flap with Vernier Caliper of T.B. Infected cattle

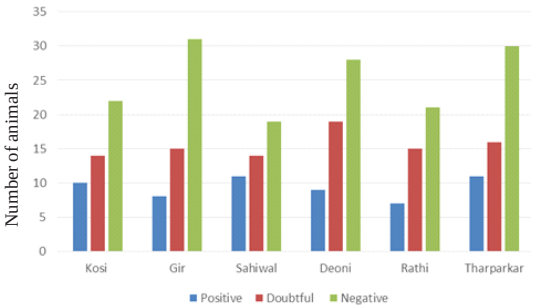

Fig. 2. Breed-wise response to Intradermal Tuberculin Test

DISCUSSION

Different workers have reported higher incidence of bovine tuberculosis with increased age[20]. It has been suggested that increased incidence of TB in older animals can be explained by a waning of protective capability in aging animals, as experimentally confirmed in the murine system. The higher incidence of the disease in older animals, may be due to prolonged close confinement with positive reactors. The increase in the likelihood of encountering *M. bovis* over a longer exposure period has been suggested[21, 22].

Identification of positive animal certain limitations associated to the immunological response against the infection and to the accuracy of the current diagnostic tools that are more evident at the final steps of the eradication process. The early detection and removal of positive animals reduces the risk from tuberculosis that they will become a source of infection for other cattle. These results have significant suggestions for farmers, national policy makers and bTB samples. We accept that the SICTT has a much higher sensitivity when used as a herd-level test. Private veterinary practitioners (PVPs) and farmers should be made aware of the increased risk associated with disease animal. These and earlier outcomes provide strong sign of bTB infection in some SICTT lacking reactor animals, in support of imperfect test sensitivity and residual bTB infection in individual animals in the Indian cattle population.

The use of tuberculin skin test is the most accurate method for diagnosis of bovine TB in cattle, therefore more studies should be implemented to further development. The intradermal tuberculin test has demonstrated to be an adequate diagnostic tool at herd level, and several countries have achieved the eradication based on this test. Future studies are essential to recognize the difference among cellular and humoral

immunities during the course of bTB infection. A number of studies have determined the contribution of Th1 and Th2 responses to the protective immunity and pathology of bovine tuberculosis infection[23]. A complete information of the skin test involved in all phases of the bovine TB immune response of naturally infected cattle is essential for the optimal exploitation of diagnosis and vaccination models.

CONCLUSION

We should initiate on a priority basis, comprehensive disease surveillance and control program, keeping the public health risk. This situation poses a threat to the farm workers, animal handlers and the consumers of milk and milk products of the farm. Testing and eradication of the infected animals is the current method of control, though additional research is currently being explored in the areas of vaccinations and other possible preventative measures.

Conflict of Interest

None of the authors of this paper have a financial or personal relationship with other people or organization that could inappropriately influence or bias the content of the paper.

ACKNOWLEDGMENTS

I thanks the study participants who contributed to this work and I appreciate the staff of IVRI, Izatnagar and dairy farm of Barsana, Mathura, Uttar Pradesh. This study was funded (SB/YS/LS-78/2013) by DST-SERB (Department of Science and Technology-Science and Engineering Research Board) New Delhi, and I am highly thankful to Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly for providing research place for this work.

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