Detection of Brucellosis in dairy cows at Goshta and Mohmand Dara Districts of Nangarhar Province, Afghanistan

Faisal Mohammad Nazar [1*], Rahimi Najibullah [2], Banuree Sayed Attaul Haq [3]

[1]Para-Clinic Department, Faculty of Veterinary Science, Nangarhar University, Jalalabad 2601, Afghanistan.
[2]Animal Production Department, Faculty of Veterinary Science, Nangarhar University, Jalalabad 2601, Afghanistan.
[3]Pre-Clinic Department, Faculty of Veterinary Science, Nangarhar University, Jalalabad 2601, Afghanistan.

[*]Corresponding author Email: nfaisal2006@yahoo.com

ABSTRACT

Background: Brucellosis is an infectious zoonotic diseases caused by a gram-negative, facultative, intracellular bacterial organism of the genus Brucella that is harmful to many animal and human species. The purpose of this study was to assess the detection of Brucellosis as well as potential risk variables linked with districts, breeds (Local, HF, and Jersey) and age (2-4, 5-7, and more than 7 years).

Materials and Methods: In the current investigation, 150 blood and milk samples were obtained from the districts of Goshta and Mohmand Dara and analyzed for Brucella antibodies using the Milk Ring Test (MRT), the Rose Bengal Plate Test (RBPT), and the Standard Tube Agglutination Test (STAT).

Findings: A total of 150 samples were evaluated for this investigation, and 30 (20%) of them tested positive for brucellosis. Among these 30 positive cases, 19 (63.33%) were positive in Goshta district and 11 (15.7%) were positive in Mohmand Dara district. Out of 30 positive samples, 19 (63.33%) were positive in local dairy cows, 5 (16.66%) in HF, and 6 (20.00%) in Jersey breeds. According to results of present study, a significant association was documented between brucellosis and age of the dairy cows as higher prevalence was recorded for 5-7 years old ($X2=10.500$, $P=0.005$). In a comparison of serum and milk antibody detection assays, it was found that serum had a greater antibody detection rate than milk.

Conclusion: This study uses MRT, RBPT, and STAT as screening tools to add some information about the frequency of brucellosis in dairy cows breeds that are available in the Mohmand Dara and Goshta areas. The prevalence of brucellosis was higher in local dairy cows compared to other dairy breeds. Brucellosis' significance to the dairy sector and its possible effects on people's health such as zoonotic effects, antibiotics resistance, relapse of the diseases, prolonged treatment duration, side effects of antibiotics and limited treatment options during pregnancy.

Keywords: Prevalence, Brucella antibodies, MRT, RBPT, STAT

INTRODUCTION

A zoonotic disease produced by brucella, brucellosis is extremely contagious. Bang's sickness is sometimes referred to as Crimean fever, Gibraltar fever, Malta fever, Maltese fever, Mediterranean fever, Rock fever, or Undulant fever. However, this sickness is referred to as "Lazybones disease" in China. It is classified as a B-class disease by the World Organization for Animal Health (OIE), which means it must be reported in accordance with Chinese law on infectious disease prevention and control (Deqiu et al., 2002).

Gram-negative bacteria belonging to the genus Brucella are responsible for brucellosis, one of the most widespread yet little-known zoonosis in the world. The three most common species that cause brucellosis in humans are *Brucella suis* (main reservoir: pigs), *Brucella abortus* (major reservoirs: cattle/other Bovidae), and *Brucella melitensis* (major reservoirs: goats and sheep). The most typical ways that people get infected are through direct contact with sick animals, consuming contaminated food (especially unpasteurized milk and milk products), or inhaling contagious aerosols (Bedi et al., 2022; Hassan et al., 2020).

Estimates indicate that brucellosis costs India US$58.8 million annually (Kollannur et al, 2007). Animals expel a vast number of organisms throughout this gestation period via milk, fetal membranes, amniotic fluid, and uterine discharge. Cattle become infected mostly through ingestion of contaminated feed and water containing aborted fetuses, fetal membranes, and uterine secretions (Terefe et al., 2017). In developing countries awareness of Brucellosis is low grade between farmers and healthcare staff, consequently, based on the common clinical signs and the scarcity of diagnostic facilities, the diagnosis is challenging in rural areas (De Glanville et al., 2017).

Serological, bacteriological, allergic skin response, and molecular techniques are used to diagnose brucellosis. Bacteriological diagnosis is the most essential confirmatory approach of Brucella infection since its specificity is substantially better than that of other diagnostic methods, and it is used as the gold standard diagnostic method. The occurrence of various Brucella biotypes among Brucella spp., as well as their identification, is critical for confirming infection and tracing the source of infection. Because of the difficulties in distinguishing between infected and vaccinated animals using conventional serological tests, bacteriological isolation and identification of biotypes of the etiological agent are required steps in the design of epidemiological and eradication programs. Molecular diagnostic approaches are also available (Geresu et al., 2016). However, this test require a lot of time, and is also risky for laboratory workers during the culture, due to non- availability of the BSL-3 laboratory. However, due to financial constraints, particularly in Afghanistan, PCR is not appropriate for screening herds. Most frequently used for screening and tracking Brucella infections in dairy cattle, a serological test called the milk ring test (MRT) is affordable (Alton et al., 1988). Zoonotic diseases cause severe issues for the families by causing both human and animal sickness, resulting in decreased revenues, and transmitting the disease to humans and animals (Grace et al, 2017).

Available published information about brucellosis in Afghanistan in dairy cows is limited, so this study aimed to estimate the detection of Brucellosis and possible risk factors associated with breeds and ages in Goshta and Mohmand Dara districts of Nangarhar province, which are near the Pakistan border, using various serological tests, viz., milk ring test (MRT), Rose Bengal Plate Test (RBPT), and Standard Tube Agglutination Test (STAT) methods.

MATERIALS AND METHODS

Study Area

The present research was carried out in the districts of Goshta and Mohmand Dara of Nangarhar province. This area is home to several farm animal species, and pasture land with vegetation in the form of grasses and herbs serves as a source of feed.

Samples Collection

150 dairy cows were chosen at random from two distinct places, the Goshta and Mohmand Dara areas, and samples of their milk and blood serum were taken from each. The samples were collected according to standard procedures reported by (Office International des Epizooties OIE, 2008). To accomplish this, the first two to three streams of milk were discarded before the genuine sample was taken and placed in a 50 ml collecting tube. Samples were delivered to the Faculty of Veterinary Science Laboratory at Nangarhar University as soon as they were collected, keeping them chilled at 4°C. On the other hand, for serum collection, 10 ml of blood was collected aseptically from each cow's jugular vein in a vacuette containing serum clot activator. For roughly 2 hours, the vacuettes were maintained upright at room temperature (20-22 degrees Celsius). The separated serum was collected in screw-capped plastic vials and delivered to the laboratory of Nangarhar University's Veterinary Science Faculty. The serum of blood samples were separated and maintained at -20 degrees Celsius until they were subjected to serological tests, specifically the Rose Bengal Plate Test (RBPT) and the Standard Tube Agglutination Test (STAT). Utilizing pre-tested questionnaires, information was gathered during sample collection about the dairy cow breeds Holstein Friesian, Jersey, and Local.

The Veterinary Research Institute in Lahore, Pakistan, provided the antigen to *B. abortus* for MRT, RBPT, and STAT. Milk samples were examined using established methods (Babu, 2004).

Statistical Analysis

IBM SPSS Statistics v23 x64 free available was used to analyze the data. Chi-square was used to investigate the association between independent and dependent variables. $P<0.05$ was set for significant association between the variables. The obtained data is presented as percentages and frequencies.

RESULTS

The contagious zoonotic disease known as brucellosis is caused by the gram-negative, facultative, intracellular Brucella bacterium, which is harmful to many animal and human species. The goal of this study was to assess the detection of brucellosis and potential risk factors.The prevalence of *Brucella abortus* antibodies determined for dairy cows for each districts (Goshta, Mohmand Dara), using RBPT, MRT and STAT is given in Table 1. Out of 150 milk and blood serum samples from dairy cows, 30 were found to be seropositive.

Table 1. Prevalence of *Brucella abortus* antibodies detected by RBPT, MRT and STAT in relation to sampling area (district)

District	Samples Examined	Positive	Prevalence (95% CI)	Chi-square test
Goshta	80	19	63.33	X2= 1.507, df=1, P=0.220
Mohmand Dara	70	11	36.66	

Dairy cows raised in Goshta appeared to have high prevalence (63.33%) compared to those in Mohmand Dara (36.66%) (X2= 1.507, df=1, P=0.220). In Table 2, it was discovered that local-bred dairy cows (63.33%) had higher seropositivity rates than HF (16.66%) and Jersey (20.00%) breeds (X2= 8.576, df=2, P=0.014).

Table 2. Prevalence of *Brucella abortus* antibodies by breed.

Breed	Samples Examined	Positive	Prevalence (%) (95% CI)	Chi-square test
Local	60	19	63.33	X2= 8.576, df=2, P=0.014
Holstein Friesian (HF)	45	5	16,66	
Jersey	45	6	20.00	

Moreover, according to the results (Table 3), dairy cows aged 5-7 years (30%) had a greater rate of brucellosis than cows aged 2-4 (6%) and more than 7 years (20%) (X2=10.500, df = 2, P=0.005).

Table 3. Age-related prevalence of *Brucella abortus* antibodies.

Age	Samples Examined	Positive	Prevalence (95% CI)	Chi-square test
2-4 years old	50	3	10	X2=10.500, df = 2, P=0.005
5-7 years old	70	21	70	
More than 7 years	30	6	20	

In this study, the serum and milk of the same dairy cows were subjected to the detection of antibodies. The antibodies in serum were detected by RBPT and STAT, whereas MRT was used for the detection of antibodies in milk. The details of the samples with results are given in Table 4. When comparing the detection of antibodies in serum versus milk using any of the tests, serum had a higher frequency of positive results.

Table 3. Tests to compare the presence of antibodies in milk and serum.
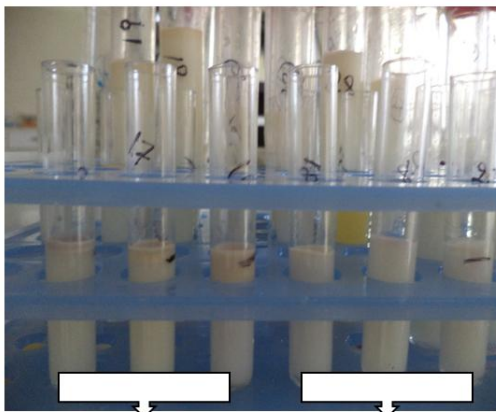
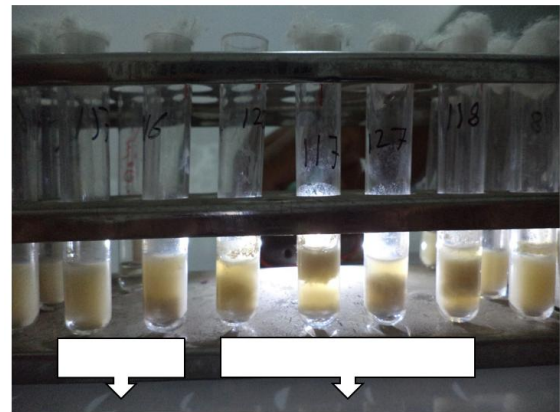| Tested samples	Positive samples		
	Positive Serum samples		Positive Milk samples
	RBPT Positive	STAT Positive	MRT positive
150	30	30	23

Milk Sample Collection

Blood Sample Collection

Positive   Negative

Mil Ring Test (MRT)

Negative   Positive

Standard Tube Agglutination Test (STAT)

DISCUSSION

Brucellosis is a zoonotic infectious illness that affects both animals and humans worldwide. It is caused mostly by *Brucella abortus* in large ruminants, *B. melitensis* in small ruminants, and *B. suis* in swine (Elhaig and Wahdan, 2023). Two traditional tests are most effective in underdeveloped countries for screening individual animals and herds, such as the Rose Bengal test (RBT and MRT), which are generally used for sero-diagnosis of brucellosis in dairy animals and are also simple and less expensive (Alton et al., 1988). For serological diagnosis and prevention of brucellosis Complement Fixation (CF), RBT and STAT tests are recommended (OIE, 2008). *Brucella abortus* antibodies were found in 20% of the dairy cows in the current investigation. Similar results have been reported from Ludhiana (Punjab), where 15.1% of cattle were discovered to have antibodies against *Brucella abortus* (Holt et al., 2021).

The current study found that brucellosis is endemic in Mohmand Dara and Ghoshta districts, although the overall prevalence of brucellosis was 20% in this study. When compared to (Khan et al., 2016; Jittapalapong et al., 2008), the significant sero-prevalence of Brucellosis antibodies in these two districts (Goshta, Mohmand Dara) was unexpected. Previously, employing MRT in Egypt, Cattle were shown to have a greater prevalence of brucellosis (51% vs. 49.8% in buffalo) (Ibrahim et al., 2012). The highest sero-positive rate (31.7%) was found

in local varieties of dairy cows when this study evaluated the sero-prevalence of Brucella antibodies in various breeds of dairy cows. According to a study done in Baluchistan (Khan et al., 2016), 5.0% of the local breed had *Brucella abortus*. Using the affordable milk ring test (MRT), a study was carried out in the cities of Islamabad Capital Territory (ICT), Rawalpindi, and Attock. According to breed, cross-breeds were more positive (7.8%), but exotic and local breeds were 6.0% and 4.4%, respectively (Ali et al., 2013). The reasons for the high prevalence in local breeds could be the small number of samples relative to the results of Ali et al., 2013.

Comparing dairy cows raised in Mohmand Dara and Goshta districts, it was discovered that the local had higher seropositivity. The observed difference, though, was not statistically significant. A significant study revealed that Rawalpindi has a high incidence of *Brucella abortus* antibodies ($P<0.003$), from Islamabad ($P>0.272$) and Attock (Ali et al., 2013).

Dairy Cows between the ages of 5-7 had the highest seroprevalence (30.0%) compared to cows with 2-4 and more than 7 years (6%, 20%). This means that the 5-7 year olds were more significant ($P=0.005$) than the 2-4 and more than 7 year olds in both districts, Similar findings have been reported from Ivory Coast's central savannah-forest area, where cattle beyond the age of 5 were found to have a higher risk of being seropositive than cattle under the age of 3 (Sanogo et al., 2012). The study's limitation was that the results were not based on the very sensitive and accurate ELISA and PCR testing, which could lead to some false positive or false negative results.

CONCLUSION

This study offers some information about brucellosis prevalence in available breeds of dairy cows in Nangarhar, Afghanistan, using MRT, RBPT, and STAT as diagnostic techniques. Being a zoonotic illness, Techniques for illness prevention in animals must be developed, particularly common food-producing animals such as cattle, buffaloes, sheep, and goats. These animals' milk serves as a common food source and is used to create dairy products. This infection from these animals is transmitted to humans through unpasteurized milk and causes disease in humans which can cause major health issues. People in Nangarhar, particularly in Goshta and Mohmand Dara districts, who keep Dairy animals to suit their requirements, are more vulnerable to brucellosis. Effective programs should be implemented in Nangarhar, as well as throughout Afghanistan.

Authors Contributions: Faisal MN designed the research project, collected data, was in charge of the laboratory, and wrote the first draft of the manuscript; Rahimi N entered the data into SPSS, analyzed the data, assisted with manuscript writing, and reviewed the manuscript; and Banuree SA assisted with data analysis and reviewed the manuscript. The article was read and approved by all authors.

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