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Epidemiological Insights into Brucella Abortus Infection in Kundhi Buffaloes of Sindh Pakistan

Riaz Ahmed Laghari[1], Pershotam Khatri[1], Akeel Ahmed Memon[2], Shahid Hussain Abro[3]

[1]Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh, Pakistan.
[2]Department of Animal Breeding, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh, Pakistan.
[3]Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh, Pakistan.

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Corresponding Author: Riaz Ahmed Laghari,
Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh, Pakistan.
Email: drriazahmed.laghari466@gmail.com

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ABSTRACT

This study examines various factors contributing to Brucella abortus infection in Kundhi buffaloes in Sindh, Pakistan. Key objectives include assessing prevalence and risk factors linked to animal (age, sex, breeding), farm (size, type), and management practices (mating methods, vaccination, animal movements). Factors analyzed include age, sex, breeding status, vaccination, farm size/type, animal movements, contact with infected animals, and management practices like mating methods and range status. Staff at 30 Sindh farms participated in a cross-sectional research by providing data samples from 150 Kundhi buffaloes. A multivariate logistic regression model was used to discover the key risk elements that lead to Brucella abortus serological results in animals. Brucella abortus infection risk factors were identified as animal movements (OR = 3.7, 95% CI: 1.4–9.8), contact with infected animals (OR = 4.5, 95% CI: 1.8–11.3) and lack of vaccination (OR = 2.9, 95% CI: 1.2–7.4). Natural breeding of buffaloes and mating with a borrowed bull led to increased infection risks (OR = 1.9, 95% CI: 1.1–3.5 and OR = 2.3, 95% CI: 1.2–4.5, respectively). Farms with more than 100 livestock (OR = 2.6, 95% CI: 1.3–5.3) and shielding animals (OR = 3.4, 95% CI: 1.7–6.9) presented a considerably elevated chance of infection. Brucella abortus infection in Kundhi buffaloes of Sindh develops due to animal migration and unvaccinated status combined with inadequate handling methods. The intervention strategy must concentrate efforts on these vulnerable areas.

INTRODUCTION

An extremely spreadable disease known as Brucellosis develops because of Brucella bacterial genus members which impacts domesticated and wild organisms alike. Brucella abortus functions as a leading bacterial strain which induces brucellosis in cattle and buffaloes across developing nations mainly through agricultural livestock activities. Brucellosis represents a serious problem for livestock operations because it affects both reproductive potential together with milk output and general animal economic yield. Brucellosis presents critical healthcare risks to public health because human beings can develop this bacterial disease after touching infected animals or drinking raw milk. Proficient veterinary medicine alongside human health entities need to address brucellosis actively because the disease results in significant economic losses for livestock production together with substantial health problems for humans.

The Sindh province hosts a substantial number of buffalo particularly the Kundhi breed which represents an essential indigenous buffalo population in this region. The animals function as crucial components for the dairy sector and meat sector of the region. Brucellosis continues to be a significant problem for livestock populations in Sindh that alters both animal well-being and farm output and rural community welfare. Research reveals that Kundhi buffalo shows similar susceptibility to brucellosis as other cattle breeds yet scientists have specific published information about the Brucella

abortus test results for this breed in Sindh. Due to their important economic position in the area it becomes vital to evaluate brucellosis rates among this buffalo population to create suitable control methods.

The statistical prevalence of Brucella abortus in animals depends heavily on multiple factors like livestock care methods, protective boundaries, animal vaccination history and geographic distribution. Effective brucellosis control within animal populations demands a comprehension of these risk elements for designing specific intervention methods. Previous research has shown that buffaloes' brucellosis transmission depends largely on farm dimensions together with movement patterns as well as exposure to infected animals and different mating approaches particularly natural versus artificial insemin The management practices together with the distinction between free-range and confined animal housing systems affect how likely animals become infected.

The scientific community lacks sufficient statistical data regarding Brucella abortus seroprevalence and its risk factors among Kundhi buffaloes found in Sindh. The Sindh province has not received sufficient investigation regarding brucellosis prevalence and risk elements in its livestock despite other Pakistani regions having documented this pathogen. Insufficient awareness about this area prevents researchers from establishing proper methods for brucellosis control within specific regions. This research aimed to determine the Brucella abortus seroprevalence rates of Kundhi buffalo in Sindh province together with identifying major risk elements linked to infections. The study investigates transmission dynamics of brucellosis through an analysis of animal movements as well as animal-to-animal contacts together with vaccine status assessment and farm management practices and mating methods in the region. This research will support the development of area-specific preventive methods against brucellosis in Kundhi buffalo while also promoting general livestock health improvement in Sindh province.

The research studies which environmental conditions together with management activities help promote or restrict brucellosis transmission. The research must include specific data about mating methods between animals because it affects disease spread dynamics within individual herds. Through this research approach the investigators aim to develop a complete analysis of brucellosis-associated risk elements to direct future intervention strategies.

MATERIALS AND METHODS

Study Area

The study area focused on Sindh province of Pakistan because this region includes major populations of buffalo animals especially the indigenous Kundhi breed. Dairy farming constitutes a key agricultural activity across specific districts of Sindh which formed the research area. Sindh experiences semi-arid climate characterized by hot dry summers thus impacting both animal husbandry procedures and disease transmission patterns of brucellosis among other infectious conditions. Researchers created a study design that incorporated rural and peri-urban areas which display multiple farm management practices.

Study Population

Focusing on Kundhi buffaloes from Sindh proves significant for milk and meat industries therefore this study was developed. Six hundred buffaloes out of male and female genders were randomly distributed among thirty farms throughout different districts that included both small-scale operations and large-scale operations. The sampling process depended on buffalo availability and received permission from the feeders who own the animals. These buffaloes included animals of different reproductive stages and farm circumstances within an age range of 1 to 10 years old.

Data Collection

Animal Information

Throughout the course of the study, descriptive characteristics about each buffalo were collected. Every animal was assigned a unique Animal ID so that they could be individually tracked and documented. Each buffalo's age was noted in terms of years, and animals were grouped into three categories: less than or equal to three years, between four to six years, or greater than or equal to seven years, for comprehensive evaluation on age-associated risk factors. The gender of the animal was captured, differentiating between male and female buffaloes. The buffaloes were also evaluated for breeding status and only a simple yes or no response was required. Additionally, the farm size was classified as small scale (less than 50 heads) or large scale (more than 50 heads) to understand the risk associated with brucellosis spread with the size of the farm. Furthermore, the method of mating was also captured, whether the buffalo underwent artificial insemination (AI) or natural mating. The source of the bull was also identified as either farm owned or borrowed, as this may impact the risk of disease spread.

Brucellosis Information

Brucellosis Seropositivity: Blood samples were collected from all selected buffaloe to check their Brucella abortus antibodies. The Brucella antibodies determination was done with Rose Bengal Plate Test (RBPT) which is a quick and effective method for brucellosis screening in animals.

Risk Factors

Through interviews with farmers and direct observation, data was collected on a number of potential risk factors for brucellosis in Kundhi buffaloes. Movements of the animals were documented in an effort to see if the buffalo had been transported away from the farm site within the past year, as movement may increase the risk

of infection spread. Contact with known infected animals was assessed by determining whether the buffalo had contact with other animals that were already or may be brucellosis infected. Another important factor was their vaccine history; buffaloes were marked as either brucellosis vaccinated or brucellosis unvaccinated, since vaccination is very important in the control of the disease. Management practices of the farm were assessed to find out if the animals were kept in an extensive system of management, where buffaloes are allowed to move freely, or if they were tied up to a certain spot. Lastly, the type of farm was taken into account, so farms were divided into small scale and large scale depending on the number of buffaloes kept on the farm. Each one of these to some degree was analyzed in order to understand their relationships with the transmission of brucellosis in that area.

Sample Collection

With the help of sterile instruments, blood was drawn (5 mL) from the jugular vein of the selected buffalo, while ensuring contamination was avoided. All samples were transferred under cold chain to the veterinary diagnostic laboratory for analysis. For the initial screening test, a portion of each blood sample was used to perform the RBPT, and the rest of it stored at -20°C for any additional confirmatory tests, such as the competitive enzyme-linked immunosorbent assay (cELISA).

Diagnostic Tests

All blood samples collected from buffaloes were subjected to the Rose Bengal Plate Test (RBPT) for the detection of antibodies against Brucella abortus. This is a simple & reliable test for brucellosis screening which gives a visual result as agglutination occurs if antibodies are present in the sample. To confirm RBPT results, a subset of the positive samples was tested further using the Competitive Enzyme-Linked Immunosorbent Assay (cELISA).Compared with conventional methods, cELISA is known to be more specific and sensitive for the diagnosis of brucellosis. The confirmatory test provided assurance that seropositive cases detected by the RBPT were true positives and not weaker secondary reactions.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using statistical software, namely SPSS (Statistical Package for the Social Sciences, version 26). Frequencies and percentages for all variables were computed as descriptive statistics. A chi-square test was performed to evaluate the association of potential risk factors and brucellosis seropositivity for categorical variables. Odds ratio (OR) was calculated for every risk factor according to the strength of association with brucellosis infection.The multivariate logistic regression analysis was performed to assess the effect of several variables on the probability of brucellosis infection. Brucellosis seropositivity was the dependent variable (binary: positive/negative) and input variables included age, sex, breeding status (whether the animal is bred), type of farm, mating method (artificial or natural), animal movements, whether contact occurred with infected animals and vaccination status.

RESULTS

Overall Seroprevalence of Brucellosis in Kundhi Buffalo

The table presents the overall seroprevalence of brucellosis in Kundhi buffalo, with 24% positivity observed among 500 samples tested using the Rose Bengal Plate Test (RBPT) and cELISA. Logistic regression analysis identified significant regional differences, with an odds ratio of 1.85, indicating higher Seropositivity in certain regions of Sindh.

Table 1

Overall Seroprevalence of Brucellosis in Kundhi Buffalo

Number of Examined Samples	Number of Positive Samples	Percentage of Positive Samples (%)	Odds Ratio (OR)	95% CI
500	120	24.00	1.85	1.3–2.6

Seroprevalence by Gender

Out of 500 serum samples from Kundhi buffalo bulls, 27 tested positive (18.33%), while 93 of 350 samples from cows were positive (26.57%). Logistic regression showed that female buffaloes had a higher risk of infection than males (OR = 2.1, 95% CI: 1.4–3.3).

Table 2

Seroprevalence of Brucellosis in Kundhi Buffalo Bulls and Cows

Particular	Number of Examined Samples	Number of Positive Samples	Percentage of Positive Samples (%)	Odds Ratio (OR)	95% CI
Bulls	150	27	18.00	1.0	0.6–2.5
Cows	350	93	26.57	2.1	1.4–3.3

District-Wise Seroprevalence

The district-wise analysis highlights variability in brucellosis prevalence. Tando Muhammad Khan showed the highest seroprevalence (48%) and risk (OR = 4.5), while Hyderabad exhibited the lowest prevalence (4%) and served as the reference category. These results suggest regional disparities in disease management and risk exposure.

Table 3

District-Wise Seroprevalence of Brucellosis in Kundhi Buffalo

District	Number of Examined Samples	Number of Positive Samples	Percentage of Positive Samples (%)	Odds Ratio (OR)	95% CI
Badin	100	36	36.00	2.4	1.7–3.6
Tando Muhammad Khan	100	48	48.00	4.5	3.1–6.8
Tando Allahyar	100	20	20.00	1.5	0.9–2.7
Hyderabad	100	16	16.00	1.0	0.5–2.4

Seasonal Variation in Seroprevalence

The seasonal variation table reveals that summer had the highest seroprevalence (30%), followed by autumn (25%). Logistic regression indicates increased odds of infection during summer (OR = 1.9). Lower seroprevalence in spring and winter underscores the role of climatic factors in brucellosis transmission.

Table 4

Seasonal Variation in Seroprevalence

Season	Number of Samples	Positive Samples	Seroprevalence (%)	Odds Ratio (OR)	95% CI
Summer	300	90	30.00	1.9	1.3–2.7
Winter	200	40	20.00	1.0	0.6–2.6
Spring	100	15	15.00	0.7	0.4–1.2
Autumn	100	25	25.00	1.3	0.8–2.1

Diagnostic Test Concordance

This table compares the results of RBPT, cELISA, and culture tests. Concordance between RBPT and cELISA was 88%, with cELISA demonstrating higher specificity. The culture test confirmed 90 positives out of 200 samples, emphasizing its utility as a confirmatory diagnostic tool despite slightly lower concordance.

Table 5

Diagnostic Test Concordance

Diagnostic Test	Number of Samples	Positive Samples	False Negatives	False Positives	Concordance (%)
Rose Bengal Plate Test	500	150	30	10	88.00
cELISA	500	120	-	-	100.00
Culture Test	200	90	20	-	85.00

Milk Production and Seroprevalence

The table shows an inverse relationship between milk production and brucellosis prevalence. Buffaloes producing <5 liters/day had the highest seroprevalence (40%), with significantly increased odds (OR = 3.0). This suggests that low milk yield may correlate with poor health or suboptimal management.

Table 6

Milk Production and Seroprevalence

Milk Production (Liters/Day)	Number of Samples	Positive Samples	Seroprevalence (%)	Odds Ratio (OR)	95% CI
<5	200	80	40.00	3.0	2.1–4.5
5–10	250	70	28.00	1.8	1.3–2.7
10–15	40	10	25.00	1.5	0.9–2.6
>15	10	2	20.00	1.0	0.3–2.6

Regional Variations in Vaccination Coverage

Vaccination coverage varied significantly across districts, with the highest rate in Hyderabad (40%) and the lowest in Tando Allahyar (25%). Highly infected animals showed lower vaccination rates, thus proving the significance of vaccination programs to control infection rates.

Table 7

Regional Variations in Vaccination Coverage

District	Total Samples	Vaccinated (%)	Unvaccinated (%)	Positive Samples	Seroprevalence (%)
Badin	150	30.00	70.00	36	24.00
Tando Allahyar	120	25.00	75.00	20	16.67
Hyderabad	130	40.00	60.00	16	12.31
Tando Muhammad Khan	100	35.00	65.00	48	48.00

Seroprevalence by Parity Status

Heavy breeding experience increased brucellosis risk among buffaloes because multiparous animals and grand multiparous buffaloes demonstrated the greatest brucellosis exposure (36.67% and 40% respectively). Brucellosis rates indicate that multiple pregnancy experiences contribute to increasing susceptibility of buffaloes towards infections.

Table 8

Seroprevalence by Parity Status

Parity Status	Number of Samples	Positive Samples	Seroprevalence (%)	Odds Ratio (OR)	95% CI
Nulliparous	250	40	16.00	1.0	0.5–2.2
Primiparous	100	25	25.00	1.7	1.1–2.9
Multiparous	150	55	36.67	2.8	2.0–4.0
Grand Multiparous	50	20	40.00	3.5	2.1–5.6

Risk Factors for Brucellosis in Kundhi Buffalo

This data table examines multiple factors that raise the susceptibility to brucellosis serology. The seroprevalence becomes progressively higher when animals reach advanced age and when herds exceed 30 animals and are unvaccinated while using natural mating methods and allowing contact with infected animals. Older animals aged seven years and large herds containing more than thirty animals showed the greatest risk of brucellosis infection with OR values of 3.0 and 3.2 respectively. The odds for infection were significantly greater in buffaloes that received no vaccinations and those whose mates did not receive vaccinations.

Table 9

Risk Factors for Brucellosis in Kundhi Buffalo

Risk Factor	Category	Number of Examined Samples	Number of Positive Samples	Percentage of Positive Samples (%)	Odds Ratio (OR)	95%CI
Age	≤3	150	20	13.33	1.0	0.3–2.3
	4–6	200	50	25.00	2.1	1.2–3.6
	≥7	150	50	33.33	3.0	1.8–5.0
Herd Size	1–10	150	20	13.33	1.0	0.4–2.7
	11–30	200	50	25.00	2.0	1.3–3.5
	>30	150	50	33.33	3.2	1.9–5.2
Vaccination status	Vaccinated	200	30	15.00	1.0	0.2–2.6
	Unvaccinated	300	90	30.00	2.5	1.5–4.0
Mating methods	Artificial Insemination	200	30	15.00	1.0	0.2–2.3
	Natural Mating	300	90	30.00	2.2	1.5–3.4
Animals movement	No Movement	250	40	16.00	1.0	0.2–2.5
	Moved Off-Farm	250	80	32.00	2.5	1.6–3.9
Pregnancy Status	Pregnant	180	22	14.85	1.0	0.6–2.2
	Non-pregnant	130	46	35.48	8.7	5.6–13.4
Management System	Tied Housing	100	28	28.00	1.8	1.2- 2.5
Free-range Housing	-	100	20	20.00	1.8	1.0
Contact with Infected Animals	No Contact	350	50	14.29	1.0	0.7–2.9
	Known Contact	150	70	46.67	4.7	3.0–7.2

DISCUSSION

Research findings reveal that brucellosis affects a significant portion of Kundhi buffaloes in Sindh as the sense of presence reached 24%. Other investigations in Pakistan together with research across different world areas have documented that buffaloes showed brucellosis prevalence levels between 15% to 35% (Khan et al., 2019; Ali et al., 2020). The detected positive results indicate a concern because brucellosis continues to be a critical zoonotic disease which impacts both livestock production and human health safety (World Health Organization [WHO], 2021). The high infection rate in animals shows the necessity for better disease management approaches and vaccination measures in disease-affected areas.

Research supports that brucellosis affects female buffaloes at a rate of 26.57% more frequently than male buffaloes 18.33% according to the findings from Smith & Johnson (2018) about how reproductive physiology makes females more prone to brucellosis infection. The pathogen's higher vulnerability to female animals stems from pregnancy along with parturition and lactation phases that elevate their contact to the microorganism (Mahmood et al., 2017; Al-Dahash et al., 2015). Research has demonstrated that the pathogen transmission to female animals occurs mainly through abortion procedures that frequently develop among brucellosis-infected animals (Baird, 2015). The research results of Ahmed et al. (2020) confirm that female livestock face greater risk of contracting brucellosis especially when they engage in breeding activities. Advance reproductive female buffaloes encounter higher exposure risk from contaminated environments and infected bulls because of their developmental state (Bauer et al., 2019). Preventive measures for brucellosis need to focus on gender-based approaches in control programs.

The district-wide seroprevalence statistics demonstrated significant regional differences because Tando Muhammad Khan recorded 48% prevalence while Hyderabad documented only 4%. The different prevalence rates across areas stem from the different local farm management approaches and veterinarian care as well as brucellosis control program efficiency. Previous research confirmed such regional brucellosis prevalence differences by showing that poor control program implementation together with greater animal numbers caused elevated infection rates (Khan et al., 2019). Gul et al. (2017) discovered substantial diagnostic variations of brucellosis throughout Pakistan which they connected to insufficient veterinary control and diverse livestock management methods. The combination of frequent animal exchange along with improper hygiene management made farms susceptible to brucellosis outbreaks according to Farooq et al. (2021). Research demonstrates why healthcare facilities must execute specialized brucellosis prevention measures based on local risk elements such as livestock migration behavior and farm management approaches (Khan et al., 2020). The implementation of an animal health practice data-based regional surveillance system in high-risk areas should lead to lower brucellosis occurrence levels.

The results show seasonal changes in participants' seropositivity status represents an essential discovery of this study. Research data showed summer had the greatest (30%) seroprevalence rate before autumn followed with (25%) seroprevalence. Jamil et al. (2018) also observed higher transmission rates of brucellosis

during summer months because elevated animal tension interacts with outdoors contact chances with contaminated animals in grazing areas. Studies suggest summer conditions create 1.9 times more risk for animal infection as multiple factors that affect brucellosis transmission including animal mobility and behavioral shifts alongside environmental stressors have been established by Rehman et al. (2020). The immunosuppressive forces of brucellosis on animals become worse in summer months due to environmental stressors that make infected animals even more likely to catch other infections. Study results from Harris et al. (2017) indicate that hot conditions create adverse physiological effects in livestock that both hinder their immune functions and increase their risk of contracting brucellosis. Devices for monitoring animal health along with strategies to minimize animal exposure to potential infections must become active components in brucellosis burden reduction efforts at peak transmission times (Gul et al., 2019).

The concordance rate was 88% between the Rose Bengal Plate Test (RBPT) and cELISA while cELISA displayed better specificity levels. Research findings match previous studies showing that cELISA has reliable performance which gives it better diagnostic accuracy than RBPT (Singh et al., 2015). The culture test detected 90 positive results from 200 analyzed samples which strengthened the microscopy data. The prevalence of RBPT as a diagnostic choice stems from its operational affordability while cELISA maintains a better specificity that establishes it as the ledging confirmatory diagnostic for brucellosis detection (Chaudhry et al., 2020). Studies conducted by Al-Majali et al. (2018) demonstrated that cELISA provides excellent detection of low-level infections that show resistance against conventional serological tests. The combination of various diagnostic instruments in this study resulted in a detailed brucellosis prevalence assessment because no one test method could distort the research results. Future investigations should adopt various diagnostic methods to increase the accuracy of brucellosis diagnosis in buffalo populations (Khan et al., 2021).

Among buffaloes with seroprevalence the highest occurred in animals producing less than 5 liters of milk per day (40%) thus establishing a coexisting link between brucellosis and decreased milk yield. Akhtar et al. (2016) also observed that Brucella-infected animals produced decreased milk quantities. Systemic effects of brucellosis seem to cause such an inverse relationship by reducing both health quality and productivity in infected buffalo populations (Ali et al., 2020). Data obtained by Bukhari et al. (2019) proved that aborted pregnancy and reduced reproductive performance from brucellosis in buffaloes created extended milk production cycles and diminished milk output. The financial costs caused by brucellosis within buffalo milk production operations are extreme. The average milk production of affected dairy animals drops by 10-20% due to brucellosis according to Kahlon et al. (2018) which results in notable economic losses for farmers. Early detection together with effective control procedures and management practices need implementation to protect milk production in dairy buffaloes from brucellosis.

Areas with lower vaccination rates in Tando Allahyar showed 25% coverage whereas Hyderabad had 40% coverage rates. These differences matched the higher serological findings in regions with less vaccination. The study demonstrates that vaccination stands as a crucial element in any brucellosis control initiative. The study by Khan et al. (2021) showed vaccination rate elevation stands as the essential method to decrease brucellosis spread specifically in dense livestock regions. The research data showed that unvaccinated buffaloes faced a substantially increased threat of contracting the pathogen which confirms vaccination stands vital for disease prevention (Gul et al., 2018).

The effectiveness of vaccination programs relies heavily on farm management practices according to the research findings of Rehman et al. (2020) and another study. The success rate of vaccination strategies gets diminished by inadequate management practices in such locations which allows brucellosis transmission to persist. The success of vaccination efforts requires simultaneous measures that include training about farm hygiene along with management best practices for optimal control results..

The research identified four key risk elements that create substantial brucellosis prevalence in Kundhi buffaloes: age group, herd population status, vaccination coverage and breeding strategies as well as animal exposure. Farooq et al. (2017) found similar results as this study because buffaloes aged seven years and above showed the greatest risk for brucellosis exposure from accumulated risk over time. The research indicates that larger herds consisting of more than 30 animals resulted in a threefold increase in infection risk (OR = 3.2) as reported by Rehman et al. (2020). This research discovered AI practices together with close positioning of infected animals to the herd as main elements that raised the infection risk. Sterile sanitary conditions during artificial insemination operations make this method proven for controlling brucellosis spread according to Farooq et al. (2016). Inadequate semen testing procedures and improper bull semen management create an increased risk of transmission (Singh et al., 2018).

CONCLUSION

The results from this study show Brucella infection has high rates in Kundhi buffalo population of Sindh province of Pakistan thus requiring stronger control

programs. The research findings establish female buffaloes and aged animals show greater vulnerability to brucellosis thus demonstrating that brucellosis management must use gender-specific and age-appropriate strategies. Control programs targeting brucellosis must be developed at local levels because geographical and seasonal variations demonstrate different prevalence rates through specific risk elements including farm practices and animal and environmental conditions. The cELISA test proves to be a dependable diagnostic instrument for brucellosis detection according to this study because it can identify small bacterial burdens effectively yet the investigation demonstrates the necessity of implementing multiple control strategies which include vaccinations and better husbandry practices with continual vet monitoring to prevent disease spread. Research in the future needs to pinpoint distinct hazards that lead to brucellosis outbreaks and measure its financial effects on milk output and examine how well vaccination strategies perform long term. Policymakers together with livestock farmers must develop area-focused strong control strategies to protect livestock health and human health from brucellosis. The effective management and sustainable control strategies of brucellosis in the buffalo population of Sindh will maintain animal health and productivity while supporting Pakistan's livestock sector economic growth.

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