A study on the prevalence of Bovine Tuberculosis in farmed dairy cattle in Himachal Pradesh

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Abstract

A study was conducted on 440 dairy cattle in six organized dairy farms in the state of Himachal Pradesh, India using tuberculin skin testing (TST) to determine the prevalence of bovine tuberculosis. An overall animal prevalence of 14.31% (63 of 440 animals) and a farm prevalence of 16.67% (1 of 6 farms) were recorded in 6 dairy farms by the TST. Of the six dairy farms studied, one of the farms showed prevalence of 34.42% (63/183). There were also marked differences in the prevalence of the disease within the breeds (pure bred and their crosses) and the different age groups. The findings were also corroborated with isolation of the organism and IFN-γ assay. The prevalence of bovine tuberculosis in one farm under study signifies potential health risk.

Keywords: Bovine Tuberculosis, Dairy Cattle, Skin testing, IFN-γ, Prevalence.

Introduction

The livestock is an important segment of expanding and diverse agricultural sector of Indian economy in general. Nearly 70% of the population in India is dependent on the agriculture and rearing of livestock, contrary to less than 3% in many developed countries. Though India is the largest milk producer in world i.e. about 100 million tons and also stands first in terms of milch cattle population with 11.54 million cattle (Basic Animal Husbandry Statistics 2006) still milk production per animal is far below the developed countries (Hemme et al., 2003). Infectious diseases are one major reason for the economic losses in the dairy sector. "Bovine Tuberculosis" causes great economic losses and poses an enormous public health threat as well (O'Reilly and Daborn 1995).

Bovine Tuberculosis caused by Mycobacterium bovis (M. bovis) has no geographical boundaries and infection occurs in diverse group of animals, which includes farm animals of economic importance, wildlife and humans (Grange 2001; Pavlik et al., 2002). It has been included in the "List B" diseases of Office Internationale des Epizooties (OIE) (OIE 2008). Zoonotic bovine TB is present in many developing countries where surveillance and control activities are often inadequate or unavailable (Cosivi et al., 1998). India is one of these countries, and many epidemiological and public health aspects of the infection remain largely unknown.

The present study was formulated with the purpose to investigate the distribution of bovine TB in organized dairy farms in the state of Himachal Pradesh. Till date, sufficient data about the actual prevalence of bovine tuberculosis in the state is not available. Although there are two case reports related to the problem from the state (Katoch et al., 2004; and Katoch et al., 2006). The present study describes the first ever report on the status of bovine tuberculosis in dairy cattle in the state confirmed through skin testing, culture and IFN-γ assay.

Materials and methods

Study Subjects

The study was conducted on 440 dairy cattle belonging to six different organized dairy farms of the State (Table 1). All of the six dairy farms were located in different terrains and agro-climatic zones of the state resulting in a random distribution. No prior information was available on the occurrence of bovine TB in the farms under study or in cattle in areas surrounding the farms except in one farm located in the campus of Himachal Pradesh Agricultural University, Palampur with two reports of bovine tuberculosis (Katoch et al., 2004; and Katoch et al., 2006). The animals were screened for the prevalence of bovine tuberculosis with single intradermal tuberculin test (SID) and comparative cervical tuberculin test (CCT). All animals that were more than six months of age were included in the study. To assess the response of animals to tuberculin skin test (TST), the animals were divided into different categories according to breed and age.

The cattle in the dairy farms belonged to three breeds namely Holstein Friesian (H-F), Jersey, and Jersey Cross-bred. On the basis of age, the animals were divided into six groups i.e. 0-3 yrs, 3-6 yrs, 6-9 yrs, 9-12 yrs, 12-15 yrs and 15-18 yrs. The first age group i.e. 0-3 yrs primarily included animals over six months of age as TST was carried out only in animals over 6 months in age. Additionally, only the non-pregnant animals were screened to avoid false negative reactions.

Management of Farms

All the cattle were being kept in semi-intensive type of housing system and were for dairy purpose only. All the animals were tested for their parasitic status, body temperature range along with presence of concurrent infections to rule out any abnormality before screening by TST.

Intradermal Tuberculin Skin Test

For SID, a site at the side of neck at the border of the anterior and middle third of the neck of the animal was shaved and skin thickness was measured (in millimeters) with calipers before injection of tuberculin. A 0.1 ml of 2,000 I.U. / animal (1 mg protein/ml) bovine PPD tuberculin (Indian Veterinary Research Institute (IVRI), Bareilly, U.P.) was injected into the dermis of the site. After 72 hours, the thickness at the injection sites was measured again and interpreted according to OIE (OIE 2008). For CCT, two sites 10 cm apart on the side of neck were shaved and skin thickness was measured. A 0.1 ml of 2,000 I.U. / animal (1 mg protein/ml) bovine PPD tuberculin (IVRI) and 0.1 ml of 2,000 I.U. / animal (1 mg protein/ml) avian PPD tuberculin (IVRI) were injected into the dermis at these sites and interpretation was done after 72 hours as per OIE manual.

Culture

Milk samples were collected from 15 cattle found positive on CCT. However, lungs and lymph nodes samples could be collected only from four cattle which died during this study. These four cattle were otherwise positive with skin testing but were not showing any signs of tuberculosis during routine clinical examination in the farm. After processing, the samples were inoculated on Lowenstein-Jensen (L-J) medium with or without pyruvate for primary isolation with incubation for a maximum period up to 8 weeks.

IFN-γ assay

The release of IFN-γ in the blood of the reactor cattle was determined by IFN-γ assay using a commercial Bovine IFN-γ ELISA kit purchased from BioSource Europe S.A., Belgium. The whole blood culture was performed in accordance with the method described by Cagiola et al., 2004. A total of 23 blood samples which comprised of 10 from skin test positive animals, 12 from doubtful and one negative animal. Of the 75 cattle from the comparative testing, IFN-γ assay was carried out in only 23 animals because of the availability of a single IFN-γ ELISA kit at that particular moment of time.

Results

Out of the total 440 cattle screened with SID, 102 cattle were found to react to bovine PPD tuberculin. Incidentally, all of these reactor cattle belonged to only one livestock farm located at Himachal Pradesh Agricultural University, Palampur. Of the 183 cattle of this Livestock farm, 63 reacted positively, while, 39 were found to be doubtful. The rest of the 81 cattle did not show reaction to bovine tuberculin and were classified as non-reactors. Among the 102 reactors to SID, comparative testing could only be done on 75 cattle owing to the death or disposal of rest of the animals. Cattle which died didn't reveal tuberculous lesions on post mortem examination. Of these 75 cattle, 47 were positive while 28 were doubtful with SID. On comparative testing, only 15 animals were found to be positive, 20 doubtful and 40 negative (Table 2).

Comparison of the positive cattle from our data on SID and CCT revealed a, relatively higher incidence of disease in the crossbred as compared to the pure bred animals (Table 3). Breed-wise detail of results of skin testing after 72 hours revealed a pattern as depicted through Figure 1. The comparison of the response of the animals as per their age group (Table 4) is also represented through Figure 2. Highest positive responses on skin testing were recorded in cattle in the age groups of 6-9 and 9-12 years compared to other age groups. Of the 23 animals tested with IFN- γ assay, 18 were found positive and 5 negative. All 10 animals positive on skin test were also found to be positive on IFN- γ assay. However, among the 12 animals doubtful on skin testing 8 were found to be positive with IFN- γ assay. Table 5 represents the comparison of the results of the SID, CCT and IFN- γ assay.

After 8 weeks post inoculation on L-J slants small, moist, shiny and fragile colonies appeared from lung and lymph node samples which were confirmed to be M. bovis through standard biochemical tests. However, there was no isolation from the milk samples collected (Table-6). Isolates were also confirmed through PCR-RFLP of hsp65 gene at National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra.

Discussion

Of the total 440 dairy animals screened, 14.31% (63/440) tested positive for bovine PPD tuberculin 72 hrs after administering SID test. Among the six dairy farms tested, a farm prevalence of 16.67% (1/6) was recorded based on SID. The study established the overall prevalence of 34.42% (63/183) in one of the

Table-1. Details of Single Intradermal Tuberculin (SID) Test

Place of Tuberculin Skin Testing	Number of Animals tested
Livestock Farm, Himachal Pradesh Agricultural University, Palampur, H.P.	183
Govt. Cattle Breeding Farm Palampur, Palampur Section, Distt. Kangra, H.P.	26
Govt. Cattle Breeding Farm Palampur, Banuri Section, Distt. Kangra, H.P.	32
Govt. Livestock Farm, Kothipura, Distt. Bilaspur, H.P.	62
Govt. Livestock Farm, Bagthan, Distt. Sirmaur, H.P.	25
Govt. Livestock Farm, Kamand, Distt. Mandi, H.P.	112
TOTAL	440

farms based on SID. High prevalence rates have been reported from southern Indian states (Nalini et al., 1998) and from Giza (Eid et al., 2001).

To rule out false positive reactors, 75 out of 102 reactor cattle were subjected to CCT. The prevalence of bovine tuberculosis infection as determined by CCT was 20.00 per cent (15/75), whereas the non-specific reaction was observed in 26.67 per cent (20/75). A prevalence rate of 18.58 per cent from 1087 cattle in Mali (Sidibe et al., 2003) and 14.5 per cent from 1813 cattle in Eritrea (Omer et al., 2001) has been reported based on CCT.

In the present study, there was a higher incidence of reactors in cattle over 6 years old than in cattle between 1 and 6 years old. Different workers have reported higher incidence of bovine tuberculosis with increased age (Milian et al., 2000; and Munroe et al., 2000). The reason for lower incidence in young calves could be the possible influence of γ-T cells, which are predominantly found in the circulation of young calves (Mackay and Hein 1989). Previous studies have shown the role of γ-T cells in anti-mycobacterial immunity (Stamp 1948). 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 (O'Reilly and Daborn 1995). The higher incidence of the disease in older animals in the present study 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 (Barwinek and Taylor 1996). The difference in results between cattle of different ages could also be a result of the slow progression of disease to a detectable level.

Likewise, in the present study there was a measurable difference in incidence of bovine tuberculosis between cattle with mixed blood (Jersey crosses) as compared to the pure bred animals. A higher incidence of bovine tuberculosis among crossbred cattle in tropical countries particularly India has been reported earlier (Selman 1981). Also various workers have recorded significant differences of the incidence of bovine TB among crossbred and pure bred cattle (Hemme et al., 2003; and Ameni et al., 2003). There is a need to evaluate the prevalence of other important diseases amongst cross bred viz-a-viz pure bred cattle.

In the present study, a total of 23 blood samples were tested in duplicate with IFN- γ assay using both bovine and avian PPD tuberculin. Out of 23 animals, 18 were found to be reactive to M. bovis by the IFN- γ assay using bovine PPD, while 4 were negative. Cattle with positive results to both skin testing and IFN- γ assay were considered reactor animals. Eight animals in the current study which were doubtful on CCT were found to be positive on IFN- γ assay. M. bovis infection in skin test-negative cattle with an assay for bovine interferon-γ has been recorded (Neill et al., 1994).

Observations from the study show a relatively higher prevalence of TB in University dairy farm in Palampur based on tuberculin test. The incidence of TB only on one farm in the state could not be explained. This may be due to the fact that the animals are held in a head-to-head arrangement at the said positive farm, predisposing the animals to infection by aerosolization. Since there is hardly any culling of animals in-production, the likelihood of spread of infection

Table-2. Comparative analysis of SID with the results of CCT of Livestock Farm, Himachal Pradesh Agricultural University, Palampur

Total no. of animals	SID Response	No. of animals	No. of animals tested by CCT	CCT Response	No. of animals
183	Positive Doubtful Negative	63 39 81	75*	Positive Doubtful Negative	15 20 40

* Only 75 animals were left in the farm at the time of testing

Table-3. Breed-wise response to Intradermal Tuberculin Test

Breed	Positive	Doubtful	Negative
Holstein- Friesian	4	3	26
Jersey	18	12	30
Jersey Cross	41	24	25

Table-4. Age-wise response to Intradermal Tuberculin Test

Age group	Positive	Doubtful	Negative
0-3 years	3	13	32
3-6 years	4	5	24
6-9 years	26	10	8
9-12 years	20	7	9
12-15 years	7	3	6

increases as the healthy animals are held in close confinement with the infected ones for a longer duration. This also partly justifies the higher incidence in the older animals.

The situation is alarming because of the "no-slaughter of cattle" policy in India. Additionally, given the scarce resources in the state, there is a tendency to keep animals with a long production life without culling, increasing their chance of participation in spread of bovine TB. This situation poses a threat to the farm workers, animal handlers and the consumers of milk and milk products of the farm. Although the numbers of positive animals were few, pooling milk from the farm does pose a great public health risk to milk consumers as it has been shown that one cow can excrete enough viable bacilli to contaminate the milk from up to 100 cows when their milk is pooled (Kleeberg 1984). Besides, this farm supplies milk to all the households in and around the university campus. The situation could be even graver, given the fact that infected animals are allowed to stay in the same farm along with the healthy animals. The personnel employed in the farm may play a possible role in transmission of infection and steps must be taken to screen them to rule out TB. Further studies are required to establish the actual cause of the prevalence of TB in the said farm and whether the animals are infected with *M. tuberculosis* and humans with *M. bovis* or vice versa and to what extent. Meanwhile, a comprehensive disease surveillance and control program, keeping the public health risk in mind should be initiated as a priority.

Acknowledgement

Authors would like to thank the authorities of the Department of Animal Husbandry, Government of Himachal Pradesh for allowing access to the various dairy farms of the State included in the study. Also we are thankful to Dr. V. M. Katoch, Ex-Director, National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra for the molecular confirmation of the isolates.

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Table-5. Comparative results of Single Intradermal Tuberculin test (SID), Comparative tuberculin test (CCT) and IFN-γ assay

Total no.of animals	SID Response	No. of animals	No. of animals tested by CCT	CCTResponse	No. of animals	No. of animals tested by IFN-γ ELISA	IFN-γ ELISA response	No.of animals
183	Positive	63	75*	Positive	15	23†	Positive	18
	Doubtful	39		Doubtful	20		Doubtful	4
	Negative	81		Negative	40		Negative	1

* Only 75 animals were left in the farm at the time of testing, † Only 23 animals were tested by IFN-γ assay

Table-6. *M. bovis* isolation from various samples

Samples	Samples processed	*M. bovis* isolation
Milk	15	-
Lung	4	+
Lymph Node	4	+

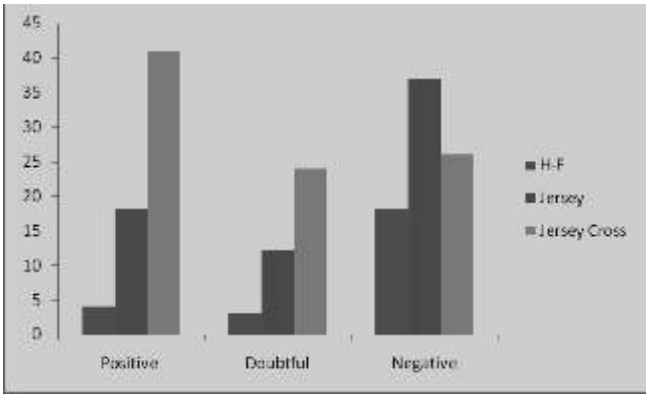

Figure-1. Breed-wise response of animals to tuberculin test

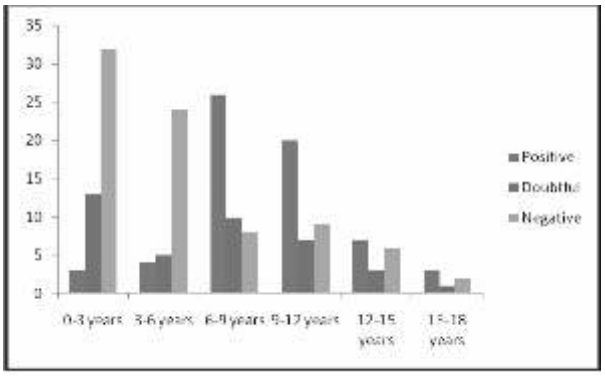

Figure-2. Age-wise response of animals to tuberculin test

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