Journal of Animal Research: v.8 n.3, p. 531-535. June 2018

DOI: 10.30954/2277-940X.06.2018.32

Sero-prevalence of Brucellosis in Small Ruminants and Human in Chhattisgarh

Pankaj Sai[1], Sanjay Shakya[1]*, Choodamani Chandrakar[1] and S.L. Ali [2]

[1]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Chhattisgarh Kamdhenu Vishwa Vidyalaya, Anjora, Durg, Chhattisgarh, INDIA
[2]Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Chhattisgarh Kamdhenu Vishwa Vidyalaya, Anjora, Durg, Chhattisgarh, INDIA

**Corresponding author: S Shakya; E-mail: shakyadurg@gmail.com*

Received: 06 Oct., 2017 **Revised:** 19 March, 2018 **Accepted:** 25 March, 2018

ABSTRACT

Brucellosis is a major cause of direct economic losses resulting from clinical disease, abortion, neonatal losses, reduced fertility, decreased milk production, emergency slaughtering of infected animals and treatment costs. It is a zoonotic bacterial disease caused by *Brucella spp.* and humans are accidental hosts. A total of 600 sera samples from goats (n=470), sheep (n=30) and in contact human (n=100) were collected from the five districts of Chhattisgarh, India and tested for the presence of anti-brucella antibodies. The overall prevalence of brucellosis in goats and sheep was found 14.00%, 11.20% and 6.66% by RBPT, STAT and I-ELISA respectively. In case of human, 7.00%, 4.00% and 0% sera samples were found positive by RBPT, STAT and I-ELISA respectively. The sero-prevalance was significantly higher in female than in male animals by RBPT, STAT and I-ELISA whereas higher sero-prevalence was recorded in man than in woman by RBPT and STAT. The sero-prevalence of brucellosis was higher in adult animals than young animals. Poor awareness about the disease and lack of vaccination plan for small ruminants pose a serious risk for small ruminants as well as their owners.

Keywords: *Brucella spp.*, goats, sheep, I-ELISA, Chhattisgarh

In India, brucellosis in sheep and goats is a major cause of abortion. Brucellosis is most neglected zoonotic disease in various parts of world and mostly persists in the poorest and vulnerable human populations. Various sero-prevalence studies in animals indicated that almost all Indian states are endemic for brucellosis, which may become an emerging serious threat to small ruminants and in contact humans (Khurana *et al.,* 2012). *B. melitensis* is most pathogenic species of brucella for human and cause severe health hazard (Abeer *et al.,* 2003; Mantur *et al.* 2008) . Ingestion of contaminated feeds and water, coitus and direct contact with aborted materials are the various mode of transmission of disease in animals (Radostits *et al.,* 2007). In endemic area human get infected via contact with infected animal or consumption of their produce *viz.* from unpasteurized milk etc. (Seleem *et al.,* 2010). The predominant extensive husbandry practice of the country provides a good opportunity for mixing of different animals species at communal grazing areas and watering points. These facilitate the transmission of disease among various animal species (Chand *et al.,* 2013; Tegegn *et al.,* 2016).

Brucellosis causes direct economic losses by death in lambs and kids as well as reduction in meat and milk production. These losses are additional to the economic and social consequences of the disease in human. It also plays a significant role as a barrier for international trade of live animal (Singh *et al.,* 2015).

Serodiagnosis of brucellosis is very important to know the status of disease in small ruminants, because control of brucellosis in animal reservoirs has a corresponding and great reduction in human brucellosis. Further data on status of animal and human brucellosis in Chhattisgarh is scanty. In view of the above facts, the present investigation was undertaken to assess the seroprevalence of brucellosis

in goats, sheep and incontact human population in five districts of Chhattisgarh.

MATERIALS AND METHODS

Sample collection

A total of 600 sera samples from goats (n= 470) sheep (n=30) and in contact human being (n=100) were collected randomly from Ambikapur, Durg, Jashpur, Raigarh and Rajnandgaon districts of Chhattisgarh during March to May 2015. After proper restraining of the animal, about 5 ml blood was collected from jugular vein using disposable syringe. In human, 5ml blood was collected from medianpubital vein. The blood was allowed to clot, then serum was separated and samples were stored in 2ml vial at -20 $^{\circ}$C in laboratory till further analysis. Human sera samples were collected with the help of local medical practitioner and paramedical staff.

Serological assays

The OIE prescribed Rose Bengal Test (RBPT), Standard Tube Agglutination Test (STAT) as well as Indirect Enzyme Linked Immunosorbent Assay (I- ELISA) were used for diagnosis of brucellosis in animals and humans.

Serodiagnosis by RBPT

The RBPT antigen was procured from Division of Biological Products, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.). Sera samples were tested as per protocol outlined in manual provided along with RBPT antigen. One drop (0.03 ml) of serum sample and one drop of antigen were taken on glass slide, mixed thoroughly with a spreader and observed for the noticeable agglutination after 4 min. The human sera samples were also screened for the presence of antibody against *Brucella spp.* using the RBPT antigen.

Serodiagnosis by STAT

The standardized STAT antigen was procured from Division of Biological Products, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.). Sera samples were tested as per protocol described by OIE (2008). In brief five sugar tubes were arranged in a rack and 0.8 ml of phenol saline in the first tube and 0.5 ml in remaining tubes were dispensed. In first tube 0.2 ml test serum sample was added after proper mixing, 0.5 ml of suspension from first tube was transferred to second tube and this process was continued up to the fifth tube and finally 0.5ml of suspension from last tube was discarded. Thereafter 0.5ml STAT antigen was added to each tube to get the final dilutions of 1:10, 1:20, 1:40, 1:80 and 1:160 in first, second, third, fourth and fifth tube respectively. For goats and sheep sera samples showing agglutination at 1:20(40 IU titer) and for human 1:40 (80 IU titer) or above was considered as positive.

Serodiagnosis of samples by I-ELISA

The sera samples were tested using Protein-G based indirect ELISA kit for goat and sheep brucellosis procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru. The procedure outlined in the instruction manual was followed. The percent positivity (PP) values which were used for the diagnostic interpretations were calculated as per the following formula:

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

Sample that gave more than 55% PP value was considered as positive, below 55% was considered as negative and sample showed PP value 55% was re-tested. The human sera samples were tested using the diagnostic kits procured from Nova Tec Immunodiagnostica GmbH and the test was carried out as per manufacturer's instructions. The mean absorbance value of cut-off controls and patient's samples was determined. The results were expressed in Nova Tec-Units (NTU) and a titer of 11 NTU or more was considered as positive.

Statistical analysis

Data obtained from serological tests were analyzed using SPSS version 20.0 for windows. Chi square test was used for analysis of risk factors like age, species and sex. The method described by Snedecor and Cochran (1994) was followed and the difference was considered statistically significant when the $p < 0.05$.

RESULTS AND DISCUSSION

Out of 500 goat and sheep sera samples, 14.00%, 11.20%, and 8.40% samples were found positive for brucellosis by RBPT, STAT and I-ELISA respectively (Table 1). The seroprevalnence of 13.28%, 11.48% and 8.51% in goats and 16.66%, 6.66% and 6.66% in sheep was recorded by RBPT, STAT and I-ELISA respectively. The similar findings were reported by Sadhu *et al.* (2015) who recorded 8.15%, 7.96% and 6.02% seropositivity in caprine by RBPT, STAT and I-ELISA. Rahman *et al.* (2011) reported higher prevalence of brucellosis in caprine than ovine. However Sadhu *et al*. (2015) and Shome *et al.* (2006) reported higher seropositivity in ovine than caprine sera samples.

Analysis of human sera samples revealed that 7.00%, 4.00% and 0% sample were positive by RBPT, STAT and I-ELISA respectively (Table 2). The findings of present study are in accordance with the findings of Kumar *et al.* (2015) who reported 9.3% and 5.3% of samples positive by RBPT and STAT respectively. However higher prevalence of 9.91% by RBPT, 9.09% by STAT and 16.52% by I-ELISA in human sera samples in Jammu was reported, by Sharma *et al.* (2016).

In the present study, highest seropositive samples were detected by RBPT (14.00%) followed by STAT (11.20%) and I-ELISA (8.40%). Sadhu *et al.* (2015) also reported highest seroprevalence of brucellosis by RBPT (11.30%) followed by STAT (11.10%) and lowest by I- ELISA (8.80%) in sheep and goats which is in agreement with present findings. Rahman *et al.* (2011) also recorded greater seroprevalence by RBPT (5.83%) followed by STAT (4.17%) and the least by I-ELISA (2.50%) in goats. However, Reddy *et al.* (2014) reported higher seroprevalence by I- ELISA (9.52%) followed by STAT (6.34%) and the lowest by RBPT (5.15%) in sheep and goats. This variation in results of various tests might be due to ability of each test to detect different antibody classes. RBPT detects both IgG and IgM, STAT also detects IgM and IgG class of antibody but mainly IgM where as I- ELISA is very specific and only detects IgG. Beside this, RBPT is highly sensitive but it is heterospecific in nature thus it can cross reacts with antibodies against *Vibrio cholera, Yersinia enteroclitica* 0:9, *Pasteurella spp.* (Sadhu *et al.* 2015).

Table 1: Seroprevalence of brucellosis in goats and sheep of Chhattisgarh region

Factor	Category	Sera sample tested	RBPT positive(%)	STAT positive (%)	I-ELISA positive (%)
Species	Goats	470	65 (13.28%)	54(11.48%)	40(8.51%)
	Sheep	30	5(16.66%)	2(6.66%)	2(6.66%)
	Total	500	70(14.00%)	56(11.20%)	42(8.40%)
	χ^2 test (p value)		0.186 (0.66)	0.659(0.41)	0.125(0.72)
Sex	Male animal	93	7(7.52%)	5(5.37%)	1(1.07%)
	Female animal	407	63(15.47%)	51(12.53%)	41(10.07%)
	χ^2 test (p value)		3.976* (0.04)	3.896*(0.04)	7.966*(0.005)
Age	Young animal	34	4(11.76%)	3 (8.82%)	2(5.88%)
	Adult animal	466	66 (14.16)	53(11.37%)	40(8.58%)
	χ^2 test (p value)		0.203(0.65)	0.207(0.64)	0.301(0.58)

*indicates significant at $p \leq 0.05$

Table 2: Seroprevalence of brucellosis in human of Chhattisgarh region

Human	Sera sample tested	RBPT positive (%)	STAT positive (%)	I-ELISA positive (%)
Male	76	6(9.21%)	4(5.26%)	0
Female	24	1(4.16%)	0	0
Total	100	7 (7%)	4(4%)	0
χ2 test (p value)		0.389 (0.53)	-	-

Table 3: District wise seroprevalence of brucellosis in animal and human

District	Seroprevalence in animal				Seroprevalence in human			
	Sera sample tested	RBPT positive (%)	STAT positive (%)	I-ELISA positive (%)	Sera sample tested	RBPT positive (%)	STAT positive (%)	I-ELISA positive (%)
Ambikapur	100	10 (10%)	11(11%)	1(1%)	20	0	0	0
Jashpur	100	16 (16%)	13(13%)	14(14%)	20	1(5%)	0	0
Raigarh	100	15(15%)	13(13%)	6(6%)	20	4(20%)	3(15%)	0
Durg	100	10(10%)	10(10%)	7(7%)	20			
Rajnandgaon	100	19(19%)	9(9%)	14(14%)	20			
Total	500	70(14.00)	56(11.20%)	42(8.40%)	100			
χ2 test (p value)		5.150(0.272)	2.875 (0.579)	16.272(0.003)		—	—	—

A significantly (P<0.05) higher seroprevalence was recorded in female animals than in male animals (Table 1). These findings are in accordance with the observation of Sharma *et al.* (2016) and Tegegn *et al.* (2016) they reported higher prevalence in female goats than male goats. The higher prevalence in female animals may be due to high concentration of erythritol in reproductive organs, which is a scarcely present in male animals (Colmenero *et al.,* 2007).

In human seroprevalence was higher in males (9.21% by RBPT and 5.26% by STAT) than female (4.16% by RBPT and 5.26% by STAT) (Table 2). Sharma *et al.* (2016) also reported higher seroprevalence of human brucellosis in man (12.24%, 10.20%, and 20.41% by RBPT, STAT, and I-ELISA, respectively) than woman (0% by RBPT, 4.35% by STAT and 0% by I-ELISA). The difference in seroprevalence between man and woman is statistically non-significant which may be due to less number of female samples analyzed during the present study.

A statistically non–significant higher prevalence of brucellosis was recorded in adult animals than young animals (Table1). Sharma *et al.* (2016) and Tegegn *et al.* (2016) also recorded higher prevalence in adult animal than young animals. Brucellosis mainly, affects sexually mature and pregnant animals (Radostits *et al.,* 2007).

District wise higher prevalence of brucellosis in sheep and goats was recorded in Rajnandgaon and Jashpur whereas in, Raigarh and Ranandgaon districts higher prevalence of brucellosis was recorded in human (Table 3).

CONCLUSION

Present studies place on record the prevalence of brucellosis in small ruminants in Chhattisgarh region. Seroprevalence was more in goats than sheep. Significantly higher seropositivity was recorded in female animal than male animals. In case of human, none of the sera sample was found positive by I-ELISA and in contrast to animal population seroprevalence was more in male than female. Although more intense study for further evaluation of samples for bacteriological isolation from positive animal and human is warranted. To control this disease joint venture among veterinary and public health professionals are very essential. Because human brucellosis can be reduced by controlling this disease in animal population. Vaccination is the best way to control the spared of disease in heathy stock. The awareness campaign among high risk group *viz.* veterinarian and livestock owner may be helpful in control of human brucellosis.

ACKNOWLEDGEMENTS

The authors are highly thankful to the Dean, College of Veterinary Science and Animal Husbandry, Chhattisgarh Kamdhenu Vishwa Vidyalaya, Anjora, Durg, Chhattisgarh, India, for providing necessary financial assistance and instrumentation facilities to carry out this research work.

REFERENCES

Abeer H.A., Lafi, S.Q. and Al-Tarazi, Y. 2003. Epidemiology of ovine brucellosis in Awassi sheep in Northern Jordan. *Prev. Vet. Med.,* **60**(4): 297-306.

Chand, P. and Chhabra, R. 2013. Herd and individual animal prevalence of bovine brucellosis with associated risk factors on dairy farms in Haryana and Punjab in India. *Trop. Anim. Health Prod*., **45**(6): 1313-1319.

Colmenero, J.D., Munoz-Roca, N.L., Bermudez, P., Plata, A., Villalobos, A. and Reguera, J.M. 2007. Clinical findings, diagnostic approach, and outcome of Brucella melitensisepididymo-orchitis. *Diagn. Microbiol. Infect. Dis*., **57**(4): 367-372.

Khurana, S.K., Srivastava, S.K., Prabhudas, K. 2012. Seroprevalence of bovine brucellosis in Haryana by avidin-biotin serum ELISA and its comparison with RBPT and SAT. *Indian J. Anim. Sci*., **82**(5): 448-450.

Kumar, M.S., Sindhib, S.H., Dhanzea, H. and Mathapatib, B.S. 2015. Sero-prevalence of Brucellosis among Veterinarians and Livestock in Junagadh Region of Gujarat State. *J.F. Z. D.*, **3**(2): 23-26.

Mantur, B.G. and Amarnath, S.K., 2008. Brucellosis in India—a review. *J. Biosci*., **33**(4): 539-549.

OIE, 2008. Bovine brucellosis, manual of standards for diagnostic tests and vaccines. OIE, Paris., 624-659.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. 2007. Veterinary medicine-a textbook of the diseases of cattle, sheep, goats, pigs and horses. 10th Ed., Edinburgh: Saunders, Elsevier.

Rahman, M.S., Hahsin, M.F.A., Ahasan, M.S., Her, M., Kim, J.Y., Kang, S.I. and Jung, S.C., 2011. Brucellosis in sheep and goat of Bogra and Mymensingh districts of Bangladesh. *Korean J. Vet. Res.*, **51**(4): 277-280.

Reddy, D.A., Kumari, G., Rajagunalan, S., Singh, D.K., Kumar, A. and Kumar, P.P., 2014. Seroprevalence of caprine brucellosis in Karnataka. *Vet. World*, **7**(3): 182-188.

Sadhu, D.B., Panchasara, H.H., Chauhan, H.C., Sutariya, D.R., Parmar, V.L. and Prajapati, H.B., 2015. Seroprevalence and comparison of different serological tests for brucellosis detection in small ruminants. *Vet. World*, **8**(5): 561-566.

Seleem, M.N., Boyle, S.M. and Sriranganathen, N., 2010. Brucellosis: a re-emerging zoonosis. *Vet. Microbiol*., **140**(3): 392-398.

Sharma, H.K., Kotwal, S.K., Singh, D.K., Malik, M.A. and Kumar, A., 2016. Seroprevalence of human brucellosis in and around Jammu, India, using different serological tests. *Vet. World*, **9**(7): 742-746.

Sharma, H.K., Kotwal, S.K., Singh, D.K., Malik, M.A., Kumar, A., Katoch, R. and Singh, M., 2016. Seroprevalence Studies on Brucellosis in Goats of Jammu Region. *Indian J. Small Rumin.*, **22**(2): 198-201.

Shome, R., Shome, B.R., Deivanai, M., Desai, G.S., Patil, S.S., Bhure, S.K. and Prabhudas, K., 2006. Seroprevalence of brucellosis in small ruminants. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*., **27**(1): 13-15.

Singh, B.B., Dhand, N.K. and Gill, J.P.S., 2015. Economic losses occurring due to brucellosis in Indian livestock populations. *Prev. Vet. Med*., **119**(3): 211-215.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods. Eighth edition, Iowa State University Press, Iowa, USA.

Tegegn, A.H., Feleke, A., Adugna, W. and Melaku, S.K., 2016. Small ruminant brucellosis and public health awareness in two districts of Afar Region, Ethiopia. *J. Vet. Sci. Technol*., **7**(335): 1-5.