

Short Communication



Comparative Study of Brucellosis in Different Breeding Practices of Cattle and Buffaloes

ABDUL BASIT^{1*}, KASHIF RAHIM¹, MUHAMMAD SHAHID², SHAMIM SALEHA¹, SIRAJ AHMAD², MIRZA ALI KHAN²

¹Department of Microbiology, Kohat University of Science and Technology (KUST), Khyber PakhtunKhwa, 26000, Pakistan; ²Microbiology and Biotechnology Center, Veterinary Research Institute (VRI), Khyber PakhtunKhwa, Peshawar, 25000, Pakistan.

Abstract | Brucellosis is considered the contagious and zoonotic bacterial infection of livestock that causes serious economic losses in animal production sector. Here, we investigated the prevalence of brucellosis and its relationship with different breeding practices of cattle and buffaloes, natural breeding and artificial insemination. The study was carried out in different private farms of district Peshawar in Khyber PakhtunKhwa, Pakistan. Blood and milk samples of 200 cattle and buffaloes were initially screened with serum plate agglutination test (SPAT) and milk ring test (MRT) and were further tested by polymerase chain reaction (PCR). A total 34 (17%) and 20 (10%) were found positive in SPAT and MRT, respectively. The overall prevalence of brucellosis was found to be 7% based on PCR. The prevalence was found relatively higher in buffaloes as compared to cattle (9% vs 5%). Further, prevalence in natural inseminated cattle and buffaloes was found higher than artificially inseminated cattle and buffaloes (4% vs 2%, $P > 0.05$). Awareness campaign should be made through extensive veterinary education among dairy farmers and veterinary assistants/professionals to control brucellosis.

Keywords | Brucellosis, Cattle, Buffaloes, Artificial insemination, Natural breeding, PCR

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***Correspondence** | Abdul Basit, Kohat University of Science and Technology (KUST), Khyber PakhtunKhwa, 26000, Pakistan; **Email:** abdul_9090@yahoo.com

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Brucellosis is a contagious and zoonotic bacterial disease of livestock, caused by various strains of *Brucella* species (Gul et al., 2007). *Brucella abortus* and *Brucella melitensis* are the principal cause of brucellosis in cattle and buffaloes (Radostits et al., 2000; Karaca et al., 2007). It is found worldwide and causes significant economic losses including abortion, loss in milk production, low fertility rates, and cost of replacement of animals (Radostits et al., 2000).

Source of transmission includes ingestion, inhalation of contaminated secretions and excretions of an infected animal in a herd. The uterus of the pregnant animals is the common site of infection which leads to necrotizing placentitis. Moreover Bulls discharge semen infected with *Brucella* are also an important source for the transmission of disease, especially when the semen of infected bull is

used for artificial insemination (Abubakar et al., 2011).

Signs and symptoms of brucellosis are nonspecific, therefore for diagnosis of bovine brucellosis there is needed a combination of serological and molecular methods. In diagnostic laboratories, sera usually are screened with any simple serological test of high sensitivity. Tests for detection of *Brucella* antibodies in milk are considered the principal method for detecting infected herds and for diagnosing brucellosis in an individual animal (Godfroid and Kasbohrer, 2002). Rarely, the clinical samples after initial screening by conventional methods are subjected to PCR for confirmation of disease (Abubakar et al., 2011). Amplification of DNA by PCR is currently used to diagnose several infectious diseases caused by fastidious or slowly growing bacteria. Due to their potential to detect very small numbers of organisms, PCR-based assays can

be applied for diagnosis (Rabab et al., 2000).

Livestock is an important sector of agriculture in Pakistan and has a large, well-adapted livestock population. People practice mixed crop-livestock farms where usually breed 90% buffaloes and 10% cattle, for dairy products and milk production production (Afzal and Naqvi, 2004). In Pakistan, several studies have reported the high incidence rates of brucellosis in livestock farms in government and private sector in different districts and provinces of Pakistan (Rabab et al., 2000; Iftikhar et al., 2007; Mukhtar and Kokab, 2008; Abubakar et al., 2010; Shafee et al., 2011; Omer et al., 2010). Moreover, many studies also reported that the natural and artificial insemination are the risk factor for the brucellosis transmission. Furthermore it has been reported that uniform supply of semen from genetically superior bulls for artificial insemination is very limited. Most of the animals are bred naturally. Reasons for not using artificial insemination are many but dislikeness and unavailability are the main issues (Khan et al., 2008).

The objective of the present study therefore was to use to estimate the level of seroprevalence in cattle and buffalos, combined with sensitive and specific diagnostic tests. A secondary objective was to estimate and compare seroprevalence of brucellosis among the natural and artificial inseminated in cattle and buffaloes and its relationship among breeding practices in District Peshawar Khyber PakhtunKhwa, Pakistan.

In the present study, a total of 200 milking cattle and buffaloes (100 each) were selected randomly from private dairy farms in district Peshawar of Khyber PakhtunKhwa, Pakistan. Blood and milk samples were collected from each cattle and buffalo. These samples were first subjected for serodiagnosis by using Serum Plate Agglutination Test (SPAT) and Milk Ring Test (MRT), and then finally confirmed by Polymerase chain reaction (PCR).

The serum samples were subjected to SPAT for screening *Brucella* antibodies as described by Alton et al. (1988). The results of agglutination were recorded. A titer of 1:80 or above was considered positive for brucella infection. Moreover, Milk ring test was conducted on milk samples as de-

scribed by Alton et al. (1988). The positive samples were differentiated on the basis of blue ring present on the top of milk after overnight reaction.

DNA was extracted from blood and milk samples by using DNA isolation kit FERMENTAS, USA. PCR was carried out by using set of primer B4 (5'-TGGCTCGGTTGCCAATATCAA -3') and B5 (5' GCGCTTGCCTTTCAGGTCTG-3'), for detection of target sequence of 223-bp within a gene code for the production of a 31-kDa membrane protein specific to the genus *brucella* as described by (Rabab et al., 2000). The amplification was performed in a DNA thermal cyler (Multi-gene Labnet international Inc.USA). Initial denaturation was carried out at 94°C for 2 minutes, and then for 35 cycles the sample DNA was denatured at 93°C for 15 seconds, annealed at 55°C for 30 seconds, and extended at 72°C for 30 seconds. The final incubation was done at 72°C for 10 minutes. For positive controls, DNA extracted from Rose Bengal antigen supplied by Veterinary Research Institute, Lahore. However, for negative control, distilled water was used. The PCR products were resolved and analysed by using 1.5% of agarose gel electrophoresis and photographed on UV photo-documentation system (Multi-gene Labnet international Inc.USA). The clear bands of *Brucella* species DNA were considered as positive results (Bailey et al., 1992).

All the recorded data were analyzed through statistical analysis using SPSS 16.0 software. Chi square test was utilized to measure the association. P-value of less than 0.05 was considered for statistically significant difference.

Out of the 100 blood and milk samples of cattle, a total of 15 and 09 samples were found positive on basis of SPAT and MRT respectively. However on the basis of PCR total 05 samples were found positive (serum=04 and milk=01) as shown in Table 1 and Figure 2. Similarly blood and milk samples of 100 buffaloes, a total of 19 and 11 samples were found positive on basis of SPAT and MRT respectively. However on the basis of PCR total 09 samples were found positive (serum= 07 and milk= 02), as shown in Table 1 and Figure 2. The overall prevalence of brucellosis in cattle and

Table 1: Prevalence of brucellosis on the basis of SPAT, MRT and PCR in cattle and buffaloes

Species	Breeding practices	Total samples	SPAT	MRT	PCR positive			P-value
					Serum	Milk	Total positive	
Cattle	Artificial	50	05	03	02	0	02	P> 0.05 (P=1.00)
	Natural	50	09	06	02	01	03	
Buffaloes	Artificial	50	09	04	03	01	04	
	Natural	50	11	07	04	01	05	
Total		200	34	20	11	03	14	

buffaloes was recorded 7% (14/200) on the basis of PCR (Table 1).

In present study comparison was made on the prevalence of brucellosis in artificial and natural inseminated cattle and buffaloes, the result revealed the prevalence of brucellosis was found higher in natural inseminated cattle and buffaloes (8%) followed by artificially inseminated animals (6%) with statistically non significance association among them (Table 1 and Figure 1).

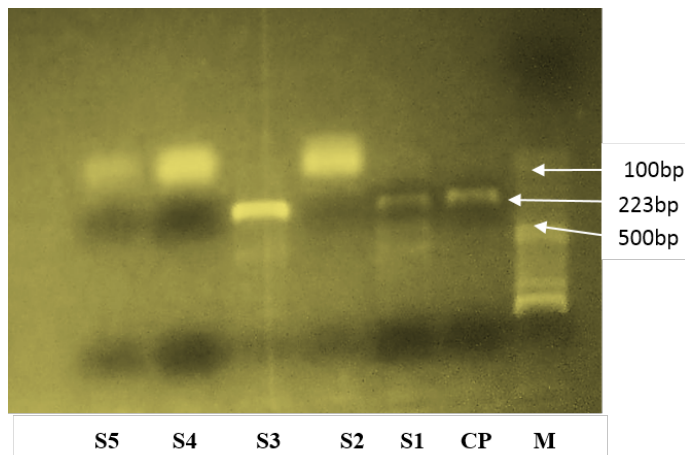


Figure 1: Gel electrophoresis of PCR products M= Marker; CP= Control Positive; S1=positive sample; S2=Negative sample; S3=Positive Sample; S4 and S5= Negative samples

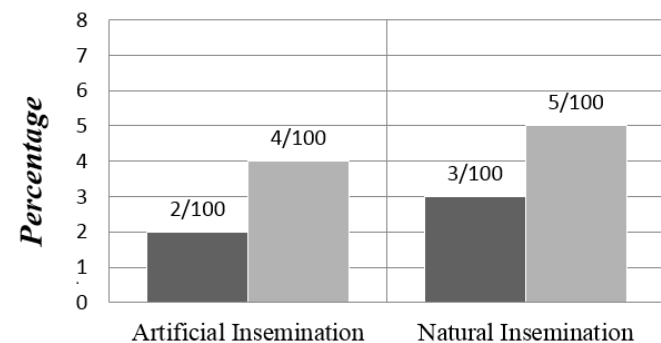


Figure 2: Comparison of the prevalence of brucellosis on the basis of breeding pattern

Brucellosis has been recognized as an important zoonotic disease as it concerned with both animal and human health. As this disease make vulnerable economic losses to the country particularly to livestock industry. Currently, veterinary diagnostic laboratories utilize Milk Ring Test for diagnosis of brucellosis in bovine milk samples, which indirectly identifies *Brucella* species in the host (Godfroid and Kasbohrer, 2002). The PCR is currently used for the diagnosis of many diseases including Brucellosis, as PCR is more reliable and sensitive due to its ability for antigenic detection than antibody detection (Akhtar et al., 2010). The inconsistent results in terms of the sensitivity and specificity of MRT and SPAT in bovines suggested that

these tests may be used for routine screening of herds but not for confirmatory diagnosis of brucellosis in individual animals. Moreover serological tests have higher sensitivities, but their specificities are generally low (Al-Attas et al., 2000). Also serological tests can be nonspecific due to cross reaction or high immunity reactions, depending on subclinical or endemic prevalence of the disease (Abubakar et al., 2011). Therefore, after initial screening by MRT and SPAT, afterwards the confirmation by PCR is needed for accurate diagnosis of Brucellosis in livestock diagnostic laboratories. Furthermore, as PCR is more reliable in terms of its sensitivity for antigenic detection than antibody detection, it may be included as a regular screening test in clinical practice in farms animals irrespective of high cost as compared to conventional tests, in order to reduce economic losses in Pakistan.

In the present study the overall prevalence of brucellosis was found 7% and the prevalence was relatively higher in buffaloes (9%) compared to cattle (5%). Abubakar et al. (2010), in their study also reported the relatively high prevalence of brucellosis in buffaloes (7.74%) compared with cattle 5.06%. Similarly in another study Shafee et al. (2011) also determined the similar findings regarding the prevalence of Brucellosis. In their study they determined the high prevalence in (8.5%) in buffaloes as compared in cattle (3%). This might be due to the area size, stocking density, artificial insemination with poor hygienic precaution, the size of the investment in livestock.

In present study, the prevalence of brucella infection was found high (8%) in naturally inseminated cattle and buffaloes as compared to artificially inseminated cattle and buffaloes (6%). Yohannes et al. (2012) in their study reported the high prevalence of brucellosis in cross-bred animals (3.64%) than indigenous ones (1.7%) in East Wollega zone. While Jergefa et al. (2008) reported in their study that artificial insemination were an important risk factor in prevalence of bovine brucellosis in Ethiopia. However, the prevalence of brucellosis infection in the present study was found low in artificially inseminated animals than naturally inseminated. This might be due to fact that the farmers in that area more preferred the natural breeding compared to artificial insemination. Moreover the bulls may be acted as carrier of brucella infection to other female animals through natural breeding practice. This disease can be transferred to all those animals which were mounted by infected bulls. In present study it was observed that in villages or at herd mostly there were only one bull there that was used for breeding practice. This could be the main reason for high prevalence of brucellosis in natural inseminated cattle and buffaloes in this study. The results of present study could not identified artificial insemination as important risk factor in incidence of brucellosis in both cattle and buffaloes. However brucellosis can also be transferred

through artificial insemination by using contaminated needles or poor management practices.

In conclusion, the result of current study showed that prevalence of brucellosis in District Peshawar is somehow prevalent. The risk factor like breeding pattern might be involved regarding this prevalence rate. Therefore there is a need to design and implement control measures aiming at preventing further spread of the disease in the Region through the use of better management practices.

CONFLICT OF INTEREST

No conflict of interest are declared by authors for the contents in the manuscript

AUTHORS CONTRIBUTION

Basit A and Rahim K designed the study and experiment work. Technical support was provided by Shahid M. Saleha S supervised the work performed and participated in its design and coordination. Ahmad S helped in collection of samples. Khan MA was the principal investigator of this project and took part in critical analysis of data. All authors read and approved the final manuscript.

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