

## Sero-epidemiology of Brucellosis in Pulikulam Cattle Herd of Tamil Nadu, India

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**ABSTRACT:** A total of 129 serum samples were collected from Pulikulam cattle herd with a history of stray abortions in Sivaganga district, Tamil Nadu, India. All samples were tested using the Rose Bengal Plate Test (RBPT) and indirect Enzyme-Linked Immunosorbent Assay (iELISA). All seropositive animals were females, with an overall prevalence of 12.40% among females. Age-wise analysis revealed seroprevalence rates of 4.65% in the 4–6 years age group and 7.75% in animals older than 6 years. No seropositive cases were found in animals aged 3–6 months, 6–12 months, 1–2 years, or 2–4 years. Additionally, no male animals tested positive for brucellosis. In comparison with iELISA, the RBPT showed a sensitivity of 88.89% and a specificity of 98.20% for detecting the brucellosis in the Pulikulam cattle herd. The high prevalence observed among adult females may be associated with sexual maturity and the longer duration of exposure to infection. As the Pulikulam cattle are predominantly managed under a nomadic grazing system and rely solely on natural mating, the risk of transmission from infected to non-infected animals is likely increased. This study highlights the need for targeted control and prevention strategies for brucellosis among Pulikulam cattle herds in the southern districts of Tamil Nadu.

**Keywords:** Brucellosis, Pulikulam cattle, RBPT, iELISA, Sensitivity, Specificity.

### INTRODUCTION

Brucellosis is a widespread yet often neglected zoonotic disease that continues to pose a significant threat to both public health and livestock productivity globally (WHO, 2005). It is primarily caused by bacteria of the genus *Brucella*, with *Brucella abortus* being the principal etiological agent in cattle. Other species such as *B. melitensis* and *B. suis* can also infect cattle, albeit less frequently (OIE, 2016). The disease manifests predominantly in the reproductive system of sexually mature female cattle, leading to placentitis, late-term abortions, stillbirths, and infertility. These reproductive failures not only compromise animal welfare but also lead to substantial economic losses through reduced calving rates, decreased milk production, and long-term infertility (Godfroid *et al.*, 2010).

Although most cows abort only once following the initial infection, they can become persistent carriers of the organism, shedding *Brucella* spp. intermittently through milk, vaginal discharges, and uterine fluids, posing a continual risk of transmission (Islam *et al.*, 2013). In many cases, especially after the first abortion or in non-pregnant females, the infection becomes subclinical and remains undetected, complicating disease control efforts. Adult bulls, on the other hand, may develop testicular infections such as orchitis or epididymitis, potentially leading to chronic infertility and further economic implications.

The economic burden of brucellosis is twofold: direct losses from reproductive failure and milk yield

reduction, and indirect costs due to veterinary interventions, vaccination programs, diagnostic testing, and culling of infected animals (Godfroid *et al.*, 2010; Islam *et al.*, 2013). Despite these impacts, brucellosis continues to be under-reported in many developing regions due to lack of awareness, limited diagnostic infrastructure, and the subclinical nature of the disease in carrier animals.

The Pulikulam breed is an indigenous zebu cattle breed native to Tamil Nadu, India, particularly known for its agility and endurance. These animals are traditionally reared in large herds comprising 200 to 800 individuals, especially in the southern districts of Tamil Nadu. Pulikulam cattle hold cultural significance and are extensively used in the traditional bull-taming sport known as *Jallikattu*. Despite their cultural and genetic value, systematic scientific studies on disease prevalence, especially reproductive disorders like brucellosis, in this breed remain sparse.

While brucellosis has been extensively documented in commercial and crossbred cattle as the pooled prevalence of brucellosis in livestock of India was estimated to be 12% (Suresh *et al.*, 2022) and Bwas first reported in India in the early 1900 (Smits and Kadri 2005) and it is now considered endemic in most parts of the country there is a noticeable gap in research concerning its prevalence and impact in native breeds like Pulikulam.

To date, there have been no published reports focusing on the occurrence of brucellosis in Pulikulam cattle,

particularly in relation to unexplained abortions. Considering the breed's unique management system and sociocultural importance, understanding the cause of reproductive failures in these animals is crucial. Therefore, the present study was undertaken to investigate the cause of stray abortions observed in a Pulikulam cattle herd. This represents the first systematic attempt to assess brucellosis or related etiologies in this indigenous breed, and aims to contribute valuable data to the limited pool of literature available on native Indian cattle health and disease epidemiology.

## MATERIALS AND METHODS

The blood samples were collected from a herd having 129 Pulikulam cattle in the vacutainers without anticoagulant with different age groups *viz.* 3 to 6 months, 6 months to 12 months, 1 to 2 years, 2 to 4 years, 4 to 6 years and above 6 years. The blood samples were centrifuged at 3000 rpm for 10 minutes to extract clear serum and stored it at -20°C until further use. The RBPT was performed according to the procedures recommended by the OIE (2016); and the *Brucella abortus* S99 strain antigen was sourced from Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. The indirect ELISA was performed using IDEXX-CHEKIT, Brucellose serum (Netherland) and the iELISA was validated using positive and negative sera samples. The sera samples were tested to iELISA in according to the manufacturers protocol.

## RESULTS AND DISCUSSION

Among the 129 serum samples tested, 20 samples (15.50%) were positive for brucellosis by the Rose Bengal Plate Test (RBPT), whereas the indirect ELISA (iELISA) detected a seropositivity rate of 12.40% (Table 1) and this align with patterns observed in Haryana (6.05% vs 6.86%) and Rajasthan (5–7%) (Meena *et al.*, 2023 whereas Lalrinzuala *et al.* (2023) reported 14.0% of seroprevalence in cattle in India by their metanalysis the data for the period of 1970 to 2020. In comparison with iELISA, the RBPT showed a sensitivity of 88.89% and a specificity of 98.20% in the

present study. These findings are in agreement with the report of Legesse *et al.* (2023). However, in contrast, Getachew *et al.* (2016) reported a lower specificity (84.5%) and a slightly higher sensitivity (89.6%) for RBPT in cattle.

The Rose Bengal Plate Test (RBPT) exhibited false-positive results in 1.55% of the samples, which were not confirmed by the indirect ELISA (iELISA). This observation is supported by the findings of Godfroid *et al.* (2010), who noted that RBPT has limitations in distinguishing true *Brucella* infections from cross-reacting agglutinating reactions caused by other bacteria, such as *Yersinia enterocolitica*. In contrast, iELISA offers higher specificity and sensitivity, as it can detect even minute quantities of antibodies during the early stages of infection where the levels that may not be detected by RBPT Legesse *et al.* (2023). In the present study, animals aged above 6 years and those in the 4–6 years age group showed the highest seropositivity rates, at 7.75% and 4.65%, respectively (Table 2).

As age advances, the susceptibility to clinical brucellosis may increase, potentially due to sexual maturity influenced by sex hormones and the presence of placental erythritol, both of which are known to affect the pathogenesis of the disease. In the present study, animals younger than four years of age showed zero prevalence of brucellosis, which might be attributed to their sexual immaturity or limited duration of exposure to infection risk. This observation is in full agreement with the findings of Asmare *et al.* (2013) and this parallels meta-analysis findings indicating increased seropositivity with age across livestock species (Suresh *et al.*, 2022). Similarly, Meena *et al.* (2023) reported a slightly higher cumulative seroprevalence in animals aged 4–9 years compared to younger animals, with rates of 3.71% in the 0.1–4.0 years age group, 3.81% in the 4.1–9.0 years group, and 2.76% in animals older than 9.0 years. In contrast, Kumar *et al.* (2016) observed a higher seroprevalence in younger calves (10%) compared to older animals (9%) and concluded that age may not have a positive correlation with seropositivity.

**Table 1: Prevalence of brucellosis in a Pulikulam Cattle herd.**

No. of samples tested	RBPT Positive	iELISA Positive	RBPT Positive iELISA negative	RBPT negative iELISA positive	RBPT positive iELISA positive
129	20 (15.50%)	16 (12.40%)	2 (1.55%)	2 (1.55%)	14 (10.85%)

**Table 2: Age-wise and Sex-wise prevalence of brucellosis in Pulikulam Cattle herd.**

Age	No. of animals tested	No. of animal positive	Percentage of positives
3-6 months	6	-	-
6-12 months	17	-	-
1 to 2 years	35	-	-
2 to 4 years	7	-	-
4 to 6 years	40	6	4.65
Above 6 years	24	10	7.75
Total	129	16	12.40

The present study found that only female animals exhibited seropositivity for brucellosis (13.22%) across different age groups (Table 3) while no male animals tested seropositive. This finding is in partial agreement with previous reports by Dhand *et al.* (2005); Islam *et al.* (2013), who also observed a higher seroprevalence in female animals compared to males. This finding consistent with report from Rajasthan and larger Indian datasets where reproductive physiology increases exposure and immunological response in females (Meena *et al.*, 2023).

**Table 3: Sex-wise prevalence of brucellosis in Pulikulam Cattle herd.**

Sex	No. of animals tested	No. of animal positive	Percentage of positives
Male	8	-	-
Female	121	16	13.22

Purebred cattle were found to be more susceptible to brucellosis compared to zebu cattle. However, Indian zebu breeds such as Deoni, Gir, Sahiwal, and Hallikar also showed seropositivity for brucellosis, as reported by Shome *et al.* (2023). Notably, the seroprevalence of brucellosis was detected for the first time in Pulikulam cattle of India.

The Pulikulam cattle are traditionally reared in large herds under a nomadic grazing system and main breeding is carried out by natural service which might be the reason for the occurrence of brucellosis as per the earlier report as the nomadic grazing and natural mating practices likely elevate horizontal transmission risk where reproductive issues sharply increased brucellosis prevalence (Krishnamoorthy *et al.*, 2015). Several factors may contribute to the occurrence of brucellosis in this breed, including close confinement, poor management of aborted materials, introduction of animals from herds with unknown or positive brucellosis status, and inadequate hygiene practices. Although the present study did not detect seropositivity in male animals, natural service remains the sole method of breeding in Pulikulam cattle, as frozen semen is currently unavailable for this breed.

## CONCLUSIONS

This study is the first to report seroprevalence of brucellosis among Pulikulam cattle, revealing a notable 12.40% prevalence by i-ELISA, predominantly affecting mature females. RBPT showed excellent diagnostic performance (sensitivity 88.89%, specificity 98.20%). The findings underscore that nomadic management and natural mating practices contribute meaningfully to disease transmission in this indigenous breed.

## FUTURE SCOPE

This pivotal study provides preliminary insights that can support future longitudinal and molecular research to identify specific *Brucella* species and transmission patterns. The findings may also inform the development of prevention and control programs, not only for the Pulikulam cattle breed but also for other indigenous

breeds under similar management systems, thereby contributing to state-wide control strategies.

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**Conflict of Interest.** None.

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