OCCURRENCES OF *BRUCELLA ABORTUS* IN SERUM AND MILK SAMPLES OF CATTLE IN LORALAI, BALOCHISTAN (A CAE STUDY)

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ABSTRACT: *The prevalence of Brucella abortus in cattle was determined using 400 samples (200 serum and 200 blood samples) from organized farms and union councils (Zangiwal Jogezai, Zangiwal Kudezai, Lahore, Ponga, Tora Thana, Waraigee Kakaran, Kuch amackzai, Makhtar, China Alazai and Bawar Nasaran) and Main City of District Loralai. The detection of Brucella abortus was determined using serological tests; Milk Ring Test (MRT), Serum Agglutination Test (SAT) and Rose Bengal Plate Test (RBPT). The prevalence of Brucella abortus was found 3.50 in cattle of study regions of Loralai. However, the prevalence of Brucella abortus in local breed was found 5.0, 3.50 and 2.50 using the serological tests. While, the prevalence in Holstein Friesian breed was observed as 02.0, 02.0 and 01.0% respectively. Generally, age wise comparison showed prevalence of Brucella abortus in local and Holstein was 01, 01 and 01% in 1-4 years of cattle. The 5-8 years age of cattle showed 6.0, 3.50 and 2.50% prevalence of the organism. Overall, Brucella abortus was detected in serum and milk samples of cattle in district Loralai, Balochistan. It was observed that the old age group cattle were more susceptible to Brucella abortus in comparison to young animals. Local breed of cattle was more susceptible to Brucellosis than Holstein Friesian.*

Keywords: *Brucella abortus*, bovine, age, sex, Loralia

1. INTRODUCTION

Baluchistan province is comprised of mountainous and arid zone and approximately 70% population is scattered in rural area and most of the people practice nomadic life. Loralai is the second largest district of Baluchistan having a total area of 9830 km square and it is 4700 feet above the sea level, divided into three subdivisions/Tehsils (Bori, Dukki, Mekhtar).

Brucellosis is an important zoonotic and contagious bacterial infection of cattle. This disease is a great health concern and economic importance around the globe [1]. Brucellosis is also known as, Malta fever, Bang's disease, contagious abortion, infectious abortion, undulant fever, and Mediterranean fever. Brucellosis is caused by gram negative bacteria known *as Brucella abortus*. This organism is also important causal agent and produce infection in animals and humans [2]. The *Brucella abortus* is non-motile, non-sporting, aerobic and small Gram-negative rods or coccobacilli shape bacteria. The exact prevalence of the brucellosis in cattle is unknown in Pakistan; but several studies have been reported that disease is prevalent from 3.25% to 4.4% in different areas of Pakistan [3].

Brucella abortus is mostly transmitted through abrasion, lacerated skin or contact with fetus, placenta, fetal and vaginal fluids from infected animals. Major cause of bacteria is either abortion or full-term parturition. This organism may also found in the feces, milk and semen. Infected animals become chronic carriers for causing disease. Brucellosis may ener in animal through mucous membranes or ingestion and was alos observed that the infection may cause by direct contact or subsequent shedding of the organisms in the milk [4]. *Brucella abortus* produce infections in different organs e.g. uterus, udder and testes. The brucellosis may involve in mastitis, orchitis, abortion, arthritis and infertility in cattle [5]. The disease has been noted as endemic in many parts of the world. However, it has been reduced and eradicated in some developed countries through proper management [6].

In Pakistan, previous studies reported that cattle were more resistant to brucellosis as comparison to buffaloes. The incidences of brucellosis were increasing in Pakistan; particularly in large animals due to poor manage mental conditions. Several previous studies reported that the outbreak of brucellosis prevalent in government and private animal farms in different regions of Pakistan [7-11]. Brucellosis could be controlled in Pakistan through applying proper farm management, routine screening and animal vaccination programs [12]. In routine practice confirmatory laboratory diagnosis is not performed due to non availability of specific diagnostic facilities and having financial limitations. Different serological tests such as milk ring test (MRT), Rose Bengal Precipitation Test (RBPT), and serum plate agglutination test (SPAT) are suitable for diagnosis of *Brucella* infection in cattle [13-14]. There was no such official policy and measures have been taken to diagnose and eradicate the disease in Pakistan [15]. The exact figure about the prevalence of brucellosis in District Loralai is unknown. Therefore, the current study will provide the insight of brucellosis in cattle at District Loralai and the information related to the prevalence of this disease and its relation to age and with different breeds farmed in district Loralai, Baluchistan, Pakistan.

2. MATERIALS AND METHODS

A total of 400 samples, blood sample (n = 200) and milk samples (n = 200) were collected from different breeds of cattle at District Loralai. The susceptibility of the organism on age and breed will be examined by collected date from animal and the data were generated from laboratorial examination of the samples. The blood samples (n = 200) will be obtained from animals through jugular vein by using disposable sterilized plastic syringes. Before the collection of blood samples, the site of collection was cleaned with a cotton swab immersed in the spirit and rubbed on the place from where the blood was collected. The vein were located and the needle were introduced into the vein and the plunger were driven back and the blood will be collected in a tube, and then left it in slanting position to clot at least for half an hour. After that, blood was kept in refrigerator overnight. On the next day, the serum were collected in clean screw caped plastic cry vials and were brought to the Disease Investigation Livestock & Dairy Development Department Baluchistan, in the cool chain container for further analysis of *Brucella* species antibodies. Following diagnostic tests were carried out to identify the positive samples. Similarly the milk samples (n = 200) were collected from cattle at district Loralai. Before sample collection, the teats of animal were properly disinfected by alcohol and then make teats to dry. Then put the first discharged milk into sterile tubes. These Tubes were put in place in ice and transferred to laboratory for diagnostic purpose.

Overall sampling collecting from study area

The data in Table-1 indicates that overall 200 serum and 200 milk samples for detection of *Brucella abortus* were obtained from union councils of district Loralai including Zangiwal Jogezai, Zangiwal Kudezai, Lahore, Ponga, Tora Thana, Waraigee Kakaran, Kuch amackzai, Makhtar, China Alazai and Bawar Nasaran, respectively. Likewise, 100 serum samples were obtained from the Main City of district Loralai (Table 1).

Table-1. Serum and milk samples collection area

Farms	Union councils	Serum samples	Milk samples
Local farms	Zangiwal Jogezai	10	10
	Zangiwal Kudezai	10	10
	Lahore	10	10
	Ponga	10	10
	Tora Thana	10	10
	Waraigee Kakaran	10	10
	Kuch Amackzai	10	10
	Makhtar	10	10
	China alazai	10	10
	Bawar Nasaran	10	10
Total Main city Loralai		100	100
Sub total		200	200
Total		400	

1. Rose Bengal Plate Test (RBPT)

The Rose Bengal stained *Brucella* antigen will be purchased from Veterinary Research Institute Lahore. The antigens were used as recommended by the Veterinary Research Institute, Lahore and test procedures were adopted as described by Gabbar [16]. A drop of 0.03ml of the serum, using serological pipette were placed on the center of a square of clear transparent glass slide. A drop of negative control and a drop of positive sera were placed separately on the square of the same slide. A drop of 0.03ml quantity of antigen suspension were taken from the vial and placed near to the drop of sera on the square. Using an applicator stick, the serum and antigen was thoroughly mixed and each mixture was spread in the form of a circle over an area of a approximately 1.5cm radium. The slide was then be gently rocked back and forth for no longer than four minutes. The slide was then microscopically examined, the positive interaction between antigen and serum are the appearance of granules with different intensity that indicates the level of antibodies in the serum of the animal infected with specific species of bacterial organism.

2. Serum Agglutination Test (SAT)

Five sterilized test tubes were placed in a test tube rack and labeled. 0.8ml of normal saline added with 0.5% phenol. Following the addition of 0.2ml serum solution was mixed thoroughly (1/5dilution). 0.5ml was drawn from the first test tube, mixed it and transferred into the second tube. After mixing well, 05.ml were transferred to the third and so on up to the last fifth tube, and mixed gently, 0.5ml were drawn and discarded from last fifth tube (two fold dilutions). 0.5ml of the standardized *B. abortus* concentrated antigen dilution (1:2) were added to each tube containing serum dilution giving a series of final dilutions of 1/20,1/40,1/60 and 1/80; then mixed thoroughly from the highest to the lowest dilution. The antigen and serum was mixed gently and incubated at 37^{o}C for overnight. After the overnight incubation, the tubes were removed. All tubes examined using an indirect source of light against a dark background and antibody titers were recorded.

3. Milk Ring Test

Milk ring test (MRT) was performed according to procedure described by Morgan *et al.* [17]. Milk and sufficient amount

of antigen were brought at room temperature before performing the test. Antigen was shaken gently to ensure homogeneity. 1ml milk sample was transferred into a tube and mixed well after addition of 30-50 µl of antigen. Samples were incubated for 1 hour at 37°C. Sample was considered positive following the formation of a blue ring above milk column indicating the presence of agglutinins in the milk and negative otherwise.

RESULTS AND DISCUSSION

In order to study the prevalence of *Brucella abortus* in cattle at district Loralai, a total of 400 hundred samples (200 serum and 200 milk samples) were obtained from local and organized farms of union councils (Zangiwal Jogezai, Zangiwal Kudezai, Lahore, Ponga, Tora Thana, Waraigee Kakaran, Kuch amackzai, Makhtar, China Alazai and Bawar Nasaran) and in City of District Loralai. The overall, breed wise and age wise prevalence of *Brucella abortus* was determined using MRT, RBPT and SAT. Fourteen samples from local breed and Holstein Frazier cattle were found positive for the *Brucella abortus* in district Loralai, while 386 samples were found negative for the organism in selected cattle samples in district Loralai (Table-2). Over all prevalence of *Brucella abortus* in that particular area was 3.5%, using RBPT, MRT and SAT test. The result fo of this study is in close agreement with Shafee *et al*., (2012) [18], who reported 3% prevalence in different regions of Baluchistan.

Table -2. Prevalence of *Brucella abortus* in samples of cattle of district Loralai

No of samples	Positive samples (%)	Negative samples (%)
400	**14 (3.5)**	**386**

The results of this study indicated a comparatively lower prevalence of brucellosis in cattle as supported by previously reported 3.97 % prevalence using different serological tests [19]. The result of this study suggested that RBPT was comparatively more sensitive test then MRT and SAT. However sensitivity test may needed to evaluate the comparative efficacy of the diagnosis of the organism. The finding of current studying regarding the prevalence of Brucellosis are also in close agreement with Shafee et al., (2011) [20], who recorded the positive causes of brucellosis in cattle farms in Quetta as 4.6% and 1.7% in cattle and buffalo respectively. In another study 6.79 and 6.84% prevalence of brucellosis were recorded in cattle and buffaloes, in Pothohar Plateau, Pakistan [21].

Prevalence of *Brucella abortus* in local and Holstein breeds of cattle

A total of 5.0, 3.50 and 2.50% prevalence of *Brucella abortus* were detected in both Local breed. While, the organism 04, 02 and 02% was prevalent in both Holstein Friesian breed (Table-3). The results indicated that local breed was higher susceptible to *Brucella abortus* in comparison to Holstein Friesian cattle. The results further indicated that RBPT test was more effective for the detection of *Brucella abortus* in serum and milk samples. This difference could be in relation to MRT may produce false–negative reactions when the milk samples contain small quantities of antibodies IgA and IgM or deficiency of the fat clustering factor [22].

Prevalence of *Brucella abortus* in different age group of local and Holsten breeds of cattle

Two percent prevalence of *Brucella abortus* was detected in 1-4 years of cattle breed. While 12, 07 and 05% prevalence of *Brucella abortus* was recorded in 5-8 years age breed (Table-4). Abubakar *et al*. (2010) [23], investigated that the incidence of brucellosis increased with age, and the infection rate was higher in old age cattle. Similar findings were noted for brucellosis in cattle in the Punjab, India [24]. Our results indicated that *Brucella abortus* was more prevalent in 5-8 years of cattle in comparison to 1-4 years age group of cattle.

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

Breed	No. of cows tested	RBPT positive	Prevalence on RBPT %	MRT positive	Prevalence on MRT %	SAT positive	Prevalence on SAT %
Local breed	200	10	5%	7	3.5%	05	2.5%
Holstein friesian	200	4	2%	2	1%	02	1%

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

Age	No. of cows tested	RBPT positive	Prevalence on RBPT%	MRT positive	Prevalence on MRT%	SAT positive	Prevalence on SAT %
1-4 years	200	02	01%	02	01%	02	01%
5-8 years	200	12	06%	07	3.5%	05	2.5.%

CONCLUSIONS

In summary, the evidence of *Brucella abortus* was detected in serum and milk samples of cattle in district Loralai, Balochistan. *Brucella abortus* prevalence was observed more susceptible in the age group of 5-8 years in comparison to animals' age 1-4 years. Local breeds were more susceptible *to Brucella abortus* infections in comparison to Holstein Friesian.

REFERENCES

1. Munir R, Afzal M, Hussain M, Naqvi S M S, Khanum A Outer membrane proteins of *B. abortus* vaccinal and field strains and their immune response in buffaloes. *Pakistan Veterinary Journal* **30**: 110-114 (2010)
2. Gul ST, Khan A. Epidemiology and epizootology of brucellosis: A review. *Pakistan Veterinary Journal* **27**:145-151. (2007)

3. Naeem, K.; Akhtar, S.; Ullah, N. The serological survey of bovine brucellosis in Rawalpindi-Islamabad districts. *Pakistan Veterinary Journal* **10**(4),154-156 (1990)
4. Stableforth A W (1959). Brucellosis, infectious diseases of animals: diseases due to bacteria. *Reviews of Science Technology* **1**: 153-159.
5. Nicoletti P, Brucella W. J., In diagnostic procedures in veterinary bacteriology and mycology. 5th Edition: 95-104 (1990)
6. Hungerford T. G. Diseases of livestock. 9th Edi. McGraw-Hill Book Company, Sydney. 1302-1304 (1990)
7. Abubakar M, Arshed MJ, Hussain M, Ehtisham–ul–Haq and Ali Q. Serological evidence of Brucella abortus prevalence in Punjab province, Pakistan a cross – sectional study. *Transboundary Emerging Diseases* **57**(6):443–447(2010)
8. Hamidullah M, Khan R and Khan I (2009). Seroprevalence of brucellosis in animals in district Kohat NWFP and comparison of two serological tests. *Pakistan Journal of Sciences* **61**(4):242–243.
9. Iftikhar H, Arshad MI, Mahmood MS and Akhtar M. Seroprevalence of Brucellosis in Human, Cattle, and Buffalo Populations in Pakistan. Turkish Journal of Veterinary and Animal Sciences **32**(4): 315–318 (2008)
10. Rabab A, Attas A, Khalifa MA, Al Qurashi AR, Bada M and Al Gulay N. Evaluation of PCR, culture and serology for the diagnosis of acute human Brucellosis. *Annals of Saudi Medicine* **20**(34): 224-228 (2000)
11. Shafee M, Rabbani M, Sheikh AA, Ahmad MD, Razzaq A. Prevalence of bovine brucellosis in organized dairy farms, using milk ELISA, in Quetta City, Balochistan, Pakistan. *Veterinary Medicine International* **37**: 1-3 (2011)
12. Abubakar M, Mansoor M and Arshed MJ. Bovine Brucellosis. Old and new concepts with Pakistan perspective. *Pakistan Veterinary Journal* **32**(2): 1–9 (2011)
13. Asif M, Awan AR, Babar ME, Ali A, Firyal S and Khan QM. Development of genetic marker for molecular detection of Brucella abortus. *Pakistan Journal of Zoology* **9**: 267–271 (2009)
14. Gul ST, Khan A. Epidemiology and epizootology of brucellosis: A review. *Pakistan Veterinary Journal* **27**:145-151 (2007)
15. Akhtar R, Chaudhry ZI, Shakoori AR, Ahmad M and Aslam A. Comparative efficacy of conventional diagnostic methods and evaluation of polymerase chain reaction for the diagnosis of bovine brucellosis. *Veterinary World* **3**(2): 53–56 (2010)
16. Gabbar K M A (1992). Procedure Veterinary Microbiology. 2nd Ed. 241-242.
17. Morgan W J B, Davidson I, Herbert C N (1978). The use of second international standard for anti-*Brucella abortus* serum in the complement fixation test. *Journal of Bio Standardization* **1**: 43-60.
18. Shafee M, Rabbani M, Ahmad M U D, Muhammad K, Sheikh A, Awan M A, Shabbir M Z. Sero-prevalence of Bovine Brucellosis using indirect ELISA in Quetta Baluchistan, Pakistan. *Journal of Animal and Plant Sciences* **22**: 125-127 (2012)
19. Faqir, M. Seroepidemiological survey of bovine brucellosis associated with reproductive disorder in Quetta district, Balochistan. M.Sc. (Hons) Thesis. Animal Reproduction, CVS, Lahore, pp: 80-81 (1991)
20. Shafee M, Rabbani M, Ahmad M U D, Muhammad K, Sheikh A, Awan M A, Shabbir M Z. Prevalence of Bovine Brucellosis in Organized Dairy Farms, Using Milk ELISA, in Quetta City, Baluchistan, Pakistan. *Veterinary Medicine International* **22**: 1-3 (2011)
21. Ali S, Ali Q, Abatih E N, Nemat U, Muhammad A, Khan I, Akhter S. Sero- prevalence of *Brucella abortus* among dairy cattle and buffaloes in Pothohar Plateau, Pakistan. *Pakistan Journal of Zoology* **45**: 1041-1046 (2013)
22. O'Leary S, Sheahan M and Sweeney T. *Brucella abortus* detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. *Research in Veterinary Sciences* **81**(2): 170–176 (2006)
23. Abubakar M, Arshed MJ, Hussain M, Ehtisham –ul–Haq and Ali Q. Serological evidence of *Brucella abortus* prevalence in Punjab province, Pakistan–a cross –sectional study. *Transboundary Emerging Diseases* **57**(6):443–447. (2010)
24. Aulakh, H. K., P. K. Patil, S. Sharma, H. Kumar, V. Mahajan, K. S. Sandhu. A Study on the Epidemiology of Bovine Brucellosis in Punjab (India) Using Milk-ELISA. *Acta Veterinary Brno* **77**: 393-399 (2008)