Int.J.Curr.Microbiol.App.Sci (2020) *9*(6): 4283-4288

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 6 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.906.502

Serological Detection of Brucellosis among Small Ruminants in Tirunelveli District, Tamil Nadu, India

A. Sundar[1*], S. Rajagunalan[1] and R. Sangameswaran[2]

[1]Department of Veterinary Public Health and Epidemiology,
[2]Department of Veterinary and Animal Husbandry Extension, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal sciences University (TANUVAS), Tirunelveli, India

**Corresponding author*

Keywords

Livestock species, gestation, stillbirths and reproductive failures

Article Info

Accepted:
12 May 2020
Available Online:
10 June 2020

A B S T R A C T

Brucellosis is an economically important zoonotic disease that causes huge economic loss to farmers. In the present study we aimed to estimate the prevalence of brucellosis among small ruminants of Tirunelveli district of Tamil Nadu, a total of 410 serum samples (Sheep-206 & Goat - 204) were collected randomly and tested for brucellosis by Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Microagglutination test (MAT) & Indirect-Enzyme Linked Immunosorbent Assay (I-ELISA). Out of 204 Goat sera, 12 animals (5.88%) were positive by all serological tests and among 206 sheep, seroprevalence was found to be 18.44%, 16.99%, 16.99% and 22.33% by RBPT, STAT, MAT and I-ELISA, respectively. Thus, the overall seroprevalence in sheep and goat was found to 19.25% and 5.88%, respectively. This study concludes higher prevalence of brucellosis among sheep and goat in Tirunelveli district highlighting the need for implementation of intensive surveillance and control measures against brucellosis.

Introduction

Small ruminants are socioeconomically important livestock species, ubiquitously reared as primary source of animal food and more than five million households in the country are engaged in rearing of small ruminants. India is a rich niche of ovine germplasm and accounts for more than 6.8% and 20% of world sheep and goat population respectively (FAO, 2000; FAOSTAT, 2010). One of the major contagious endemic bacterial diseases of small ruminants in the country is brucellosis, characterized by loss of productivity, abortion in the fourth or fifth month of gestation, stillbirths and reproductive failures. Brucellosis is also an important zoonotic disease (ILRI, 2012). In India brucellosis due to *B. melitensis* is most widespread, a highly pathogenic organism for humans and a major cause of abortion in small ruminants (Mantur *et al.*, 2008). In humans, brucellosis is usually marked by an intermittent or remittent fever accompanied by sweats, fatigue, malaise, anorexia, weight loss, headache, arthralgia and back pain, may

persist for weeks or months in the absence of specific treatment (Corbel, 2006). The transmission of *Brucella* infection and its prevalence in a region depends upon several factors like food habits, methods of processing milk and milk products, social customs, husbandry practices, climatic conditions, socioeconomic status, and environment hygiene (Tikare *et al.,* 2008). Most data and evidence on the economic burden of brucellosis and its control is from the developed world even though the losses are believed to be higher in the developing countries (McDermott *et al.,* 2013). This disease in cattle and buffalo accounted for 95.6% of the total losses occurring in livestock populations. These losses are additional to the economic and social consequences of the disease in humans (Singh *et al.,* 2015). The gold standard for the diagnosis of brucellosis is isolation of *Brucella* bacteria. However, to isolate *Brucella* bacteria is time consuming and resource-intensive. However, alternative methods have been developed which include, identification of nucleic acid from the bacterium by molecular biology technology and a large number of serological tests. Use of battery of serological tests increases the efficiency of diagnosis of brucellosis (Sundar *et al.,* 2015)

As per the 19th census, it is evident that Tirunelveli region has the highest recorded small ruminant population - sheep (3, 03,105) & goat (3, 30,230). Due to paucity of information regarding prevalence of brucellosis among small ruminants in and around Tirunelveli region, an initiative is needed for the screening of these small ruminant populations for Brucellosis.This study was conceived to address insufficient data on seroprevalence of Brucellosis among sheep and goat in Tirunelveli region, Tamil Nadu by various serological test viz., RBPT, STAT, I-ELISA and MAT

Materials and Methods

Collection of serum samples

A total of 410 serum samples (204 from goat and 206 from sheep) were collected from various organized and unorganized farms in Tirunelveli district . About 4 ml of blood was collected in clot activator vacutainers and centrifuged at 3000 rpm for 15 minutes. Serum was separated , and stored at – 20℃ in 2 ml sterile microcentrifuge tubes after adding 0.01% sodium azide.

Serological testing

The collected sera samples were subjected to different serological tests as described below.

Rose Bengal Plate Test (RBPT)

The RBPT was performed according to the method described by Alton *et al.,* (1975). The Rose Bengal Antigen was procured from the Division of Biological Products, IVRI, Izatnagar. Before the test, both serum and antigen were allowed to come at room temperature. Then, the test was performed by mixing 30 µl each of serum and antigen on a clean, grease free glass plate. With continuous shaking, the plates were observed for any appearance of agglutination. Appearance of agglutination within 4 minutes of mixing of reagents was considered as positive while absence of agglutination was recorded as negative result.

Standard Tube Agglutination Test (STAT)

The test was performed in clean glass tubes (13 mm x 100 mm) according to the method described by Alton *et al.,* (1975). The *B. abortus* plain antigen for the test was procured from the Division of Biological Products, IVRI, Izatnagar. For the test proper, 5 test tubes set for each sample was arranged.

For high titre sera, more dilutions were prepared in order to achieve end point titre. An amount of 0.8 ml phenol saline (diluent) was added to the first tube and 0.5 ml in subsequent tubes. Then 0.2 ml serum was added in the first tube and mixed thoroughly to make 1:5 dilutions. From the first tube 0.5 ml was transferred to the second tube to make 1: 10 dilution and subsequent transfers were made as like from the first tube to make 1:20, 1:40 and 1:80 dilutions while from the last tube 0.5 ml of final dilution was discarded.

After making dilutions, 0.5 ml antigen was added to each tube to give final dilutions as 1:10. 1:20, 1:40, 1:80 and 1:160. The contents were mixed thoroughly and incubated overnight at 37°C. The degree of agglutination was judged by opacity of the supernatant fluid. The highest serum dilution showing 50 percent or more agglutination (50 % clearing) was considered as the titre of the serum. The titre so obtained was expressed in the unit system by doubling of the serum titre as International Unit (I.U.) per ml of serum. A titre of 1:20 or 40 I.U. and above was considered as positive in sheep and goat.

I-ELISA

The I-ELISA was performed using the Protein-G based kit for Caprine and Ovine Brucellosis manufactured by GenomixCarl Private Limited, Kadappaas per the manufacturer's instructions

Microagglutination test (MAT)

MAT was performed with slight modification as described by Baum (1995). All the serum samples were tested with a minimum of 8 dilutions in microtitre plate with V shaped well. Microtiter plate was appropriately labelled and 80 µL of 0.85% normal saline was added to the first row and 50 µL to the rest of the rows. Each well of the first row was added with 20 µL of test serum samples and mixed thoroughly and 50 µL of this mixture was transferred to the corresponding well in the second row using multichannel pipette. The process was repeated until the last row and from the last row 50 µL of the mixed contents was discarded. This was followed by addition of 50 µL of plain antigen to all the well. The microtiter plate was incubated at 37 °C for 24 h before the results were read. Controls were run using known positive and known negative sera. Interpretation of the results was based on the formation of an agglutination matrix (mat formation) or button formation at the bottom of the well. The titer so obtained was expressed in the unit system by doubling of the serum titer as International Units (I.U.) per milliliter of serum; 40 I.U. (≥1:20) or above was considered positive for brucellosis.

Results and Discussion

In the present study, a total of 410 (204 from goat and 206 from sheep) serum samples were collected randomly from different breeds of sheep and goats in both organised and un organised farms of Tirunelveli district and subjected to different serological tests. The overall seroprevalence was found to be 19.25% among sheep population and 5.88% among goat population.

RBPT

Among 206 sheep serum samples tested by RBPT, 38 (18.44%) samples were found positive and among 204 goat serum samples tested 12 (5.88%) samples were found positive.

Table.1 Prevalence of Brucellosis is Sheep by serological tests

Diagnostic test	RBPT	I-ELISA	STAT	MAT
No. of positive	38	46	35	35
% positivity	18.45%	22.33%	16.99%	16.99%

Table.2 Prevalence of Brucellosis is Goat by serological tests

Diagnostic test	RBPT	I-ELISA	STAT	MAT
No. of positive	12	12	12	12
% positivity	5.88%	5.88%	5.88%	5.88%

STAT

Among sheep 35(16.99%) animals were positive for Brucellosis. Among goat samples 12(5.88%) animals were positive by STAT for Brucellosis

I-ELISA

In indirect ELISA, among 206 sheep serum samples 46 (22.33%) were found positive and 12 (5.88%) serum samples were positive among 204 goat serum tested.

Microagglutination test (MAT)

Among sheep, 35(16.99%) samples were positive for Brucellosis. Among goat samples tested 12(5.88%) were positive by MAT.

Brucellosis, an economically important reproductive disease of livestock, is prevalent in most developing countries, including India and also constitutes an important public health problem (Corbel, 2006). In the present study, overall seroprevalence in sheep and goat was found to 19.25% and 5.88%, respectively which was higher than overall seroprevalence of the country as reported as 5.5% & 2.3% in sheep and goat, respectively by Shome *et al.*, (2015). This study also reports that higher seroprevalence among sheep than goats. Similar kind of findings were also reported in different parts of our country (Suryavanshi *et al.*, 2014; Sadhu *et al.*, 2015 and Shome *et al.*, 2015). But Sonawane *et al.*, (2011) reported higher prevalence in goats compared to sheep which may due to improper sample size between sheep and goats. In our study, seroprevalence rate among sheep was observed to be 18.44%,16.99%, 16.99% & 22.33% by RBPT, STAT, MAT and I-ELISA, respectively which corroborates well that of Sharma *et al.*, 2016 who reported13.82%, 16.13% and 23.13% seroprevalence by RBPT, STAT and I-ELISA, respectively. Similar prevalence was also reported by Shome *et al.*, (2006); Maher-Sulima and Venkataraman (2007); Sonawane *et al.*, (2011) and Kanani *et al.*, (2018). Our study reports the prevalence of 5.88% among goats by all the serological tests done. Our study report corroborates with study conducted state level reports 4.6% prevalence among goats by Shome *et al.*, (2015). Saikia and workers (2019) reported a lower prevalence of 1.72% among 1514 samples whereas Kaur *et al.*, (2019) reported higher prevalence of 18.7% among 277 samples. This variation may be due to sampling difference which was rectified in our study by proper sampling resulting more or less similar to our state level prevalence reported earlier.

This study concludes higher seroprevalence of brucellosis among sheep and goat which needs to be addressed immediately by

adopting suitable control strategy for brucellosis. At field level, control of brucellosis can be achieved by conducting RBPT, a cost effective and rapid tests performed even in fields to screen brucellosis before purchasing new animals to the farm. This study also recommends vaccination of sheep and goats is also crucial and should be included in National Animal Disease Control Programme for Foot and Mouth Disease (FMD) and brucellosis (NADCP). Since brucellosis is one of the important zoonotic diseases, the higher seroprevalence in animals suggests that the disease may also be prevalent in humans especially among animal handlers, veterinarian, butcher and farmers. Hence proper monitoring and surveillance is also recommended for human brucellosis.

Acknowledgement

The authors are thankful to TANUVAS authorities for providing the necessary funding for carrying out the research work.

References

Alton G G, Jones L M and Pietz D E. (1975). Laboratory Techniques in Brucellosis. Monograph Series. World Health Organization, Geneva, Switzerland.

Baum, M., Zamir, O., Bergman-Rios, R., Katz, E., Beider, Z., Cohen, A., & Banai, M. (1995). Comparative evaluation of microagglutination test and serum agglutination test assupplementary diagnostic methods for brucellosis. Journal of Clinical Microbiology, 33(8),2166–2170. https://doi.org/10.1128/jcm.33.8.2166-2170.1995

Corbel, M. M. J. (2006). Brucellosis in humans and animals. WHO Library Catalogue in Publication Data, 1–88. https://doi.org/10.2105/AJPH.30.3.299

FAO, World Watch List for domestic animal diversity, 3rd edn. Information Division, FAO, 2000; Rome.

FAOSTAT. 2010. FAOSTAT Agriculture Data. Food and Agriculture Organization Statistics, Rome, Italy.

International Livestock Research Institute (ILRI), Institute of Zoology, Hanoi School of Public Health, 2012. Mapping of Poverty and Likely Zoonoses Hotspots. Zoonoses Project.4. Report to the Department for International Development, UK. ILRI, Nairobi.

Kanani A, Dabhi S, Patel Y, Chandra V, Vinodh Kumar O R, Shome R (2018) Seroprevalence of brucellosis in small ruminants in organized and unorganized sectors of Gujarat state, India, Veterinary World, 11(8): 1030-1036.

Kaur N, Sharma NS, Kaur P, Sandhu Y, Kashyap N. Seroprevalence Studies on Caprine Brucellosis in Punjab (India). Int. J. Curr. Microbiol Appl Sci. 2019; 8(12):851–859.

Maher-Sulima and Venkataraman, K. S. Seroprevalence of *Brucella melitensis* in goats. Ind.Vet J., 2007; 84: 986-987

Mantur B. G, Amarnath S. K. Brucellosis in India – A review. J. Biosci. 2008;33:539–547.

McDermott, J., Grace, D., Zinsstag, J., 2013. Economics of brucellosis impact and control in low-income countries. Rev. Sci.Technol. Off.Int. Epiz. 32(1), 249–261.

Sadhu, D. B H., Panchasara, 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. Veterinary World8(5):561-566.

Saikia G K, Konch P, Boro A, Shome R, Rahman H, Das S K. Seroprevalence of caprine brucellosis in organised farms of Assam, India. ~ 21 ~ J

EntomolZool Stud 2019;7(1):21–25

Sharma, H. K., Kotwal, S. K.,Singh, D. K., Malik, M. A., Kumar, A., Katoch, R., & Singh, M. (2016). Sero-prevalence of sheep brucellosis in Jammu. Journal of Pure and Applied Microbiology, 10(3), 2277–2282.

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

Shome, R., Triveni, K., Padmashree, B. S., Swati Sahay., Krithiga, N., Shome, B. R., P Krishnamoorthy, P and Rahman, H. (2015). Spatial Distribution of Brucellosis in Small Ruminants of India using indigenously Developed ELISA Kit Journal of Pure and Applied Microbiology,9(3): 1-7.

Singh, B. B., Dhand, N. K., & Gill, J. P. S. (2015). Economic losses occurring due to brucellosis in Indian livestock populations. Preventive Veterinary Medicine, 119(3–4), 211–215.https://doi.org/10.1016/j.prevetmed.2015.03.013

Sonawane G G, Tripathi S, Dubey S C. Sero-incidence of brucellosis in small ruminants of semiarid Rajasthan. Indian JAnim Sci 2011;81(4):327–329

Sundar, A., Sinha, D. K., and Singh, D. K. 2015. Comparative Study on Seroprevalence of Bovine Brucellosis by Serological Tests and Serum based PCR. Journal of Veterinary Public health, 13 (1): 9-13.

Suryawanshi S N, Tembhurne P A, Gohain S and Ingle V C. 2014. Prevalence of Brucella antibodies in sheep and goats in Maharashtra. Indian Research Journal of Extension Education1. 4(4)(Special Issue): 75-77.

Tikare N V, Mantur B G and Bidari L H 2008 Brucellar meningitis in an infant – evidence for human breast milk transmission; J. Trop.Pediatr.54 272–274.

How to cite this article:

Sundar, A., S. Rajagunalan and Sangameswaran, R. 2020. Serological Detection of Brucellosis among Small Ruminants in Tirunelveli District, Tamil Nadu, India. *Int.J.Curr.Microbiol.App.Sci.* 9(06): 4283-4288. doi: https://doi.org/10.20546/ijcmas.2020.906.502