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Research Article

Prevalence of brucellosis in sheep and goat in Mumbai and Pune region by serological methods

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Abstract

The present investigation was undertaken for the detection of brucellosis in small ruminants by using RBPT, LFA and indirect ELISA. Five hundred and fifty serum samples were collected in blood collection vials from slaughterhouses, and sheep and goat farms located in and around Mumbai and Pune city. All the sera samples of sheep and goats were tested for *Brucella* antibodies using RBPT. All the samples were found negative by RBPT. Out of the total 550 samples, representative 10 samples which found positive for brucellosis by using i-ELISA were used for the presence of *Brucella* antibodies by Lateral flow assay (LFA). All those 10 samples selected for this serological test were tested positive by LFA. Out of 550 sera samples, 113 animals (20.54%) were found positive for brucellosis by i-ELISA while, 437 (79.45%) tested negative. The highest numbers of i-ELISA positive animals were observed in sheep and goat farms of Mumbai (25.19%), while the lowest in the slaughterhouse of Mumbai (17.39%). A comparison of i-ELISA findings in sheep and goats revealed that the proportion of brucellosis in goats (23.09%) was more than in sheep (11.11%). ELISA can be used for herd testing of brucellosis in small ruminants. The overall, i.e. 20.54% seroprevalence of brucellosis in sheep and goats in the studied locations needs the right preventive measures to control the disease.

Key words: Brucellosis, ELISA, Serological methods, Small ruminants

Highlights

- Five hundred and fifty sera samples of sheep and goats were tested for *Brucella* antibodies using RBPT and the results of the test indicates that the RBPT is not useful for screening of sheep and goats for brucellosis.
- Lateral flow assay can serve as diagnostic test for quick detection of brucellosis and segregation of infected animals to prevent disease transmission to other animals and strengthen the disease reporting from livestock species in the country.
- i-ELISA showed 20.54% serological prevalence of brucellosis in sheep and goats at Mumbai and Pune region of Maharashtra. A comparison of i-ELISA findings in sheep and goats revealed that the proportion of brucellosis in goats (23.09%) was more significant than in sheep (11.11%). This prevalence of brucellosis warrants the implementation of proper preventive measures.
- The highest numbers of i-ELISA positive animals were observed in sheep and goat farms of Mumbai (25.19%), while the lowest in the slaughterhouse of Mumbai (17.39%). Prevalence of brucellosis in slaughterhouses (17.39%) in Mumbai region indicates transmission risk to the butchery personnel and consumers, hence slaughterhouse testing and monitoring for brucellosis is needed to prevent the spread of human infection.
- i-ELISA is a better serological test as compared to other serological tests, and it is useful for detection of *brucella* antibodies in sheep and goats.

INTRODUCTION

Sheep and goats play an essential role in the rural economy in India (Sen *et al*., 2004).

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These are considered the primary source of livelihood, employment and income for the millions of poor rural households and jobless women. There is a tremendous demand for small ruminant production in India. Still, diseases like brucellosis hamper productivity due to the loss of milk production, abortion at late pregnancy, stillbirth and reproduction failure, and limit economic return from small ruminants (Megid *et al*., 2010).

Brucellosis is a zoonotic disease of animals prevalent throughout the world. The causative agents of brucellosis are facultative intracellular gram-negative, non-spore forming, minute coccobacillary bacteria belonging to the genus *Brucella*. Caprine brucellosis, caused by *Brucella melitensis*, is a common cause of abortions in goats. The disease is manifested by late term abortions, weak calves, infertility and characterized mainly by placentitis, epididymitis and orchitis. Sheep and goats usually have only one abortion however, reinvasion of the uterus and organism shedding can occur during future pregnancies. Some infected animals carry the pregnancy to term and then shed the organism. There is the reduction in milk production in affected animals.

Traditionally, serological tests have been widely used to screen animals for brucellosis. These tests are affordable, quick, and sensitive. Because of its affordability, sensitivity, specificity, speed, repeatability and ease of interpretation via colourimetric end product, Enzyme-linked immune-sorbent assay (ELISA) has surpassed other serological tools to detect brucellosis. Strict cleanliness and immunisation programmes are used as control measures. National Control Programme on Brucellosis (NCBP) is implemented to control brucellosis in India. It suggests periodical surveillance using milk ring test and ELISA for herd screening, and also a vaccination programme is implemented. Considering the above facts, the present study was undertaken on the prevalence of brucellosis in small ruminant populations in Mumbai and Pune region using serological methods.

MATERIALS AND METHODS

Ethical permission: All samples were collected as per standard procedures given by CPCSEA without causing any stress to animals. Prior consent of the farm owner was obtained for the visit and collection of samples.

Sample collection: Five hundred and fifty serum samples were collected from the slaughterhouse, and the sheep and goat farms located in and around Mumbai (449) and Pune city (101) and stored at -20°C until required for further investigations and diagnostic purposes.

Procedure of Rose Bengal plate test (RBPT): The coloured antigen required for the RBPT test was purchased from the Indian Veterinary Research Institute, Division of Biological Products, Izatnagar. 30 µL of serum was put on a clean grease-free glass slide, and an equal amount of coloured antigen was added and thoroughly mixed. The mixture was observed for clumping/agglutination, and then the results were recorded as agglutination (+) or no agglutination (-).

Procedure of Lateral flow assay (LFA): Antigen Rapid GS Brucella Ab Test Kit (Cat. No. RB2306DD) manufactured by BionoteInc, Republic of Korea was used for detecting antibodies in serum against *Brucella*. The test was done as per manufacturer's instructions.

Procedure of Indirect enzyme-linked immunosorbent assay (i-ELISA): Asur Dx™ Brucella Antibodies ELISA Test Kit (Bovine/ Ovine/Caprine) manufactured by Biostone Animal Health LLC, Dallas, Texas USA (Cat. No. 10022-05) was used for detection of *Brucella* antibodies. The test was performed as per the manufacturer's instructions. A sample having a PP (Percent Positivity) value equal or over 40% was taken as positive, while below 40% was termed as negative.

RESULTS

RBPT: Five hundred and fifty sheep and goat

Table 1. Detection of *Brucella* antibodies by i-ELISA in sheep and goats

Place/farm	Sheep	Goat	Total	Sheep	Goat	Total
Slaughterhouse, Mumbai	68	254	322	3 (4.41)	53 (20.86)	56 (17.39)
Sheep and Goat farm, Mumbai	49	78	127	10 (20.40)	22 (28.20)	32 (25.19)
Goat farm, Pune	_	101	101	_	25 (24.75)	25 (24.75)
Total	**117**	**433**	**550**	**13 (11.11)**	**100 (23.09)**	**113 (20.54)**

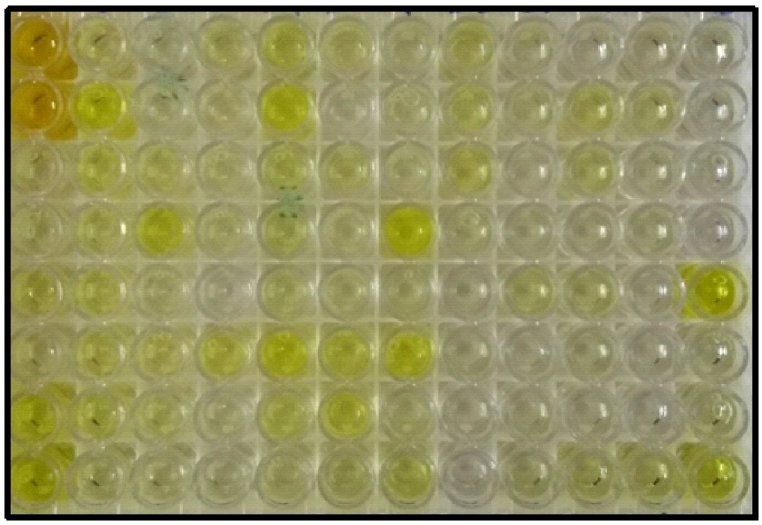

Fig. 1. Detection of *Brucella* spp. antibodies by ELISA

sera samples were checked for *Brucella* antibodies using RBPT. All the samples were found negative by RBPT.

LFA: Representative 10 ELISA positive sera samples (10/550) were used for the detection of *Brucella* antibodies by Lateral flow assay. All those 10 samples selected for this serological test were found positive by LFA.

i-ELISA: Five hundred and fifty sera samples of sheep and goat were tested for the presence of *Brucella* antibodies using i-ELISA. A total of 113 animals (20.54%) were positive for i-ELISA, whereas 437 (79.45 %) tested negative (Fig. 1). The results of i-ELISA are shown in Table 1. The highest numbers of i-ELISA positive animals were observed in sheep and goat farms of Mumbai (25.19%), while the lowest in the slaughterhouse of Mumbai (17.39%). A comparison of i-ELISA findings in sheep and goats revealed that the proportion of brucellosis in goats (23.09 %) was greater than in sheep (11.11%).

DISCUSSION

RBPT is a cheaper, sensitive, readily available field screening test used for diagnosis of brucellosis. But it is not useful test for screening of sheep and goats for brucellosis.

Several workers in India have studied the prevalence of brucellosis in small ruminants by RBPT. In the present study, RBPT results showed all sera samples negative. RBPT was routinely used for screening large animals for brucellosis. Despite fewer data available, this test was used for detection of brucellosis in sheep and goats. The main hurdle in using this test in small ruminants is standardized conditions of RBPT antigen (Garin-Bastuji

et al., 1998). In bovines, an equal proportion of serum and antigen, i.e. 30 µL each (1:1) was used in RBPT to diagnose brucellosis. In the present study, the same protocol was used, and we found unexpected results, i.e. all the sera samples showed negative results by RBPT. Hence, the proportion of serum and antigen in the modified state (3:1) should be used in sheep and goats to improve the results of RBPT (Blasco *et al*., 1994). The test was routinely used to detect brucellosis in bovines and human, but not useful in detecting brucellosis in sheep and goats due to conflicting results (WHO, 1986; Sharma, 2000; Thakur and Thapliyal, 2004).

Ebid *et al.* (2020) reported lower prevalence of 0.73% in the Arabian Gulf. Rahman *et al.* (2019) reported prevalence of 3.9% in China; Olufemi *et al.* (2018) reported prevalence of 15%, 6.6% and 7.6% in Puje, Avyi, and Hospital in Taraba state, Nigeria.

Lateral flow assay can serve as diagnostic test for detection of brucellosis and suggested for adaptation on farm, slaughter, market, clinical and pre-purchase surveillance of brucellosis in country. The present study showed all 10 sera samples positive by LFA. The seroprevalence of brucellosis by LFA in small ruminants has been studied by several workers in India. Kavya *et al.* (2017) reported 8.57% and 2.73% prevalence in sheep and goats respectively by blood-LFA and 10.71% and 3.19% in sheep and goats respectively by serum-LFA in Karnataka. Kathiriya *et al.* (2020) reported 15 (13.39%) and 25 (16.03%) samples positive in sheep and goats respectively by LFA in Gujarat. Manasa *et al*. (2019) found 8 (18.1%) samples positive from local organized farms of Andhra Pradesh and Telangana regions by LFA. Shome *et al.* (2018) found 25/298 (8.38%) goat samples positive for brucellosis in Bengaluru.

ELISA is a diagnostic method that is widely used in detection of brucellosis in animals. In the present study, 20.54% prevalence of brucellosis was reported in Mumbai and Pune region by i-ELISA. These results of ELISA are concordant with the findings of Kanani *et al.* (2018) and Manasa *et al.* (2019). Kanani *et al.* (2018) found prevalence of 24.44%, 17.24% and 7.50%, 9.35% in sheep and goat of unorganized and organized sector respectively. While, Manasa *et al.* (2019) found prevalence of 20.45% and 22.7% in sheep and goats by i-ELISA and c-ELISA in Andhra Pradesh and Telangana regions of India. Sonekar *et al*. (2018) found higher i.e 66.24% prevalence of brucellosis in sheep flock in Maharashtra. Sumathi *et al*. (2018) found 47.27% of prevalence in sheep and goats in Karnataka. In contrast to that, Sadhu *et al*. (2015) reported less i.e 8.80% prevalence of brucellosis in small ruminants by i-ELISA in Banaskantha district of North-Gujarat. Patel *et al*. (2017) found prevalence of 7.41% by i-ELISA, while Bait *et al*. (2019) reported 10.66% seroprevalence of brucellosis in small ruminants of western Maharashtra.

The reason for this variation in seroprevalence in small ruminants may be due to the introduction of new animals without proper quarantine and serological testing, frequent mixing of infected flock with a healthy one, mixing of different species, lack of bio waste and biohazard waste disposals such as aborted foetus and foetal tissues and exposure of a healthy animal to contaminated food.

The prevalence of brucellosis in slaughterhouses in the Mumbai region indicates a transmission risk to butchery personnel and consumers. Therefore, accurate testing of animals before slaughter and marketing is needed to prevent the spread of human infection. Our findings correlate with Ilhan *et al.* (2008), who found blood and lymphoid tissue samples positive for brucellosis in slaughterhouses in Turkey. Hence it is important to monitor the infection in slaughterhouses and serological testing mainly ELISA is useful tool for detection of brucellosis.

I-ELISA showed 20.54% serological prevalence of brucellosis in sheep and goats at the studied locations. Seroprevalence of brucellosis was greater in goats (23.09%) than in sheep (11.11%). Prevalence of brucellosis in slaughterhouses (17.39%) in the Mumbai

region indicates transmission risk to the butchery personnel and consumers; hence slaughterhouse testing and monitoring for brucellosis is needed to prevent the spread of human infection. Thus, i-ELISA is a useful serological test, and it is advocated for screening and diagnosis of brucellosis in small ruminants. Aside from that, control and prevention initiatives at the state and national levels should be initiated to reduce brucellosis.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: SSS: Writing,original draft preparation; VDT: Writing, review and editing, conceptualization and data curation; SBM: Project administration and supervision; RRS: Visualization and Investigation.

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