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Research Article

ASSESSMENT OF SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH BRUCELLOSIS IN GOAT

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Abstract- The study was carried out to know the seroprevalence and risk factors associated with occurrence of brucellosis in goats. Total 515 serum samples were randomly collected from goats having above 6 months of age during March, 2013 to March, 2014 and screened by Rose Bengal Plate Test, Standard Tube Agglutination Test and Indirect – Enzyme Linked Immunosorbent Assay. Out of total 515 sera samples, 42 (8.15%) were positive by RBPT, 41 (7.96%) by STAT and 31 (6.02%) by I- ELISA. Seroprevalence significantly ($P < 0.05$) higher found in sirohi followed by marwadi, surati, zalawadi and mehasani breed by RBPT and STAT but insignificant ($P > 0.05$) by I-ELISA. Seroprevalence insignificantly ($P > 0.05$) higher in female than male by all three test. Seroprevalence significantly ($P < 0.05$) higher found in adult age group than young age group; mixed flock than single flock; unorganized rearing practice (Migratory and Panjarapol) than organized; with abortion history than without abortion history and with carpal hygroma than without carpal hygroma by all three serological test. Seroprevalence significantly ($P < 0.05$) higher found with orchitis than without orchitis by RBPT and STAT but insignificant ($P > 0.05$) by I-ELISA.

Keywords- Brucellosis, Caprine, Risk factors, Seroprevalence, I-ELISA.

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Introduction

Brucellosis is a zoonotic bacterial disease caused by Brucella spp. and is primarily a disease of animals whereas humans are accidental hosts [1]. WHO regarded that it is a neglected zoonosis [2]. There are six identified species and numerous biotypes of Brucella spp. *B. melitensis* causes disease primary among sheep and goats and is also the most pathogenic for humans. Brucella spp. bacteria are host specific but cross-species infections may occur, particularly with *B. melitensis* [1]. The disease in goats is usually more severe and prolonged than in sheep due to the fact that the susceptibility to *B. melitensis* is generally higher in goats compared to in sheep [3]. *Brucella melitensis* leads to abortion, decreased fertility, increased neonatal losses and emergency slaughtering of the infected animals [4]. Transmission occurs mainly after abortion when the bacteria can be found in fluids and tissues connected with pregnancy like the placenta, dead fetuses and the udder [1-2]. Caprine brucellosis is widespread in the India which is a major cause of abortion in goats and also causes of large number of brucellosis cases in human [5-6]. Annually about 500,000 new cases of human brucellosis found worldwide and making it the commonest zoonosis [7]. Some risk factors which is associated with brucellosis infection in small ruminants include large herd size, lack of housing hygiene, breed, high animal stock density, frequent animal movements, shared watering spots, shared common pastures, intermixing of flocks and wandering or transhumant flocks [8-11]. In India, Free grazing and movement with frequent mixing sheep and goats flocks also impart to the high prevalence and wide distribution of brucellosis in them [12]. Commonly, diagnosis of brucellosis was based on the detection antibodies in serum sample followed by cultural isolation of the bacteria [13-14]. But cultural isolation diagnostic method has lack of sensitivity and is not a practical and dependable method in large-scale diagnostic programs [13, 15]. These disadvantages make serology the most useful diagnostic tool for Brucella infection [16-17]. Most widely used serological diagnostic tests for brucellosis are Rose Bengal Plate Test, Standard Tube Agglutination Test and Enzyme Linked Immunosorbent Assay. Some workers have tried to ascertain the seroprevalence of brucellosis in goats of Banaskantha district of Gujarat, India but systematic work has been not carried out. Thus, the aims of the this study were to determine seroprevalence of brucellosis by using serological tests like RBPT, STAT and I-ELISA and to assess the risk factors associated with occurrence of brucellosis in goats.

Materialsand Methods

Study area: The assessment of seroprevalence and risk factors associated with brucellosis in goat is carried out in Banaskantha district of Gujarat (India). Banaskantha district located at 23.03-24.45 North latitude and 71.21-73.02 East longitude of North western part of the Gujarat. Normally district has mainly three seasons *viz.* summer, monsoon, and winter. In monsoon, normal rainfall of the district is 601 mm.

Study period: The study was conducted during March, 2013 to March, 2014.

Serum sample collection and examination: Total 515 serum samples of goats were randomly collected from Banaskantha district of North Gujarat. Goats above 6 months of age were included in this study. The information regarding risk factors of brucellosis was noted in previously decided questionnaire. The risk factors like breed, sex, age, flock type, animal rearing practice and clinical status of animal i.e., abortion history, orchitis and carpal hygroma, were recorded. Age of animals

was categorized into < 1, 1 -2, 2 - 3, 3 -4 and >4 years; flock type into single or mixed (more than one species kept together)and animal rearing practice into organized and unorganized (including panjarapol and migratory). Whole blood was collected from the jugular vein of each animal randomly selected from the flock and the blood was stored at room temperature until the serum was separated (3-4 hours on average). The sera were stored at -20°C in the laboratory until tested by three serological tests namely, Rose Bengal Plate Test, Standard Tube Agglutination Test and Indirect – Enzyme Linked Immunosorbent Assay for the presence of Brucella antibodies.

Rose Bengal Plate Test (RBPT)

The RBPT was done as per the protocol described by Alton et al. [18]. The RBPT antigen purchased from the Institute of Animal Health and Veterinary Biological, Hebbal, Bangalore, Karnataka, India. The antigen and serum brought to the room temperature before test performed. Well shaken the antigen containing bottle solution till the homogenous suspension became. After that, took one drop (0.03 ml) of serum sample and one-drop (0.03 ml) antigen on the same slide using different micropipette and mixed thoroughly by using a spreader. Then slide rotated for 4 minute and saw immediately after that for results. When there was visible agglutination found after 4 minute then the test considered as positive [Fig-1].

Fig-1 Rose Bengal Plate Test (RBPT)

Standard Tube agglutination Test (STAT)

The STAT was done as per the procedure described by Alton et al. [18]. The Brucella abortus plain antigen purchased from Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, Karnataka, India. To perform the test, took 0.8 ml of 0.5% phenol saline in the first agglutination tube whereas 0.5 ml in remaining four agglutination tubes. After that 0.2 ml serum sample added in the first tube and mixed well by shaking. The 0.5 ml diluted serum from first tube transferred to the second tube and the process repeated up to the fifth tube. The 0.5 ml diluted serum discarded from the last tube and 0.5 ml Brucella abotus plain antigen added to each tube to get final dilution of 1:10, 1:20, 1:40, 1:80, and 1:160 in first, second, three, four, and fifth tube, respectively. A control tube made by mixing 0.5 ml antigen and 1.5 ml of 0.5% phenol saline in an agglutination tube. All six tubes incubated at 37°C for 20 hour. Serum samples considered as a positive if showing agglutination at 1:20 titer per ml of serum or above [Fig-2].

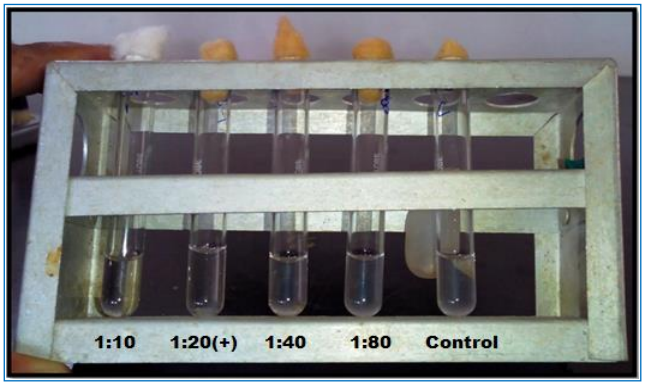

Fig-2 Standard Tube Agglutination Test; Tube No. 1,2=Positive reaction, Tube No. 3, 4=Negative reaction, Tube C = Control

Indirect – Enzyme Linked Immuno Sorbent Assay (I-ELISA)

This test performed as per procedure described by manufacturer by Protein-G based kit for Caprine and Ovine Brucellosis [Fig-3]. The I-ELISA manufactured by Project Directorate on Animal Disease Monitoring and Surveillance, Hebbal, Bangalore, Karnataka, India using smooth lipo-polysaccharide for coating. Samples, reagents, and plates brought to room temperature before starting the test. 100 µl of antigen transferred into each well of the microtiter plate by using a micropipette. The sides of the plate tapped for even distribution of the antigen at the bottom of each well. The plate covered with aluminium foil/lid and incubated at 4°C in the refrigerator for overnight. The plate washed with 100 µl of washing buffer after incubation and the washing procedure was repeated for 2 times. The 100 µl of diluted serum sample transferred in duplicate wells (two-wells) and three control sera (High, moderate, and negative sera) in quadruplicate wells (four-wells) of the microtiter plate and incubated at 37°C for 1 hour on the ELISA plate shaker @ 300 rpm. The plate washed three times with washing buffer after removing from the shaker. Then 100 µl of working diluted solution of chromogen added to each wells of the microtiter plate and incubated at room temperature in dark place for 7 min or until a visible color developed in the strong positive wells by covering with aluminium foil. Immediately after the color development, further reaction discouraged by adding 50 µl stopping solution to each well of the microtiter plate. Immediately after that, the plate read in the ELISA plate reader at 492 nm wavelength. Percent positivity (PP) value was calculated as follows:

$$PP = \frac{\text{Average OD value of test serum}}{\text{Median OD of the strong positive sera}} \times 100$$

Any sample positive when PP value was more than 55%, negative when below 55% and a sample retested when 55%.

Fig-3 Microtiter plate showing the results of I-ELISA; Well A1 and B1- Conjugate control; Well C1 and D1 – Strong positive control; Well E1 and F1 - Moderately positive control; Well indicate positive reaction of field sera - H1, B2,C3, D3, B4, C4, D4, F4, B5, D5, B6, A7, B7, B10, C10, A11, C11, D11, F11, H11, B12, and C12; rest of well – indicate negative reaction.

Statistical analysis:

The data were entered in Excel (Microsoft) and analysed by chi-square test (X^2). A Chi square (X^2) test was used to compare the prevalence between different risk factors associated with brucellosis. Analysis performed with Microsoft excels and Graph Pad Prism 5.03 software for window and the difference statistically significant if the p-value was < 0.05 at 5% level.

Results

Seroprevalence:

Out of total 515 goats, there were 42 (8.15%), 41 (7.96%) and 31 (6.02%) goats found positive by RBPT [Fig-1], STAT [Fig-2] and I- ELISA [Fig-3], respectively. Similar seroprevalence was reported by Khaleket al.(2012) [19] in goat. On the other hand, Sulima et al. (2010) [20] reported higher prevalence in goat while Tayshete (2001) [21] reported slight lower seroprevalence in the goats of North

Gujarat. Hamidullah et al. (2009)[22] noted that reason of this discrepancy in variation could be due to variation in management practices and frequent introduction of new animals without proper serological testing and there is no practice of detection and removal of animals with high incidence of abortions.

Risk factors associated with brucellosis:

Breed: Seroprevalence was higher in Sirohias 15.18% by RBPT, 16.07% by STAT and 09.82% by I-ELISA followed by Marwadi as 10.40% by RBPT, 9.60% by STAT and 8.00% by I-ELISA, Surati as 7.57% by RBPT, 6.06% by STAT and 6.06% by I-ELISA, Zalawadi as 3.45% by RBPT, 3.45% by STAT and 3.45% by I-ELISA and Mehasani breed as 3.28% by RBPT, 3.28% by STAT and 2.73% by I-ELISA [Table-1]. These differences in seroprevalence among different breeds of goats were statistically significant (P <0.05) by RBPT and STAT but insignificant (P >0.05) by I-ELISA. Similarly, Brisibe et al. (1996) [23] found non-significant difference in seroprevalence of brucellosis among different breeds of goat. Rahman et al. (2012) [24] also found insignificantly higher prevalence in crossbred of Black Bengal goat than pure Black Bengal goat. Brucellosis is not breed-specific [25]. The difference may be due to sample size variations among different breeds.

Table-1 *Breed and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
Marwadi	125	13 (10.40)	12 (9.60)	10 (8.00)
Mehasani	183	6 (3.28)	6 (3.28)	5 (2.73)
Sirohi	112	17 (15.18)	18 (16.07)	11 (9.82)
Surati	66	5 (7.57)	4 (6.06)	4 (6.06)
Zalawadi	29	1 (3.45)	1 (3.45)	1 (3.45)
Total	515	42 (8.15)	41 (7.96)	31 (6.02)
X^2 test (P value)		14.91** (P = 0.0049)	17.12* (P = 0.0018)	7.56^{NS} (P = 0.1090)

Sex: Seroprevalence was insignificantly (P >0.05) slight higher in female as 8.37% by RBPT, 8.13% by STAT and 6.69% by I-ELISA than the male as 7.22% by RBPT, 7.22% by STAT and 3.09% by I-ELISA [Table-2]. Similar result found by Rahman et al. (2012) [24] who got insignificantly (P >0.05) higher prevalence in female than the male of Black Bengal goat by I-ELISA. Similar result also recorded by Arshad et al. (2011) [26]. Actually, brucellosis is not known breed specific and not sex specific [25], the detection of slight high antibody in female animals than in males suggests the presence of suitable factors such as erythritol which assist in the growth of brucella organisms. Erythritol is a sugar synthesized in the placenta and promotes the growth of virulent strains of brucella organisms and localized within the placenta [27].

Table-2 *Sex and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
Male	97	7 (7.22)	7 (7.22)	3 (3.09)
Female	418	35 (8.37)	34 (8.13)	28 (6.69)
Total	515	42 (8.15)	41 (7.96)	31 (6.02)
X^2 test (P value)		0.14^{NS} (P = 0.7077)	0.09^{NS} (P = 0.7636)	1.81^{NS} (P = 0.1786)

Age: Seroprevalence of brucellosis was significantly (P<0.05) higher in more than 4 year age group as 13.71% by RBPT, 13.71% by STAT and 10.48% by I-ELISA followed by in 3 to 4 year age group as 11.02% by RBPT, 10.24% by STAT and 6.29% by I-ELISA, 2 to 3 year age group as 6.87% by RBPT, 6.87% by STAT and 5.34% by I-ELISA, 1 to 2 year age group as 2.25% by RBPT, 2.25% by STAT and 3.37% by I-ELISA and no seroprevalence in below 1 years of age group [Table-3]. Solorio-Rivera et al.(2007) [28] reported a significantly (P<0.2) higher prevalence of brucellosis in goat aged >36 months (12%) followed by aged between 24-36 months (11%) and aged ≤ 24 months (6%). Asmare et al. (2013) [29], Arshad et al. (2011) [26], Rahman et al. (2011a) [30], Maheshwari et al. (2012) [31] and Islam et al. (2010) [32] also noted that the seroprevalence of brucellosis to be associated with age as low prevalence in young stock age) than the adults (more than 48 months age). This could be due to the fact that brucellosis is essentially the disease of sexually matured animals and susceptibility increases with sexual maturity due to the influence of sex hormones and erythritol on the pathogenesis of brucellosis [33].

Table-3 *Age and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
< 1	44	0 (0.00)	0 (0.00)	0 (0.00)
1 - 2	89	2 (2.25)	2 (2.25)	3 (3.37)
2 - 3	131	9 (6.87)	9 (6.87)	7 (5.34)
3 – 4	127	14 (11.02)	13 (10.24)	8 (6.29)
> 4	124	17 (13.71)	17 (13.71)	13 (10.48)
Total	515	42 (8.15)	41 (7.96)	31 (6.02)
X^2 test (P value)		14.85* (P = 0.0050)	14.47* (P = 0.0059)	9.81* (P = 0.0437)

Flock type: Seroprevalence was significantly (P < 0.05) higher in mixed type flock as 10.24% by RBPT, 10.24% by STAT and 6.48% by I-ELISA than single type flock as 5.40% by RBPT, 4.95% by STAT and 5.40% by I-ELISA [Table-4]. The finding of the current study was in agreement with the finding of Asmare et al. (2013) [29] who observed higher recovery of seroreactors in flocks where other livestock species (cattle, sheep or camel) were kept together with goats (P=0.002). Megersa et al. (2011) [34] noted that the prevalence of brucellosis more in larger and mixed flocks. This could be due to goats, sheep and other species animals were kept together in mixed type flock. Hence, infected animals in mixed type flock were increase in frequency and rate of contact with the rest of the healthy animals of flocks.

Table-4 *Flock type and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
Single	222	12 (5.40)	11 (4.95)	12 (5.40)
Mixed	293	30 (10.24)	30 (10.24)	19 (6.48)
Total	515	42 (8.15)	41 (7.96)	31 (6.02)
X^2 test (P value)		3.94* (P = 0.0472)	4.81* (P = 0.0283)	10.27* (P = 0.0059)

Animal rearing practice: Seroprevalence was significantly (P < 0.05) high in unorganized sector (Migratory flock as 8.03% by RBPT, 8.03% by STAT & 8.83% by I-ELISA and Panjarapol as 13.66% by RBPT, 13.04% by STAT & 5.59% by I-ELISA) than organized farm flock as 0.00% by RBPT, STAT and I-ELISA [Table-5]. Similar result was obtained by Lone et al.(2013) [35] in sheep who recorded higher prevalence of brucellosis in unorganized sector (14.14%) as compared organized sector (3.23%). But contrast result was obtained by Singh et al. (1998) [36], who reported incidence of brucellosis higher in organized state government goat farms (4.9%) than farmer's goat flocks (0.8%) by dot-Elisa and SAT. In this study higher seroprevalence found in unorganized sector (Migratory flock and Panjarapol) compare to organized sector. This difference could be due to variations in animal management and production systems. There was frequent mixing of flocks, no segregation of infected flocks and absence of hygiene measures viz., the use of isolated kidding areas, appropriate disposal of aborted materials and removal of foci of infection could aggravate the spread of infection in unorganized sector (Migratory flock and Panjarapol) compare to organized sector.

Table-5 *Animal rearing practice and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
Migratory	249	20 (8.03)	20 (8.03)	22 (8.83)
Organized farm	105	0 (0.00)	0 (0.00)	0 (0.00)
Panjarapol	161	22 (13.66)	21 (13.04)	9 (5.59)
Total	515	42 (8.15)	41 (7.96)	31 (6.02)
X^2 test (P value)		15.85* (P=0.0004)	14.76* (P=0.0006)	10.27* (P=0.0059)

Abortion history: Seroprevalence of brucellosis was significantly (P < 0.05) higher with abortion history as 31.03% by RBPT, 24.14% by STAT and 20.69% by I-ELISA than without abortion history as 6.68% by RBPT, 6.94% by STAT and 5.65% by I-ELISA [Table-6]. This result was in agreement with the finding of Rahman et al. (2011a) [30], Rahman et al. (2011b) [37] and Tesfaye et al. (2012) [38] whom found significant association between abortion and occurrence of brucellosis in goats and sheep.

Table-6 *Abortion history and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
Yes	29	9 (31.03)	7 (24.14)	6 (20.69)
No	389	26 (6.68)	27 (6.94)	22 (5.65)
Total	418	35 (8.37)	34 (8.13)	28 (6.69)
X^2 test (P value)		20.86* (P < 0.0001)	10.68* (P = 0.0011)	9.76* (P = 0.0018)

Orchitis: Seroprevalence was significantly (P < 0.05) higher with orchitisthan without orchitis by RBPT and STAT but insignificant (P > 0.05) by I-ELISA [Table-7]. Chand et al. (2002) [39] tentatively identified *B. melitensis* in swab sample from 3 breeding ram of an organized sheep farm with abortion history and also found development of epididymo-orchitis in 4 out of 9 serologically brucellosis positive ram. Curro et al. (2012) [40] collected 50 testis samples from serologically Brucella positive sheep and goats and isolated Brucella spp. in four samples. According to Godfroid et al.(2004) [41] and OIE (2009) [42], *B. melitensis* may localize in the testis, epididymis and accessory sex glands, causing orchitis and epididymitis and ultimately infertility and the shedding of bacteria in semen.

Table-7 *Orchitis and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
Yes	4	2 (50.00)	2 (50.00)	0 (0.00)
No	93	5 (5.38)	5 (5.38)	3 (3.22)
Total	97	7 (7.22)	7 (7.22)	3 (3.09)
X^2 test (P value)		11.41* (P = 0.0007)	11.41* (P = 0.0007)	0.13^{NS} (P = 0.7152)

Hygroma: Out of total 515 goats, one goat found positive for brucellosis with carpal hygroma [Fig-4]. Seroprevalence was significantly (P < 0.05) higher with carpal hygroma than without carpal hygroma by RBPT, STAT and I-ELISA [Table-8]. Ramadan et al. (1991) [43] collected serum and Hygroma fluid from three rams and one ewe afflicted with unilateral carpal Hygroma and found all four sheep serologically positive by both RBT and TAT. Godfroid et al. (2004a) [44] told that Uni- or bilateral hygromas, especially of the carpal joints and other bursae, may be evident in some animals in chronically infected herds.

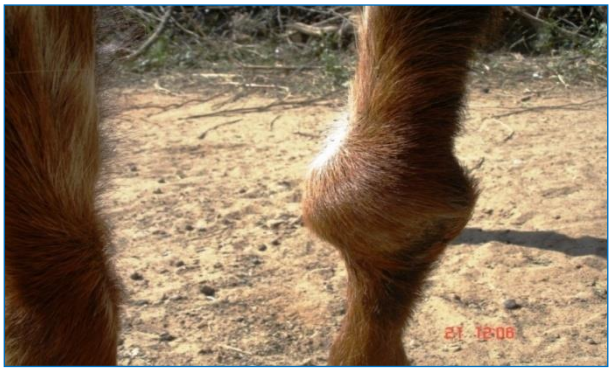

Fig-4 Serologically positive goat showing the Carpal hygroma

Table-8 *Carpal Hygroma and their association with occurrence of brucellosis*

Risk factors	No. of serum tested	No. of RBPT Positive (%)	No. of STAT Positive (%)	No. of I-ELISA Positive (%)
Yes	1	1 (100)	1 (100)	1 (100)
No	514	41 (7.98)	40 (7.78)	30 (5.84)
Total	515	42 (8.15)	41 (7.96)	31 (6.02)
X^2 test (P value)		11.28* (P = 0.0008)	11.58* (P = 0.0007)	15.64* (P < 0.0001)

* Significant at 5% Level (P < 0.05), NS – Non Significant

Conclusion:

This study was carried out in Banaskantha district of Gujarat, India. This study showed that brucellosis exists in goats of this area and may serve as source of brucella infection to human being either by consumption of raw milk and meat or by improper handling of placenta and aborted fetus. Brucellosis causes the high economic losses to livestock sector due to loss of animal protein and also serves as hazard to human health. So that, timely awareness programme should be facilitated. Adaptation of proper prevention and control strategies are also needed. There are several risk factors like age, flock type, animal rearing practice, abortion history, orchitis and carpal hygroma are significantly (P < 0.05) associated with occurrence of brucellosis.

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Conflict of Interest: None declared

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