Full Length Article

SEROPREVALENCE AND SPATIAL DISTRIBUTION OF BRUCELLOSIS IN LARGE AND SMALL RUMINANTS IN GADAG DISTRICT, KARNATAKA

Santosh Sajjan[1]*, Shashidhar Ballari[2],and Asif Hebbal[3]

Department of Veterinary Public Health and Epidemiology
Veterinary College
Karnatka Veterinary, Animal and Fisheries Sciences University
Gadag - 582 101, Karnataka

ABSTRACT

The present study was conducted to determine the seroprevalence and spatial distribution of brucellosis in small and large ruminants in the Gadag district of Karnataka, India. The study was carried out under the National One Health Programme for Prevention and Control of Zoonoses (NOHPPCZ) as part of the Sentinel Surveillance Site activities. A total of 250 serum samples from bovinae (cattle and buffaloes, n=80) and caprinae (sheep and goats, n=170) were collected and tested for anti-Brucella antibodies using the Rose Bengal Plate Test (RBPT) and confirmed by indirect Enzyme-Linked Immunosorbent Assay (i-ELISA). The overall seroprevalence of brucellosis was found to be 6.25% in bovinae and 11.76% in caprinae. Geographic Information System (GIS) mapping was employed and revealed distinct spatial clustering of positive cases in specific talukas. The results confirm a significant circulation of Brucella antibodies, particularly in small ruminants, which poses a greater risk for occupational transfer of zoonotic brucellosis to the human population. The findings support the need for targeted, GIS-guided intervention strategies in the identified hotspots.

Keywords: Brucellosis, seroprevalence, GIS mapping, sentinel surveillance.

Received : 20.06.2025 Revised :09.12.2025 Accepted : 15.12.2025

[1]Associate Professor (I/C) and Head
* Corresponding Author Email : dr.santoshsajjan@gmail.com
[2]Assistant Professor and Head, Dept. of Veterinary Pathology,
[3]Assistant Professor (Contractual)

INTRODUCTION

Brucellosis is a highly contagious and one of the most widespread zoonotic diseases globally, reported to be endemic in many developing countries of Africa, Asia, and Latin America (Dean *et al*., 2012). It is characterized by chronic infection,

manifesting as placentitis, abortion in late pregnancy, infertility, and reduced milk production in livestock (Godfroid *et al.,* 2010; Singh *et al.*, 2015). Despite its high burden, brucellosis remains a neglected zoonosis by World Health Organization (WHO) and World Organization for Animal Health (WOAH) (Franc *et al*., 2018; OIE, 2017).

In India, where livestock contributes significantly to the economy, brucellosis is endemic in both organized and unorganized sectors. The disease poses a severe occupational hazard to veterinarians, farmers, and abattoir workers who are in close contact with infected animals (Hull & Schumaker, 2018). Recent national surveys have highlighted the persistence of the disease (Shome *et al.*, 2019). Specifically, in Karnataka, seroprevalence has been reported in small ruminants, attributed to factors such as unrestricted animal movement and lack of quarantine measures (Natesan *et al.*, 2021; Reddy *et al.*, 2014).

However, data regarding the specific prevalence and, critically, the spatial distribution of brucellosis in the Gadag district of Karnataka remains scanty. To address this gap, the Department of Veterinary Public Health and Epidemiology at Veterinary College, Gadag, was designated as a Sentinel Surveillance Site under the National One Health Programme for Prevention and Control of Zoonoses (NOHPPCZ), funded by the National Centre for Disease Control (NCDC), New Delhi.

This study, conducted during 2024-25, aimed to estimate the seroprevalence of brucellosis in large and small ruminants in Gadag using a dual-testing strategy (RBPT and i-ELISA). Furthermore, this study employed Geographic Information System (GIS) mapping to visualize the spatial distribution of seropositivity, providing a novel epidemiological baseline for targeted control strategies in the district.

MATERIALS AND METHODS

Study Area and Sentinel Surveillance Site

The study was conducted in the Gadag district of Karnataka, located in the Deccan Plateau. The study was carried out under the aegis of the NOHPPCZ, funded by the NCDC. The Department of Veterinary Public Health and Epidemiology, Veterinary College, Gadag, functioned as the Sentinel Surveillance Site for priority zoonoses, including Brucellosis.

Sampling Strategy and Sample Collection

A cross-sectional surveillance study was conducted from August 2024 to March 2025. A total of 250 serum samples were collected from randomly selected animals in villages across the Gadag, Mundaragi, Ron, and Shirahatti talukas. The sample distribution included: Bovinae (cattle and buffaloes): n=80; Caprinae (sheep and goats): n=170. Approximately 5–7 ml of blood was collected aseptically from the jugular vein of each animal. Serum was separated by centrifugation at 1000g for 10 minutes, labelled, and stored at -20°C until further analysis.

Serological Assays

A parallel testing strategy was employed using a screening test followed by a confirmatory assay (Godfroid *et al*., 2010).

Rose Bengal Plate Test (RBPT)

RBPT was performed as per the procedure described by Alton *et al*. (1988). RBPT antigen obtained from the ICAR-Indian Veterinary Research Institute, Izzatnagar, Uttar Pradesh, was used. An equal volume of 0.03 ml of serum sample and antigen was taken on the slide and mixed thoroughly. The appearance of definite clumping/agglutination within 3 min was considered a positive reaction while no clumping/ agglutination as negative.

Indirect Enzyme- linked Immunosorbent Assay (I-ELISA)

For serodiagnosis of brucellosis Protein-G based indirect ELISA (i-ELISA) kit for bovine brucellosis and i-ELISA Kit for sheep and goat brucellosis were procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru (Indian Patent No. 250709). The procedure outlined in the instruction manual was followed for detection of antibodies in samples. The results were expressed as percentage positivity (PP) values. The PP values used for the diagnostic interpretations were calculated using the following formula:

$$PP = \frac{\text{(Average OD value of test serum X 100)}}{\text{(Median OD of the strong positive control)}}$$

Bovine sera sample that gave PP value 55% to 65% is considered moderate positive and more than 65% as strong positive. Sample showed a PP value of below 55% is taken as negative and sample with PP value of only 55% was re-tested. Goats and sheep sera sample with PP value of more than 54% was considered positive, below 54% was considered as negative and sample showed PP value 54% was re-tested.

GIS Mapping and Data Analysis

The geographic coordinates (latitude and longitude) of the sampling villages were recorded. These coordinates, along with the seropositivity data, were used to generate a spatial distribution map in QGIS software. Statistical analysis was performed to calculate the prevalence percentage:

$$\text{Prevalence (\%)} = \frac{\text{(Number of Positive Animals)}}{\text{(Total Number of Animals Tested) X 100}}$$

The agreement between RBPT and i-ELISA was assessed using Kappa (κ) statistics, where κvalue of <0.20, 0.21-0.40, 0.41- 0.60, 0.61-0.80, and 0.81-1.0 indicated the strength of agreement as poor, fair, moderate, good, and very good, respectively.

RESULTS

Seroprevalence Findings

The study analyzed a total of 250 serum samples collected from large and small ruminants across four talukas of the Gadag district between August 2024 and March 2025.

In the large ruminant (bovinae) population, 5 out of 80 samples tested positive for *Brucella* antibodies using both the Rose Bengal Plate Test (RBPT) and the indirect Enzyme-Linked Immunosorbent Assay (i-ELISA). This indicates an overall seroprevalence of 6.25% (5/80). In the small ruminant (caprinae) population, 20 out of 170 samples were found positive by both assays, resulting in an overall seroprevalence of 11.76% (20/170). The species-wise distribution of seropositivity is detailed in Table 1.

Diagnostic Agreement and Spatial Distribution

The agreement between the two serological assays was assessed using Kappa statistics. In both cohorts, a Kappa (κ) value of 1.0 was calculated, indicating perfect agreement between the RBPT (screening) and i-ELISA (confirmatory) tests.

The integration of serological results with geographic coordinates using QGIS software revealed a heterogeneous distribution of cases. The spatial analysis (Figure 1) confirmed that the seropositive cases were not uniformly distributed but exhibited distinct spatial clusters or hotspots, predominantly localized to villages within the Ron and Mundaragi talukas. The map illustrates that these high-prevalence areas correlate visually with regions of high small ruminant density and known livestock trading routes, indicating localized risk factors influencing disease spread.

The Kappa (κ) statistic demonstrated perfect agreement (κ =1.0) between the RBPT and i-ELISA results for both bovinae and caprinae samples.

DISCUSSION

This study, conducted as part of the NOHPPCZ Sentinel Surveillance, establishes a specific epidemiological baseline for brucellosis in the Gadag district. India possesses the world's largest dairy herd and ranks first in total milk production, contributing significantly to the global supply. However, this intensive production is threatened by endemic zoonoses like brucellosis.

The observed seroprevalence of 6.25% in large ruminants aligns with the lower end of national trends. For instance, a large random sampling survey across 15 states in India reported a seroprevalence of 8.3% in cattle and 3.6% in buffaloes (Shome *et al*., 2019). While lower than the 13.01% reported in high-burden states like Chhattisgarh (Jain *et al*., 2019), the presence of infection in Gadag confirms a sustained level of circulation.

The most significant finding is the high seroprevalence of 11.76% in small ruminants. This rate is notably higher than recent surveillance data from other parts of Karnataka, where seroprevalence in sheep and goats was reported at 8.29% and 5.82%, respectively (Natesan *et al*., 2021). It is, however, comparable to high-prevalence states like Gujarat, where rates of 13.60%

have been recorded (Kanani *et al.,* 2018). The elevated rate in Gadag's small ruminants is likely driven by the extensive, free-range grazing practices common in this semi-arid zone, which facilitates rapid transmission (Sharifi *et al*., 2015).

Comparing these findings with broader surveillance data underscores the variability of brucellosis burden across India. The identification of specific hotspots in Ron and Mundaragi talukas via GIS mapping is a critical output of this study. It suggests that risk is not uniform and that control efforts,such as vaccination and movement restrictions, should be spatially targeted rather than applied broadly (Veeramani *et al*., 2022).

The perfect diagnostic agreement (κ=1.0) reinforces the reliability of the two-stage testing protocol for field surveillance (Sadhu *et al.,* 2015). Given that small ruminants are often reservoirs for *B. melitensis*, the most pathogenic species for humans, the high prevalence in this sector (11.76%) highlights a significant occupational risk to the farming community in Gadag. These findings support the NOHPPCZ strategy of using sentinel sites to guide precision-based interventions.

CONCLUSION

Based on the serological and spatial analysis, this study confirms the presence of circulating brucella antibodies, with a seroprevalence of 6.25% in large ruminants and a higher rate of 11.76% in small ruminants, in the Gadag district of Karnataka.

The implementation of GIS mapping identified distinct geographic hotspots of infection in the Ron and Mundaragi talukas, which should be the primary focus for targeted disease control measures. As a designated Sentinel Surveillance Site under the NOHPPCZ, this study confirms the small ruminant population as a significant reservoir and highlights a clear occupational and public health risk to farmers and other livestock handlers in the district.

To mitigate this zoonotic threat, we recommend a focused one-health approach, involving: (1) Targeted Vaccination of small ruminant flocks in the identified hotspots and (2) Public Awareness campaigns about hygienic practices and safe dairy consumption. This multi-sectoral strategy is essential for reducing the burden of brucellosis in both the animal and human populations of Gadag.

ACKNOWLEDGMENT

The authors are thankful to National Centre for Disease Control (NCDC), New Delhi for providing financial assistance under National One Health Programme for Prevention and Control of Zoonoses (NOHPPCZ)scheme to carry out the present work.

Table.1. Seroprevalence of brucellosis in large and small ruminants in Gadag district.

Species Category	Total Samples Tested	RBPT Positive (%)	i-ELISA Positive (%)
Large Ruminants (Bovinae)	80	5 (6.25%)	5 (6.25%)
Small Ruminants (Caprinae)	170	20 (11.76%)	20 (11.76%)
Total	**250**	**25 (10.00%)**	**25 (10.00%)**

RBPT: Rose Bengal Plate Test; i-ELISA: Indirect Enzyme-Linked Immunosorbent Assay. Agreement between tests was perfect (κ =1.0).

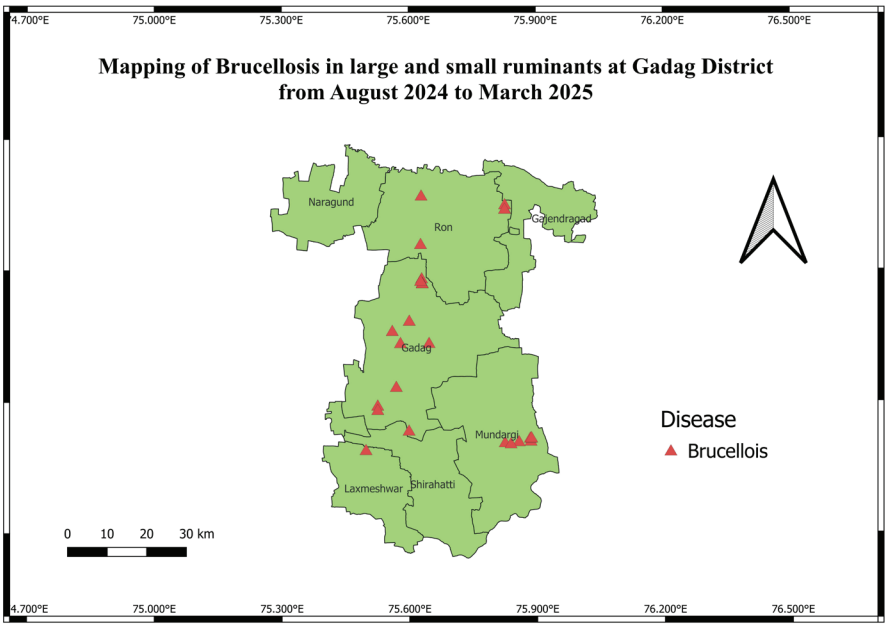

Fig.1. Mapping of Brucellosis in large and small ruminants at Gadag District

REFERENCES

Alton, G.G., Jones, L.M., Angus, R.D. and Verger, J.M. (1988) Techniques for Brucellosis. Paris: Institute National de le Recherché Agronomique.

Dean, A.S., Crump, L., Greter, H., Schelling, E. and Zinsstag, J. (2012) Global burden of human brucellosis: a systematic review of disease frequency. *PLoS Neglected Tropical Diseases*, **6**(10): e1865.

Franc, K.A., Krecek, R.C., Hasler, B.N. and Arenas-Gamboa, A.M. (2018) Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health*, **18**(1): 125.

Godfroid, J., Nielsen, K. and Saegerman, C. (2010) Diagnosis of brucellosis in livestock and wildlife. *Croatian Medical Journal*, **51**: 296-305.

Hull, N.C. and Schumaker, B.A. (2018) Comparisons of brucellosis between human and veterinary medicine. *Infection Ecology and Epidemiology*, **8**(1): 1500846.

Jain, L., Kumar, V., Chaturvedi, S., Roy, G. and Barbuddhe, S.B. (2019) Seroprevalence of brucellosis in bovines of Chhattisgarh, India. *Indian Journal of Animal Research*, **53**(2): 255-59.

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

Natesan, K., Kalleshamurthy, T., Nookala, M., Yadav, C., Mohandoss, N., Skariah, S., Sahay, S., Shome, B.R., Kumar, O.R.V., Rahman, H. and Shome, R. (2021) Seroprevalence and risk factors for brucellosis in small ruminant flocks in Karnataka in the Southern Province of India. *Veterinary World*, **14**(11): 2855-2862.

OIE (World Health Organization for Animal Health) (2017) World Animal Health Organization. One Health: Neglected Zoonoses-FAO-APHCA/ OIE Regional Technical Workshop on the Prevention and Control of Animal Brucellosis and Tuberculosis in Asia 11–13 September, 2017.

Reddy, D.A., Kumari, G., Rajagunalan, S., Singh, D.K., Kumar, A. and Kumar, P. (2014) Seroprevalence of caprine brucellosis in Karnataka. *Veterinary 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. *Veterinary World*, **8**(5): 561-66.

Sharifi, H., Mashayekhi, K. and Tavakoli, M.M. (2015) Risk factors of small ruminant brucellosis: a cross-sectional study in Southeast Iran 2012. *Human and Veterinary Medicine International Journal of the Bioflux Society*, **7**: 42-45.

Shome, R., Triveni, K., Swati, S., Ranjitha, S., Krithiga, N., Shome, B.R., Nagalingam, M., Rahman, H. and Barbuddhe, S.B. (2019) Spatial seroprevalence of bovine brucellosis in India - a large random sampling survey. *Comparative Immunology, Microbiology and Infectious Diseases*, **65:** 124-27.

Singh, B.B., Dhand, N.K. and Gill, J.P.S. (2015) Economic losses occurring due to brucellosis in Indian livestock populations. *Preventive Veterinary Medicine,* **119**(3-4): 211-15.

Veeramani, P., Dhayanithi, N., Suresh, K.P. and Natesan, K. (2022) Risk factors and spatial distribution of brucellosis in dairy animals in Tamil Nadu, India: a cross-sectional study. *Transboundary and Emerging Diseases*, **69**(3): 1443-1454.