



Detection of Brucella Antibodies in Sheep with Special Aspect of Clinicalstatus and Breed

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Abstract | Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals. To ascertain the serological status of the disease, a total of 1373 samples were screened for the presence of *Brucella* antibodies by RBPT and i-ELISA. Overall seroprevalence recorded in sheep was 6.84% (94 samples out of 1373) and 4.80% (66 out of 1373) by RBPT and i-ELISA respectively. Clinical status wise seroprevalence was recorded in heifer 4.65% and 6.97%, clinically healthy animals 2.62% and 1.79%, animals with the history of abortion 16.58% and 13.98%, hygroma 27.27% and 18.18%, pregnant 4.59% and 2.29%, non-pregnant 6.80% and 4.18%, status unknown 10.78% and 6.86%, still birth 17.64% and 5.88%, retention of placenta 20.00% and 20.00%, orchitis 16.12% and 9.67% respectively by RBPT and i-ELISA, respectively. Breedwise highest seroprevalence was recorded in Patanwadi 7.82% and 5.99% followed by Marwadi 6.35% and 4.16%, Magra 3.84% and 1.28%, Avikalin 4.76% and 0.00% and Chokhla 1.78% and 0.00% by RBPT and i-ELISA, respectively. Present study revealed that the Patanwadi breed of the sheep is relatively more susceptible and high rate of seroprevalence was observed from the animals having history of abortion, retention of placenta and hygroma.

Keywords | Seroprevalence, Sheep, Brucellosis, RBPT, I-ELISA

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INTRODUCTION

Brucellosis is caused by various bacterial species of the genus *Brucella*, which is the second most widespread zoonosis worldwide (Dawood, 2008). It is one of the infectious diseases, which poses major constraints for animal production. The disease is an important public health problem in many parts of the world including India (Pal, 2007; Hadush and Pal, 2013). The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis, epididymitis and orchitis, with excretion of the organisms in uterine discharges and milk (England et al., 2004). In addition to

its direct effects on animals, brucellosis causes economic losses through abortions, stillbirths or the death of young stock. The disease can also have a blow on exports and have negative impact on the efforts to improve breeding. Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Maadi et al., 2011). As signs and symptoms of brucellosis are unspecific, culture and serology are necessary for diagnosis (Colmenero et al., 1996). Although India is largest milk producer in the world (102 million tons), animal resource in the country is threatened by reproductive disorders viz., infertil-

ity, retained placenta, abortion, endometritis *etc.*, causing considerable economic losses. Brucellosis has been one of the most important reproductive diseases among different livestock species as well as animal handlers (Verma et al., 2000; Chahota et al., 2003). It is an emerging disease since the discovery of *Brucellamelitensis* as the cause of Malta fever by Bruce in 1887 and the isolation of *B. abortus* from aborted cattle by Bang in 1897 (McMahan, 1944). Brucellosis is essentially a disease of sexually matured animals this indicates its impact on human and animal health, as well as socioeconomic status, especially, where rural income relies largely on animal husbandry and animal by-product industry. Brucellosis is found worldwide. Though it has been eradicated from many countries, it is one of the most serious diseases in developing countries. The rate of infection varies greatly from one country to another and between regions within the country, with highest prevalence in dairy cattle. In India, brucellosis was first reported in 1942 and is now endemic throughout the country (Renukaradhya et al., 2002). In general, risk factors such as unrestricted trade and movements of animals, free grazing and movement with frequent mixing of flocks of sheep and goats also attribute to the high prevalence and wide distribution of brucellosis. In addition, increasing demand for dairy products and animal protein, changing agricultural methods and movement of animals have caused high prevalence of disease. Accurate diagnosis of brucellosis is essential for institution of control strategies, either disease as a whole or as species-specific. The most widely used serological tests for diagnosis of brucellosis in animals are rose bengal plate test (RBPT), standard tube agglutination test (STAT) and enzyme linked immunosorbent assay (ELISA). Since, neither a single serological test nor a combination of tests detects all infected animals, nor also due to high homology among *Brucella* species, the detection of brucellosis remains a major problem. Hence the present study was planned with an objective to study the seroprevalence of Brucellosis among various sheep breeds and their correlation with clinical signs. Present study will help to identify the resistant breed/animal, strong positive reactor from the heathy heard, which may help in segregation of such animals which facilitate the easy control of diseases and on long run become economic to farmer. For these reason we focus on the detection of *Brucella* antibodies with special aspect of clinical status and breed.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

A total of 1373 sera were collected from sheep screened by RBPT and i-ELISA for detection of *Brucella* antibodies. Approximately 10 ml of blood was collected from individual animal aseptically from jugular vein using plain vacutainer (BD vacutainer). The vacutainer tubes were kept

in slanting position at room temperature for two hours and centrifuged at 3000 rpm for 10 minutes. The separated serum was collected in screw capped plastic vial and held at -20°C temperature till further use.

ROSE BENGAL PLATE TEST (RBPT)

The RBPT antigen was procured from the Institute of Animal Health and Veterinary Biological (IAH and VB), Hebbal, Bangalore. One drop (0.03 ml) of serum was taken on a glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (0.03 ml) of the antigen was added. 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) Indirect ELISA kit was procured from National institute of Veterinary Epidemiology and Disease informatics (NIVEDI) Bangalore, and used as per manufacturer's protocols. The kit detects the antibodies against *Brucella lipopolysaccharide* (LPS) in serum samples.

PROCEDURE

- Antigen from stock (Stored at -20°C) was added @ 40 μl /12 ml of coating buffer.
- Diluted antigen was dispensed @ 100 μl into each well of the microtitre plates after proper mixing kept for incubation over night at 4°C in refrigerator.
- After incubation, plates are washed three times using 100 μl wash buffer and tap on a tissue paper to remove residual wash buffer.
- Control and test sera were diluted in blocking buffer separately in perplex plates for which 5 μl of test and control sera were diluted in 500 μl blocking buffer.
- The diluted 100 μl test sera samples in duplicate wells and three controls (high, moderate and negative sera) in quadruplicate wells were transferred from the perplex plates to the antigen coated microtiter plates.
- The plates were incubated at 37°C for one hour on the ELISA plate shaker at 300 rpm.
- The plates were washed three times with washing buffer as mentioned earlier.
- The working dilution of the conjugate (Protein G HRP conjugate) was made by adding 1.5 μl of the conjugate to 12 ml of blocking buffer (1:8000 dilution).
- Then 100 μl of the working dilution of conjugate was added to each well and incubate at 37°C for one hour on the shaker at 300 rpm.
- The plates were washed three times with washing buffer.
- Substrate/chromogen solution was prepared by adding 1 OPD tablet (5 mg) to 12 ml distilled water followed

by the addition of 50 µl hydrogen peroxide (3 %).

- Then 100 µl chromogen solution was added to all the well and incubated at room temperature for seven minutes in dark place.
- After the color developed, immediately stopped the reaction by adding 50 µl of stopping solution to all wells.
- The plates were read in the ELISA reader (Thermo Scientific MULTISCAN GO) at 492 nm immediately.

INTERPRETATION OF RESULTS

Percent positivity (PP) values which were used for acceptance of test sera data and for diagnostic interpretation are calculated as follows:

$$PP = (\text{Average OD value of test wells} / \text{Median OD of C}^{++} \text{ well (Positive control)}) \times 100$$

The mean of any sample that gave a PP value between 55 to 65 per cent was moderate positive and below 55 per cent was taken as negative. If sample showed a PP value of that below 55 per cent was negative. On the other hand, when a sample showed a PP value of 54 per cent, then, it was retested.

RESULTS AND DISCUSSION

OVERALL SEROPREVALENCE

In the present study RBPT and i-ELISA used for detection of *brucella* antibodies in sheep sera. Out of 1373 sera tested, 94 (6.84 %) and 66 (4.80 %) were found to be positive for *Brucella* antibodies by RBPT and i-ELISA, respectively (Figure 1 and Figure 2). Similarly, Lone et al., (2013) reported 6.50% and Hassanain and Ahmed (2012) reported 5.71% seroprevalence in sheep by RBPT. Panchasara et al. (2015) recorded more or less similar overall seroprevalence was 10.66%, 10.29% and 9.38% by RBPT, STAT and i ELISA, respectively in North Gujarat. Tayshete (2001) detected 4.0% seroprevalence in north Gujarat by i-ELISA.



Figure 1: Rose Bengal test

In contrast to the present findings Awandkae et al. (2012), Azmi (2012) and Raju (2005) reported 28.10%, 21.10%

and 12.00% seroprevalence in sheep by RBPT, respectively. Sulima et al. (2010) and Al-Mariri et al. (2011) was detect 20.35% and 60.00% seroprevalence in sheep by i-ELISA, respectively.

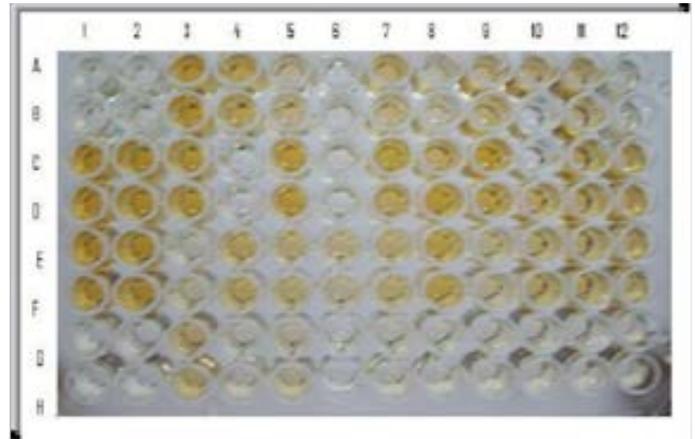


Figure 2: i-ELISA: Wells A1, B1, A2, B2: Negative control; Wells C1, D1, C2, D2: Moderately positive control; Wells E1, F1, E2, F2 : Strong positive control; Wells A3, B3, C3, D3, C5, D5 etc. field sera indicate positive reaction

CLINICAL STATUS WISE SEROPREVALENCE

In the present study, serum sample collected from sheep showing different clinical condition. The rate of seroprevalence was highest in hygroma (27.27% and 18.18%), followed by retention of placenta (20.00% and 20.00%), still birth (17.64% and 5.88%), abortion (16.58% and 13.98%), orchitis (16.12% and 9.67%), status unknown (10.78% and 6.86%), non-pregnant (6.80% and 4.18%), pregnant (4.59% and 2.29%) and Clinically healthy status (2.62% and 1.79%) of sheep in Gujarat by RBPT and i-ELISA, respectively (Table 1). Patel (2015), who found highest seroprevalence was obtained in abortion of 57.14% and 46.43% followed by hygroma (50.00% and 37.50%) and orchitis (40.00% and 20.00%) by RBPT and i-ELISA, respectively.

BREED WISE SEROPREVALENCE

In the present study, five breeds of sheep were included. The rate of seroprevalence was highest in Patanwadi (7.82% and 5.99%), followed by Marwadi (6.35% and 4.16%), Magra (3.84% and 1.28%), Avikalin (4.76% and 0.00%) and Chokhla (1.78% and 0.00%) by RBPT and i-ELISA, respectively (Table 2). In support to present findings of Patel (2015) highest seroprevalence was obtained in patanwadi sheep of 14.80% and 13.27% followed by 12.50% and 11.41% in Marwari; 8.82% and 5.88% in Magra; 6.45% and 3.23% in Avikalin and no seroprevalence in Chokhla breed by RBPT and I-ELISA, respectively. However, Kotadiya (2012) reported 15.68% and 23.52% seroprevalence of brucellosis in Marwari breed followed by Patanwadi breed (9.60 and 15.25%) and Magra breed (8.82% and 11.76%) of sheep in Gujarat by RBPT and i-ELISA, respectively.

Table 1: Clinical status wise seroprevalence

| Status | No. of tested | RBPT Positive | i-Elisa Positive |
|-----------------------|---------------|------------------|------------------|
| Clinically healthy | 725 | 19(2.62) | 13(1.79) |
| Abortion | 193 | 32(16.58) | 27(13.98) |
| Hygroma | 22 | 06(27.27) | 04(18.18) |
| Pragnant | 87 | 04(4.59) | 02(2.29) |
| Non-pragnant | 191 | 13(6.80) | 08(4.18) |
| Status unknown | 102 | 11(10.78) | 07(6.86) |
| Still birth | 17 | 03(17.64) | 01(5.88) |
| Retention of Placenta | 05 | 01(20.00) | 01(20.00) |
| Orchitis | 31 | 05(16.12) | 03(9.67) |
| Total | 1373 | 94(6.84%) | 66(4.80%) |

Table 2: Breedwise seroprevalence

| Breed | No. of tested | RBPT Positive | i-Elisa Positive |
|--------------|---------------|------------------|------------------|
| Patanwadi | 767 | 60(7.82%) | 46(5.99%) |
| Marwadi | 456 | 29(6.35%) | 19(4.16%) |
| Magra | 78 | 03(3.84%) | 01(1.28%) |
| Chokhla | 56 | 01(1.78%) | 00(0.00%) |
| Avikalin | 21 | 01(4.76%) | 00(0.00%) |
| Total | 1373 | 94(6.84%) | 66(4.80%) |

CONCLUSIONS

The present study indicates prevalence of brucellosis in sheep in Gujarat. Overall seroprevalence detected in sheep was 6.84% and 4.80% respectively by RBPT and i-ELISA. The high rate of prevalence recorded might be the reason of samples collected from the herds/flocks having history of reproductive disorder. Moreover, the variation in breed wise seroprevalence rate of sheep might be due to disease resistance.

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CONFLICT OF INTEREST

There is no conflict of interest with any of the party either directly or indirectly to the content of this article.

AUTHORS CONTRIBUTION

Kirit B. Patel carried out the experiment, analyzed epidemiological data and prepared the draft manuscript, Harshad

C. Chauhan and Bharatsingh S. Chandel provided guidance and technical support, Bharat K. Patel carried out the experiment and analyzed epidemiological data, Sandip S. Patel and Arun C. Patel edited the manuscript. Mehul D. Shrimali carried out the sample collection.

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