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INCIDENCES OF BRUCELLA ABORTUS IN SERUM AND MILK SAMPLES OF CATTLE IN RAWALPINDI

Saba Naazir[1], Nida Naazir[2], Tehmina Naazir[2], Adnan Yousaf[1*], Abdul Wakeel[3], Bismillah Noori[1], Hira Aijaz[1], Shaheena Kiran Tunio[1], Faiza Habib[3]

[1]Sindh Agriculture University Tandojam, Pakistan
[2]Department of Livestock and Dairy Development, Punjab, Pakistan
[3]Department of Livestock and Dairy Development, Balochistan, Pakistan

Abstract. The prevalence of Brucella abortus in cattle was determined using n = 800 samples (n = 400 serum and n = 400 blood samples) collected from organised dairy farms in various areas (Chakri, Taxila, Gujar Khan, Kallar Syedan, Dhamial, Kahuta, Kotli Sattian, Murree, Rawat, and Ganda Kass) and the Rawalpindi Main City. Serological techniques such as the Milk Ring Test (MRT), Serum Agglutination Test (SAT), and Rose Bengal Plate Test were used to diagnose Brucella abortus (RBPT). Brucella abortus was discovered in 5.38 % of cattle in Rawalpindi research areas. Using serological tests, the prevalence of Brucella abortus in local breeds was determined to be 6.5 %, 4.75 %, and 4.75 %, respectively. In the Holstein Friesian breed, the prevalence was found to be 4.25 %, 2.25 %, and 1.75 %, respectively. In general, Brucella abortus RBPT (3.25 %), MRT (1.75 %), and SAT (1.75 %) were found in cattle aged 1 to 5 years. The bacterium was shown to be prevalent in cattle aged 6-9 years (7.5 %), MRT (5.25 %), and SAT (4.75 %). In Rawalpindi, Brucella abortus was found in serum and milk samples from cattle. Cattle in the older age groups were shown to be more sensitive to Brucella abortus than those in the younger age groups. Brucellosis was more common in local cattle than in Holstein Friesian cattle.

Keywords: *Brucella abortus, bovine, age, sex, Rawalpindi.*

Corresponding Author: *Adnan Yousaf, Sindh Agriculture University Tandojam, Pakistan, e-mail: dr.adnan011@gmail.com*

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1. Introduction

Brucellosis is a zoonotic and highly contagious bacterial infection that affects cattle (Yousaf *et al*., 2017). This disease is a major health and economic concern all across the world (Munir *et al*., 2010). Malta fever, Bang's illness, contagious abortion, infectious abortion, undulant fever, and Mediterranean fever are all names for Brucellosis. Brucellosis is caused by Brucella abortus, a gram-negative bacteria (Yousaf *et al*., 2015). This organism is also a significant cause of diseases in both animals and humans (Gul et al., 2007). Brucella abortus is a Gram-negative rod or coccobacilli-shaped bacteria that is non-motile, non-sporting, aerobic, and non-motile (Yousaf *et al.*, 2016). The actual frequency of brucellosis in cattle in Pakistan is unknown; however, multiple studies have suggested that the disease is present in Pakistan at rates ranging from 3.25% to 4.4% in various places (Naeem *et al.*, 2012). Brucella abortus is spread mostly through abrasion, lacerated skin, or contact with infected animals fetus, placenta, fetal, and vaginal secretions. Abortion or full-term parturition are the two most common causes of bacteria. This bacteria can also be detected in faeces, milk, and sperm (Habib *et al*., 2019, Yousaf *et al*., 2017). Infected animals become disease carriers for the rest of their lives. Brucellosis can be transmitted to animals by mucosal membranes or ingestion, and the infection can also be spread through direct contact or subsequent shedding of the

organisms in milk (Stableforth *et al*., 2012). Brucella abortus causes infections in a variety of organs, including the uterus, udder, and testes (Yousaf *et al*., 2018). Brucellosis in cattle can cause mastitis, orchitis, abortion, arthritis, and infertility (Nicoletti *et al*., 2012). In several places of the world, the disease has been identified as endemic (Khan et al., 2016). However, with good management, it has been decreased and eradicated in certain developed countries (Hungerford *et al.*, 2012). Previous studies in Pakistan found that cattle were more resistant to brucellosis than buffaloes. Brucellosis was on the rise in Pakistan, especially in large animals, due to poor mental health management (Abubaker *et al*., 2011). Several earlier research found brucellosis outbreaks in government and private livestock farms across Pakistan (Abubaker *et al*., 2017, Hamidullah *et al*., 2019). In Pakistan, brucellosis could be controlled by implementing effective farm management, routine screening, and animal vaccination programmes (Iftikhar *et al*., 2008, Rabab *et al*., 2000). Confirmatory laboratory diagnosis is not performed in ordinary practice due to a lack of appropriate diagnostic facilities and financial constraints (Shafee *et al*., 2011). Brucella infection in calves can be diagnosed using a variety of serological assays, including the milk ring test (MRT), the Rose Bengal Precipitation Test (RBPT), and the serum plate agglutination test (SPAT) (Asif *et al*., 2009, Gul *et al*., 2007). In Pakistan, there was no such governmental policy, although steps were conducted to diagnose and eradicate the condition (Akhtar *et al.*, 2010). The precise number of people infected with brucellosis in Rawalpindi is unclear. As a result, the current study will provide information on the prevalence of brucellosis in cattle in Rawalpindi, as well as information on the disease's relationship to age and different breeds farmed in District Rawalpindi, Punjab Pakistan.

2. Materials and methods

Sample Collection area

Overall, n = 800 samples (n = 400 serum and n = 400 blood samples) collected from organized dairy farms in various areas (Chakri, Taxila, Gujar Khan, Kallar Syedan, Dhamial, Kahuta, Kotli Sattian, Murree, Rawat, and Ganda Kass). Similarly, 200 serum samples were collected from Rawalpindi Main City (Table 1).

Table 1. Serum and milk samples collection area

Farms	**Area**	**Serum Sample**	**Milk Sample**
Local Dairy Farm	Chakri	20	20
	Taxila	20	20
	Gujar Khan	20	20
	Kallar Syedan	20	20
	Dhamial	20	20
	Kahuta	20	20
	Kotli Sattian	20	20
	Murree	20	20
	Rawat	20	20
	Ganda Kass	20	20
Total Main City Rawalpindi		200	200
Sub Total		400	400
G.Total		**800**	

Collection of blood Sample

At District Rawalpindi, a total of n = 800 samples, including blood samples (n=400) and milk samples (n = 400), were collected from various types of cattle. The organism's vulnerability to age and breed will be investigated using data obtained from animals and data acquired from laboratory evaluation of the samples. Using disposable sterilized plastic syringes, blood samples (n = 200) will be taken from animals through the jugular vein. Before taking blood samples, the collection site was cleansed with a cotton swab soaked in spirit and wiped on the area where the blood would be taken. The vein was found, the needle was inserted, the plunger was pushed back, and the blood was collected in a tube, which was then placed in a slanting position for at least half an hour to clot. After that, the blood was refrigerated for the night. The serum was collected the next day in clean screw-capped plastic cry vials and sent in the cool chain container to the SB Lab Rawalpindi for further examination of Brucella species antibodies. To identify the positive samples, diagnostic procedures were performed.

Collection of milk Sample

Milk samples (n = 400) were also obtained from the cattle in the Rawalpindi district. The teats of the animals were adequately cleansed with alcohol prior to sample collection, and the teats were subsequently dried. Then, into sterile tubes, place the first expelled milk. These tubes were placed in ice and transported to the laboratory for testing. Sampling of the research region as a whole.

Rose Bengals Plate Test (RBPT)

SB lab Rawalpindi would provide the Rose Bengal stained Brucella antigen. The antigens were utilized according to the SB Lab Rawalpindi recommendations, and the test protocols were followed as described by (Gabbar, 2016). Using a serological pipette, a drop of 0.03ml of serum was deposited in the center of a square of clear translucent glass slide. On the same slide, a drop of negative control and a drop of positive sera were placed individually on the square. From the vial, a drop of 0.03ml antigen suspension was taken and placed near the drop of sera on the square. The serum and antigen were thoroughly mixed with an applicator stick, and each mixture was distributed in the shape of a circle over a 1.5 cm radium. After then, the slide was gently bounced back and forth for four minutes. The presence of granules of varying intensities in the serum of the animal infected with specific species of bacterial organism shows the amount of antibodies in the serum of the animal infected with specific species of bacterial organism.

Test for Agglutination in the Serum (SAT)

Five sterilized test tubes were labelled and placed in a test tube rack. 0.8 mL normal saline with 0.5 % phenol added after adding 0.2 ml serum solution, the mixture was thoroughly mixed (1/5dilution). From the first test tube, 0.5 ml was drawn, mixed, and transferred to the second tube. After thoroughly mixing, 05.ml were transferred to the third, fourth, and fifth tubes, and gently mixed; 0.5 ml were extracted and discarded from the fifth tube (two fold dilutions). Each tube containing serum dilution received 0.5 ml of the standardized B. abortus concentrated antigen dilution (1:2), resulting in a series of final dilutions of 1/20, 1/40, 1/60, and 1/80, which were then well mixed from the highest to the lowest dilution. The antigen and serum were carefully mixed together and incubated overnight at 37°C. The tubes were removed after an overnight incubation period.

Antibody titers were measured in all tubes using an indirect light source against a dark backdrop.

Test for Milk Rings

(Morgan et al. 2005) described a protocol for doing the milk ring test (MRT). Before performing the test, milk and a suitable amount of antigen were warmed to room temperature. To achieve homogeneity, the antigen was gently shook. After adding 30-50 µl of antigen, 1ml of milk sample was placed into a tube and thoroughly mixed. At 37°C, the samples were incubated for 1 hour. The presence of a blue ring above the milk column indicated the presence of agglutinins in the milk, and the sample was regarded positive otherwise.

3. Discussion and results

A total of n = 800 samples (n = 400 serum and n = 400 milk samples) collected from local and organized dairy farms in various areas (Chakri, Taxila, Gujar Khan, Kallar Syedan, Dhamial, Kahuta, Kotli Sattian, Murree, Rawat, and Ganda Kass) and in the city of District Rawalpindi to study the prevalence of Brucella abortus in cattle MRT, RBPT, and SAT were used to determine the overall, breed-by-breed, and age-by-age prevalence of Brucella abortus. In district Rawalpindi, 26 samples of local breed and 17 Holstein Friesian cattle were found to be positive for Brucella abortus, while 757 samples were determined to be negative for the pathogen in selected cow samples (Table 2).

Table 2. Prevalence of Brucella abortus in of cattle

No of Sample	Positive Sample	Positive (%)
800	43	5.38

Using the RBPT, MRT, and SAT tests, the overall prevalence of Brucella abortus in that area was 5.37 %. The findings of this study are consistent with those of (Shafee *et al*., 2012), who reported a 3% prevalence in several Baluchistan districts. According to the findings of this study, cattle have a lower prevalence of brucellosis than humans, which is corroborated by previous reports of 3.97 % prevalence using several serological assays (Faqir *et al*., 1991). The findings of this study revealed that the RBPT was a more sensitive test than the MRT and the SAT. Brucella abortus was found in serum and milk samples from cattle in the Rawalpindi. In comparison to animals aged 1-5 years, Brucella abortus was shown to be more prevalent in the age group of 6-9 years. In compared to Holstein Friesian, local breeds were more sensitive to Brucella abortus infections. However, a sensitivity test may be required to assess the comparative efficacy of the organism's diagnosis. The current study's findings on the prevalence of Brucellosis are similarly in line with those of (Shafee *et al*., 2011), who found 4.6% and 1.7% positive causes of brucellosis in cattle and buffalo farms, respectively. Another study found 6.79% and 6.84% prevalence of brucellosis in cattle and buffaloes in Pakistan's Pothohar Plateau (Ali *et al*., 2013).

Brucella abortus prevalence in local and Holstein cattle breeds

Brucella abortus was found in local breeds with a prevalence of 6.5%%, 4.75%, and 4.75%, while the organisms 4.25%, 2.25%, and 1.75% were found in Holstein Friesian breeds by RBPT, MRT and SAT respectively (Table 3).

Table 3. Prevalence of Brucella abortus in milk and serum samples of local breed and Holstein Friesian cattle

Breed	No of Samples	RBPT Positive	RBPT (%)	MRT Positive	MRT (%)	SAT Positive	SAT (%)
Local Breed	400	26	6.5	19	4.75	19	4.75
Holstein Friesian	400	17	4.25	9	2.25	7	1.75

In compared to Holstein Friesian cattle, the data showed that the local breed was more sensitive to Brucella abortus. Furthermore, the results showed that the RBPT test was more successful in detecting Brucella abortus in serum and milk samples. This discrepancy could be due to MRT producing false–negative results when milk samples contain tiny amounts of antibodies IgA and IgM or a fat clustering factor deficiency (O'Leary *et al*., 2006).

Brucella abortus prevalence in different age groups of indigenous and Holstein Friesian cattle breeds

Brucella abortus was shown to have a 3.25%, 1.75%, 1.75% incidence in cattle aged 1-5 years while 7.5%, 5.25%, and 4.75% of 6-9 year-old cattle was documented by RBPT, MRT and SAT respectively (Table 4).

Table 4. Prevalence of Brucella abortus in serum and milk samples of different age group of cattle

Age	Animals Tested	RBPT Positive	RBPT (%)	MRT Positive	MRT (%)	SAT Positive	SAT (%)
1-5 Years	400	13	3.25	7	1.75	7	1.75
6-9 Years	400	30	7.5	21	5.25	19	4.75

The incidence of brucellosis increased with age, and the infection rate was higher in old age cattle, according to (Abubakar *et al*., 2010). Brucellosis in cattle was found to be similar in the Punjab region of India (Aulakh *et al*., 2010). Our findings revealed that Brucella abortus was more common in cattle aged 6-9 years compared to animals aged 1-4 years.

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