The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 9, 2025

PREVALENCE AND DETERMINATION OF RISK FACTORS FOR THE OCCURRENCE OF BRUCELLOSIS IN CATTLE AT KARACHI

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DOI: https://doi.org/10.5281/zenodo.17163971

Keywords
Brucellosis, Cattle, Risk factors, indirect ELISA, Zoonotic disease, Seroprevalence

Article History
Received: 05 July 2025
Accepted: 05 September 2025
Published: 20 September 2025

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Abstract

Background: Brucellosis remains a major zoonosis undermining cattle productivity and posing public-health risks through occupational exposure and consumption of unpasteurized dairy. This study estimated the prevalence of brucellosis and identified associated risk factors in cattle kept around Karachi, Pakistan. Methods: A cross-sectional survey was conducted in which 125 samples were collected: 100 blood samples (72 crossbreed, 28 indigenous) and 25 vaginal swabs from clinically suspect animals. Sera were screened using an indirect ELISA. Vaginal swabs were cultured on Brucella selective agar and isolates identified by microscopy and standard biochemical tests. Risk factors, locality, age groups (<4 years, >4 years) and sex were assessed using chi-square tests in SAS 8.1; significance was set at $p<0.05$. Results: Overall seroprevalence was 8.0% (8/100). Prevalence was higher in crossbreeds cattle (12.0%) than in indigenous breeds (4.0%). By sex, females showed 14.0% positivity versus 2.0% in males ($\chi^2=11.77$, $p=0.0082$). By age, prevalence was 12.0% in >4-year animals and 4.0% in <4-year animals ($\chi^2=5.42$, $p=0.1434$). Locality-wise seroprevalence was highest in Cattle Colony (16.0%), followed by Gulshan-e-Hadeed (8.0%), Sohrab Goth (4.0%) and Malir (4.0%); differences were not significant ($\chi^2=13.14$, $p=0.1564$). Brucella abortus was isolated from 1/25 (4.0%) vaginal swabs but not from blood. Isolates exhibited catalase-, oxidase- and urease-positive, and indole-, methyl-red- and Voges–Proskauer-negative profiles, consistent with B. abortus. Conclusions: Brucellosis is present in Karachi's cattle population with an 8.0% overall seroprevalence, disproportionately affecting females, older animals and crossbreds. Although locality differences were not statistically significant, the burden concentrated in the Cattle Colony warrants targeted interventions. Surveillance using ELISA, biosecurity improvements (testing of replacements, safe disposal of abortus materials), hygiene at calving, education, and milk pasteurization are recommended to reduce transmission. Future studies with larger, stratified samples and multivariable analyses are needed to quantify the independent effects of husbandry practices and to evaluate vaccination and test-and-segregate strategies suitable for peri-urban dairy systems.

INTRODUCTION

Cattle are domesticated animals that belong to the Bovidae family and are primarily raised for their meat (beef), milk, and hides. They play a vital role in agriculture and the global economy, with their contributions spanning various sectors (McLeod, 2011). Domesticated cattle are kept on farms for a variety of uses, such as producing dairy, beef, leather, and veal. Because of their well-developed sentiments and unique traits, they are known to create close relationships with other animals in their herd. Cattle spend much of their time grazing, moving many kilometers each day in their natural habitat. Since they are sociable creatures by nature, they require comfortable resting places and unrestricted mobility. Cattle that receive proper care and attention can live up to 20 years. However, the cattle in commercial beef farms only live for an average of 18 months before being slaughtered (Herrero et al., 2013).

Cattle play a significant role in global food systems, particularly in providing essential nutrients through both meat and milk. Meat from cattle is a key source of high-quality protein, essential amino acids, and micronutrients such as iron and vitamin B12, which are critical for human health (Jimmy et al., 2020). Similarly, milk from cattle is a versatile and widely consumed product, offering a rich source of calcium, vitamins D and B12, and protein, contributing to bone health and overall nutrition (McGuffey, 2017). Beyond nutrition, cattle farming has socioeconomic importance, particularly in rural economies where livestock serve as a source of income, cultural identity, and livelihood (Abu Hatab et al., 2019). With a total of 51.5 million dairy cattle, Pakistan ranks fourth in the world for milk production, producing between 21,691 and 23,357 million tons of milk annually (Ashraf et al., 2021).

Milk and meat are rich sources of essential nutrients that contribute significantly to human health. Meat, particularly beef, is a high-quality protein source that provides all the essential amino acids vital for muscular hypertrophy, tissue regeneration, and immunological efficacy (Kumar et al., 2023). It is also abundant in important micronutrients like iron, zinc, and vitamin B12, which support red blood cell formation, immune health, and cognitive function (Voronina et al., 2022). Similarly, milk offers a range of vital nutrients, including calcium, which is crucial for bone health and the prevention of osteoporosis, as well as vitamins such as B12 and D, which play key roles in metabolism and immune function (Cimmino et al., 2023).

The list of bacteria associated with milk-borne diseases is wide and includes Brucella sp., C. jejuni, B. cereus, E. coli, C. burnetii, L. monocytogenes, M. tuberculosis, M. bovis, M. avium subspecies paratuberculosis, Salmonella sp., and certain strains of S. aureus that produce highly heat-stable toxins (Dhanashekar et al., 2012). Among these, brucellosis remains one of the most significant zoonotic diseases impacting both animal productivity and public health.

Brucellosis is a milk-borne disease caused by Brucella species, primarily *B. abortus* in cattle, and can be transmitted to humans through the consumption of contaminated raw milk and dairy products or direct contact with infected animals, aborted fetuses, or contaminated materials (Corbel, 2006; Glynn & Lynn, 2008). In humans, the disease is commonly known as undulant fever, presenting as fever, malaise, muscle pain, and other systemic complications if untreated (Pappas et al., 2006). In cattle, brucellosis is characterized by reproductive failure, including abortions, stillbirths, retained placenta, infertility, reduced milk production, and extended calving intervals (Rukhshanda et al., 2010; Mellado et al., 2014).

Several risk factors contribute to the occurrence and spread of brucellosis in dairy cattle, including large herd sizes, mixed-species farming, higher stocking density, shared water sources, natural mating practices, introduction of new animals without testing, and improper disposal of aborted materials (Omer et al., 2000; Terefe et al., 2017). Moreover, individuals involved in dairy farming, veterinary work, meat processing, and abattoir operations are at increased risk of exposure to Brucella infections due to their close contact with potentially infected animals and materials (Ngenzi, 2011).

In Pakistan, brucellosis continues to be a major public health and economic concern, yet limited data are available regarding its prevalence and the risk factors associated with its occurrence in Karachi, one of the country's largest urban and dairy production centers. Understanding the burden of brucellosis in

cattle within this region is essential for improving disease surveillance, adopting effective control strategies, and safeguarding both animal productivity and human health. Therefore, the present study was conducted to isolate and identify *B. abortus*, determine its prevalence, and assess the risk factors contributing to the occurrence of brucellosis in cattle populations at Karachi.

2. Materials and methods

A study was conducted to evaluate the prevalence and risk factors linked to brucellosis. In cattle, focusing on different cross and indigenous cow breeds. Additionally, based on proof of previous abortion in cattle and the existence of questionable clinical abortion cases.

2.1 Collection of samples

A total of 125 samples, 100 blood samples (72 from Cross breed and 28 from Indigenous breed of cattle) and 25 vaginal samples were collected from different locations of Karachi. The collected samples were screened for the Prevalence and Risk factors association of brucellosis through following formula. The animals' jugular veins were used to draw blood samples using single-use, sterile plastic syringes. For sample collection, animals were divided into two age groups i.e. younger (<4 years) and older (>4 years) animals. Before collection of blood samples, a swab of cotton immersed in spirit was rubbed on the place where from the blood was collected. The vein was located and the needle was introduced into the vein and the plunger was driven a little back and the blood was collected without anticoagulant and then left in slanting position to clot at least for half an hour. The vaginal swabs were taken from cattle having an abortion or that had been aborted during sampling process. Then samples were transported to the Central Veterinary Diagnostic Laboratory (CVDL) Tandojam in serology section for microscopic, biochemical and ELISA antibody test.

$$Prevelence\ \% = \frac{No\ of\ Infacted\ Animals}{Total\ No\ of\ Animals\ Observed} \times 100$$

2.2 Examination of samples

2.2.1 Isolation and identification of *Brucella* swab samples

The process of isolating and identifying *Brucella* was conducted following the standard procedures outlined in the OIE manual (2006).

2.2.2 Media preparation and culturing

Media was prepared by suspending 2.17gm of *Brucella* selective agar (Hi Media, India) in 100ml of double distilled water and autoclaved at 15 Ibs pressure, 121°C temperature for 15 minutes and cooled at room temperature. Finally, 5% sterile horse serum and one vial of *Brucella* selective supplement were added aseptically.

Twenty-five (25) clinically suspicious vaginal swab samples were collected and cultured. Following that, these samples were inoculated in Hi Media's *Brucella* Selective Agar. For up to four (04) days, the inoculation plates containing the specimen were incubated at 37°C, and colonies were checked every twenty-four hours.

2.2.3 Microscopic examination

Brucella colonies were collected using a sterile wire loop and combined with distilled water to create a smear on a transparent glass slide. After that, the smear was dried in an incubator and heat-fixed. Ziehl-Nielsen's (differential stain) technique was used to stain the slide. The identification of *Brucella* species was determined by their Gram-negative characteristics, exhibiting a pinpoint smooth appearance and a coccobacilli shape, typically arranged singly, though occasionally found in pairs or clusters.

2.2.4 Biochemical tests

For further Biochemical characterization organism Catalase, Oxidase, Urease, Methyl Red, Voges-Proskauer test and Indole test were also performed for conformation.

2.3 Brucellosis indirect ELISA antibody test

Ninety-six (96) wells of plate were used. Briefly, 10µl of dilution buffer was used to dilute the antigen in

positive and negative control wells. After that the test samples were incubated at 21°C (±5 °C) for 45 ±4 minutes and each well was washed 3 times, with approximately 300 ml of wash solution. 100µl of conjugate was added into each well and incubated it for 30 minutes at 21 °C (±5 °C). Then each well was washed with washing solution for 3 times. 100 µl of the substrate solution was added to each well and incubated for 15 minutes ± 2 minutes at 21 °C (±5 °C) in the dark. All wells were blocked loading 100µl of the stop solution to each well in order to stop the reaction. The optimal density (OD) was interpreted at 450nm. The sample percentage inhibition value less than or equal to 50% was considered as positive and greater than 60% has considered as negative.

2.4 Determination of risk factors associated with Brucellosis in cattle

A cross-sectional study was conducted to identify the risk factors related with brucellosis occurrence in cattle. Data was gathered using standardized questionnaires distributed to cattle farmers, as well as through laboratory testing of blood samples from cattle for serological evidence of *Brucella* infection. Potential risk factors, including Locality (Cattle colony, Gulshan-e-Hadeed, Sohrab goth, Malir), Age (> 4 years and < 4 years), Sex (Male, Female) were assessed.

2.5 Statical analysis

Statistical Analysis System (SAS) version 8.1 software was used to analyse the data collected on the risk variables for brucellosis in cattle. The chi-square test was applied to categorical data in order to investigate correlations between various risk factors and the incidence of brucellosis. At a p-value of less than 0.05, statistical significance was established.

3. Results

3.1 The overall prevalence of brucellosis in cattle

During present study, 100 blood samples were obtained from cattle breeds (cross breed and indigenous breed) examined by indirect ELISA technique for the prevalence of brucellosis. Table 1 shows that out of 100 blood samples 8 were identified as positive for brucellosis. The overall prevalence of brucellosis in cattle was determined as 8.00%. The highest prevalence was observed in cross breeds 12.00%, followed by Indigenous breeds 4.00%.

Table 1. The overall seroprevalence of brucellosis in cattle

Breeds	Indirect ELISA		
	No of Samples Examined	No of Positive samples	% of positive samples
Cross Breeds	50	06	12.00
Indigenous Breeds	50	02	4.00
Total	100	8	8.00

3.2 Identification of *B. abortus*

In present study, *B. abortus* was isolated from the vaginal swabs and blood. Out of total 25 samples, (01) sample was found positive from vaginal swab. While no isolate was identified from (100) blood samples (Table 2).

Table 2. Isolation and Identification of *B. abortus*

Sample type	No of sample cultured	No of isolates	Percentage (%)
Vaginal swab	25	01	4
Blood	100	00	0
Total	125	01	0.8

3.3 Biochemical characteristics of *B. abortus*

Brucella organisms stained red against blue back ground, under modify Ziehl-Neelsen stain. The present *Brucella* species were found to be catalase, oxidase and urease positive. While indole, methyl red and Voges-Proskauer test negative (Table 3).

Table 3. The biochemical characteristics of *B. abortus*

S. No	Tests	Results
1	Oxidase	+ ve
2	Urease	+ ve
3	Catalase	+ ve
4	Indole test	-ve
5	Methyl red test	-ve
6	Voges-Proskauer test	-ve

3.4. The gender wise prevalence of brucellosis in cattle

Fig. 1 shows that out of 100 blood samples comprising 50 females and 50 males were collected and tested. The prevalence of brucellosis was identified 14.00% in females, followed by 2.00% in males.

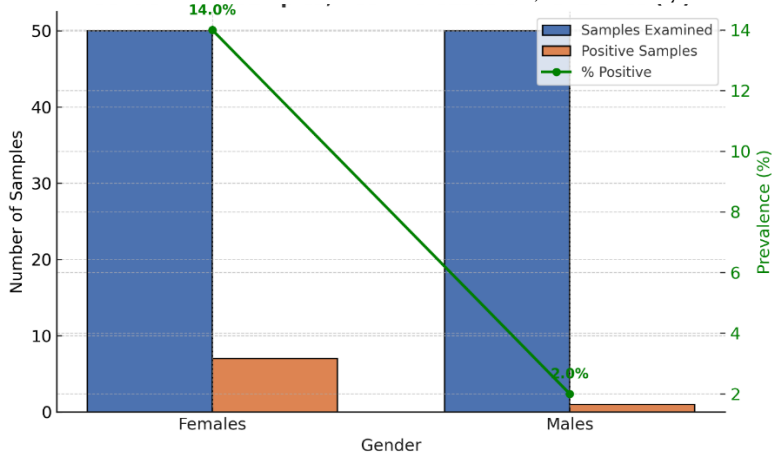

Fig. 01 The gender wise prevalence of brucellosis in cattle

3.5 The age wise prevalence of brucellosis in cattle

Out of 100 blood samples 50 from cattle older than 4 years and 50 from younger than 4 years of age were examined. The highest prevalence recorded among older animals (>4 years) was 12.00%. While the lowest prevalence was observed in youngest animals (<4 years) 4.00% and is showed in fig. 2.

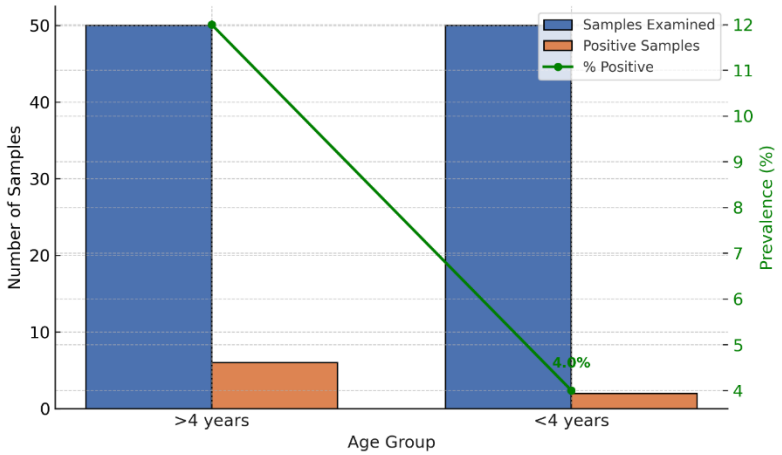

Fig. 2. The age wise prevalence of brucellosis in cattle

3.6 Assessment of risk factors associated with the seropositivity of Brucellosis in Karachi

Using chi-square test factors were assessed for the prevalence of Brucellosis. Among them, one was found extremely significant and two was found non-significant.

3.7 Locality

Information with respect to assessment of the locality as risk factor is exhibited in fig 3. Data regarding association between prevalence of brucellosis and different localities of Karachi shows that Cattle colony had the highest prevalence rate 16.00% followed by Gulshan-e-Hadeed 8.00%, Sohrab goth 4.00% and Malir 4.00%. There was no significant variation ($p>0.05$) observed within the brucellosis outbreak and localities of Karachi.

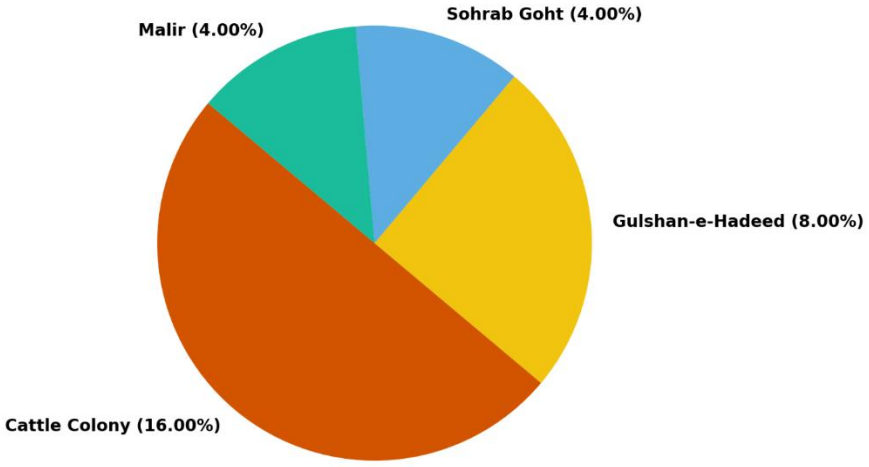

Fig. 3. Association between locality and brucellosis in cattle

3.8 Sex

The findings regarding association between sex and prevalence of brucellosis in cattle are shown in Table 4.7. The study indicated that the prevalence of brucellosis was higher in females 14.00%, followed by males 2.00%. The data reveals a highly significant variation ($p<0.05$) between both male and female.

Table 4. Association between sex and brucellosis in cattle

Sex	No of Animals Examined	No of Animals affected	prevalence (%)	X^2 (p-value)
Females	50	07	14.00	11.77(0.0082)
Males	50	01	2.00	
Total	100	8	8.00	

3.9 Age

Age of the animals were analyzed in two categories i.e. below 4 year and above 4 years (Table 4.8). According to data the prevalence rate of brucellosis was high 12.00 % in >4 years age group and least 4.00% <4 years of age group. There was no significant ($p<0.05$) difference between the age categories of cattle.

Table 5. Association between age and brucellosis in cattle

Age	No of Animals Examined	No of Animals affected	prevalence (%)	X^2 (p-value)
> 4 years	50	06	12.00	5.42(0.1434)
< 4 years	50	02	4.00	
Total	100	08	8.00	

4. Discussion

Brucellosis is a contagious bacterial infection caused primarily by *B. abortus*. This disease leads to reproductive issues such as abortions, stillbirths, and reduced milk production, significantly impacting livestock productivity and economic losses for farmers. It is a zoonotic disease, presenting a risk to human health by direct contact with diseased animals or the ingestion of contaminated dairy products. Effective control measures include vaccination, regular testing, and culling of infected animals to prevent the spread of the disease (Food and Agriculture Organization FAO (2023).

The present study was directed to evaluate the prevalence of brucellosis in Karachi. Out of 100 blood samples, 08 were identified as positive for brucellosis. The overall prevalence of brucellosis in cattle was determined as 8.00%. The highest prevalence was observed in cross breeds 12.00%, followed by Indigenous breeds 4.00%. Similarly, Awais et al. (2024) and Shehzad et al. (2021), identified the prevalence rate of brucellosis in Indigenous breeds as 5.0% to 15.0%, followed by cross breeds as 2.05% to 28.90% respectively. Bovine brucellosis in India has been documented, with a national average seroprevalence of 5% in cattle (Renukaradhya et al., 2002). The incidence of brucellosis infection in cattle in Punjab, India, was reported to be 9.9% (Dhand et al., 2005). Kamwine et al. (2017) documented a prevalence of 26.5% of *Brucella* in Uganda. Iran and Sri Lanka have shown an extensive range of prevalence estimations, with an overall brucellosis prevalence of 0.7% in cattle (Ahasan et al., 2017). The incidence of brucellosis in cross-breed cattle was 23.6%, while in indigenous cattle it was 13.8% (Khan et al., 2021).

In the present study, 4.00% *B. abortus* was isolated from the Vaginal swab samples. While no isolate was identified from blood samples. The single positive result from the vaginal swab indicates that *Brucella* can be localized in reproductive tissues, aligning with the known pathophysiology of brucellosis, where the bacteria primarily affect the reproductive organs of infected animals (Ali et al., 2014). Geresu et al. (2016) and Dag et al. (2012), conveyed that *B. abortus* were isolated from vaginal swab as 8.69% and 9.57%.

Present study showed that *Brucella* organisms stained red against blue back ground, under modify Ziehl-Neelsen stain. In present study *Brucella* species were found to be catalase, oxidase and urease positive. While indole, methyl red and Voges-Proskauer test

negative. Corbel, (2006), Ali et al. (2014) and Anbazhagan (2024), reveled that positive findings for catalase, oxidase, and urease tests align with the biochemical characteristics of *B. abortus*, the species predominantly linked to cattle, while indole and methyl red test negative. Similarly, the biochemical characterization of isolates was oxidase-positive, catalase-positive and urease positive (Kim et al., 2009). The biochemical characterization of isolates was oxidase-positive, catalase-positive, urea of basic fuchsin dye medium (Dag et al., 2012).

During this study, 100 blood samples comprising 50 females and 50 males blood samples were collected and tested. The prevalence of brucellosis was identified 14.00% in females, followed by 2.00% in males. Efrem et al. (2023) and Van Loo, (2023) identified that the prevalence rate of brucellosis 2.2% and 2.4% in females, which are more suspectable due to the factors such as hormonal changes, reproductive statue and close contact with infected animals. In contracts, male cattle generally exhibit lower prevalence rates as 0.5% and 1.4% due to their lesser involvement in the transmission cycle through abortion or calving.

In the existing investigation old animals above 4 years of age seem to be more susceptible to brucellosis. The highest prevalence recorded among older animals (>4 years) 12.00%. While the lowest prevalence was observed in youngest animals (<4years) 4.00%. Comparable results have been accounted by Rahman et al. (2011) and Aulakh et al. (2008) who reported that growing up animal >4 years of age groups appeared to be more suspectable to *B. abortus as* 3.54% and 22.2. while the lowest seroprevalence was observed in youngest animals <4 years as 1.45% and 12.3% respectively.

During the present study, data regarding association between prevalence of brucellosis and different localities of Karachi shows that Cattle Colony had the highest prevalence rate 16.00% followed by Gulshan-e-Hadeed 8.00%, Sohrab Goth 4.00% and Malir 4.00%. There was no significant variation ($p>0.05$) observed within the brucellosis outbreak and localities of Karachi. Awais et al. (2024) The study indicated that the prevalence at the farm level was highest in Tehsil Multan at 43.75%, followed by Shujabad at 31.03% and Jalalpur Pirwala at 27.27%; however, the differences were not statistically significant (P=0.545). similarly Segwagwe et al. (2018) reported that in India, Katabagemu had the highest overall prevalence of brucellosis at 28.6%, followed by Rwimiyaga at 17.3%, Gatunda at 14.3%, and Karangazi at 5.6%. The statistical study applying the chi-square test for independence indicated no significant variation in brucellosis prevalence across the various industries ($p > 0.05$).

The findings regarding association between sex and prevalence of brucellosis in cattle indicated that the prevalence of brucellosis was higher in females 14.00%, followed by males 2.00%. The data reveals a highly significant variation ($p<0.05$) between both male and female. Rajeswari et al. (2014) reported that sex-wise prevalence of brucellosis in males 6.45%, followed by females 6.62%. The prevalence of brucellosis in male animals was 16.7%, according to Segwagwe et al. (2018), whereas in female animals it was 19%. There was a statistically significant difference between the proportion of female animals tested positive for *Brucella* and those tested negative (81.0 vs. 19.0%). In terms of total seropositivity, there is no statistically significant difference between male animals (12.05%) and female animals (12.59%) (Bayemi et al., 2009). Although there was a statistically insignificant correlation between the sexes, 25.4% of female animals and 6.5% of male animals tested positive (Khan et al., 2021).

In the current study the age of the animals was analyzed in two categories i.e. below 4 year and above 4 years. According to our findings the prevalence rate of brucellosis was high 12.00 % in >4 years age group and least 4.00% in <4 years of age. There was extremally significant ($p<0.05$) difference between the age categories of cattle. The age groups of cattle showed a highly significant difference ($p<0.05$), as reported by Khan et al. (2021). The results of the multivariate analysis showed that the seroprevalence was 27.1% in adults and 4.7% in younger animals, with a significant difference ($p < 0.001$). The disease prevalence varied with age, according to Kumar et al. (2005) the lowest prevalence was 0.6% in the up to two years age group, followed by 11.8% in the two to four years age group, 12.4% in the four to six years age group, and 15.8% in six to eight years age group. In animals older than eight years, the seroprevalence was 13.6%. Various age groups had statistically

varied prevalence rates when all data were considered ($p = 0.04$).

5. Conclusion

The findings of this study indicate that brucellosis is widespread among all cattle breeds in the province of Sindh. The highest occurrence of brucellosis was noted in cross breeds in contrast to local cattle breeds of Sindh province. The only bacterial species identified from cattle in the current study was *B. abortus*. The study also concluded that female cattle are at a higher risk of brucellosis compared to male cattle. In the course of the current investigation, the indirect ELISA emerged as the most effective technique, demonstrating superior sensitivity, specificity, and accuracy in the detection of *Brucella* antibodies in cattle sera.

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