

# Comparative prevalence of tuberculosis in two dairy herds in India

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## Summary

Holstein Friesian cows and their crossbred progeny on a farm in northern India were tested for tuberculosis (TB) infection using a single intradermal tuberculin test. The results showed that the animals persistently harboured TB infection for periods of two to four years. The recent comparative intradermal tuberculin test revealed that at least five out of nine of these cattle reacted positively to bovine tuberculin purified protein derivative. A high (15.76%) prevalence rate resulted because none of the infected animals had been segregated or culled from the herd since the first incidence was detected in 1992. In contrast, another farm in western India that practiced segregation and culling was able to contain the level of prevalence of TB between 0.65% and 1.85%. These findings call for stricter regulations on the management of TB at farm, state and country level and a revision in the mode of breeding programmes adopted by farms.

## Keywords

Cattle – Comparative intradermal skin test – Farm – India – *Mycobacterium bovis* – Single intradermal skin test – Tuberculosis.

## Introduction

Guidelines from the World Organisation for Animal Health (OIE) (7) and the Bureau of Indian Standards (BIS) (2) specify that artificial insemination centres and breeding establishments must be free of tuberculosis (TB). The single intradermal tuberculin test (SITT) with bovine tuberculin purified protein derivative (PPD) is the OIE recommended test for screening against TB and this test was used in a comparative study of tuberculosis prevalence on two farms in India. The first farm, located in northern India, maintains Holstein Friesian (HF) cattle and crossbreeds and is engaged in frozen semen production, embryo transfer, maintenance of a unit for the production of breeding bulls, and the sale of milk. The TB status of the herd was monitored between 1994 and 1996 and the data were compared with the results of tests conducted by farm authorities in 1990, 1992 and 1993. The second farm, located in western India, is involved in similar activities but practices periodic culling of reactors. The TB status of the herd was monitored for 4 years, from 2002 to 2005.

## Materials and methods

### Animals

Twelve HF cows certified free from diseases, including bovine TB, were imported from Germany in 1990 and were maintained on the first farm along with 204 indigenous cows (Sahiwal, Kankrej, Gir). Different crossbred progenies (Sahiwal × HF, Kankrej × HF and Gir × HF) were produced through artificial insemination using semen from certified indigenous bulls located in a farm in western Indian. Hence, by 1992, 339 females were available. However, 155 animals were sold, and by 1994 there were only 184 females (including the 12 imported HF) in the herd.

The farm located in western India had 455 crossbred animals in 2002. The farm introduced animals into the main herd under various breeding programmes and also sold animals periodically, hence the population of crossbreeds tested in this farm varied. In 2002, 2003, 2004

and 2005 the number of animals tested was 455, 722, 410 and 545, respectively.

### Tuberculosis screening tests

On the farm in northern India, screening of HF cows and their crossbred progeny against TB was carried out each year (with the exception of 1991) by SITT between 1990 and 1996. Bovine tuberculin PPD brew nos. 1/1989-1990, 1/1991-1992, 1/1993-1994, 1/1994-1995 and 1/1995-1996 (2,000 tuberculin units per 0.1 ml) were obtained from the Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh. The test was conducted as per the manufacturer's instructions: a 2.5 cm<sup>2</sup> area in the middle third of the neck was shaved and 0.1 ml of bovine tuberculin PPD was injected intradermally. A positive reaction to tuberculin was recorded 72 h post intra-dermal injection if there was an increase of 4 mm or more in skin thickness with or without clinical signs (4). In July 1996, more than 120 days after the annual SITT screening in March, comparative intradermal tuberculin tests (CITT) employing bovine tuberculin PPD (batch no. 0412-7360; 1 mg equivalent to 50,000 international units/ml) and avian tuberculin PPD (batch no. 0408-32501; 1 mg equivalent to 25,000 international units/ml) (Commonwealth Serum Laboratories, Australia) were also conducted according to the manufacturer's instructions (6). For CITT two areas of 2.5 cm<sup>2</sup> each, located diagonally opposite each other in the middle third of the neck (at least 10 cm apart) were shaved. One area was inoculated with 0.1 ml of bovine tuberculin PPD and the other with 0.1 ml of avian tuberculin PPD.

The animals on the farm in western India were tested by SITT alone as described above using bovine tuberculin PPD brew nos. 1/2001-02 and 1/2003-04

(2,000 tuberculin units per 0.1 ml) obtained from the IVRI, Uttar Pradesh.

## Results

On the first farm, the imported animals and the resident cattle were TB negative by SITT in 1990, but four imported HF dairy cows (identification nos. 49, 25, 119 and 37) and one home-bred HF crossbred cow (identification no. S 51) tested positive in 1992. One of the imported cows tested positive again in 1993. In 1994, all the cows that had been positive in one or more of the previous years tested positive again. In addition, 23 previously TB-negative cows tested positive (which included 15 home-bred females), bringing the total to 28. All 28 of these females continued to be tuberculin reactors in 1995 (12 imported cows and 16 home-bred animals), another home-bred female also tested positive for the first time in that year (identification no. 24) (Tables I and II). The prevalence of TB in this herd during 1995 as detected by SITT stood at 15.76%. Two TB reactors (identification nos. 49 and 119) that were positive on three occasions died with typical lesions suggestive of pulmonary TB before the screening scheduled for March 1996. Out of 29 animals tuberculin positive in 1995, nine animals remained positive in 1996. One HF cow (identification no. 25) was positive to bovine tuberculin PPD for four consecutive years.

The available data revealed that at least nine females were persistent TB reactors (i.e. they tested positive in three or more consecutive years) between 1990 and 1996, and five of the nine reacted to bovine tuberculin PPD as per the 'severe interpretation criteria' in the CITT in 1996. In

**Table I**  
**Imported Holstein Friesian dairy cows on a farm in northern India persistently reacting to bovine tuberculin purified protein derivative (PPD)**

Identification numbers of tuberculin reactors by SITT	Year of screening by SITT and reactor status <sup>(a)</sup>					March 1996	CITT <sup>(b)</sup> status in July 1996
	1990	1992	1993	1994	1995		
57	-	-	-	+ 16.44	+ 21.46	NA	NA
49	-	+ 4.94	-	+ 6.62	+ 16.40	NA	NA
25	-	+ 8.62	+ 5.56	+ 7.56	+ 6.34	-	-
214	-	-	-	+ 15.28	+ 8.27	NA	NA
48	-	-	-	+ 6.08	+ 18.35	NA	NA
205	-	-	-	+ 22.00	+ 10.02	NA	NA
119	-	+ 4.22	-	+ 14.18	+ 23.50	NA	NA
23	-	-	-	+ 22.50	+ 18.35	+ 8.34	B + 3.36
190	-	-	-	+ 11.00	+ 6.93	NA	NA
191	-	-	-	+ 6.84	+ 5.88	NA	NA
37	-	+ 7.84	-	+ 16.64	+ 8.77	NA	NA
8	-	-	-	+ 17.24	+ 8.90	+ 6.24	B + 4.24

**Table II**  
**Crossbred\* dairy cows on a farm in northern India persistently reacting to bovine tuberculin purified protein derivative (PPD)**

Identification numbers of tuberculin reactors by SITT	Year of screening by SITT and reactor status <sup>(a)</sup>						CITT <sup>(b)</sup> status in July 1996
	1990	1992	1993	1994	1995	March 1996	
S 51	NA	+ 5.72	–	+ 9.04	+ 5.60	NA	NA
S 131	NA	–	–	+ 12.36	+ 10.38	NA	NA
S 348	NA	NA	NA	+ 24.23	+ 8.36	NA	NA
S 393	NA	NA	NA	+ 13.43	+ 29.80	+ 8.92	B + 3.9
S 390	NA	NA	NA	+ 13.56	+ 10.87	+ 11.45	–
S 399	NA	NA	NA	+ 15.54	+ 15.74	+ 13.44	A + 5.16
S 302	NA	NA	NA	+ 11.84	+ 10.69	NA	NA
S 344	NA	NA	NA	+ 7.36	+ 13.25	+ 7.70	– <sup>(c)</sup>
S 372	NA	NA	NA	+ 7.08	+ 30.85	NA	NA
S 337	NA	NA	NA	+ 14.52	+ 18.83	NA	NA
S 322	NA	NA	NA	+ 24.7	+ 33.74	+ 17.80	B + 7.18
S 405	NA	NA	NA	+ 14.18	+ 10.41	+ 12.80	B + 6.84
S 442	NA	NA	NA	+ 19.12	+ 17.36	NA	NA
S 411	NA	NA	NA	+ 6.76	+ 21.59	NA	NA
S 436	NA	NA	NA	+ 8.86	+ 8.13	NA	NA
S 359	NA	NA	NA	+ 18.60	+ 25.02	NA	NA
24	NA	NA	NA	NA	+ 7.12	NT	B + 4.22
S 521	NA	NA	NA	NA	NA	10.50	B + 5.77

\* Holstein Friesian (HF) × Sahiwal; HF × Kankrej; HF × Gir  
 SITT: single intradermal tuberculin test  
 CITT: comparative intradermal tuberculin test  
 a) skin thickness in mm 72 h post inoculation with bovine PPD (SITT)/bovine PPD and avian PPD (CITT)  
 b) CITT positive by 'severe interpretation criteria'  
 c) inconclusive by CITT

A +: avian tuberculin PPD positive by CITT  
 B +: bovine tuberculin PPD positive by CITT  
 NA: not available  
 NT: not tested  
 -: negative  
 +: positive

addition, 28 animals were TB positive for two consecutive years, 1994 and 1995 (Tables I and II). Indecision about removal of these reactors jeopardised the health of the rest of the herd and resulted in a TB prevalence of 15.76% in 1995 (Table III). In contrast, the survey conducted between 2002 and 2005 in the farm located in western India showed that the prevalence of TB varied from 0.65% to 1.85% when reactors were regularly culled from the herd (Tables IV and V).

## Discussion

In addition to the high prevalence rates reported on the first farm in this study, high prevalence rates for TB have been reported in other parts of India. For example, a high prevalence of TB was detected by SITT in farms of southern India in the states of Tamil Nadu (34.58%) (3) and Karnataka (30% to 35%) (5). In the current study the prevalence was lower (15.76%) but considerably alarming. Apart from not removing the reactors from the herd, another factor that could have contributed to elevated prevalence levels could be the stress levels of cows under tropical conditions, as their average daily milk yield exceeded 20 l.

**Table III**  
**Distribution of tuberculin reactors on a farm in northern India from 1990 to 1995\***

Year of testing	1990	1992	1993	1994	1995
Number tested/ number positive	216/0	339/5	184/1	184/28	184/29
Percentage positive	Nil	1.70	0.54	15.21	15.76

\*data for 1996 was not included because a lack of antigens meant that not all the animals were tested that year

**Table IV**  
**Distribution of tuberculin reactors on a farm in western India where reactors are routinely culled**

Year of testing	2002	2003	2004	2005
Number tested/ number positive	455/3	722/6	410/9	545/7
Percentage positive	0.65	0.83	1.85	1.28

**Table V**  
**Details of tuberculin reactors in crossbred cattle and buffalo positive by SITT on a farm in western India from 2002 to 2005**

Identification numbers of tuberculin reactors by SITT	Year of screening by SITT and reactor status <sup>(a)</sup>			
	2002	2003	2004	2005
7045	+ 6.60	C	NA	NA
7205	+ 6.60	C	NA	NA
BM 171	+ 15.00	C	NA	NA
3583	–	+ 6.90	C	NA
1745	–	+ 4.64	C	NA
9156	–	+ 7.59	C	NA
3207	–	+ 8.68	C	NA
2305	–	+ 4.73	C	NA
902	–	+ 9.41	C	NA
3095	–	–	+ 9.36	C
189	–	–	+ 4.25	C
1943	–	–	+ 5.13	C
3038	–	–	+ 5.52	C
2888	–	–	+ 5.14	C
7238	–	–	+ 5.93	C
2476	–	–	+ 4.21	C
2217	–	–	+ 4.50	C
1303	–	–	+ 7.12	C
HF 83	NA	NA	–	+ 4.90
HF 54	–	–	–	+ 4.14
BM 5146	–	–	–	+ 4.90
SAGAR	–	–	–	+ 4.68
BM 144	–	–	–	+ 4.45
JRS 03	NA	–	–	+ 4.49
3186	NA	–	–	+ 4.74

a) skin thickness in mm 72 h post inoculation of bovine purified protein derivative (SITT)

C: culled

NA: not available

SITT: single intradermal tuberculin test

–: negative by SITT

+: positive by SITT

In contrast, when the farm located in western India was followed from the year 2002 to 2005 for the purposes of estimating the prevalence of TB, it was evident that the periodical test and culling method adopted was able to minimise the prevalence to a level below 2%. This farm periodically introduced new animals as part of the breeding programme, after they were initially screened for brucellosis, TB and Johne's disease. Thereafter, the animals were quarantined for 45 days and re-tested before being introduced into the herd. However, some animals might have evaded identification by SITT. Avoiding periodical introduction of animals for breeding purposes could result in this farm becoming free from TB.

This study clearly indicates that if a farm does not follow the recommendations of the OIE or the BIS for the control of TB, a high prevalence situation could be generated over a period of time. The Animal Husbandry Department in the southern Indian state of Karnataka reported a prevalence of TB infection in farms varying between 4.9% and 18.2% between 1989 and 1994; they also observed that a lack of immediate action to segregate and cull the reactors after diagnosis had contributed to a high prevalence of the disease (1). The present comparative study of the two farms shows that detection and culling can reduce the prevalence but not eliminate it, unless a change is made in the breeding strategies and policies.

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## Comparaison de la prévalence de la tuberculose bovine dans deux troupeaux de vaches laitières en Inde

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### Résumé

Des vaches Holstein Frisonne ainsi que leurs descendants issus de croisement provenant d'une ferme laitière du nord de l'Inde ont été soumis au test intradermique simple à la tuberculine pour le diagnostic de la tuberculose. Les résultats ont révélé que ces animaux étaient atteints d'infection persistante sur des périodes allant de deux à quatre ans. Le test comparatif intradermique à la tuberculine récemment mis au point a révélé que cinq bovins sur neuf réagissaient à la tuberculine dérivée de protéines purifiées (PPD). Le taux de prévalence élevé (15,76 %) s'explique par le fait qu'aucun animal infecté n'a été séparé du troupeau ni abattu depuis l'apparition des premiers cas en 1992. En revanche, dans une autre ferme laitière de l'ouest de l'Inde, la mise à l'écart et l'abattage des vaches atteintes ont permis de ramener la prévalence à un taux compris entre 0,65 % et 1,85 %. Ces observations militent en faveur d'une réglementation plus rigoureuse de la gestion de la tuberculose bovine au niveau des fermes, des états et du pays, ainsi que d'une révision des programmes d'élevage appliqués dans les fermes.

### Mots-clés

Bovin – Ferme – Inde – *Mycobacterium bovis* – Test comparatif intradermique à la tuberculine – Test intradermique simple – Tuberculose bovine.



## Prevalencia comparativa de tuberculosis en dos rebaños lecheros de la India

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### Resumen

El autor describe el análisis de vacas frisonas y de su progenie cruzada mediante la prueba intradérmica sencilla de la tuberculina en una explotación situada en el norte de la India con el fin de detectar en los animales una posible tuberculosis. Los resultados pusieron de manifiesto que los animales albergaban la infección de modo permanente durante periodos de entre dos y cuatro años. La reciente prueba intradérmica comparativa reveló que al menos cinco de nueve ejemplares presentaban reacción positiva al derivado proteico purificado (PPD) de la tuberculina. Se observó una elevada tasa de prevalencia (15,76%) porque no se había sacrificado o separado del resto del rebaño a ninguno de los animales infectados desde que en 1992 se detectó la primera incidencia. En cambio, otra explotación del oeste del país donde se practicaban la segregación y el sacrificio fue capaz de mantener la prevalencia de tuberculosis en índices de entre un 0,65% y un 1,85%. Tales conclusiones ponen de relieve la necesidad de instaurar reglas más estrictas de lucha contra la infección, tanto en las explotaciones como en los estados y el conjunto de la nación, y de revisar los programas de cría que se aplican en las explotaciones.

### Palabras clave

Ganado vacuno – Granja – India – *Mycobacterium bovis* – Prueba intradérmica comparativa – Prueba intradérmica sencilla – Tuberculosis.



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