



Sero-incidence of brucellosis in small ruminants of semi-arid Rajasthan

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ABSTRACT

The present study was taken up to ascertain the prevalence of brucellosis in sheep and goats of semi-arid region of Rajasthan by Rose Bengal Plate Test and commercially available ELISA kit. RBPT detected brucella antibodies in 94 (8.82%) out of 1065 and ELISA in 118 (11.47%) out of 472 sheep serum samples tested. The highest seroprevalence was recorded in Tonk (24.47% and 43.29%), followed by Ajmer district (11.16% and 14.06%) by RBPT and ELISA, respectively. RBPT detected *Brucella* antibodies in 12 (1.65%) out of 723 and ELISA detected 19 (4.67%) out of 406 goat serum samples. The incidence in Tonk district was 1.31% and 10.44%, in Ajmer 2.20% and 2.63%, in Bundi 1.75% and 1.85%, and in Bhilwara 3.4% and 5.47%, by RBPT and ELISA, respectively. The combined incidence in sheep and goats of semi-arid Rajasthan was 5.92% by RBPT and 15.6% by ELISA. Our results showed higher seroprevalences in small ruminants of Tonk, Bhilwara and Ajmer districts. There is a need to screen human population dealing with husbandry, health and marketing of small ruminants and to develop effective control and prevention strategies for sheep and goats of this area against brucellosis.

Key words: Brucellosis, Rajasthan, Sero-incidence, Small ruminants

Brucellosis, a contagious and communicable bacterial disease affecting a large number of domestic animals *i.e.* cattle, sheep, goats, swine, yaks, horses, dogs and humans, leads to abortion in late pregnancy, still birth and infertility. In human's brucellosis is one of the most wide spread serious zoonosis transmitted from animals by direct contact with blood, placenta, fetus, uterine secretions or contaminated food, water, environment and through consumption of infected and raw animal products (especially milk and milk products). Reporting from livestock-human interface have received less attention than it actually deserves (Kumar *et al.* 1997, Rathore *et al.* 2002, Bandyopadhyay *et al.* 2009).

Rajasthan, one of the largest states, harbours a huge small ruminant population (sheep-10.05 million and goats-16.8 million) reared generally under extensive system of management. Rearing of sheep and goat is a centuries old sustainable livelihood for millions of small holders who raise these animals on common resources mostly on zero input basis (Mann *et al.* 1980). Unchecked intermixing of flocks during grazing lands and also during distant migration causes disease transmission through close contact, aborted fetuses, dead animals and breeding of local females with good rams/buck. Information about brucellosis of sheep and goats at

field level in the state is not clear. The present study was an attempt to demonstrate incidence of brucellosis in small ruminants of semi-arid Rajasthan by using advance and reliable diagnostic tools to evaluate more realistic picture by screening a sizeable population of small ruminants.

MATERIALS AND METHODS

Collection of sera samples: As per the agro-climatic distribution of the state, 5 districts of semi-arid zone *i.e.* Tonk, Ajmer, Jaipur/Sawai Madhopur, Bhilwara and Bundi were selected for collection of serum samples from sheep and goats elapsing the period between 2003–05. Samples (sheep, 1065; goats, 723) were collected from villages of 5 districts with history of abortion and abortion free flocks as well. History of the animals regarding age, sex, owner, clinical signs, and number and frequency of abortions was noted during sample collection. Blood samples were collected in sterile plastic syringes and transported on ice up to the laboratory. Sera were separated aseptically in sterile plastic vials and stored at –20 °C until use.

Rose Bengal plate test (RBPT): The RBPT antigen was procured from Indian Veterinary Research Institute (IVRI), Izatnagar and the test was performed as per Alton *et al.* (1975). Primary screening was carried out using RBPT. The samples from flocks having history of abortions and showing positivity to RBPT were selected for ELISA to compare the tests and determine the status of disease incidence. RBPT-

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negative samples and without history of abortion were also tested randomly by ELISA.

Indirect ELISA: The test was performed as per manufacturer's protocol of ELISA kits. The intensity of the color was measured by taking OD at 450 nm on ELISA reader. The controls included positive and negative serum. The S/P% was calculated as per the formula given in protocol with the help of computer.

$$S/P\% = 100 \times \frac{(\text{OD}_{450} \text{ of test sample}) - (\text{OD}_{450} \text{ of negative control})}{(\text{OD}_{450} \text{ of the mean positive control}) - (\text{OD}_{450} \text{ of mean negative control})}$$

Serum samples whose S/P% was equivalent or lower than 110%, in between 110% - 120% and above 120%, were considered as negative, doubtful and positive, respectively.

RESULTS AND DISCUSSION

A comparative study of seroprevalence for brucellosis in various districts of semi-arid Rajasthan in sheep and goats by RBPT and ELISA is presented in Tables 1 and 2. RBPT detected *Brucella* antibodies in 94 (8.82%) out of 1065 and ELISA in 118 (25.0%) out of 472 sheep sera tested. The highest seroprevalence was in Tonk (24.47% and 43.29%), followed by Ajmer district (11.16% and 14.06%) by RBPT and ELISA, respectively. Out of 564 sheep tested by RBPT and 113 tested by ELISA, none of the sheep sera from Jaipur/Sawai Madhopur, Bhilwara and Bundi was found positive for brucellosis. The present findings indicated that active infection was not present in the tested sheep flocks of these districts. The history collected during sample collection revealed no abortions in these flocks further supporting our findings. The overall seroprevalence (8.82%) by RBPT, recorded in sheep in the present study was in agreement with those reported by previously (Kumar *et al.* 1997, Shome *et al.* (2006) . However, seroprevalence by ELISA in the present study was two times higher (25%) in comparison to the reports of Kumar *et al.* (1997) (10.31%) and Shome *et al.* (2006) (5.66%) in Rajasthan. In a previous study carried out more than 2 decades ago Dubey and Mathur (1980) reported very low prevalence of 1.75% in flocks of Tonk and Ajmer districts indicated a significant increase in the incidence of

brucellosis among sheep of this area over the last two-and-half decades. The higher prevalence recorded in sheep could be of great concern from public health point of view as most of the human cases of brucellosis were reported from the areas that also had higher incidence of brucellosis in sheep (Mathur 1968) .

Out of 723 goat sera tested from semi-arid region, RBPT detected 12 (1.65%) as *Brucella* positive. The ELISA detected 19 (4.67%) out of 406 goats tested. The incidences as detected by RBPT and ELISA were respectively 1.31% and 10.44% in Tonk, 2.20% and 2.63% in Ajmer, 1.75% and 1.85% in Bundi and 3.4% and 5.47% in Bhilwara districts. None of the goats from Jaipur/Sawai Madhopur districts were found positive by RBPT, however, one (1.58%) of 63 goats tested positive by ELISA. Incidence in goats by RBPT observed presently was almost similar to those reported in goats of Rajasthan by the previous workers (Kumar *et al.* 1997, Kulshreshtha *et al.* 1978). Shome *et al.* (2006) however reported a higher incidence in goats of Rajasthan by RBPT (5.09%). The incidence in present study by ELISA was almost comparable with the previous reports (Mathur 1968, Sharma *et al.* 1979 and Shome *et al.* 2006). Serological tests are useful in the surveillance of brucellosis and the reliability of the test is of paramount importance. Low incidence by RBPT in goats as compared to the findings of previous workers could be due to the large sample size covering different locations of semi arid Rajasthan.

The combined incidence of brucellosis in sheep and goats of semi-arid Rajasthan was 5.92% by RBPT and 15.6% by ELISA. In the diagnosis of caprine and ovine brucellosis the efficacy of the RBPT was considered doubtful (WHO 1986), however, in view of cost consideration, feasibility and reliability as field diagnostic test, RBPT has been found to be much cheaper, easier and convenient to perform than the ELISA. The test has been considered the second most sensitive test for detecting reactors in cattle, sheep and humans next to ELISA and dot-ELISA (Stemshorn 1985, Sharma 2000, Thakur and Thapliyal 2004). Hence, this test can be used for screening of herd in the remote places with subsequent confirmation in state/central diagnostic

Table 1. *Brucella* testing in sheep and goats of semi-arid Rajasthan

Districts	Sheep				Goats			
	RBPT		ELISA		RBPT		ELISA	
	ST	SP	ST	SP	ST	SP	ST	SP
Tonk	286	70 (24.47)	231	100 (43.29)	228	03 (1.31)	67	07 (10.44)
Ajmer	215	24 (11.16)	128	18 (14.06)	136	03 (2.20)	76	02 (2.63)
Jaipur/SMP	175	0	33	0	155	0	63	01 (1.58)
Bhilwara	290	0	56	0	147	05 (3.40)	146	08 (5.47)
Bundi	99	0	24	0	57	01 (1.75)	54	01 (1.85)
Total	1065	94 (8.82)	472	118 (25.0)	723	12 (1.65)	406	19 (4.67)

ST, sample tested; SP, sample positive, figures in parenthesis indicated per cent positivity for brucellosis.

Table 2. Comparison of RBPT and ELISA with respect to species

Species	RBPT		ELISA	
	ST	SP	ST	SP
Sheep	1065	94 (8.82)	472	118 (25.0)
Goat	723	12 (1.65)	406	19 (4.67)
Total	1788	106 (5.92)	878	137 (15.60)

ST, sample tested; SP, sample positive; figures in parenthesis indicated per cent positivity for brucellosis.

laboratories by ELISA which requires sophisticated equipment (Shome *et al.* 2006).

Our results showed that Tonk, Bhilwara and Ajmer districts of Rajasthan were at higher risk, as the disease incidences in these districts were higher in sheep and goats. Infection in sheep population of these districts was greater than the goat population. Though the incidence of brucellosis was comparatively low in Jaipur and Bundi districts, irrespective of the magnitude it could be a matter of serious concern for the public health services because of the potential danger of transmission of infection to human and cattle population (Mathur 1968). In a recent study, 155 out of 175 (88.5%) human patients of brucellosis in Bikaner district were from rural areas who were occupationally involved with animals in field, handling of animals and during parturition (Kochar *et al.* 2007). Humans said to be the dead end host for brucellosis, the infection causes painful symptoms including headaches, bodyaches, shivering, orchitis and loss of labor (Mantur and Amarnath 2008). Hence, it is required to create awareness in the public about the disease and to screen the veterinary/medical professionals, shepherds and butchers for better understanding of the transmission of *brucella* infection from animals to humans (Agasthya 2007).

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