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Research Article

Seropositivity of Brucellosis in swine population of selected Indian states: warranting the need for future surveillance

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Abstract

Brucellosis is a highly contagious re-emerging zoonotic disease that affects livestock and poses significant threat to public health. It is endemic in India, with the prevalence ranging from 6.5% to 16.4% among different livestock species. The disease causes significant economic loss to the livestock sector in the country. Extensive work has been undertaken to assess the burden of brucellosis in ruminants, however, only few studies have reported the significance of swine brucellosis as a public health concern in India. Despite having the fifth highest pig population in the world, brucellosis in pigs is often overlooked in the country. Therefore, the present study was undertaken to determine the seropositivity of brucellosis in the swine population of selected Indian states. Under this study, a total of 390 swine serum samples collected randomly across 16 districts of five Indian states, namely Uttar Pradesh, Uttarakhand, Maharashtra, Assam, and Tamil Nadu were screened using RBPT. Among the 390 swine sera samples, antibodies against *Brucella* were detected in 26 samples with an overall seropositivity of 6.5% (95% CI: 4.5-9.5). Maximum seropositivity was reported from the state of Maharashtra (10.5%; 95% CI: 5.6-18.9) followed by Uttar-Pradesh (8.8%; 95% CI: 4.5-6.5), Uttarakhand (6.6%; 95% CI: 1.8-2.1), Assam (5.7%; 95% CI: 2.6-11.9), and Tamil Nadu (1.2%; 0.2-6.7). In addition to this, our findings reported the presence of brucellosis in swine population of Uttarakhand for the first time which underscores the need for further investigation of brucellosis in swine herds in the other states of India.

Keywords: Brucellosis, RBPT, Seropositivity, Swine

Highlights

- Brucellosis in swine was detected using RBPT in 390 serum samples collected across five Indian states
- Out of 390 swine samples, 26 tested positive for Brucella antibodies, with a seropositivity of 6.5%
- First report of swine brucellosis in Uttarakhand, highlighting the need for studies in other states

INTRODUCTION

Brucellosis is a re-emerging zoonosis and is among the seven most neglected diseases globally, with an estimated true incidence of 5,000,000 to 12,500,000 cases annually (Hull and Schumaker, 2018). It not only affects livestock but is highly contagious and is of major zoonotic potential (OIE, 2022). The disease is endemic in India with the prevalence ranging from 5.3% to 16.4% among different livestock species (Thoppil, 2000; Shome *et al*., 2006; Kollannur *et al*., 2007; Aulakh *et al*., 2008, Lone *et al*., 2013; Shome *et al*., 2021). Recently, a systematic review and meta-analysis study by Suresh *et al*. (2022) reported the pooled prevalence of brucellosis as 12% in livestock of India. A systematic analysis of economic loss attributed by brucellosis in Indian livestock reported a median loss of US $3.4 billion and loss of US $0.6 per pig (Singh *et al*., 2015). *Brucella* organism is an intracellular Gram-negative, non-capsulated, non-motile, non-spore cocco-bacilli belonging to genus *Brucella* (Mathew *et al*., 2015). Currently, there are 12 species of *Brucella*, of which the four commonly occurring species with zoonotic potential include *B. melitensis*, *B. abortus*, *B. suis* and *B. canis*. Brucellosis in swine is caused by *Brucella suis* which is subdivided into five biovars based on the metabolic characteristics, growth in the presence of basic fuschin or thionin dyes, and agglutination pattern by monoclonal antibodies of LPS-O side chain (Alton *et al*., 1988). In America and Asia, biovar 1 and 3 are endemic, whereas biovar 2 is reported in Egypt and Europe (Elmonir *et al*., 2022). Swine brucellosis is mainly caused by biovar 1, 2 and 3 whereas biovar 1 and 3 are pathogenic to humans. *Brucella* organisms infect pigs through several routes, including ingestion of aborted fetuses, contact with infected fetal membranes, or fluids in their living pens. Additionally, direct contact with the infected material through

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broken skin, conjunctiva, or nasal mucous membranes, as well as through natural breeding or artificial insemination, can facilitate transmission. Clinical signs of Brucellosis in sows are characterised by abortion at any stage of pregnancy, birth of dead or weak piglets, whereas in boars, infection of secondary sex organs, epididymitis, hygroma, lameness and paralysis in both the sexes (OIE, 2022). In humans, infection occurs through various pathways including exposure to infected pigs during cleaning, feeding, and slaughtering, along with occupational contact in veterinary healthcare workers, and handling of *B. suis* cultures in laboratory. Since in India, vaccination for swine brucellosis is not undertaken, increase in awareness among pig handlers and veterinarians plays a crucial role in the prevention of the disease by reducing the disease prevalence, particularly in the absence of vaccination policy (Shome *et al*., 2022).

Brucellosis in swine can be diagnosed by isolation, molecular and serological techniques. Isolation is the confirmatory and gold standard but is not suitable for testing large number of samples, and involves complex lab procedures, requires biocontainment facilities, poses risk to the laboratory personnel and is less sensitive (Kalleshamurthy *et al*., 2019). Molecular techniques cannot be used for surveillance purpose because of their high cost and less sensitivity due to the presence of inhibitors in the DNA samples (Yu and Nielsen, 2010; Wang *et al*., 2014). Therefore, serological tests are best suited for surveillance and laboratory diagnosis of brucellosis, which includes buffered *Brucella* antigen tests like buffered plate agglutination test, rose bengal plate test (RBPT), complement fixation test (CFT), enzyme-linked immunosorbent assays (ELISA), and fluorescence polarization assay. However, OIE has recommended RBPT for screening of brucellosis in animals (OIE, 2022). RBPT is a simple spot agglutination test widely used for screening of brucellosis in both animals and humans. The test offers several advantages like low cost, yields quicker results which can be read by naked eyes, does not require any equipment and can be performed with ease in field conditions without many technicalities.

In India, extensive work has been done to assess the burden of brucellosis in ruminants; however, only a few studies have been undertaken to assess the significance of brucellosis in swine as a public health concern. Although, the pig population of India is 9.06 million as per 20th livestock census (DAHD, 2019) and ranks 5th in the world, brucellosis in swine is often overlooked. Therefore, the present study was undertaken to determine the seropositivity of brucellosis in swine population of selected Indian states.

MATERIALS AND METHODS

Place of work and sample collection: The present study was carried out at Brucella laboratory, Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute, Bareilly, India. A total of 390 swine sera samples were collected randomly across 16 districts of five Indian states, namely Uttar Pradesh, Uttarakhand, Maharashtra, Assam, and Tamil Nadu. The detail of samples collected from different states is shown in the Table 1.

Rose Bengal plate test: A total of 390 field swine serum samples were screened using RBPT. The test was performed as per the protocol of OIE, 2022 using *B. abortus* Rose Bengal coloured antigen having pH 3.6 obtained from National Brucella Culture Repository, Indian Veterinary Research Institute, Izatnagar, India. For performing RBPT, the test samples and the test antigen were first brought to the room temperature. Thereafter, 30 µL of test antigen was dispensed on to the glass slide followed by addition of 30 µL of serum alongside the antigen. The serum and antigen were then thoroughly mixed, and the mixture was agitated gently for 4 minutes at room temperature. The final test results were recorded after 4 minutes and were interpreted as positive, negative, or doubtful. Samples showing no agglutination were considered as negative and marked as 0 whereas

Table 1. Details of sample collected from different states of India

S. No.	State	Districts	Number of samples collected
1.	Uttarakhand	Rudrapur, Haridwar	30
2.	Uttar-Pradesh	Gorakhpur, Deoria, Basti, Mau, Azamgarh	90
3.	Assam	Kamrup, Barpeta, Baksa, Cachar	105
4.	Maharashtra	Mumbai, Satara	85
5.	Tamil Nadu	Tiruchirapalli, Thanjavur	80

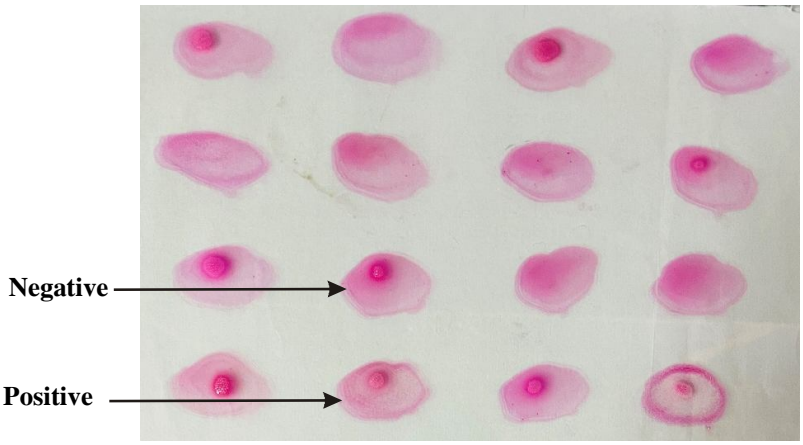

Fig. 1. Varying levels of agglutination of swine sera samples in RBPT

Table 2. Results of swine serum samples tested from different regions using RBPT.

S. No.	State	No. of samples tested	No. of samples positive	Seropositivity
1.	Uttarakhand	30	2	6.6%
2.	Uttar Pradesh	90	8	8.8%
3.	Assam	105	6	5.7%
4.	Maharashtra	85	9	10.5%
5.	Tamil Nadu	80	1	1.2%

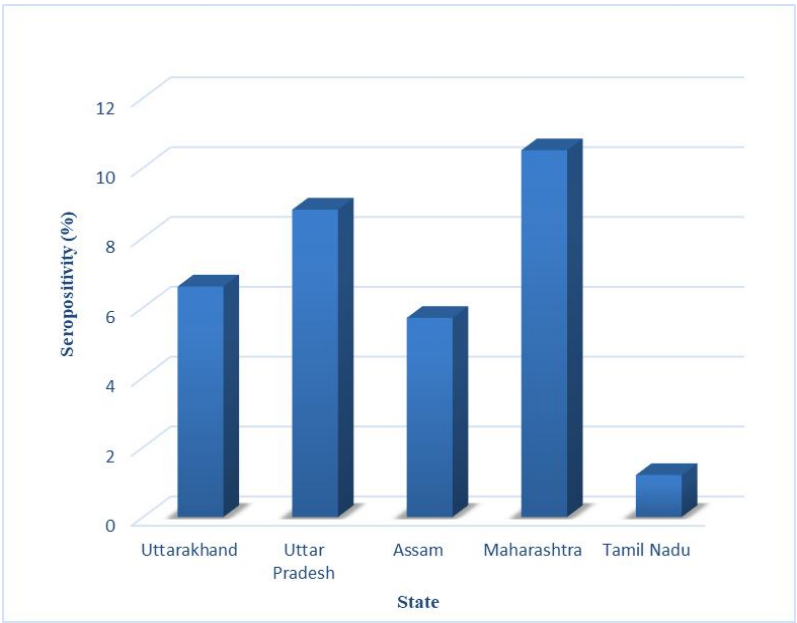

Fig. 2. Seropositivity of swine brucellosis across different states of India

samples showing agglutination were considered positive and marked as +, ++, +++ according to the level of agglutination (Fig. 1). Additionally, Chi-Square test was applied to determine any significant difference in the seropositivity rates across the states.

RESULTS

Among the 390 swine sera samples screened using RBPT, antibodies against Brucella were detected in 26 samples with an overall seropositivity of 6.5% (95% CI: 4.5-9.5). The details of swine samples tested for brucellosis from different states have been depicted in Table 2. Maximum seropositivity was reported from Maharashtra (10.5%; 95% CI: 5.6-18.9) followed by Uttar-Pradesh (8.8%; 95% CI: 4.5-6.5), Uttarakhand (6.6%; 95% CI: 1.8-2.1), Assam (5.7%; 95% CI: 2.6-11.9), and Tamil Nadu (1.2%; 0.2-6.7) (Fig. 2). The calculated Chi-Square value was 5.9 with a p-value of 0.1.

DISCUSSION

In India, limited information is available on brucellosis in swine. This can be attributed to its variable clinical picture at both individual and herd levels, complex epidemiology, broad host range, difficulties associated with the specificity of serological tests in swine along with the short commercial cycle in pig industry (Yilma *et al.*, 2016; Ducrotoy *et al.*, 2017; Preena *et al.*, 2024). Varying levels of seroprevalence of swine brucellosis has been reported from some states of India like Punjab (16.7%), Madhya Pradesh (3.2%), and Karnataka (9.5%) (Guarino *et al.*, 2000; Thoppil, 2000; Kaur *et al.*, 2020). A study by Jindal *et al.* (2017) reported a 2.72% seroprevalence of brucellosis in slaughter pigs from Punjab using RBPT. Recently, Preena *et al.* (2024) reported the seroprevalence of swine brucellosis in Tamil Nadu as 5.2%. The seropositivity of swine brucellosis in Assam was recorded as 87.2% using indirect ELISA in sows having history of abortion (Nath *et al.*, 2009). Another study by Shakuntala *et al.* (2020) reported the seroprevalence of swine brucellosis from Meghalaya as 0.36% based

on RBPT. In the present study, the seropositivity of brucellosis in swine population of selected states was 6.5% using RBPT. Similarly, Shome *et al.* (2022) reported 4.33% seroprevalence of swine brucellosis using indirect ELISA in a pan-India assessment during the year 2018-19. Methodological disparities, especially the use of different serological tests such as RBPT in the present study versus ELISA in others, may account for the variation in reported seropositivity. The chi-square value of 5.69 with a p-value of 0.1 suggests that there was no statistically significant difference between the seropositivity rates across the states in this study.

Although our study reported the presence of the *Brucella* organism in pigs, we could not determine the species of *Brucella* infecting pigs because RBPT only detects the presence of smooth lipopolysaccharide (S-LPS) antigen which is commonly shared by various species of *Brucella*, like *B. suis*, *B. abortus* and *B. melitensis*. Despite the strict host specificity of *Brucella* organism, instances of breaches in animal host boundaries have been observed, particularly in mixed or integrated farming systems and at the interface of livestock and wildlife (Fretin *et al*., 2013; Preena *et al*., 2016). Few studies have also reported the presence of *B. abortus* infection in feral swine herds, indicating that wild pigs can also act as a reservoir of *B. abortus* for domestic livestock (Stoffregen *et al*., 2007; Preena *et al*., 2020). Therefore, it is essential to monitor the transmission of brucellosis between different livestock species using precise diagnosis and identification up to the species level. Additionally, we reported the presence of brucellosis in swine population of Uttarakhand which is not reported by any study in India till date. Therefore, this study highlights the need for undertaking comprehensive serosurveillance for brucellosis in swine herds of different states of India. The strength of our study includes screening of swine serum samples for brucellosis from 16 districts across five states belonging to four different geographical zones of India, and thus generating preliminary pan-India data. A key limitation of the present study was the absence of confirmatory diagnostic testing, such as c-ELISA or PCR on RBPT-positive samples. Future investigations should incorporate confirmatory tests to improve diagnostic accuracy and provide more definitive evidence of swine brucellosis, particularly in areas where data is scarce.

Brucellosis in swine is an important zoonotic disease for the veterinarians, pig handlers, persons living in proximity to pigs and therefore its zoonotic potential should not be ignored. Based on the findings of our study, we recommend targeted awareness campaigns for pig farmers, butchers, and handlers to highlight the risks of brucellosis and the importance of preventive measures. Emphasis should be made on the use of personal protective equipment (gloves, masks and protective clothing) during high-risk activities such as slaughtering and farrowing. Additionally, implementing basic farm-level biosecurity practices such as isolating the sick animals, safely disposing the aborted materials, and regular disinfection of pig enclosures can help in reducing the risk of disease transmission. Further, the results of the present study warrant the need for investigation of brucellosis in domestic and feral swine herds in the other states of the country. Additionally, targeted molecular and serological studies would be helpful in identifying the species of *Brucella* in pigs. In India, vaccination in pigs for brucellosis is not practiced, therefore increase in awareness along with the continued serosurveillance of brucellosis in swine population of the country will aid in controlling the disease in swine herd.

Conflict of interest: The authors declare no conflict of interest.

Author's contribution: IG: Original draft writing, sampling and investigation; MSK: Provision of resources; MG, MD, ABM: Sampling; HD: Idea conceptualization and supervision of entire research.

Data availability statement: All the data supporting the research findings are included in this paper. The corresponding author can be contacted for additional information.

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