SEROPREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS IN AND AROUND HASSAN, KARNATAKA, INDIA

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ABSTRACT

Brucellosis is a contagious disease affecting animal health especially in developing countries. *Brucella melitensis* is a major cause of abortion in small ruminants and a significant public health pathogen. This study was carried out to know the sero prevalence of brucellosis in small ruminants in and around Hassan district, Karnataka, India. A total of 192 serum samples were collected from different flock of sheep & goat and subjected to RBPT and iELISA. Overall prevalence rate of brucellosis was found to be 20.83% (CI: 15.08 – 26.57%) and 16.66% (CI: 11.38 – 21.93%) by RBPT & iELISA, respectively. Prevalence rate in sheep was 25.85% (CI: 18.77% - 32.93%) and 20.40% (CI: 13.89% - 26.91%) by RBPT and iELISA respectively whereas in goats it was 04.44% (CI: 0.0 % - 10.39%) by both tests. Region wise, significantly higher prevalence was recorded in migratory flocks from Hiriyur (57.77%, 46.66%) and Sira (35.71%, 25.00%) compared to native Hassan flocks (3.36%, 3.36%) both by RBPT and iELISA, tests respectively. Significant difference in prevalence was observed between species but not between tests.

***Key words*:** Brucellosis, Seroprevalence, Small ruminants, Hassan, Karnataka

Introduction

Small ruminants are socio economically important livestock species and means of livelihood for many marginal farmers especially in agriculture based countries. India is having a rich source of small ruminants with a population of 140.5 and 71.5 million of goat and sheep which is second and third largest in the world, respectively (Saminathan *et al.,* 2016). Brucellosis is the most economically devastating contagious reproductive disease of sexually matured animals with worldwide distribution (Gogi *et al.,* 2017). Brucellosis in India is very common but often neglected disease. *Brucella melitensis* is the major causative agent of brucellosis in small ruminants (Redkar *et al.,* 2001) characterized by loss of productivity, abortion in the fourth or fifth month of gestation, stillbirths and reproductive failures (Radostits *et al.,* 2000). In humans, 90% of the brucellosis cases are said to be associated with *B. melitensis* due to its high virulence as compared to *B. abortus* (Seleem *et al.,* 2010).

Though, isolation & identification of brucella organisms is the most reliable approach for diagnosis of brucellosis (Boral *et al.,* 2009) it has some limitations like low sensitivity, time consuming, tedious, time, type of sample collection and this procedure always puts the laboratory workers under great risk of infection. Brucellosis results in varied immune responses in the host which necessitates the detection of antibodies against brucella organisms by different serological assays. The Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), Serum Tube Agglutination Test (STAT), Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) etc. are widely used in various combinations for the diagnosis of brucellosis.

In Karnataka, many studies reported the seroprevalence of brucellosis in sheep and goat (Desai *et al.,* 1995; Kumar *et al.,* 1997; Shome *et al.,* 2006; Awati *et al.,* 2011; Sripad *et al.,* 2011; Vivekananda *et al.,* 2011, Avinash *et al.,* 2014 and Muttannagouda *et al.,* 2014) but there is no literature available about the prevalence of the disease in and around Hassan district. In this context, the present study was carried out with an objective to know seroprevlenace of brucellosis in small ruminants in and around Hassan district, Karnataka state, India by using RBPT and iELISA.

Materials and Methods

Samples

Hassan is located between 12° 13‘ & 13° 33‘ north latitude and 75° 33‘ & 76° 38‘ East longitude in Karnataka state and it is having good population of small ruminants especially migratory flocks and a local unique Hassan sheep breed. A total of 192 serum samples were collected from unvaccinated migratory flock from Hiriyur, Sira, and native Hassan under sterile conditions, transported and stored in laboratory at -20°C until further use. All the samples were initially screened by RBPT and later subjected to iELISA.

Rose Bengal Plate Agglutination Test

Rose bengal plate agglutination antigen was procured from Institute of Animal Health and Veterinary Biologicals (IAH & VB), Bangalore, KVAFSU, India. The antigen was stored at 4 °C and routinely checked for the presence of autoagglutination, if any, prior to use. RBPT was conducted according Alton *et al.* (1988). Briefly, on a clean glass slide equal quantities (30 ìl) of test serum and colored RBPT antigen was added and mixed well. The results were interpreted as either positive or negative based on presence or absence of agglutination/clumping reaction. Along with the samples, Positive and negative serum controls were included in the test.

Enzyme Linked Immunosorbent Assay

The ELISA kit was procured from SVANOVIR® Brucella Ab I-ELISA O/C, Sweden and stored at 4 °C until further use. All

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serum samples were subjected to ELISA for detection of specific Ig G antibodies against *B. melitensis* as per the manufacturer's protocol. Interpretation of results was done based on the colour development, optical density (OD) values at 450nm by using BioRad® microplate absorbance reader and percent positivity. All OD values for the test samples as well as the negative control were related to the OD value of the positive control and percent positivity (PP) value was calculated by OD of sample or Negative control / OD of positive control × 100. The optical density (OD) values of the test positive control in each ELISA test performed should be Ã 1.0 and per cent positivity of negative control should be Â 10. Accordingly samples that gave more than or equal to 15% PP value was considered as positive, below 15% was considered as negative.

Statistical analysis

A Chi-square (X^2) test was done to compare the prevalence of brucellosis (in percent) between spe-cies, region and tests. Significance was determined at 5% level. The difference was considered statistically significant if the p value is <0.05.

In this present study, iELISA was taken as gold standard reference test and subsequently the relative sensitivities, specificities and overall agreement were estimated for RBPT with iELISA.

Sensitivity: It is the capacity of the test to detect diseased animals, when compared with the gold stan-dard test (A / A+C ×100).

Specificity: It is the capacity of the test to detect non-diseased animals, when compared with the gold standard test (D / B+D × 100).

Overall agreement: Is the proportional similarity of the results of both the tests (A+D / N × 100). (True positives (A), False positives (B), False negatives (C), True negatives (D), Total no. of samples (N))

Results and Discussion

Out of 192 serum samples tested, 40 and 32 samples were positive by RBPT and iELISA with an overall prevalence rate of 20.83% (CI: 15.08 - 26.57%) and 16.66% (CI: 11.38-21.93%), respectively. Species wise analysis revealed that the prevalence of brucellosis in sheep was 25.85% (CI: 18.77% - 32.93%) and 20.40% (CI: 13.89% - 26.91%) by RBPT and iELISA, respectively whereas in goats it was 04.44% (CI: 0.0 % - 10.39%) by both tests (Table 1). Sex wise analysis revealed the prevalence in female sheep as 30.70% and 25.43% whereas, in male sheep it was 9.09% and 3.03% by RBPT and iELISA, respectively. In female goats prevalence rate was 5.71% by both tests (Table 2). Out of ten, none of the male goats were positive for brucellosis by both tests. Region wise, highest prevalence was recorded in animals from Hiriyur (57.77%, 46.66%) followed by Sira (35.71%, 25.00%) and Hassan (3.36%, 3.36%) by RBPT and iELISA, respectively (Table 3). Significantly higher prevalence was observed in sheep than in goats. Both the tests, RBPT and iELISA were found to be equally effective and no significant statistical difference was observed between them (Table 1). Sensitivity, specificity and overall agreement of RBPT with iELISA, considering iELISA as a standard reference test, was 96.96%, 95% and 95.85%, respectively (Table 4).

Brucellosis is one of the five main notifiable bacterial diseases of zoonotic importance in the world. Brucellosis is a disease of animals with humans as an accidental host. *B. melintensis* is the most virulent and major cause of ovine and caprine Brucellosis. In India, on an average, the disease causes revenue losses of INR (Indian rupee) 42 per sheep and INR 30 per goat (Saminathan *et al.,* 2016). Isloor *et al.* (1998) showed prevalence rate of 7.9% and 2.2% in sheep and goat respectively by screening samples from 10 states of India which indicated the widespread prevalence of Brucellosis in small ruminants in the country.

The RBPT is often used as a rapid screening test (Ruiz-Mesa *et al.,* 2005). In contrary to our results, many studies in Karnataka showed lower serological prevalence of brucellosis in small ruminants based on RBPT ranging from 1.73% to 14.93% (1.73% and 9.01% Kumar *et al.,* 1997; 3.2% Sripad *et al.*, 2011; 4.9% and 7.6% Desai *et al.,* 1995; 5.15% Avinash *et al.,* 2015; 5.30% Awati *et al.,* 2011; 10.43% Muttannagouda *et al.,* 2014**;** 14.93% Shome *et al.,* 2006).

The efficacy of RBPT as an individual test is questionable (Teshale *et al.,* 2006). The positive predictive value of the RBPT is low and a positive test result thus requires confirmation by a more specific test (Smits and Kadri, 2005). The sensitivity of ELISA is much better than other serological methods in small ruminants (Chand *et al.,* 2005 and Teshale *et al.,* 2006). Further, ELISA has been reported to be highly sensitive and specific and it can be used for the determination of specific IgG, IgM and IgA *Brucella* antibodies in blood, serum and CSF (Nielsen *et al.,* 1996). Hence, animals detected positive in ELISA were taken as turely positive and infected with brucellosis. In contrast to our results based on iELISA Vivekananda *et al.* (2011), Shome *et al.* (2006), Avinash *et al.* (2014) and Muttannagouda *et al.* (2014) showed the lower prevalence with 1.96 %, 7.23%, 9.52% and 11.30%, respectively. However, in the present study no significant difference between RBPT and iELISA has been observed which may be attributed to small sample size.

In the present study, seropositivity of 20.83% and 16.66% was observed by RBPT and iELISA respectively which was in accordance with the results observed by Shome *et al.* (2006), Rahman *et al.* (2011), Din *et al.* (2013) and Sadhu *et al.* (2015). It was noticed that most of the RBPT positive animals were also positive in iELISA except eight may be due to the ability of each test to detect different antibody classes. The RBPT detected higher positivity of brucellosis in small ruminants compared to iELISA. This could be since iELISA is a quantitative test which detects only IgG while RBPT qualitatively detects both IgM and IgG and besides this, RBPT was highly sensi-tive but heterospecific (Sadhu *et al.,* 2015). It will show cross reactivity with bacteria that have lipopolysaccharide (LPS) O-chains similar to those of *brucellae*, which include *Vibrio cholerae* O1, *E. coli* O:157, *Salmonella* group N (O:30) and *Yersinia enterocolitica* O:9, *Campylobacter fetus* and *Bordetella bronchiseptica* (Munoz *et al.,* 2005 and OIE 2009). One sample which was iELISA positive was found to be negative by RBPT.

In comparison to males, higher seroprevalence was recorded in female sheep and goat (Table 2). Similar observations were recorded by Priya *et al.* (2010), Rahman *et al.* (2011), Sharma *et al.* (2015), Sharma *et al.* (2016) and Gogoi *et al.* (2017). This higher prevalence in females may be due to the preferential localization of brucella organisms in the uterus and high erythritol content of placenta, which stimulates

Table 1: Overall seroprevalence of brucellosis

Sl. No	Species (No. Screened)	Particulars	Positive by RBPT	Positive by ELISA	χ^2 p values
1	Sheep (147)	Number affected	38	30	0.3323
		Prevalence	25.85%[a]	20.4%[a]	
		Confidence Interval	18.77% - 32.93%	13.89%-26.91%	
2	Goat (45)	Number affected	2	2	0.99
		Prevalence	4.44%[b]	4.44%[b]	
		Confidence Interval	0.0 % - 10.39%	0.0 % - 10.39%	
Total samples (192)		Number affected	40	32	0.431
		Overall prevalence	20.83%	16.66%	
		Confidence Interval	15.08 – 26.57%	11.38 - 21.93%	
		χ^2 p values	***0.0039***	***0.0223***	

Table 2: Sex wise seroprevalence of brucellosis

S. No.	Sex	No. Screened	Sheep			No. Screened	Goat		
			Particulars	RBPT	ELISA		Particulars	RBPT	ELISA
1	Female	114	Number affected	35	29	35	Number affected	02	02
			Prevalence	30.70%	25.43%		Prevalence	5.71%	5.71%
			Confidence Interval	22.23-39.16%	17.43% - 33.43%		Confidence Interval	0.0%-13.39	0.0%-13.39
2	Male	33	Number affected	03	01	10	Number affected	00	00
			Prevalence	9.09%	3.03%		Prevalence	00	00
			Confidence Interval	0.0%- 18.89%	0.0%-8.87%		Confidence Interval	00	00
	Total	147	Number affected	38	30	45	Number affected	02	02
			Prevalence	25.85%	20.40%		Prevalence	44.4%	44.4%
			Confidence Interval	18.77%-32.92%	13.88%-26.91%		Confidence Interval	29.88%-58.91%	29.88%-8.91%

Table 3: Region wise seroprevalence of brucellosis

Sl. No.	Region	No. Screened	Particulars	Positive by RBPT	Positive by ELISA
1	Hiriyur	45	Number affected	26	21
			Prevalence	57.77%[a]	46.66%[a]
			Confidence Interval	43.33%-72.20%	32.08%-61.23%
2	Sira	28	Number affected	10	07
			Prevalence	35.71%[a]	25.00%[a]
			Confidence Interval	17.96%-53.45%	8.96%-41.03%
3	Hassan	119	Number affected	04	04
			Prevalence	3.36%[b]	3.36%[b]
			Confidence Interval	0.12%-6.59%	0.12%-6.59%
	Total	192	Number affected	40	32
			Prevalence	20.83%	16.66%
			Confidence Interval	15.08%-26.57%	11.38%-21.93%
			χ^2 p values	***<0.001***	***<0.001***

growth of this organism. Statistically significant higher seropositivity with p value <0.05 was observed among sheep population than goats which was in accordance with the results of Singh *et al.* (2000), Sadhu *et al.* (2015) and Shome *et al.* (2006).

In the present study higher prevalence was observed in migratory flocks from Hiriyur and Sira compared to native Hassan animals (Table 3). This indicates the migratory flock as the potential source for the rapid spread of infection all along its migratory path.

The relative sensitivity and specificity of RBPT in comparison with iELISA was higher in our study when compared with the results of Hassanain and Ahmed (2012), Avinash *et al.* (2014), Sadhu *et al.* (2015) and Sharma *et al.* (2016). Whereas it was lower in contrast to the studies of Aktar *et al.* (2010), Talukder *et al.* (2012) and Sharma *et al.* (2016).

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Table 4: Sensitivity and specificity of RBPT compared to iELISA

Sl. No.	Particulars	Computed Values
1.	True positives (A)	32 (16.66%)
2.	False positives (B)	08 (04.16%)
3.	False negatives (C)	01 (0.52%)
4.	True negatives (D)	152 (79.16%)
5.	Sensitivity	96.96%
6.	Specificity	95%
7.	Positive likelihood ratio	19.39
8.	Negative likelihood ratio	0.032
9.	Positive predictive value	80%
10.	Negative predictive value	99.34%
11.	Overall agreement	95.83%

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