

## SERO-PREVALENCE AND POTENTIAL RISK FACTORS OF BRUCELLOSIS IN CAMELS (*CAMELUS DROMEDARIUS*) FROM THE SOUTHERN REGION OF KHYBER PAKHTUNKHWA, PAKISTAN

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### Abstract

Brucellosis, a zoonotic bacterial disease caused by *Brucella* spp., poses a serious threat to livestock productivity and public health. In dromedary camels, it leads to reproductive disorders and economic losses, while also serving as a source of human transmission. To evaluate the sero-prevalence of brucellosis in camels and associated risk factors in the southern districts of Khyber Pakhtunkhwa (KP), Pakistan, including Tank, Dera Ismail Khan, Lakki Marwat, and Kohat. From March to May 2025, 100 camel blood samples were tested for brucellosis using RBPT and iELISA. The overall sero-prevalence was 13.00% with RBPT and 9.00% with iELISA. Based on RBPT, prevalence was highest in Tank (15.79%), followed by Kohat (14.29%), D.I. Khan (12.12%), and Lakki Marwat (10.00%), with no significant variation ( $\chi^2 = 1.29$ ,  $p = 0.731$ ). Using iELISA, Kohat (10.71%) and Lakki Marwat (10.00%) showed slightly higher rates than D.I. Khan (9.09%) and Tank (5.26%). Data was analyzed using Statistix 8.1 software. Chi-square analysis identified significant associations of brucellosis seropositivity (RBPT) with age ( $\chi^2 = 3.85$ ,  $p = 0.050$ ), abortion history ( $\chi^2 = 5.00$ ,  $p = 0.025$ ), and sex ( $\chi^2 = 4.10$ ,  $p = 0.043$ ). Prevalence was higher in camels >5 years (16.36%) than ≤5 years (8.89%), in those with abortion history (25.00%) compared to without (9.21%), and in males (14.29%) versus females (12.31%). Other factors, including body condition, herd size, mating practice, water source, and interspecies contact, showed epidemiological importance but were not statistically significant ( $p > 0.05$ ). The study revealed that brucellosis is moderately prevalent in camels of southern Khyber Pakhtunkhwa and is strongly linked to reproductive factors such as age and abortion history, with sex and body condition also influencing risk. Both RBPT and iELISA detected the disease, but neither test proved more sensitive when used alone. The findings show the need for regular serological screening, stronger biosecurity, regulated breeding, and a One Health approach to protect animals and human health.



## INTRODUCTION

According to a 2019 study by the Pakistan Bureau of Statistics, Pakistan's camel population grew steadily to surpass 1.1 million camels (1). Camel populations are growing in arid and semi-arid regions because people desire to use them for a variety of purposes. Marecha camels are beneficial for expanding dairy production in underserved areas since, according to research, they generate around 4179 liters of milk each lactation (2,3). People in hot, arid climates benefit from vitamin C in camel milk because it shields their cells from stress and nutrient deficiencies (4,5).

Since camel milk has a unique nutritional profile, more research is being done on it worldwide. Research indicates that camel milk is more effective than cow's milk since it has less lactose, which benefits those who are lactose sensitive. Camel milk has three to five times the amount of vitamin C as ordinary milk (6-8). Calcium, iron, zinc, magnesium, potassium, copper, and other essential elements are all found in camel milk (9-11). Because of these crucial components, milk is acknowledged as a functional food. People throughout the world are increasingly consuming camel milk because they believe it has therapeutic properties, although age, sex, and lactation phases also have a significant impact on milk output and composition (12-16).

Heat stress is directly proportional to the loss of products of animals such as milk, meat and fertility rate as well (17). Good nutrition, especially probiotics, prebiotics help animals to increase their byproducts (18-22)

Pakistan is the world's eighth-largest camel-raising country (23). Pakistan is home to around one million camels (Economic Survey, 2011-12). The animal's nationwide population distribution is shown in Figure 1. According to the 2006 Census, the highest percentages of one-humped camels, or dromedaries, are found in Baluchistan (41%) and Sindh (30%), Punjab (22%), and KPK (7%).

According to scientific research, camel milk helps people manage their health conditions, particularly diabetes mellitus. Patients with type 1 diabetes who consumed 0.5 liters of camel milk daily had improved blood sugar control and reduced insulin use, according to an Indian study trial (24). Ethiopians believe camel milk increases sexual desire; hence they utilize it as traditional medicine (25, 26). Like providing nourishing camels, camels have a significant economic and social impact on arid regions in Asia and Africa. The Thar and

Saharan herders rely on camels to move their commodities, make leather items, and obtain food supplies (27, 28).

Families in the Saharan and Thar deserts keep camels for their milk and meat, and they use them for transportation, riding, and leather manufacture to make ends meet (29, 30). According to research, camels can successfully survive in harsh environmental situations (31). Because they can reproduce successfully and stay healthy to generate resources while other animals die, camels prove to be flexible during drought spells. Since camels adapt their lifestyle to adapt to changing conditions, they can be used to create drought-resistant livestock programs in arid regions (32, 33). Disease transmission happens when direct contact with infected animals or consume unsafe animal food products such as undercooked meat and raw milk (34,35). People who work with livestock such as farmers, veterinarians, abattoir employees and herders are most likely to catch brucellosis (36). Because of grate public health concern, the health care professionals struggle with brucellosis diagnosis because in the developing countries they lack proper medical tests (37,38). Due to the important significance that camels (*Camelus dromedarius*) play in both commerce and culture, Pakistan's management methods for camel illnesses are insufficient (39, 40). People depend on camels to transport their belongings, gather milk, and consume meat and leather goods. Effective precautions against human and animal illnesses are necessary for those in KP and the border regions who rely on camels for their livelihood (41).

Camels are found across Pakistan's natural habitats, ranging from the deserts of Sindh and Baluchistan to the southern plains of Khyber Pakhtunkhwa (42, 43). One million camels are said to exist in Pakistan, with the majority residing in the southern districts of Dera Ismail Khan, Tank, Lakki Marwat, and Kohat, according to the 2019 Livestock Census (1). Because of local ethnic customs, studies reveal that drinking raw camel milk increases the risk of zoonotic diseases in humans (44). Camel cattle receive less medical attention than other agricultural animals, despite their crucial significance. Brucellosis has been evading detection in camel herds since 2016, according to research (45). In camels, brucellosis causes unique reproductive problems including infertility, inflammation of the testicles, contaminated placenta, and miscarriage (46).



Hidden infections are typical which makes it hard to find and handle diseased animals (47). The risk of *Brucella* passing between different animal species increases substantially when southern KP camels' pasture together and access shared water sources with various other livestock types. The spread of Brucellosis infections increases because people in the community use raw camel milk as natural medicine (48).

There are various species in the genus *Brucella*, but the three most dangerous to veterinary medicine and public health are *B. suis*, *B. melitensis*, and *B. abortus*. *B. abortus* and *B. melitensis* are the primary causes of infections in camels (49). Because of its ease of transmission and propensity for serious illness, *B. melitensis* is the most harmful of these to humans and has been designated as a category B bioterrorism agent by the Centers for Disease Control and Prevention (CDC) (36,49). Intracellular pathogens called *Brucella* organisms enter and multiply inside macrophages, eluding the host's immune system and causing persistent infections (50). Animal reproductive organs are the preferred location for the bacterium, which causes diseases such as infertility, retained placenta, and abortion that are significant causes of financial loss (51).

There are several species of *Brucella*, but only *B. suis*, *B. melitensis*, and *B. abortus* may seriously harm both human and animal health. A 2018 OIE report states that the only bacteria that cause camel diseases are *B. abortus* and *B. melitensis*. Since *B. melitensis* is a very dangerous pathogen that spreads quickly between species, the Centers for Disease Control and Prevention classify it as a category B bioterrorism agent (36). When *Brucella* germs take over macrophage cells, they may safely proliferate inside of them, resulting in infection. Chronic diseases and host immune responses are suppressed (52). The pathogen spreads readily to the reproductive organs of animals, resulting in illnesses and financial losses.

In Pakistan, brucellosis epidemics recur in different places due to a lack of veterinary assistance, a lack of knowledge about the illness, and the absence of control strategies (45). When farmers sell raw milk without enough safety procedures and barely shield animals from infection, farms contribute to the spread of illness. Even in remote areas of southern KP, medical testing facilities are insufficient. Due to supply chain and economic concerns, traditional laboratory diagnostic procedures such as serum

agglutination and RBPT are rarely employed (53). Because these sophisticated detection technologies are difficult to locate at modest clinical locations, research institutes usually have to offer molecular tests. Although camels play a significant role in Pakistan's rural economy, there aren't much comprehensive research on camel brucellosis, especially in southern KP (41). Limited and dispersed study results that use small sample groups make it hard to know the actual scope of this disease. Research on cattle and small ruminants has grown more prominent but scientists still know little about how specific risk factors affect camels in their grazing and management routines (54). The methods that spread zoonotic diseases from camels to people during milk consumption and daily work as herders remain unknown in Khyber Pakhtunkhwa.

Studying the prevalence of camel brucellosis in the region is crucial because of the animals' social and economic significance to KP communities as well as their function as disease carriers. To protect people and farmers who depend on camels for their livelihood, epidemiological study will aid in the development of efficient disease management strategies. Our local scientific evidence backs advocacy efforts to include camel brucellosis in regional and national brucellosis eradication initiatives. In the southern Khyber Pakhtunkhwa area of Pakistan, we intend to investigate the prevalence of brucellosis in camels and seek for risk factors.

## Materials And Methods

### Ethical Considerations

Camels' owners gave their free agreement, and the study was authorized by the Institutional Animal Care and Use Committee (IACUC), guaranteeing adherence to ethical guidelines for animal research. To preserve animal welfare and reduce suffering, blood was drawn in accordance with approved procedures. To avoid contamination and guarantee the safe disposal of items, stringent biosecurity and biosafety protocols were implemented.

### Study Area

Kohat, Lakki Marwat, Tank, and Dera Ismail Khan were selected for the study due to their high camel populations and documented brucellosis cases. For transportation, milk, and meat, these nomadic and agropastoral people depend on camels. Grazing interactions with other species are influenced by the dry to semi-arid environment, and regions were

chosen based on the advice of veterinary authorities about high transmission risk.

### Study Design

A cross-sectional design was used in the study, and camels from various regions and management systems were chosen at random. They included camels of all sexes and all ages, as well as those subjected to hazards such as herd movement, water, and shared pasture. To identify illness patterns and transmission factors, data on immunization, reproductive issues, and herd history were gathered.

### Restraining of Camels

The primary veterinarian used traditional techniques to properly confine camels in the field. Until the camel was sitting in the Kush posture, a soft rope was connected to one of its front legs. To minimize movement, the camel's front legs were then tied to both. Both a halter and a hand grip on the top lip were used to draw blood from the jugular vein, and camels that were hostile or apprehensive had their rear legs further restrained. In order to keep animals and workers safe while lowering stress, no pharmaceutical sedatives were administered, and all procedures adhered to animal welfare regulations.

### Sample Size and Collection

A total of 100 camels from commercial enterprises, nomadic herds, and smallholder farms with varying population densities, ages, and sexes had their blood samples taken. Using sterile vacutainer gel tubes, blood was extracted from the jugular vein while adhering to stringent biosafety regulations to avoid contamination. A unique identification number and camel data, including location and

demography, were attached to each sample. The Veterinary Research Institute in Peshawar received the samples within 24 hours after being kept at 4°C, guaranteeing precise serological findings.

### Serological Testing

Brucella antibodies were detected in camel serum by two serological techniques. Due to its affordability and quickness, the Rose Bengal Plate Test (RBPT) was used as the first screening method. To prevent false positives and identify real infections, iELISA (IDvet Serum Ab Test kit, France) was used to confirm positive RBPT samples. The combination of both tests, conducted in accordance with normal lab methods, increased diagnostic accuracy and guaranteed consistent, reproducible results.

### Risk Factor Assessment

Epidemiological data on brucellosis risk variables, including herd size, management, animal mobility, biosecurity, and reproductive history, were collected using a standardized questionnaire. Focus was placed on interaction with other cattle species, common water supplies, and grazing patterns as important pathways of transmission. In-person interviews with camel owners and herders yielded comprehensive information, which was further checked for correctness using veterinary records. Important risk variables were identified in the data, and their relationship to brucellosis was evaluated by comparing them to serological findings.

### Age Estimation

Age of the experiment camel was estimated to history taking and by dental formula given below (table. 1).

**Table 1. Dental Formula: I 1/3, C1/1, P3/2, M3/3**

Age	Dental Status
Birth 1 year	Milk teeth
1-2 years	All milk incisors present
2-3 years	First permanent incisor (One)
3-4 years	Second permanent incisor (two) erupts
4-5	Third permanent incisor (three) erupts
5-6	Canines erupt, molars complete
6-8	Ful treatment dentition, wear starts to show
10 years	Teeth yellow are missing

### Statistical Analysis

Statistix 8.1 software was used to evaluate the data, and descriptive statistics were employed to determine the overall prevalence of camel brucellosis. The relationships between risk variables and sero-positivity were evaluated using chi-square testing. When the p-value was less than 0.05, it was deemed statistically significant, indicating a strong correlation between risk variables and the incidence of illness. This research provided a detailed picture of the epidemiology of brucellosis in the study region and revealed important transmission mechanisms.

### Results

#### Overall Sero-Prevalence of Brucellosis in Camels

The Rose Bengal Plate evaluate (RBPT) and the indirect enzyme-linked immunosorbent assay (iELISA) were used to evaluate 100 camel serum samples collected from southern Khyber Pakhtunkhwa for antibodies against *Brucella* species. According to the data in table 2 shows that camels in this region had a moderate risk of brucellosis, with RBPT detecting the disease in 13.00% of them and iELISA detecting it in 9.00%.

**Table 2. Overall Sero-Prevalence of Brucellosis in Camels**

Test	Total Samples (N)	Positive Cases (n)	Prevalence (%)
RBPT	100	13	13.00%
iELISA	100	09	9.00%

#### District-Wise Sero-Prevalence of Brucellosis

Four districts were used to get camel blood samples: Tank, Lakki Marwat, Kohat, and D.I. Khan. While the iELISA findings ranged from 5.26% to 10.71%, the sero-prevalence by RBPT was between 10.00% and 15.79%. Both the D.I. Khan and Kohat regions

revealed substantial antibody presence by iELISA, while the Tank district had the highest RBPT prevalence (15.79%). Table 3, figure 1 shows that there was no significant difference in prevalence between the districts ( $\chi^2 = 1.29$ ,  $p = 0.731$ ).

**Table 3. District-Wise Sero-Prevalence of Brucellosis**

District	mples (N)	RBPT Positive (n)	RBPT Prevalence (%)	iELISA Positive (n)	iELISA Prevalence (%)	$\chi^2$ Value	p-value
D.I. Khan	33	04	12.12%	03	9.09%		
Kohat	28	04	14.29%	03	10.71%	1.29	0.731
Lakki Marwat	20	02	10.00%	02	10.00%		
Tank	19	03	15.79%	01	5.26%		
Total	100	13	13.00%	9	9.00%		

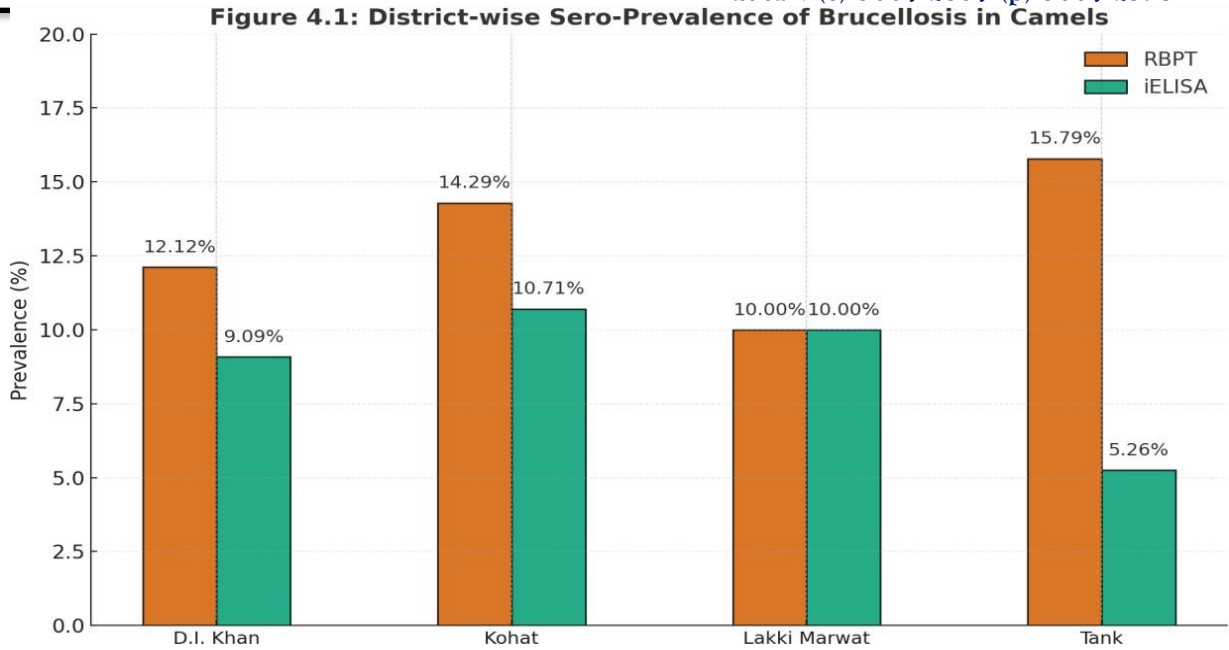


Figure 1. The RBPT and iELISA test results for brucellosis in camels at the district level.

**Risk Factors Associated with Brucellosis by RBPT**

The relationship between risk variables and brucellosis sero positive in camels using RBPT is displayed in Table 4, figure 2. Infection was substantially correlated with age group, sex, and history of abortion ( $p < 0.05$ ). The prevalence was higher in males (14.29%) than in females (12.31%). The frequency was higher in older camels (>5 years) (16.36%) than in younger ones (8.89%). The

prevalence was higher in camels with a history of abortion (25.00%) than in those without (9.21%). There were no significant relationships found between other characteristics such as body condition, herd size, mating behavior, contact with other animals, and water supply ( $p > 0.05$ ). These findings suggest that camel brucellosis risk is influenced by reproductive and demographic factors.

**Table 4. Risk Factors Associated with Brucellosis by RBPT**

Risk Factor	Category	Camels (N)	RBPT Positive (n)	Prevalence (%)	$\chi^2$	p-value
Sex	Male	35	05	14.29%	4.10	0.043*
	Female	65	08	12.31%		
Age Group	≤5 years	45	04	8.89%	3.85	0.050*
	>5 years	55	09	16.36%		
Abortion History	Yes	24	06	25.00%	5.00	0.025*
	No	76	07	9.21%		
Body Condition	Good	65	07	10.77%	0.50	0.480
	Poor	35	06	17.14%		
Herd Size	≤10 animals	67	07	10.45%	2.90	0.089
	>10 animals	33	06	18.18%		
Mating Practice	Natural Mating	75	09	12.00%	0.20	0.654
	No Mating	25	04	16.00%		
Contact with Others	Yes	65	10	15.38%	1.50	0.220
	No	35	03	8.57%		

Water Source	Communal	51	08	15.69%	2.80	0.095
	Controlled	49	05	10.20%		

\*Note: \*Significant at  $p < 0.05$

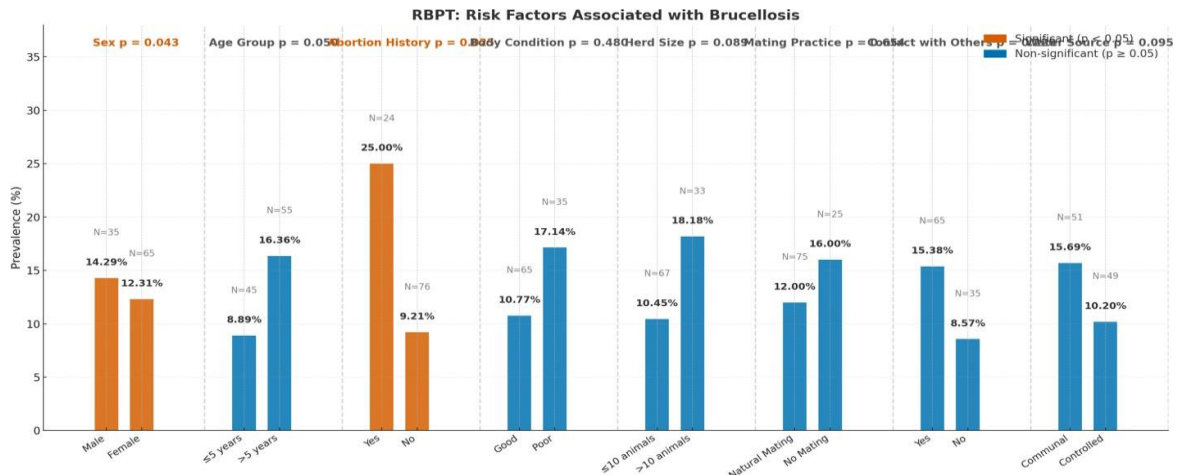


Figure 2. The prevalence of brucellosis in camels with associated risk factors as detected by the Rose Bengal Plate Test (RBPT).

### Risk Factors Associated with Brucellosis by iELISA

The risk factor analysis based on iELISA results is shown in Table 5, figure 4. Abortion history and age group were strongly associated with brucellosis infection ( $p < 0.05$ ). The incidence was higher in camels older than five years (16.36%) than in younger camels (8.89%). Camel prevalence was 20.83% among those with a history of abortion and

10.53% for those without. Sex, body condition, herd size, mating behavior, contact with other animals, and water source were among the other characteristics that did not exhibit a significant correlation ( $p > 0.05$ ). This suggests that in this population, brucellosis infection may be more significantly influenced by age and reproductive history.

Table 5. Risk Factors Associated with Brucellosis by iELISA

Risk Factor	Category	mples (N)	iELISA Positive (n)	valence (%)	$\chi^2$	p-value
Sex	Male	35	03	8.57%	2.50	0.114
	Female	65	10	15.38%		
Age Group	≤5 years	45	04	8.89%	4.00	0.045*
	>5 years	55	09	16.36%		
Abortion History	Yes	24	05	20.83%	4.10	0.043*
	No	76	08	10.53%		
Body Condition	Good	65	07	10.77%	0.60	0.440
	Poor	35	06	17.14%		
Herd Size	≤10 animals	67	07	10.45%	3.00	0.083
	>10 animals	33	06	18.18%		
Mating Practice	Natural Mating	75	09	12.00%	0.10	0.750
	No Mating	25	04	16.00%		
Contact with	Yes	65	10	15.38%	1.80	0.180
	No	49	05	10.20%		

Others	No	35	03	8.57%		
Water Source	Communal	51	08	15.69%	3.20	0.074
	Controlled	49	05	10.20%		

\*Note: \*Significant at  $p < 0.05$

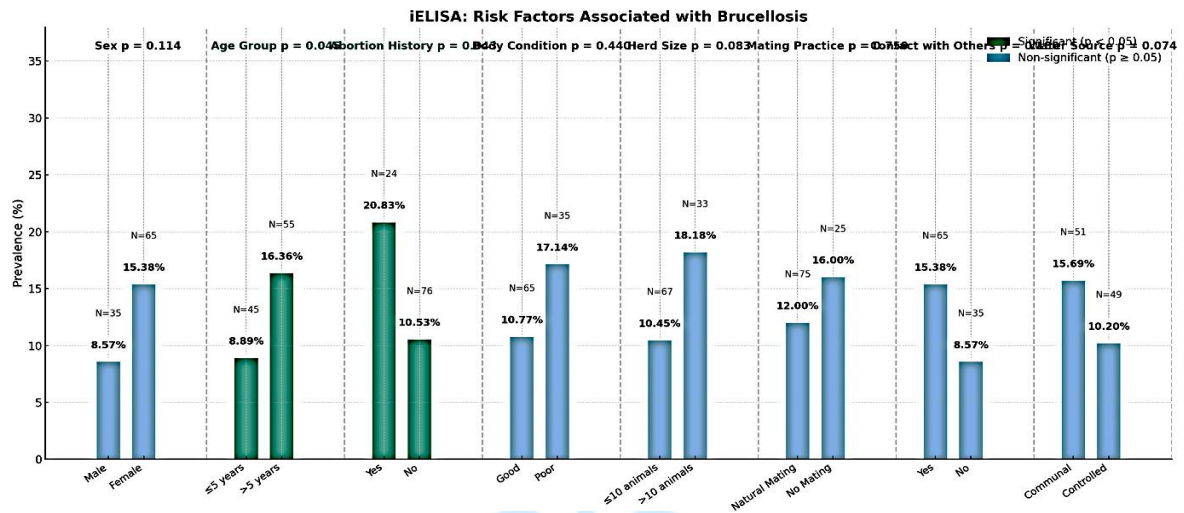


Figure 4. The prevalence of brucellosis in camels with associated risk factors as detected by the indirect Enzyme-Linked Immunosorbent Assay (iELISA).

### Discussion

The purpose of this study was to ascertain the seroprevalence of brucellosis in dromedary camels in the southern districts of Khyber Pakhtunkhwa (KPK), Pakistan, and look into the risk factors that are linked to the disease. The study's use of the indirect enzyme-linked immunosorbent assay (iELISA) and the Rose Bengal Plate Test (RBPT) shows that brucellosis is a serious and potentially underappreciated health risk in the area. In line with the literature documenting variations in diagnostic test sensitivity and specificity, the RBPT produced a greater apparent seroprevalence than iELISA (37). While iELISA showed the highest prevalence in Kohat (10.71%), RBPT recorded the highest seropositivity in the Tank district (15.79%). Notably, sex and abortion history were risk variables that were statistically significant ( $p < 0.05$ ), whereas age, body condition, herd size, mating practice, and exposure to other animals showed epidemiologically meaningful, albeit statistically non-significant, trends. These findings sound like previous studies highlighting the complex multifactorial nature of *Brucella* transmission in camel populations (55,56).

This study's RBPT and iELISA values differed, which is not uncommon and has been extensively

reported. RBPT has low specificity since it reacts with other antibodies and bacterial proteins such *Yersinia enterocolitica* O:9 and *Escherichia coli* O:157, despite being affordable and appropriate for large-scale screening (57). On the other hand, iELISA is capable of accurately and precisely detecting long-term or low-level infections (58). Consequently, combining these tests improves diagnostic confidence and minimizes false positives and negatives. The variation between RBPT and iELISA results across districts, especially in Tank and Kohat, may be attributed to differences in environmental contamination, husbandry practices, and population density of livestock. The Previous regional research studies in Punjab, Balochistan, and parts of Sindh have also reported similar variability, strength the importance of using dual diagnostic strategies for effective surveillance (59). Moreover, as *Brucella* spp. may remain undetectable in the early or latent stages, iELISA is preferred in confirming subclinical infections (60). *Brucella* seropositivity in female camels was highly correlated with their history of abortion, with a prevalence of 25.00%. The findings of (61), who observed a strong correlation between abortion and brucellosis in Somali camels, are consistent with this understanding. In the placenta and

reproductive system, brucella organisms can cause placentitis, retained fetal membranes, and late-gestation abortions (62).

In addition to lowering productivity, these reproductive symptoms are important markers for identifying Brucella infection in endemic regions. Male camels had a greater seroprevalence (14.29%) than females (12.31%), making the sex-wise factor another statistically significant variable. Additionally, the results reported in Sudan and the United Arab Emirates ascribed this tendency to the regular movement of male camels for transportation, trading, and mating (63). The likelihood of meeting contaminated settings or diseased animals rises with increased animal movement. However, the danger of horizontal and vertical transmission is increased when common breeding males are used across several herds without adequate testing (64).

The study's age-related pattern was observed, where older animals (>5 years) had a higher seroprevalence (16.36%) than younger camels (less than 5 years; 8.89%), aligns with previous findings in Saudi Arabia, Iran, and Kenya (65). As people age, their risk of contracting Brucella increases because of the disease's chronicity, cumulative reproductive cycles, and prolonged exposure. Adult females are also more vulnerable to Brucella spp., which preferentially infect reproductive tissues, because of having several pregnancies (37). Although body condition did not play a significant role in this study, the proportion of badly conditioned camels was higher (17.14%) than that of well-conditioned camels (10.77%). A brucellosis infection or other immune-system-compromising disorders that make animals more susceptible to infection may be indicated by a poor state of health (64). Herd size was found to be an epidemiologically relevant variable, while not being statistically significant ( $p > 0.05$ ). Seroprevalence was higher in camels from larger herds (>10 animals) (18.18%) than in smaller herds (10.45%). This trend is in line with research from Sudan, Nigeria, and Ethiopia that found that higher herd sizes were linked to higher brucellosis risk because of increased animal interaction, sharing grazing or watering locations, and inadequate biosecurity (65). Conversely, unrestrained animal movements and inadequate veterinarian supervision worsen disease transmission in communal herd environments (62). Additionally, mating behaviors showed significant epidemiological ramifications.

Compared to camels undergoing monitored natural mating (12.00%), those not participating in any regulated mating practices (non-mated) exhibited a higher seroprevalence (16.00%).

Using borrowed stallions or bulls in pastoral farming systems has made it easy for Brucella spp. to spread among herds (Musa et al., 2008). Four studies pointed out that breeding males played a role in brucellosis spreading among herds in dry places, for example, in the Afar region of Ethiopia and in parts of Iran (61). Brucellosis was seen more frequently (15.38%) in sheep, goats and cattle compared to other animal species studied. This is consistent with cross-species transmission dynamics reported by (37), who showed that camels often share grazing pastures with small ruminants, especially sheep and goats—frequently infected with Brucella melitensis. In KPK and adjoining tribal areas, such mixed-herd practices are common, especially in transhumant and nomadic systems. The interspecies interactions also enhance brucellosis transmission, with infected goats or cattle serving as potential reservoirs. Regarding management systems, camels managed under communal or open systems demonstrated higher brucellosis prevalence (15.69%) than those reared under controlled or tied-up systems (10.20%). This outcome supports the hypothesis that unrestricted movement and close animal-to-animal contact in traditional management settings amplify disease transmission (60).

The study's findings are consistent with those of other research projects conducted in arid and semi-arid countries that raise camels. Depending on the area and testing method, camel brucellosis has been detected in Pakistan with seroprevalence levels ranging from 6% to 18% (59). The iELISA results from this investigation are fairly similar to the 12.7% prevalence of camel brucellosis in Balochistan. Previous investigations have reported varying frequencies of brucellosis in camels in Saudi Arabia, Sudan, and Iran (2). In 1992, Radwan et al. reported that nearly 8.1% of Saudi Arabian camels tested positive for iELISA, and in 2008, Musa et al. reported that 10.9% of Sudanese camels tested positive for RBPT.

They found that the disease was up to 21% prevalent in areas of Ethiopia with high camel livestock mixing and inadequate veterinary infrastructure. These findings demonstrate that the prevalence of camel brucellosis in KPK is comparable to that in other areas with high-risk

camel farming. However, the major species of *Brucella*, the test used for identification, the age and reproduction of affected animals, and whether vaccination is being utilized can all affect the incidence of *Brucella* antibodies in each area (Nielsen & Yu, 2010). More cases of brucellosis spreading to camels occur when nations concentrate their brucellosis programs on small ruminants (37).

One of the most serious zoonotic diseases in the world is brucellosis, and its prevalence in camels poses a serious threat to public health, particularly in areas like Khyber Pakhtunkhwa where there is a high level of livestock-human contact. *Brucella* spp. can infect humans through milk, urine, aborted materials, and vaginal secretions that infected camels may produce (66). Ingestion of unpasteurized camel milk, inhalation of contaminated aerosols during animal handling or abortion, and direct contact with diseased animals or their secretions can all cause human brucellosis (67). Close contact with diseased animals and camel milk have been directly connected to several human brucellosis epidemics in the Middle East and East Africa (68). Moreover, the lack of awareness about zoonoses among camel herders, combined with poor veterinary access, contributes to the disease's silence spread in both animal and human populations (69).

Brucellosis spread from animals is considered one of the biggest zoonotic diseases and because it exists in camels, it is a major threat to health in communities that interact closely with animals such as those in Khyber Pakhtunkhwa. Infected camels may shed *Brucella* spp. in milk, urine, aborted materials, and vaginal secretions, all of which are potential sources of human infection (66,36). Brucellosis in humans can result from direct contact with infected animals or their secretions, consumption of unpasteurized milk, and inhalation of contaminated aerosols during animal handling or abortion (67). Several outbreaks of human brucellosis in the Middle East and East Africa have been linked directly to camel milk and close contact with infected animals (68,37). A lack of awareness about zoonoses among camel farmers, as well as limited access to vets, allows the disease to remain undetected among both animals and humans (69). Many cases of brucellosis in KPK are not reported since its main symptoms (like fever, tiredness, joint stiffness) can be mistaken for malaria or typhoid (70).

This study demonstrates that brucellosis frequently causes a significant amount of reproductive failure in camels since there is a direct correlation between a history of abortion and brucellosis positive results. The ability of infected males to reproduce may be diminished by orchitis or epididymitis, while infected females may undergo late-term miscarriage, infertility, and difficulty expelling the placenta (62). Chronically diseased camels may give less milk and conceive less frequently, which can significantly reduce the profitability of raising them. According to a survey conducted in Ethiopia, the annual economic losses in smallholder camel herds due to reproductive failures caused by brucellosis were estimated to be between 15% and 25% (61).

Additionally, they discovered that a significant portion of the production losses in Sudanese camel herds were caused by brucellosis. Since camels are the primary source of milk, meat, transportation, and revenue for people living in pastoral areas, these kinds of losses are particularly significant in KPK (71). Preventing brucellosis has a direct impact on poverty reduction, rural business support, and food security. Additionally, the loss of breeding camels due to brucellosis impacts both individual families and entire communities because camels are commonly utilized as dowry or trade goods (72). Due to their lengthy lifespans and slow rate of reproduction, camel populations require a long time to recover from inbreeding.

The findings indicate that brucellosis remains a prevalent issue among southern Khyber Pakhtunkhwa (KPK) camels. The seroprevalence rates found by iELISA and RBPT indicate a persistent spread of *Brucella* infections, especially in bigger, communally managed herds and those with a history of abortion. As is typical in similar communities around the world, this supports the findings of previous research and official declarations that reproductive issues and herding techniques play a major role in the development of brucellosis in camels (66). Some of the risk factors studied here did not reach statistical significance, but their significance in epidemiology supports other studies showing that practices in animal husbandry and contact between species help spread diseases (59). Animals found to be seropositive and especially those with reproductive issues must be quarantined or removed to keep horizontal transmission cycles stopped during vital reproductive stages. Brucellosis, being a zoonotic

disease, is a major concern for public health. Drinking raw camel milk and not being aware of how to stay safe among herders helps to maintain the virus in people (73). For this reason, well-targeted efforts should be made to raise awareness of biosecurity and safe ways to handle animals. Vaccination strategies, although currently lacking camel-specific options, might benefit from pilot trials using vaccines developed for small ruminants, particularly in mixed-species farming systems endemic for *Brucella melitensis* (67).

To effectively and sustainably control brucellosis, a broader One Health approach is required. Disease surveillance, outbreak management, and risk communication can all be enhanced when veterinarians, doctors, epidemiologists, and environmental professionals collaborate (69). To safeguard the public and stop the spread of disease, authorities must mandate testing before animals are moved and prohibit the sale of unpasteurized milk from herds that have not been tested (70,73). Overall, this study offers vital epidemiological data that helps with resource sharing and veterinary public health decisions in Pakistan's arid and semi-arid regions, with the goal of reducing the long-term risk of camel brucellosis to humans and animals.

### Conclusion

The study in southern Khyber Pakhtunkhwa, Pakistan, found camel brucellosis prevalence of 9%

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by iELISA and 13% by RBPT, showing moderate occurrence. Camels aged 5 years, those with abortion history, and males (due to herd movement) had higher seropositivity. Other factors like herd size, body condition, and communal water access showed epidemiological relevance but not always statistical significance. iELISA proved more reliable than RBPT, highlighting the need for awareness, better management, and a One Health approach for effective control.

### Author's contribution

**Conceptualization:** MS, AZ

**Methodology:** MS, AB

**Formal analysis:** JK, IS

**Writing reviews and editing:** MU, AUR

All authors have read and agreed to the published version

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All authors declare no conflict of interest

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and promising therapeutic/medicinal application of camel milk as a functional food in human and animals: A comprehensive review. *Animal Biotechnology*. 2023 Oct 2;34(6):1988-2005.

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