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Seroprevalence of Brucellosis in Sheep and Goat Population Using Rose Bengal Test in District Tank, Khyber Pakhtunkhwa

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ABSTRACT

Background: Brucellosis is a contagious, economically significant zoonotic disease affecting livestock reproductive performance and posing a public health risk. This study investigated the seroprevalence and associated risk factors of brucellosis in sheep and goats in District Tank, Khyber Pakhtunkhwa, using the Rose Bengal Plate Test (RBPT). **Methods:** A cross-sectional study was conducted on 400 animals (200 sheep and 200 goats) selected from multiple union councils of District Tank. Blood samples were collected and analyzed using RBPT. Epidemiological data including animal demographics, reproductive history, and herd practices were recorded. **Results:** The overall seroprevalence was 13.75%, with 13.0% in goats and 14.5% in sheep ($p > 0.05$). Seropositivity was significantly higher in females (14.6%), animals aged over 3 years (20.7%), and those bred via natural service (19.1%). Autumn showed the highest seasonal prevalence (17.0%). Reproductive disorders such as abortion (25.0%), retained placenta (22.9%), and mastitis (19.0%) were significantly associated with infection. Orchitis in males showed a strong association with seropositivity (31.8%). **Conclusion:** Brucellosis is endemic in small ruminants of District Tank, with clear links to demographic, seasonal, and reproductive factors. The findings support the implementation of targeted control measures, reproductive health monitoring, and awareness programs to mitigate both economic losses and zoonotic risk.

INTRODUCTION

Brucellosis is a chronic infectious disease of considerable veterinary and public health importance, particularly in developing countries where animal husbandry forms the backbone of the rural economy [1]. Caused by facultative intracellular, gram-negative coccobacilli of the genus Brucella, the disease manifests predominantly as reproductive failure in animals, including abortions, infertility, retained placenta, orchitis, and reduced milk production. Its zoonotic nature also makes it a significant threat to human health, primarily among individuals in direct contact with infected animals or animal products such as raw milk and unpasteurized dairy [2-3].

Pakistan, with an estimated livestock population of 186.2 million animals, holds the sixth-largest livestock-based economy globally. Livestock contributes approximately 62.68% to the agriculture sector and 14.36% to the national GDP [2-4]. Over 8 million families rely directly on livestock for their livelihoods, particularly in rural and semi-urban areas. Despite this sector's importance, infectious diseases like brucellosis continue to compromise animal productivity and public health. In Pakistan, where diagnostic infrastructure is limited and veterinary oversight is inconsistent across districts, the control of brucellosis remains challenging [5].

Brucella melitensis, the primary agent of caprine brucellosis, and *Brucella ovis*, which affects sheep, are endemic in several parts of the country [6]. These pathogens exhibit tropism for the reproductive organs and are transmitted via venereal contact, contaminated feed, water, or through direct contact with aborted fetuses, fetal membranes, and uterine discharges [7]. Erythritol, a sugar alcohol found in high concentrations in the gravid uterus, plays a key role in bacterial multiplication, particularly during the last trimester of pregnancy. Vertical transmission through colostrum and milk further amplifies the risk to neonates and humans [5-7].

The disease in humans typically presents as undulant fever, fatigue, arthritis, or reproductive complications, with transmission primarily occurring through consumption of unpasteurized dairy products or direct exposure to infected animals [8]. Recent epidemiological studies from Pakistan have indicated a brucellosis prevalence of up to 17.7% in occupational groups such as farmers, veterinary staff, and abattoir workers. However, most rural areas still lack region-specific data, and control measures are hindered by insufficient awareness, diagnostic delays, and a lack of vaccination campaigns [9-10].

The present study was designed to investigate the seroprevalence of Brucella spp. in sheep and goats using RBPT, assess associations with key demographic and reproductive factors, and identify potential zoonotic implications. By addressing this regional data gap, the findings aim to contribute to the development of evidence-based strategies for disease control and prevention in small ruminants, ultimately reducing the risk to both animal and human populations in this endemic setting.

MATERIALS AND METHODS

Study Area

This cross-sectional study was conducted in District Tank, located in the southern region of Khyber Pakhtunkhwa, Pakistan. The district is predominantly rural and agro-pastoral, with livestock rearing as a key economic activity. The geographical and climatic conditions of the area favor mixed herding of small ruminants, often under extensive or semi-intensive management systems. Veterinary healthcare infrastructure is limited, and disease surveillance is minimal, making the area epidemiologically significant for zoonotic diseases like brucellosis.

Study Population and Sampling Criteria

The target population included indigenous sheep and goats reared by smallholder farmers. A total of 400 animals were selected for sampling, comprising 200 goats and 200 sheep. Animals were selected based on a combination of purposive and random sampling, ensuring representation across different union councils (UCs) of the district. Priority was given to animals with a reproductive history suggestive of brucellosis, such as abortion, stillbirths, mastitis, retained placenta, orchitis, and infertility. However, asymptomatic animals from the same herds were also included to assess subclinical infection prevalence.

Data Collection and Animal-Level Information

Each animal was assigned a unique identification tag, and a structured proforma was used to collect data on multiple variables, including:

- Species (Sheep/Goat)
- Sex (Male/Female)
- Age Group (<1 year, 1–3 years, >3 years)
- Breed (Damani, Waziri, Balkhi, or crossbred)
- Breeding Method (Natural Service)
- Season (Winter, Spring, Summer, Autumn)
- Geographic Location (Union Council-level classification)
- Reproductive Disorders (abortion, mastitis, retained placenta, orchitis)
- Animal owners' information and herd-level data were also recorded to trace back epidemiological linkages and assess potential zoonotic exposure risk.

Sample Collection and Transportation

From each animal, 3-5 mL of blood was collected aseptically from the jugular vein using a sterile vacutainer containing a clot activator. Samples were labeled, kept in insulated cool boxes (2–8°C), and transported to the Laboratory facility, within 4–6 hours of collection. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until serological testing.

Serological Testing: Rose Bengal Plate Test

All sera were subjected to RBPT, a rapid slide agglutination test recommended by the World Organization for Animal Health (OIE, 2016). The following SOPs were followed:

1. Equal volumes (30 µL) of Rose Bengal antigen and serum sample were mixed on a clean white tile.
2. The mixture was gently rotated manually for 4 minutes.
3. Results were interpreted based on the degree of agglutination:
   - 0: No visible agglutination – negative
   - +: Weak agglutination – equivocal
   - ++/+++: Moderate to strong agglutination – positive

Samples graded ++ or +++ were considered seropositive. Equivocal results (+) were excluded from final prevalence estimation to avoid misclassification.

Data Management and Statistical Analysis

All data were coded and entered into Microsoft Excel 2010, and exported to SPSS version 21.0 for statistical analysis. Descriptive statistics were computed for overall and group-wise prevalence. The Chi-square test was employed to assess associations between categorical risk factors and brucellosis seropositivity. Furthermore, one-way Analysis of Variance (ANOVA) was performed to determine statistical differences in infection rates among different levels of continuous or grouped variables such as age and season. A p-value < 0.05 was considered statistically significant.

Quality Assurance and Biosafety

Standard biosafety protocols were observed during sample collection, handling, and laboratory procedures. All personnel were equipped with gloves, masks, and protective clothing, and the lab environment was routinely disinfected. Positive control sera were run in parallel to validate RBPT antigen performance.

RESULTS

Regarding the distribution of brucellosis seroprevalence in goats and sheep based on RBPT results, out of 200 goats tested, 26 were seropositive, resulting in a prevalence rate of 13.0% with a 95% confidence interval (CI) ranging from 8.6% to 18.4%. Similarly, out of 200 sheep, 29 animals were found positive, yielding a slightly higher prevalence of 14.5% (95% CI: 9.8%–20.0%). The overall prevalence across both species was calculated at 13.75% (95% CI: 10.4%–17.9%). The p-value (>0.05) indicates that the difference in prevalence between goats and sheep was not statistically significant. This suggests that both species are equally susceptible to brucellosis under the management conditions of the study area, likely due to shared environments and husbandry practices (Table 1).

The association of various risk factors; sex, age, breeding method, and season was evaluated with brucellosis seropositivity. A statistically significant association was observed between sex and seropositivity (p = 0.042), with female animals showing a higher prevalence (14.6%) compared to males (10.5%). Age also influenced infection rates significantly (F = 3.51, p = 0.033), with animals older than 3 years exhibiting the highest prevalence (20.7%), whereas younger age groups (<1 year and 1–3 years) had lower rates of 10.0%. The breeding method showed a strong correlation with seroprevalence (χ^2 = 6.12, p = 0.025); animals bred by natural service had a significantly higher infection rate (19.1%) compared to those bred through artificial insemination (7.2%). Seasonal variation was also notable (F = 4.09, p = 0.041), with the highest seroprevalence observed in autumn (17.0%), followed by summer (16.0%), while winter (10.0%) recorded the lowest prevalence. These findings imply that biological, management, and environmental factors significantly influence brucellosis transmission dynamics in small ruminants (Table 2).

The relationship between specific reproductive disorders in female animals and seropositivity for brucellosis was evaluated. Females with a history of abortion had a notably high prevalence of 25.0%, with an odds ratio (OR) of 2.39 (95% CI: 1.02–5.56), indicating a statistically significant association (p < 0.01). Retained placenta was associated with a 22.9% prevalence (OR: 2.21; 95% CI: 0.85–5.73; p < 0.05), while mastitis was linked to a 19.0% prevalence (OR: 1.63; 95% CI: 0.64–4.18; p < 0.05). In contrast, animals without any reproductive disorder showed a baseline prevalence of 9.5%. The elevated odds ratios and statistically significant p-values confirm that reproductive pathologies, particularly abortion and placental retention, are strong indicators of underlying brucella infection in females. These disorders likely reflect the tropism of Brucella spp. for reproductive tissues and emphasize the importance of reproductive history as a screening criterion in endemic areas (Table 3).

The link between orchitis and brucellosis seropositivity in male animals was also investigated. Among the 22 males clinically diagnosed with orchitis, 7 tested positive for brucellosis, yielding a prevalence of 31.8%. In contrast, among the 64 males without orchitis (control group), only 2 were seropositive, resulting in a much lower prevalence of 3.1%. The calculated odds ratio was 14.6 (95% CI: 2.9–73.5), with a p-value < 0.05, indicating a strong and statistically significant association. These findings suggest that orchitis is a critical clinical indicator of brucellosis in male small ruminants and should prompt diagnostic testing. The high odds ratio also supports the hypothesis that Brucella organisms preferentially colonize testicular tissues, leading to inflammation and impaired fertility (Table 4).

Table 1

Seroprevalence of Brucellosis by Species

Species	No. Tested	No. Positive	No. Negative	Prevalence (%)	95% CI	P-value
Goat	200	26	174	13.0	8.6–18.4	>0.05
Sheep	200	29	171	14.5	9.8–20.0	
Total	400	55	345	13.75	10.4–17.9	

Table 2

Association between Risk Factors and Brucellosis Seroprevalence

Factor	Category	No. Tested	Positive (n)	Prevalence (%)	χ^2	P-value
Sex	Male	86	9	10.5	4.16	0.042
	Female	314	46	14.6		
Age Group	<1 year	100	10	10.0	3.51	0.033
	1–3 years	160	16	10.0		
	>3 years	140	29	20.7		
Breeding Method	AI	180	13	7.20	6.12	0.025
	Natural	220	42	19.1		
Season	Winter	100	10	10.0	4.09	0.041
	Spring	100	12	12.0		
	Summer	100	16	16.0		
	Autumn	100	17	17.0		

Table 3

Reproductive Disorders and Brucella Seropositivity in Female Animals

Disorder	Total Cases	Positive Cases	Seroprevalence (%)	Odds Ratio	95% CI	P-value
Abortion	48	12	25.0	2.39	1.02–5.56	<0.01
Retained Placenta	35	8	22.9	2.21	0.85–5.73	<0.05
Mastitis	42	8	19.0	1.63	0.64–4.18	<0.05
No Disorders (n=189)	—	18	9.5	—	—	—

Table 4

Orchitis and Brucellosis in Male Animals

Parameter	Value
Total Males with Orchitis	22
Brucella Positive among them	7
Prevalence (%)	31.8
Control Group (no Orchitis)	64
Positive in Control Group	2
Control Prevalence (%)	3.1
Odds Ratio	14.6
95% CI	2.9–73.5
P-value	<0.05

DISCUSSION

This study provides a critical insight into the seroprevalence of brucellosis in small ruminants (sheep and goats) in District Tank, Khyber Pakhtunkhwa, using the Rose Bengal Plate Test (RBPT). With an overall seroprevalence of 13.75%, the findings confirm that brucellosis is endemic in the region's small ruminant population. This is consistent with previous studies conducted in other parts of Pakistan. For instance, Shehzad et al. (2021) reported a brucellosis prevalence ranging from 14% to 17.74% in small ruminants across various provinces of Pakistan, demonstrating that this zoonotic disease remains a persistent threat nationwide [11].

The slightly higher seroprevalence observed in sheep (14.5%) compared to goats (13.0%) was not statistically significant, suggesting both species are equally susceptible to Brucella infection under the shared environmental and management conditions prevalent in rural Tank. This finding is in agreement with observations by Mikolon et al. (1998), who reported that mixed herding practices, communal grazing, and water sources facilitate cross-species transmission of Brucella melitensis and B. ovis [1].

Sex-wise analysis revealed that female animals had a significantly higher seroprevalence (14.6%) than males (10.5%), a result consistent with previous literature. The increased susceptibility in females is likely linked to the presence of erythritol and hormonal changes during pregnancy, which promote the growth and persistence of Brucella spp. in reproductive tissues [12-15]. Similar patterns have been reported by Letesson et al. (2017), where Brucella displayed genital tropism, particularly in the gravid uterus [10].

Age was also a significant determinant of seropositivity. Animals older than three years exhibited a seroprevalence of 20.7%, significantly higher than younger animals. This aligns with findings from Sarker et al. (2014), who suggested that older animals accumulate more exposure opportunities, leading to higher infection risk [12]. Age-related vulnerability underscores the importance of targeted screening protocols in older breeding animals [16].

Breeding method emerged as another important risk factor. Animals bred through natural service showed significantly higher seropositivity (19.1%) compared to those bred by artificial insemination (7.2%). This supports findings from Radostits et al. (2000), who highlighted venereal transmission of Brucella through infected semen or contaminated genital secretions during natural mating [17]. These results underline the importance of promoting AI practices, especially in endemic regions, to mitigate disease spread.

Seasonal variation was also found to influence seroprevalence significantly. The highest prevalence was recorded in autumn (17.0%), followed by summer (16.0%), with the lowest in winter (10.0%). This trend is comparable to the findings of Poester et al. (2013), who noted that environmental stress and reproductive synchronization during specific seasons enhance disease transmission [7, 18]. The congregation of animals during limited grazing periods may also increase exposure in autumn.

One of the most important associations observed was between reproductive disorders and brucellosis seropositivity in females. Animals with a history of abortion, retained placenta, or mastitis exhibited significantly higher infection rates. Abortion cases had a seroprevalence of 25.0%, with an odds ratio of 2.39, confirming a strong association. These findings are consistent with those of Seleem et al. (2010), who emphasized that reproductive failures are hallmark manifestations of Brucella infection in small ruminants [19]. Infected animals often shed the pathogen in high quantities during parturition, making them critical sources for horizontal transmission [20-21].

Similarly, orchitis in male animals was found to be strongly associated with brucellosis. Males with orchitis showed a 31.8% seroprevalence, with an odds ratio of 14.6, significantly higher than the 3.1% prevalence in the control group. This aligns with findings from Mainar-Jaime et al. (2005), who reported Brucella ovis to be a common etiological agent of orchitis in rams, resulting in reduced fertility and chronic carrier states [14]. This further supports the need for andrological examination as part of disease surveillance in breeding males.

From a zoonotic perspective, these findings carry serious public health implications. Brucellosis is a re-emerging zoonosis, and its persistence in rural livestock populations increases the risk of spillover to humans. Aessopos et al. (2007) documented that 24% of human cases had direct contact with animals, while 76% were linked to consumption of raw dairy products [4]. In Pakistan, where raw milk consumption is common, the risk is elevated. As such, both veterinary and public health sectors must collaborate to implement control measures, including public awareness campaigns, milk pasteurization, and targeted human screening [22-23].

In light of the evidence, the study emphasizes the utility of the Rose Bengal Plate Test as a cost-effective screening tool for field-level diagnosis. Although RBPT may not differentiate between active and past infection, its high sensitivity makes it suitable for surveillance and outbreak monitoring. The test's practicality in remote and resource-constrained settings justifies its use as the primary diagnostic approach, as also recommended by the OIE [18].

Despite the comprehensive data gathered, the study is not without limitations. The cross-sectional nature of the study precludes temporal interpretation of infection dynamics, and the reliance on a single serological test without confirmatory ELISA or PCR may under- or overestimate true prevalence. Nonetheless, the findings are critical for guiding local disease control policies.

CONCLUSION

The present study confirms that brucellosis is endemic in the sheep and goat populations of District Tank, with an overall seroprevalence of 13.75% detected via RBPT. Significant associations were observed with sex, age, breeding method, season, and reproductive disorders, highlighting key epidemiological risk factors. The strong link between reproductive abnormalities and seropositivity underscores the disease's impact on animal fertility. These findings emphasize the need for targeted surveillance, biosecurity measures, and public health education to mitigate both animal productivity losses and zoonotic transmission risk in rural livestock systems.

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