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Seroprevalence of Brucellosis in Bovine

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A B S T R A C T

Brucellosis is an important zoonotic disease and it cause significant reproductive losses in animals. The present study envisaged the appraisal of seroepidemiology of brucellosis in cattle and buffaloes by i-ELISA and RBPT. In present study a total 550 bovine serum samples screened for detection of Brucella specific antibodies. Out of 550 samples, 112 (20.36%) and 75 (13.64%) sera samples were detected positive for Brucella antibody by RBPT and i-ELISA, respectively. Species-wise seroprevalence was detected 21.67% and 14.55% in cattle and 18.50% and 12.33% in buffaloes by RBPT and i-ELISA, respectively. Sex wise higher rate of seroprevalance observe in Female 20.71% and 14.47% then males 18.81% and 9.90% by RBPT and i-ELISA, respectively. Overall sensitivity 74.67%, specificity 90.45% and overall agreement 88.21% of RBPT by comparing with i-ELISA for detection of Brucella antibodies.

Introduction

Bovine Brucellosis is an infectious disease caused by *Brucella abortus*. It is a small, non-motile, coccobacilli, Gram negative, facultative, intracellular bacterial organisms of the genus *Brucella*. *Brucella* is named after Sir David Bruce who, in 1886, first isolated the organisms from the spleen of a soldier at post-mortem with what was then called Malta fever. The isolation of *B. abortus* from aborted cattle was carried out by Bang in 1897 (Mcmahan, 1944). The disease has a considerable impact on human and animal health, as well as socioeconomic impacts, especially in which rural income relies largely on livestock breeding and dairy products. It causes significant reproductive losses in sexually mature animals (Wadood *et al.*, 2009). According to OlE, it is the second most

important zoonotic disease in the world after rabies. The disease affects cattle, swine, sheep, goats, camels and dogs.

Brucella infection occurs through inhalation or ingestion of organisms *via* the nasal, oral and pharyngeal cavities or through conjunctiva or skin abrasions. The disease can also be transmitted by direct or indirect contact with infected animals, fetal membranes (Godfroid *et al.,* 2005).

Humans are commonly infected through ingestion of raw milk, cheese or meat or through direct contact with infected animals, products of conception or aniaml discahrges (*e.g.,* among shepherds, farmers and veterinarians) and through inhalation of infectious aerosols by workers in abattoirs and microbiology laboratories.

Brucellosis is a major cause of reproductive losses, abortion, placentitis, epididymitis and orchitis in animals. The symptoms and signs most commonly reported in human are fever, fatigue, malaise, chills, sweats, headaches, myalgia, arthralgia and weight loss (Kochar *et al.,* 2007 and Mantur *et al.,* 2007)

Diagnosis of a disease is of prime significance in order to identify, prevent and control of a disease. As signs and symptoms of brucellosis are unspecific, culture and serology are necessary for diagnosis (Colmenero *et al.,* 1996). Cultural isolation and identification of the agent is the gold standard test for *Brucella* diagnosis, however, this process is risky, time consuming, laborious and not suitable for disease surveillance. Moreover, it is a high risk biological pathogen that requires laboratories with qualified staff and facilities and class 3 personal protective equipment - PPE (Lage *et al.,* 2008 and Poester *et al.,* 2009). The Brucellosis diagnosis and surveillance almost entirely on serological tests, *e.g.,* Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), indirect Enzyme Linked Immunosorbent Assay (i-ELISA), and Complement Fixation Test (CFT), that detect antibodies against *Brucella* antigens including lipopolysaccharides (LPS) and give indirect evidence of *Brucella* infection (Adone and Pasquali, 2013). Serological tests are relatively easy to perform and provide a practical advantage in detecting the prevalence of *Brucella* infection.

Materials and Methods

A total of 550 serum samples were collected from cattle (323) and buffaloes (227) from rural areas and organized farms belonging to five districts of Gujarat (Table 1).

Rose Bengal Plate Test (RBPT)

One drop (0.03 ml) of serum was taken on a clean glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (0.03 ml) of the RBPT antigen (IAH and VB, Hebbal, Bangalore). The antigen and serum were mixed thoroughly with sterile tooth picks and then the slide was rotated for four minutes and result was read immediately. Definite clumping/agglutination was considered as positive reaction, whereas no clumping/agglutination was considered as negative.

Indirect-Enzyme Linked Immunosorbent Assay (i-ELISA) for cattle and buffalo

Brucella Antibody Test Kit, ELISA along with the user's manual was procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) formerly Animal Disease Monitoring and Surveillance (ADMAS), Bangalore was used in the present study. The test was performed as per the protocol outlined in the user manual.

Comparison of sensitivity and specificity of i-Elisa and RBPT

The comparative efficacy of RBPT to i-ELISA was determined with regards to their sensitivity, specificity and overall agreement in the diagnosis of brucellosis for detecting antibodies. A total of 550 (323 from cattle and 227 from buffalo) serum samples were tested by i-ELISA and compared with RBPT. Cross tabulation of RBPT with i-ELISA, considering i-ELISA as a gold standard test were recorded as per Samad *et al.,* (1994) to determine relative sensitivity, specificity and overall agreement of RBPT.

Statistical analysis

The data were entered in Excel (Microsoft) and analyzed by chi-square test. Charts were constructed in Word (Microsoft) and in Excel. A Chi square (X^2) test was done to compare the prevalence of brucellosis (in per cent) between different attributes of the seroepidemiology of brucellosis. Significance was determined at 5 per cent level. The difference was considered statistically significant if the *p*-value was < 0.05.

Results and Discussion

During the present investigation 550 serum samples were screened from Cattle (323) and buffaloes (227) for the presence of *Brucella* antibodies and of these, 112 samples were found positive by RBPT (Fig. 1) and 75 by i-ELISA (Fig. 2) with the overall seroprevalence rates of 20.36% and 13.64%, respectively. Species wise seroprevalence recorded was (21.67% and 14.55%) in cattle and (18.50% and 12.33%) in buffaloes by RBPT and i-ELISA, respectively (Table 1). Hence, species wise cattle showed higher rate of Seroprevalence compared to buffaloes. However, no significant difference was observed statistically. Similarly, Aulakh (2008) reported over all Seroprevalence of 18.26% in cattle and buffaloes from Punjab by ELISA. Gumber (2004) reported over all Seroprevalence of 22.5% in cattle and buffaloes in Punjab, by RBPT. Patel *et al.,* (2015) reported 14.81% Seroprevalence by i-ELISA. However, in contrast to the present study higher rate of seroprevalence of 28.00% in cattle and buffaloes in Bangladesh have been reported by Ahmed *et al.,* (2010) by ELISA. Kushwaha *et al.,* (2015) also reported very high seroprevalence rate of 33.85%, 32.61% and 30.90% in cattle and buffaloes by ELISA, RBPT and STAT, respectively. Whereas Sharma *et al.,* (2015) detected 41.25% animals' positive by RBPT and 46.86% positive by ELISA in Gujarat. Basit *et al.,* (2015) reported relatively higher rate of seroprevalence in buffaloes (9.00%) compared to cattle (5.00%).

Sexwise sera sample were collected from males (101) and female (449). Out of 101 males, 18.81% and 9.90% and 449 females, 20.71% 14.47% detected positive by RBPT and i-ELISA, respectively. Higher seroprevalence was observed in female than male by both test which corroborates the findings of Rahman *et al.,* (2011) and Mangi *et al.,* (2015).

Clinical status wise seroprevalence ranging from (15.04% to 52.17%) and (9.95% to 34.78%) respectively by RBPT and i-ELISA. Overall clinical status wise seroprevalence observed was highest in previous history of abortion (52.17% and 34.78%) and lowest was in clinically healthy (15.04% and 9.95%), respectively by RBPT and i-ELISA. Sexwise, Specieswise and clinical status wise all showed significant differed ($P \leq 0.05$) in Gujarat. Patel *et al.,* (2015) reported 18.30% seroprevalence in animals with reproductive disorders. Dinka *et al.,* (2013) reported the overall prevalence of reproductive disorders in the study area was 18.3%, with bovine brucellosis (32.90%), repeated breeding (26.80%) and abortion (14.60%).

Table.1 Overall seroprevalence of *Brucellosis* in cattle and buffaloes

Attribute	No. of sample tested	No. of sample found positive			
		RBPT	%	i-ELISA	%
Species:					
Cattle	323	70	21.67	47	14.55
Buffalo	227	42	18.50	28	12.33
Total	**550**	**112**	**20.36**	**75**	**13.64**
Sex:					
Male	101	19	18.81	10	9.90
Female	449	93	20.71	65	14.47
Total	**550**	**112**	**20.36**	**75**	**13.64**
Clinical status:					
Previous history of abortion	69	36	52.17	24	34.78
Hygroma	17	05	29.41	04	23.52
Orchitis	12	03	25.00	02	16.66
Clinically Healthy	452	68	15.04	45	9.95
Total	**550**	**112**	**20.36**	**75**	**13.64**
Districts:					
Banaskantha	195	42	21.54	26	13.33
Patan	43	19	44.19	09	20.93
Sabarkantha	123	12	9.76	16	13.01
Surat	90	24	26.66	14	15.55
Katchchh	99	15	15.15	10	10.10
Total	**550**	**112**	**20.36**	**75**	**13.64**

Table.2 Sensitivity specificity and overall agreement of RBPT by comparing with i-ELISA for detection of *Brucella* antibodies in cattle and buffaloes

Test		i-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	**Positive**	56	56	112	74.67	90.45	**88.21**
	Negative	19	419	438			
	Total	**75**	**475**	**550**			

Table.3 Sensitivity, specificity and overall agreement of RBPT by comparing with i-ELISA for detection of *Brucella* antibodies in cattle

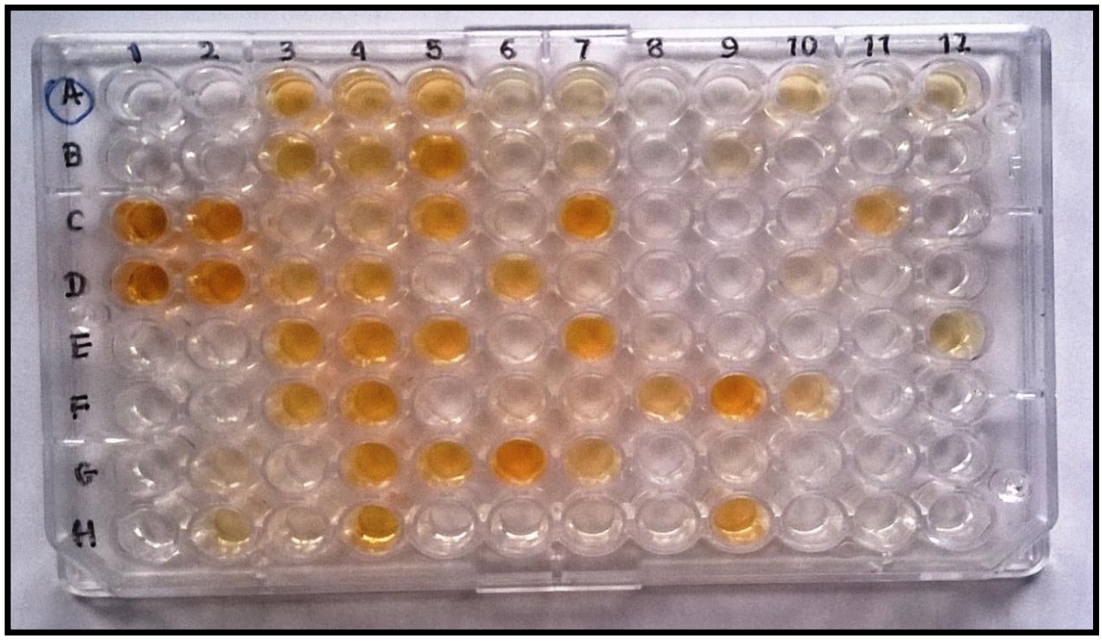
Test		i-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	33	37	70	70.21	86.59	**84.21**
	Negative	14	239	253			
	Total	**47**	**276**	**323**			

Table.4 Sensitivity, specificity and overall agreement of RBPT by comparing with i-ELISA for detection of *Brucella* antibodies in buffaloes

Test		i-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	23	19	42	82.14	90.45	**89.43**
	Negative	05	180	185			
	Total	**28**	**199**	**227**			

Fig.1 Rose Bengal Plate Test (RBPT) for detection of *Brucella* antibodies

Fig.2 i-ELISA for detection of *Brucella* antibodies



Well A1, A2,	: Conjugate control
Well B1, B2,	: Negative control
Well C1, C2, D1, D2	: Positive control
Rest of wells	: Test sera (Field)

A total of 550 samples which included five districts viz., Banaskantha (195), Patan (43), Sabarkantha (123), Surat (90) and Katchchh (99). The seroprevalence detected in Banaskantha district was (21.54% and 13.33%), in Patan district was (44.19% and 20.93%) in Sabarkantha district was (9.76% and 13.01%), in Surat district was (26.66% and 15.55%) and in Katchchh district was recorded (15.15% and 10.10%), respectively by RBPT and i-ELISA. Patan showed higher rate of seroprevalence compared to other districts. All the districts showed seroprevalence significant differed ($P \leq 0.05$) except Katchchh districts of the Gujarat.

In present study, overall sensitivity, specificity and overall agreement of RBPT were 74.67%, 90.45% and 88.21%, respectively (Table 2) and Species-wise sensitivity of RBPT found was cattle 70.21% and buffaloes 82.14 % whereas specificity was 86.59% in cattle and 90.45% in buffaloes (Table 3 and 4). Species-wise variation in sensitivity and specificity between both the tests may be due to the differences in the total number of sample tested from each species.

Various tests were compared with regards to their sensitivity and specificity in the diagnosis of bovine brucellosis. The sensitivity and specificity of RBPT were compared with i-ELISA. Indirect-ELISA is a sensitive test which can detect low concentrated antibody and test poor quality serum (Hobbs, 1985).

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