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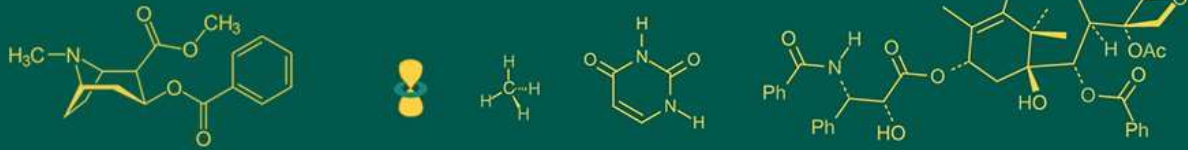
Pravin J Chaudhari
M.V.Sc, Department of
Veterinary Microbiology,
College of Veterinary Science
and Animal Husbandry,
Anand Agricultural University
Kamdhenu University, Anand,
Gujarat, India

Bharat B Bhanderi
Associate Professor,
Department of Veterinary
Microbiology, College of
Veterinary Science and Animal
Husbandry, Anand
Agricultural University
Kamdhenu University, Anand,
Gujarat, India

Yash A Desai
M.V.Sc, Department of
Livestock Production
Management, College of
Veterinary Science and Animal
Husbandry, Anand
Agricultural University
Kamdhenu University, Anand,
Gujarat, India

Prevalence of bovine tuberculosis

Pravin J Chaudhari, Bharat B Bhanderi and Yash A Desai

Abstract
The aim of this present study was to carried out the prevalence of bovine tuberculosis (bTB), using the single intradermal comparative cervical tuberculin test (SICCT), single intradermal tuberculin (SIT) test and detection of *Mycobacterium bovis* in cattle from different region (Anand, Rajkot and Mehsana districts) of Gujarat state. Total of 20 dairy farms in 800 animals (620 cattle & 180 buffalo) were studied for the prevalence of bovine TB using single intradermal comparative cervical tuberculin (SICCT) test and single intradermal tuberculin (SIT). Out of the 800 SICCT tested animals, 1.37% (11) were SICCT positive, 12.87% (103) were Avian positive (PPD-A) and 10.62% (85) were SIT positive (PPD-B). Overall prevalence of bTB in SICCT test was 1.37%. and SIT test was 10.62%. Species-wise, highest prevalence of bTB was found in cattle 1.45% (9/620) and in buffalo 1.11% (2/180) by SICCT test. Using SIT test, highest prevalence was observed in cattle 11.45% (71/620) than buffalo 7.77% (14/180). In Cattle, breed-wise highest prevalence of bTB was found in HF cross 2.00% (7/346), followed by Kankrej 1.19% (1/84) and Gir 0.52% (1/190) by SICCT test. Animals testing by SIT test, highest prevalence was observed in HF cross 14.16% (49/346) followed by Kankrej 8.33% (7/84) and Gir 7.89% (16/190). In buffalo, breed-wise highest prevalence of bTB was found in Murrah 14.28% (1/7) followed by Jaffarabadi 2.38% (1/42), Mehsana 0.00% (0/75) and Surti 0.00% (0/56) by SICCT test, whereas highest prevalence was observed in Murrah 28.57% (2/7) followed by Jaffarbadi 16.66% (7/42), Mehsana 5.33% (4/75) and Surti 1.78% (1/56) by SIT test. District-wise, highest prevalence was observed in Anand 1.54% (9/583) followed by Rajkot 1.19% (2/167) and Mehsana 0.00% (0/50) by SICCT test, whereas highest prevalence was observed in Anand 12.52% (73/583) followed by Rajkot 6.58% (11/167) and Mehsana 2.00% (1/50) by SIT test. Prevalence of bovine TB can be done in early stage in live animals with SCCIT test which showed promising results than SIT test.

Keywords: Bovine tuberculosis, single intradermal comparative cervical tuberculin test (SICCT), single intradermal tuberculin test (SIT)

Introduction

Bovine tuberculosis (bTB) is the most devastating and challenging multi host endemic, chronic (granulomatous) bacterial zoonotic disease. Also, bovine TB breakdowns are not uniform in nature; they can be categorized as "sporadic," "persistent," and "recurring" (Skuce *et al*., 2012) [11]. *M. bovis* causes mostly extra-pulmonary types of tuberculosis and spreads easily to humans by inhaling aerosols or ingestion of unpasteurized contaminated milk (Prasad *et al*., 2005) [10]. Cattle was considered as a true host and it affects other domestic animals, wildlife, and also humans with worldwide annual losses to agriculture of $3 billion (Filia *et al*., 2016) [2]. For more than 100 years, tuberculin methods developed by Von Pirquet and Mantoux in 1907-1908 are being used to diagnose tuberculosis in both humans and cattle. Single intradermal comparative cervical tuberculin test (SICCT), caudal fold test (CFT) and single intradermal test (SIT) are three types of tuberculin test. Tuberculin formulated from mycobacteria called purified protein derivative (PPD), has identified delayed-type hyper-sensitivity (OIE, 2019) [7]. False positive reactions occur by other than *M. bovis* due to cross reaction. False negative reactions may be recorded as tested before delayed-type hypersensitivity develops, factors related to the PPDs (expired product, product stored under inappropriate conditions, manufacturing errors, low potency) or to the methodology (doses, site of injection, inexperience), advanced tuberculosis or anergic condition and immunosuppression (Bezos *et al*., 2014) [1]. This zoonotic disease continues to have considerable economic and public health implications, with an impact on international trade of animals and animal products (Olea-Popelka *et al*., 2017) [9]. Since both the species of organism (MTB and *M. bovis*) pose a threat to health of animals and thereby capable of

Corresponding Author:
Pravin J Chaudhari
M.V.Sc, Department of
Veterinary Microbiology,
College of Veterinary Science
and Animal Husbandry,
Anand Agricultural University
Kamdhenu University, Anand,
Gujarat, India

infecting humans and viz. (reverse zoonosis), detection of the bacteria in the early stage is needed. Intradermal tuberculin test is recognized by the World Organization for Animal Health (OIE) as the primary screening test for detection of bTB in the early bovine stage (OIE, 2009) [8]. Thus, this study was conducted to investigate the detection of bovine TB and detection of *M. bovis* in cows from a different organized dairy farm.

2. Materials and Methods
2.1 study area and number of animals selected for study
During the period from January-2019 to February-2020, the present study was conducted in 20 dairy farms with total 1726 number of animals herd strength, out of which, 800 animals (620 cattle & 180 buffalo) from 20 farms, among which 12 were cattle farms {farms A to G in Anand (453) and H to L in Rajkot (167)} and 8 were buffalo farms {farms M to Q in Anand (130) and R to T in Mehsana (50)} were selected and screened by SICCT test as in section 3.1. Among those 20 farms, 07 farms (05 cattle farms, and 02 buffalo farms) had history of tuberculin testing in the past (Table 1). The information of the breed-wise selected animals for analysis is given in the Table 2 and Table 3.

Table 1: Details of animals selected in different district of Gujarat state

Sr. No.	Species & Districts		Farms	Total No. of animals	Animals selected for testing (Animal ID)	Total	Previous history of testing
1.	Cattle	Anand	Farm (A)	120	A1 - A57	57	No
2.			Farm (B)	71	B1 - B36	36	Yes
3.			Farm (C)	380	C1 - C213	213	Yes
4.			Farm (D)	125	D1 - D47	47	No
5.			Farm (E)	70	E1 - E27	27	No
6.			Farm (F)	75	F1 - F31	31	Yes
7.			Farm (G)	95	G1 - G42	42	No
8.		Rajkot	Farm (H)	68	H1 - H25	25	No
9.			Farm (I)	71	I1 - I27	27	Yes
10.			Farm (J)	86	J1 - J39	39	Yes
11.			Farm (K)	47	K1 - K20	20	No
12.			Farm (L)	132	L1 - L56	56	No
			Total	1340		620	
13.	Buffalo	Anand	Farm (M)	105	M1 - M47	47	No
14.			Farm (N)	22	N1 - N8	8	Yes
15.			Farm (O)	108	O1 - O50	50	No
16.			Farm (P)	50	P1 - P18	18	No
17.			Farm (Q)	20	Q1 - Q7	7	Yes
18.		Mehsana	Farm (R)	35	R1 - R15	15	No
19.			Farm (S)	28	S1 - S22	22	No
20.			Farm (T)	18	T1 - T13	13	No
			Total	386		180	
	Grand Total			1726		800	

Table 2: Details of the (n=620) cattle selected for SICCT test

Breed Wise	Cattle			Total
	Gir	Kankrej	HF cross	
Age-wise information				
Calf	32	08	38	78
Heifer	63	00	30	93
Adult	95	76	278	449
Total	190	84	346	620
Sex-wise information				
Male	10	2	24	36
Female	180	82	322	584
Total	190	84	346	620

Table 3: Details of the (n=180) buffalo selected for SICCT test

Breed wise	Buffalo				Total
	Surti	Mehsana	Jaffarabadi	Murrah	
Age-wise information					
Calf	12	1	-	-	13
Heifer	18	1	10	-	29
Adult	26	73	32	07	138
Total	56	75	42	07	180
Sex-wise information					
Male	12	2	2	07	23
Female	44	73	40	-	157
Total	56	75	42	07	180

2.2 Single intradermal comparative cervical tuberculin (SICCT) test: Materials was required in SICCT include tuberculin syringes, Vernier calliper**,** straight razor, paint marker, bovine purified protein derivative (B-PPD) and avian purified protein derivative (A-PPD) both obtained from Prionics, Lelystad, Netherlands. All the animals were subjected to comparative cervical intradermal tuberculin test as per the guidelines from the World Organization for Animal Health (OIE). Briefly, the test was carried out in the middle third of the neck of each animal where avian tuberculin PPD-2500 (PPD-A) (Prionics) and bovine tuberculin PPD-3000 (PPD-B) (Prionics) were injected (i.e., 0.1 ml of PPD) in 10 cm bellow from the crest and 12.5 cm between PPDs sites of the neck Skin thicknesses were measured with calliper before and 72 h after PPD injections. After 72 h, the thickness of the same skin fold at both sites will be measured and recorded. Bovine and avian positive reactors are obtained using the formula: $\{(B72 - B0) - (A72 - A0)\}$. B0 and A0 indicated skin thickness before injecting bovine and avian tuberculin, respectively, and B72 and A72 will be the corresponding skin-fold thickness 72 h post-injection (Fig 1).

Interpretation of the SICCT test was carried out in accordance with the OIE guideline (OIE Terrestrial Manual, 2009) [8]:

- Increase in skin-fold thicknesses of ≥4mm in bovine site than avian site was considered as a positive.
- Increase in skin-fold thicknesses between 1-4mm in bovine site than avian site was considered as an inconclusive.
- Increase in skin-fold thicknesses of bovine site ≤ avian site of injection was regarded to be a negative.

Fig 1: Intradermal Skin testing materials

2.3 Single intradermal tuberculin test (SIT)
The SIT test is similar to the SICCT test, but the difference between these two tests is that only bovine PPD (PPD-B) taken from the SICCT analysis, was used for comparative analysis between these two tests (SICCT & SIT), so the interpretation of the SIT test eventually changed as compare to SICCT test (Fig 1).
Interpretation of the SIT test was carried out in accordance with the OIE guideline (OIE Terrestrial Manual, 2009) [8]:
- Increase in skin-fold thickness of ≥ 4mm was considered to be a positive.
- Increase in skin-fold thickness between 2-4mm was considered to be an inconclusive.
- Increase in skin-fold thickness of < 2mm was considered to be a negative.

2.4 Disease prevalence by skin test
Disease prevalence

$$\text{Disease prevalence:} \frac{\text{Number of diseased animals at any given time}}{\text{Total no. of animals at any given time}} \times 100\%$$

Disease prevalence was categorized in breed-wise, age-wise, district-wise, lactation-wise and sex-wise of Gujarat state.

3. Results and Discussion
3.1 Detail information about SICCT test
In several organized dairy farms, the skin test was performed as a routine bovine TB screening procedure. In the present study, total 800 animals (620 cattle and 180 buffalo) screened by the SICCT test, 11 (1.37%) were found to be positive, 258 (32.25%) were inconclusive and 531 (66.37%) were negative (Table 4) (Fig 2).
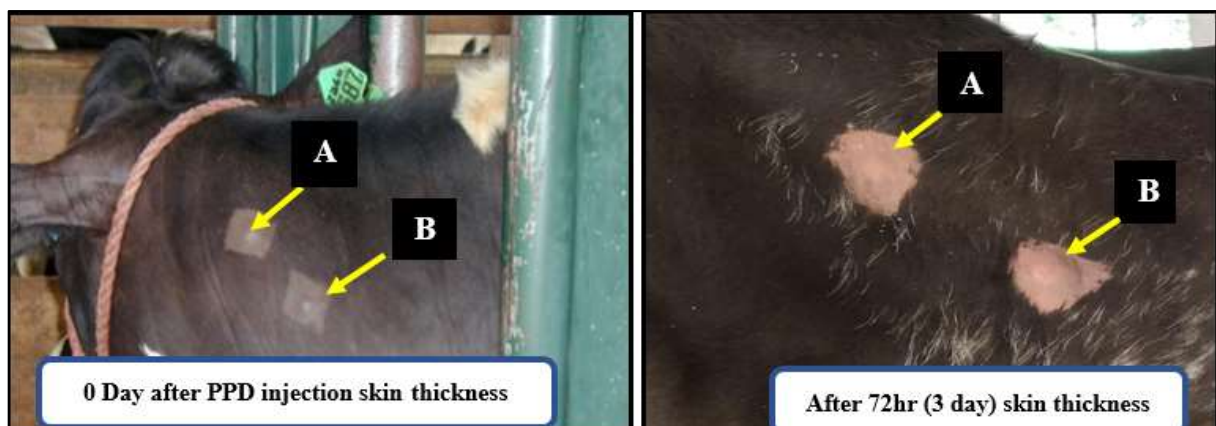

Fig 2: SICCT test positive animals

Table 4: Species wise information about SICCT tested animals

Species of animals	No. of animals	SICCT result (ΔB-ΔA) = {(B2 -B1) - (A2 -A1)} ΔB= B2 -B1 and ΔA= A2 -A1		
		SICCT positive ΔB-ΔA=(≥4 mm)	Inconclusive ΔB-ΔA=(1-4 mm)	Negative ΔB ≤ ΔA
Cattle	620	9 (1.45%)	215 (34.67%)	396 (63.87%)
Buffalo	180	2 (1.11%)	43 (23.88%)	135 (75.00%)
Total	800	11 (1.37%)	258 (32.25%)	531 (66.37%)

(A1 & B1 – Zero-day skin thickness, A2 & B2 – After 72hr skin thickness)

Of the 800 SICCT tested animals, 103 (12.87%) were Avian positive (PPD-A) and 85 (10.62%) SIT positive (PPD-B) animals. Avian positive (PPD-A) animals included 85 (13.70%) were cattle and 18 (10.00%) were buffalo, while SIT positive animals were 71 (11.45%) cattle and 14 (7.77%) buffalo (Table 5)

Table 5: Species wise information about SICCT tested animals

Species of animals	Total no of animals	Avian positive (PPD-A) ΔA= (A2-A1) = ≥4 mm	SIT positive (PPD-B) ΔB= (B2-B1) = ≥4mm
Cattle	620	85 (13.70%)	71 (11.45%)
Buffalo	180	18 (10.00%)	14 (7.77%)
Total	800	103 (12.87%)	85 (10.62%)

(A1 & B1 – Zero-day skin thickness, A2 & B2 – After 72hr skin thickness)

In the positive animals, increase in skin fold thickness were observed between 4 mm to 13 mm (Fig 2). Among the eleven SICCT positive animals, nine were cattle (seven HF Cross adult females, one Kankrej adult female and one Gir adult male) and two were buffalo (one Murrah adult male and one Jaffarabadi adult female) (Table 6).

Table 6: Information about SICCT test positive animals

Sr. No.	Animal id (Farm id)	Breed/ Age/ sex / district wise identification	SICCT test Result		
			ΔB = (B2-B1)	ΔA = (A2-A1)	ΔB-ΔA ≥4mm
		Cattle (N=09)			
1.	C-1	HF cross / Adult / Female / Anand	9	3	6
2.	C-2	Kankrej / Adult / Female / Anand	10	6	4
3.	A-1	HF cross / Adult / Female / Anand	12	2	10
4.	A-2	HF cross / Adult / Female / Anand	11	4	7
5.	A-3	HF cross / Adult / Female / Anand	14	1	13
6.	E-1	HF cross / Adult / Female / Anand	6	1	5
7.	E-2	HF cross / Adult / Female / Anand	8	0	8
8.	I-1	Gir / Adult / Male / Rajkot	12	5	7
9.	L-1	HF cross / Adult / Female / Rajkot	8	1	7
		Buffalo (N=2)			
10.	E-3	Murrah / Adult / Male / Anand	10	2	8
11.	O-1	Jaffarabadi / Adult / Female / Anand	10	1	9

3.2 Prevalence of bovine TB by SICCT test
3.2.1 Overall prevalence of bTB by SICCT test
In the present study, overall prevalence of bTB was 1.37% by SICCT test. Out of 800 animals, 1.37% (11/800) were found positive, 32.25% (258/800) were found inconclusive, and 66.37% (531/800) were negative in SICCT test (Table 7).

Table 7: Overall information about SICCT test

Cattle=620 Buffalo=180	SICCT test results (%)		
	Positive ΔB-ΔA=(≥4mm)	Inconclusive ΔB-ΔA=(1-4mm)	Negative ΔB-ΔA=(<1mm)
Total (n=800)	11 (1.37%)	258 (32.25%)	531 (66.37%)

3.2.2 Species-wise prevalence of bTB by SICCT test
The species-wise prevalence was observed in cattle 1.45% (9/620) and buffalo 1.11% (2/180). Species-wise tested animals, 34.67% (215/620) of cattle and 23.88% (43/180) of buffalo were inconclusive and 63.87% (396/620) of cattle and 75.00% (135/180) of buffalo were negative (Table 8).

Table 8: Species-wise information about SICCT test

Total animals (N= 800)	SICCT test results (%)		
	Positive ΔB-ΔA=(≥4mm)	Inconclusive ΔB-ΔA=(1-4mm)	Negative ΔB-ΔA=(<1mm)
Cattle (620)	9 (1.45%)	215 (34.67%)	396 (63.87%)
Buffalo (180)	2 (1.11%)	43 (23.88%)	135 (75.00%)
Total (n=800)	11 (1.37%)	258 (32.25%)	531 (66.37%)

3.2.3 District-wise prevalence of bTB by SICCT test

District-wise prevalence observed was 1.54% (9/583) in Anand, and 1.19% (2/167) in Rajkot, while none of the animals tested from Mehsana found to be positive. In Anand, Rajkot and Mehsana, 29.50% (172/583), 40.71%

(68/167), and 36.00% (18/50) of the animals were inconclusive and 68.90% (402/583), 58.00% (97/167) and 64.00% (32/50) of the animals were negative by SICCT test, respectively (Table 9).

Table 9: District-wise information about SICCT test

Total animals (N= 800)	SICCT test results (%)		
	Positive ΔB-ΔA=(≥4mm)	Inconclusive ΔB-ΔA=(1-4mm)	Negative ΔB-ΔA=(<1mm)
Anand (583)	9 (1.54%)	172 (29.50%)	402 (68.90%)
Rajkot (167)	2 (1.19%)	68 (40.71%)	97 (58.00%)
Mehsana (50)	-	18 (36.00%)	32 (64.00%)
Total (n=800)	11 (1.37%)	258 (32.25%)	531 (66.37%)

3.2.4 Breed-wise prevalence of bTB by SICCT test

The breed-wise prevalence was observed in cattle 1.45% (9/620) and buffalo 1.11% (2/180). In cattle, breed-wise prevalence was observed in Gir cattle 0.52% (1/190), Kankrej cattle 1.19% (1/84) and crossbred Hf cattle 2.00% (7/346). In Buffalo, breed-wise prevalence was observed in Jaffarabadi buffalo 2.38% (1/42) and Murrah male buffalo 14.28% (1/7), while in Surti and Mehsana breeds, none of the animals was found to be positive (Table 10). In SICCT tested animals, 34.67% (215/620) of cattle breeds and 23.88% (43/180) of buffalo breeds were inconclusive and 63.87% (396/620) of cattle breeds and 75.00% (135/180) of

buffalo breeds were negative. In breed-wise inconclusive animals, 43.10% (82/190) of Gir cattle, 35.70% (30/84) of Kankrej cattle, 29.76% (103/346) of HF cross cattle, 7.10% (4/56) of surti buffalo, 40.00% (30/75) of Mehsana buffalo and 21.40% (9/42) of Jaffarabadi buffalo. Breed-wise negative animals include 56.31% (107/190), 63.00% (53/84), 68.20% (236/346), 92.85% (52/56), 60.00% (45/75), 76.19% (32/42) and 85.70% (6/7) were Gir cattle, Kankrej cattle, HF cross cattle, Surti buffalo, Mehsana buffalo, Jaffarabadi buffalo and Murrah buffalo, respectively.

Table 10: Breed-wise information about SICCT test

Total animals (N= 800)		SICCT test results (%)		
		Positive ΔB-ΔA=(≥4 mm)	Inconclusive ΔB-ΔA=(1-4 mm)	Negative ΔB-ΔA=(<1 mm)
Cattle (620)	Gir (190)	1 (0.52%)	82 (43.10%)	107 (56.31%)
	Kankrej (84)	1 (1.19%)	30 (35.70%)	53 (63.00%)
	HF cross (346)	7 (2.00%)	103 (29.76%)	236 (68.20%)
	Total (n=620)	9 (1.45%)	215 (34.67%)	396 (63.87%)
Buffalo (180)	Surti (56)	-	4 (7.10%)	52 (92.85%)
	Mehsana (75)	-	30 (40.00%)	45 (60.00%)
	Jaffarabadi (42)	1 (2.38%)	9 (21.40%)	32 (76.19%)
	Murrah (07)	1 (14.28%)	-	6 (85.70%)
	Total(n=180)	2 (1.11%)	43 (23.88%)	135 (75.00%)

3.2.5 Age-wise prevalence of bTB by SICCT test

The age-wise prevalence of bTB in adult animals was 1.87% (11/587), while in calf and heifer none of the animals found to be positive. In SICCT age-wise tested animals, 25.00%

(23/91) of calf, 21.30% (26/122) of heifer and 35.60% (209/587) of adult animals were inconclusive, and 74.70% (68/91) of calf, 78.68% (96/122) of heifer and 62.50% (367/587) of adult animals were negative (Table 11).

Table 11: Age-wise information about SICCT test

Total animals (N= 800)	SICCT test results (%)		
	Positive ΔB-ΔA=(≥4 mm)	Inconclusive ΔB-ΔA=(1-4 mm)	Negative ΔB-ΔA=(<1 mm)
Calf (91)	-	23 (25.00%)	68 (74.70%)
Heifer (122)	-	26 (21.30%)	96 (78.68%)
Adult (587)	11 (1.87%)	209 (35.60%)	367 (62.50%)
Total (n=800)	11 (1.37%)	258 (32.25%)	531 (66.37%)

3.2.6 Sex-wise prevalence study of bTB by SICCT test

The sex-wise prevalence was observed in male animals 3.38% (2/59) and female animals 1.21% (9/741). Sex-wise inconclusive animals included 32.20% (19/59) of male and

32.25% (239/741) of female animals. Sex-wise negative animals include 64.40% (38/59) of male and 66.53% (493/741) of female (Table 12).

Table 12: Sex-wise information about SICCT test

Total animals (N= 800)	SICCT test results (%)		
	Positive ΔB-ΔA=(≥4mm)	Inconclusive ΔB-ΔA=(1-4mm)	Negative ΔB-ΔA=(<1mm)
Male (59)	2 (3.38%)	19 (32.20%)	38 (64.40%)
Female (741)	9 (1.21%)	239 (32.25%)	493 (66.53%)
Total (n=800)	11 (1.37%)	258 (32.25%)	531 (66.37%)

3.2.7 Lactation-wise prevalence of bTB by SICCT test
Overall Lactation-wise prevalence was 1.74% (9/515) including milch and dry animals. The lactation-wise prevalence was observed in milch animals 1.69% (6/355) and dry animals 1.87% (3/160). Out of 37.86% (195/515)

lactation-wise inconclusive animals, 42.20% (150/355) and 28.10% (45/160) were milch and dry, respectively. Out of 60.38% (311/515) lactation-wise negative animals, 56.00% (199/355) were milch and 70.00% (112/160) were dry (Table 13).

Table 13: Lactation-wise information about SICCT test

Total animals (N= 800)	SICCT test results (%)		
	Positive ΔB-ΔA=(≥4mm)	Inconclusive ΔB-ΔA=(1-4mm)	Negative ΔB-ΔA=(<1mm)
Milch (355)	6 (1.69%)	150 (42.20%)	199 (56.00%)
Dry (160)	3 (1.87%)	45 (28.10%)	112 (70.00%)
Total (n=515)	9 (1.74%)	195 (37.86%)	311 (60.38%)

3.3 Prevalence of bovine TB by SIT test
3.3.1 Overall prevalence of bTB by SIT test
In this present study, overall prevalence of bTB was 10.62%

by SIT test. Out of 800 animals, 10.62% (85/800) were found positive, 37.37% (299/800) were found inconclusive, and 52.00% (416/800) were negative in SIT test (Table 14).

Table 14: Overall information about SIT test

Cattle=620 Buffalo=180	SIT (PPD-B) test results (%)		
	Positive ΔB=B2-B1=(≥4mm)	Inconclusive ΔB=B2-B1=(2-4mm)	Negative ΔB=B2-B1=(<2mm)
Total (N= 800)	85 (10.62%)	299 (37.37%)	416 (52.00%)

3.3.2 Species-wise prevalence of bTB by SIT test
The species-wise prevalence was observed in cattle 11.45% (71/620) and buffalo 7.77% (14/180). In species-wise tested

animals, 34.67% (215/620) of cattle and 46.66% (84/180) of buffalo were inconclusive and 53.87% (334/620) of cattle and 45.55% (82/180) of buffalo were negative (Table 15).

Table 15: Species-wise information about SIT test

Total (N= 800) animals	SIT test results (%)		
	Positive ΔB=B2-B1=(≥4mm)	Inconclusive ΔB=B2-B1=(2-4mm)	Negative ΔB=B2-B1=(<2mm)
Cattle (620)	71 (11.45%)	215 (34.67%)	334 (53.87%)
Buffalo (180)	14 (7.77%)	84 (46.66%)	82 (45.55%)
Total	85 (10.62%)	299 (37.37%)	416 (52.00%)

3.3.3 District-wise prevalence study of bTB by SIT test
The district-wise prevalence was observed in Anand 12.52% (73/583), Rajkot 6.58% (11/167) and Mehsana 2.00% (1/50) in present study. In Anand, Rajkot and Mehsana, 36.00%

(210/583), 35.92% (60/167), and 58.00% (29/50) of the animals were inconclusive and 51.45% (300/583), 57.48% (96/167) and 40.00% (20/50) of the animals were negative by SIT test, respectively (Table 16).

Table 16: District-wise information about SIT test

Total animals (N= 800)	SIT test results (%)		
	Positive ΔB=B2-B1=(≥4mm)	Inconclusive ΔB=B2-B1=(2-4mm)	Negative ΔB=B2-B1=(<2mm)
Anand (583)	73 (12.52%)	210 (36.00%)	300 (51.45%)
Rajkot (167)	11 (6.58%)	60 (35.92%)	96 (57.48%)
Mehsana (50)	1 (2.00%)	29 (58.00%)	20 (40.00%)
Total	85 (10.62%)	299 (37.37%)	416 (52.00%)

3.3.4 Breed-wise prevalence study of bTB by SIT test
The breed-wise prevalence of cattle 11.45% (71/620), was higher as compare to buffalo 7.77% (14/180). In cattle, breed-wise prevalence was observed in Gir cattle 7.89% (15/190), Kankrej cattle 8.33% (7/84) and in Hf cross cattle 14.16% (49/346). In Buffalo, breed-wise prevalence was observed in Jaffarabadi buffalo 16.66% (7/42), Murrah male buffalo 28.57% (2/7), Surti buffalo 1.78% (1/56) and Mehsana buffalo 5.33% (4/75) (Table 17). In SIT, 34.67% (215/620) cattle breeds and 46.66% (84/180) buffalo breeds were inconclusive and 53.87% (334/620) cattle breeds and

45.55% (82/180) were negative. In breed-wise inconclusive animals, 24.73% (47/190) of Gir cattle, 40.47% (34/84) of Kankrej cattle, 38.72% (134/346) of HF cross cattle, 33.92% (19/56) of surti buffalo, 60.00% (45/75) of Mehsana buffalo, 42.85% (18/42) of Jaffarabadi buffalo and 28.57% (2/7) of Murrah buffalo. Breed-wise negative animals include 67.36% (128/190), 51.19% (43/84), 47.10% (163/346), 64.20% (36/56), 34.66% (26/75), 40.40% (17/42) and 42.85% (3/7) were Gir cattle, Kankrej cattle, HF cross cattle, Surti buffalo, Mehsana buffalo, Jaffarabadi buffalo and Murrah buffalo, respectively.

Table 17: Breed-wise information about SIT test

| Total animals (N= 800) | | SIT test results (%) | | |
		Positive ΔB=B2-B1 (≥4mm)	Inconclusive ΔB=B2-B1 (2-4mm)	Negative ΔB=B2-B1 (<2mm)
Cattle (620)	Gir (190)	15 (7.89%)	47 (24.73%)	128 (67.36%)
	Kankrej (84)	7 (8.33%)	34 (40.47%)	43 (51.19%)
	HF cross (346)	49 (14.16%)	134 (38.72%)	163 (47.10%)
	Total	71 (11.45%)	215 (34.67%)	334 (53.87%)
Buffalo (180)	Surti (56)	1 (1.78%)	19 (33.92%)	36 (64.20%)
	Mehsana (75)	4 (5.33%)	45 (60.00%)	26 (34.66%)
	Jaffarabadi (42)	7 (16.66%)	18 (42.85%)	17 (40.40%)
	Murrah (07)	2 (28.57%)	2 (28.57%)	3 (42.85%)
	Total	14 (7.77%)	84 (46.66%)	82 (45.55%)

3.3.5 Age-wise prevalence study of bTB by SIT test
The age-wise prevalence was observed in adult animals 13.28% (78/587), calf 2.19% (2/91) and heifer 4.00% (5/122). In age-wise tested animals, 35.16% (32/91) of calf, 38.52% (47/122) of heifer and 37.41% (220/587) of adult were inconclusive and 62.63% (57/91) of calf, 57.37% (70/122) of heifer and 49.23% (289/587) of adult were negative (Table 18).

Table 18: Age-wise information about SIT test

| Total animals (N= 800) | SIT test results (%) | | |
	Positive ΔB=B2-B1=(≥4mm)	Inconclusive ΔB=B2-B1=(2-4mm)	Negative ΔB=B2-B1=(<2mm)
Calf (91)	2 (2.19%)	32 (35.16%)	57 (62.63%)
Heifer (122)	5 (4.00%)	47 (38.52%)	70 (57.37%)
Adult (587)	78 (13.28%)	220 (37.41%)	289 (49.23%)
Total	85 (10.62%)	299 (37.37%)	416 (52.00%)

3.3.6 Sex-wise prevalence study of bTB by SIT test
The sex-wise prevalence was observed in male animals 6.77% (4/59) and female animals 10.93% (81/741). In sex-wise tested animals, 33.89% (20/59) of male and 37.65% (279/741) of female were inconclusive and 59.32% (35/59) of male and 51.41% (381/741) of female were negative (Table 19).

Table 19: Sex-wise information about SIT test

| Total animals (N= 800) | SIT test results (%) | | |
	Positive ΔB=B2-B1=(≥4mm)	Inconclusive ΔB=B2-B1=(2-4mm)	Negative ΔB=B2-B1=(<2mm)
Male (59)	4 (6.77%)	20 (33.89%)	35 (59.32%)
Female (741)	81 (10.93%)	279 (37.65%)	381 (51.41%)
Total	85 (10.62%)	299 (37.37%)	416 (52.00%)

3.3.7 Lactation-wise prevalence study of bTB by SIT test
Overall lactation-wise prevalence was 13.59% (70/515). Lactation-wise prevalence was observed in milch animals 14.00% (50/335) and dry animals 12.50% (20/160). Out of 45.63% (235/515) lactation-wise inconclusive animals, 52.10% (185/335) and 31.23% (50/160) animals were milch and dry, respectively. Out of 40.77% (210/515) lactation-wise negative animals, 33.87% (120/335) were milch and 56.20% (90/160) were dry (Table 20).

Table 20: Lactation-wise information about SIT test

| Total animals (N= 800) | SIT test results (%) | | |
	Positive ΔB=B2-B1=(≥4mm)	Inconclusive ΔB=B2-B1=(2-4mm)	Negative ΔB=B2-B1=(<2mm)
Milch (335)	50 (14.00%)	185 (52.10%)	120 (33.87%)
Dry (160)	20 (12.50%)	50 (31.23%)	90 (56.20%)
Total (n=515)	70 (13.59%)	235 (45.63%)	210 (40.77%)

Prevalence of bovine TB in the cattle and buffalo was 1.37% by SICCT test and 10.62% by SIT test in Gujarat state. Overall prevalence of bTB in SICCT test was 1.37%. These results are comparatively lower than those observed by (Vyavahare, 2012) [16]: 57.53% and (Thakur et al., 2016):13.12% in India.
Species-wise, highest prevalence of bTB was found in cattle 1.45% (9/620) and in buffalo 1.11% (2/180) by SICCT test. Using SIT test, highest prevalence was observed in cattle 11.45% (71/620) than buffalo 7.77% (14/180). In Cattle, breed-wise highest prevalence of bTB was found in HF cross 2.00% (7/346), followed by Kankrej 1.19% (1/84) and Gir 0.52% (1/190) by SICCT test. Animals testing by SIT test, highest prevalence was observed in HF cross 14.16% (49/346) followed by Kankrej 8.33% (7/84) and Gir 7.89% (16/190). In buffalo, breed-wise highest prevalence of bTB was found in Murrah 14.28% (1/7) followed by Jaffarabadi 2.38% (1/42), Mehsana 0.00% (0/75) and Surti 0.00% (0/56) by SICCT test, whereas highest prevalence was observed in Murrah 28.57% (2/7) followed by Jaffarabadi 16.66% (7/42), Mehsana 5.33% (4/75) and Surti 1.78% (1/56) by SIT test. District-wise, highest prevalence was observed in Anand 1.54% (9/583) followed by Rajkot 1.19% (2/167) and Mehsana 0.00% (0/50) by SICCT test, whereas highest

prevalence was observed in Anand 12.52% (73/583) followed by Rajkot 6.58% (11/167) and Mehsana 2.00% (1/50) by SIT test. Age-wise, highest prevalence of bTB was found in adult animals 1.87% (11/587) followed by calf 0.00% (0/91) and heifer 0.00% (0/122) by SICCT test. Highest prevalence was observed in adult animals 13.28% (78/587) followed by heifer 4.00% (5/122) and calf 2.19% (2/91) by SIT test. Sex-wise, more prevalence of bTB was found in male animals 3.38% (2/59) than in female animals 1.21% (9/741) by SICCT test and more prevalence was observed in female animals 10.93% (81/741) than in male animals 6.77% (4/59) by SIT test.

Tshopp *et al*. (2011) concluded that the overall prevalence of bovine TB was 0.90% in skin test-positive local zebus cattle. Using a cut-off of >2 mm, the overall prevalence increased to 4%. In southern Ethiopia, Gumi *et al*. (2011) [4] concluded that the prevalence of cattle 4.40% and 6.10% respectively while using the >4-mm and >2-mm cut-offs. For Using systemic review and meta-analysis from 1942 to 2016, estimated the prevalence of bTB in Gujarat and India was: 3.60% & 7.30% respectively (Srinivasan *et al*., 2018) [12]. Age-wise, highest prevalence was observed in adults, followed by calves and heifers. Regarding age wise prevalence, our results were in accordance with the previous study reported by Linton and Dorshkind (2004) [6], Frasca *et al*. (2011) [3] and Vyavahare (2012) [16]. Sex-wise, highest prevalence was observed in male than female animals by SICCT test. In contrast, in SIT sex-wise more prevalence was observed in male than female in present study. Regarding sex-wise prevalence, our SICCT results were in accordance with the previous study reported by Kazwala *et al*. (2001) [5] and Vyavahare (2012) [16].

3.4 Comparative study
3.4.1 Comparison between intradermal skin test
Out of 800 animals in the farm, 11 (1.37%) animals were positive in SICCT test, while 85 (10.62%) animals positive in SIT test. SIT test positive animals higher as compare to SICCT test positive animals (Table 21). In present study, number of SICCT test positive animals were lower than SIT test positive animals because, PPD-A was used in SICCT test has cross reactivity with pathogenic and non-pathogenic mycobacteria therefore, the chances of false positive results are reduced in SICCT test.

Table 21: Comparison between SICCT and SIT test

Species-wise tested animals	Tuberculin skin test results (%)	
	SICCT Positive ΔB-ΔA= (≥4mm)	SIT Positive ΔB= B2-B1 (≥4mm)
Cattle (n=620)	9 (1.45%)	71 (11.45%)
Buffalo (n=180)	2 (1.11%)	14 (7.77)
Total (n=800)	11(1.37%)	85(10.62%)

3.4.2 Statistical analysis
Comparison of intradermal skin test (SICCT & SIT) status carried out using chi-square test. The level of chi square test was 5%. The chi square test was carried as per Thrusfield *et al*. (2005) [14].

Following is the formula for Chi-square test: $\chi2=\sum \frac{(O-E)^2}{E}$

Where,
O is observed frequencies and E is expected frequencies.

E is calculated using following formula: $E_{XY}= \frac{(C_Y)*(R_X)}{N}$

Where,
E_{XY} is Expected frequency of Xth row under Y^{th} column; C_y is the total sum of observed frequency under Y^{th} column; R_x is the total sum of observed frequency under X^{th} row and N is the total number of observations.

3.4.2.1 Comparison of intradermal skin test (SICCT and SIT)
To compare the tuberculin skin test proportion of diseased animals, chi-square test was carried out by analyzing the 2*2 contingency table (disease prevalence row was not considered while applying chi-square test). The level of chi square test was 5%.
The chi square value obtained between skin test and disease status was 60.68 which indicated that difference in skin test and disease status was significant. ($\chi2$tab) < ($\chi2$cal) indicated alternate hypothesis is accepted, hence there is significance difference between both intradermal skin tests and disease status (Table 22).

Table 22: Comparison of skin test by chi-square test

Skin test Disease Status	SICCT test	SIT test
Positive	11	85
Negative	789	715
Disease Prevalence	1.37%	10.62%

(Degree of Freedom:1, Table value of Chi-Square ($\chi2$tab):3.84)

4. Conclusions
Prevalence of bovine TB in the cattle and buffalo was 1.37% by SICCT test and 10.62% by SIT test in Gujarat state. District-wise higher prevalence was observed in Anand followed by Rajkot and Mehsana. Highest prevalence of bTB was found in cattle compared to buffalo. In cattle, breed-wise prevalence was higher in HF cross followed by Kankrej and Gir. In buffalo, breed wise prevalence was higher in Murrah followed by Jaffarabadi, Mehsana and Surti. Age-wise highest prevalence was observed in adult animals followed by calf and heifer. Number of SICCT positive animals were lower than SIT positive animals because, PPD A used in SICCT test has cross reactivity with pathogenic and non-pathogenic mycobacteria therefore, the chances of false positive results are reduced in SICCT test.

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