

Short Communication

Sero-Prevalence of Brucellosis in Cattle in Southern Area of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Brucellosis considered as the most important zoonotic disease worldwide. The diagnosis of brucellosis in bovines requires the use of more than one serological test for routine diagnosis. Present study was conducted to estimate the occurrence and prevalence of brucellosis in the lactating dairy cattle located in Bannu and Lakki Marwat districts of Khyber Pakhtunkhwa, Pakistan. A total of 302 blood and milk samples were collected from different breed of cattle. The blood and milk samples were screened for brucellosis on the basis of serum plate agglutination test (SPAT) and Milk Ring Test (MRT), respectively. The overall prevalence of brucellosis was found 5.2% and 10.9% in District Bannu and Lakki Marwat, respectively, moreover prevalence of brucellosis on basis of SPAT and MRT showed 7.94% and 3.97% respectively. On the basis of breeding pattern, prevalence of brucellosis was found 10.2%, 19% and 13% in local breed, Friesian cross and Jersey cross respectively and no significant association was found among them ($P > 0.05$). Higher prevalence of brucellosis was found in cattle with age group of greater than five years with statistically significant association ($P < 0.05$). Moreover, in case of breeding practice the prevalence of brucellosis was found higher within unknown source of breeding followed by community bull and artificial insemination ($P > 0.05$). Furthermore, higher prevalence of brucellosis was found in animals that were set free for grazing compared to stall feeder animals ($P > 0.05$). It can be therefore concluded that regular screening of animals for brucellosis in these areas is required to control this disease.

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Brucellosis is the contagious and zoonotic disease of humans and animals worldwide especially in the Middle East, Africa, Asia, South and Central America (Yohannes et al., 2012 and Munir et al., 2010). It is an infectious bacterial disease caused by member of genus *Brucella*, *Brucella melitensis* and *Brucella abortus* are the principal cause of brucellosis in bovines (Karaca et al., 2007 and Radostits et al., 2000). Brucellosis is primary reproductive disease with potential of great economic loss in bovines resulting abortion, death of young stock, birth of weak calves, stillbirth, delayed calving and infertility in males and reduced milk production in females (Abubakar et al., 2011 and Maadi et al., 2011). This disease occurs by means of contaminated water or feed, inhalation and via conjunctiva, and it can be transmitted through direct or indirect contact with infected animals or animal secretions. Moreover ingestion of the excretion in uterine discharge and milk of infected animals also the source of transmission (Yohannes et al., 2012).

Signs and symptoms of this disease are nonspecific or may be atypical. Laboratory confirmation is therefore

essential for the diagnosis of brucellosis. Moreover, combinations of serological, cultural and molecular techniques are essential for diagnosis. Serological blood tests and Milk ring test (MRT) are mostly used for diagnosis of brucellosis (Al-Mariri and Haj-Mahmoud, 2010). Detection of *brucella* antibodies in milk considered the principal technique for screening and monitoring the infected dairy herds or an individual animal for diagnosing brucellosis at regular intervals (Godfroid and Kasbohrer, 2002). Serological tests are comparatively easy to carry out and provide a practical benefit in estimating the prevalence of *Brucella* infection in bovines (Abubakar et al., 2011).

Brucellosis is one of the major disease problems in Pakistan. Many studies documented the prevalence of brucellosis in livestock of different districts and provinces of Pakistan (Rabab et al., 2000; Iftikhar et al., 2008; Mukhtar and Kokab, 2008; Abubakar et al., 2010; Shafee et al., 2011; Ullah et al., 2013). Moreover, livestock populations of Pakistan are large and well adapted to the local environmental conditions. Therefore this disease has a

significant impact on human, animal health and socioeconomic aspects; where the people of rural areas of Pakistan mainly dependent on livestock, land cultivation and their domestic animals (Shafee et al., 2011 and Maadi et al., 2011). Due to the lack of diagnostic facilities and financial limitations in Pakistan, veterinarians generally prefer conventional tests for screening of brucellosis. Serum Agglutination Test, Rose Bengal Test and Milk Ring Test are usually performed at both private and government livestock laboratories (Asif et al., 2009; Gul and Khan, 2007).

This study was carried to estimate the prevalence of brucellosis in milk and serum samples collected from of different breed of cattle located in Bannu and Laki Marwat area of Khyber PakhtunKhwa region of Pakistan.

The study was conducted on the lactating dairy cattle in District Bannu and Lakki Marwat, Khyber PakhtunKhwa Pakistan, from May to July, 2011(Figure 1).

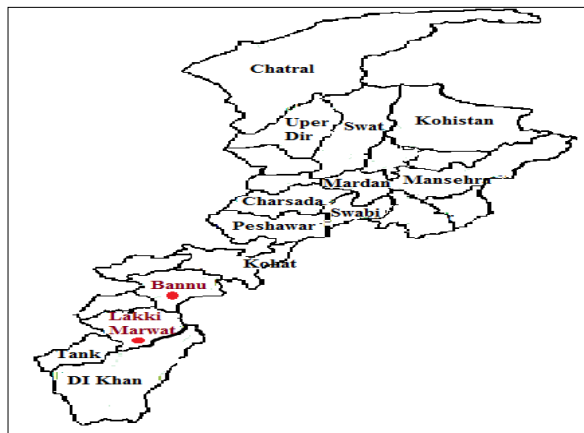


Figure 1: Map of Khyber PakhtunKhwa province showing the study area (Highlighted Red)

A total (n = 302) blood sera and milk samples of Cattle were randomly collected. Approximately 5 mL of milk was collected from four quarters of each cattle into sterile glass bottle. All the blood serum and milk samples were then kept in an ice box and transported directly to the VRI, Peshawar.

The serum samples were subjected to SPAT for screening brucella antibodies as described by Alton et al., (1975). The results of agglutination in SPAT were recorded. A titer of 1:80 or above was considered positive for brucellosis according to the instruction of the antigen manufacturer (Global invitro.LLP Sudbury Hill, London UK).

Milk ring test was conducted on milk as describe by Alton et al., (1988) with slight modification. The antigen hematoxylin used in this test was supplied by Veterinary Research Institute (VRI) Peshawar. The positive samples were differentiated on the basis of blue ring present on the top of milk after overnight reaction.

Statistical analysis was executed by using SPSS (version 16.0) software. By using chi square test significance of difference was determined. Value of $p < 0.05$ considered as statistically significant.

In the present study, from 302 samples total 170 and 132 blood and milk samples were collected from District Bannu and District Lakki Marwat respectively. Among 170 samples from District Bannu, 13 (7.64 %) and 5 (2.94%) cattle were found positive for brucellosis on the basis of SPAT and MRT respectively (Table 1). Similarly from 132 samples from District Lakki Marwat, (11 8.33%) and 7 (5.30%) samples were positive on the bases of SPAT and MRT respectively (Table 1). With respect to the districts, incidence rate of brucellosis was found higher in Lakki Marwat (10.8%) compared to Bannu district (5.64%) (Table 1).

Table 1: Prevalence of brucellosis in the cattle located in Bannu and Lakki Marwat Districts, Khyber PakhtunKhwa Pakistan

| Technique used | District Bannu | | District Lakki Marwat | |
|----------------|---------------------|-----------------------|-----------------------|-----------------------|
| | Total no of samples | No of Positive Sample | Total no of samples | No of Positive Sample |
| SPAT | 170 | 13(7.6%) | 132 | 11(8.33%) |
| MRT | 170 | 5 (2.9%) | 132 | 7 (5.30%) |
| Total | 340 | 18 (5.29%) | 164 | 18 (10.9%) |

Table 2: Prevalence of brucellosis in locally available breed of the cattle, in Bannu and Lakki Marwat distracts, Khyber PakhtunKhwa Pakistan

| Breed | Total sample | Positive on SPAT | Positive on MRT | Total | p- value |
|----------------|--------------|------------------|-----------------|-------------|----------|
| Local cross | 176 | 14 (7.95%) | 4 (2.27%) | 18 (10.2 %) | P > 0.05 |
| Friesian cross | 68 | 7 (10.29%) | 6 (8.82%) | 13 (19 %) | |
| Jersey cross | 37 | 3 (8.10%) | 2 (5.40%) | 5 (13%) | |
| Sahiwal | 21 | 0 | 0 | 0 | |
| Total | 302 | 24 (7.94%) | 12 (3.97%) | 36 (11.9%) | |

From total 302 Cattles, 176 were local breed, 68 were Friesian cross, 37 were Jersey cross and 21 were Sahiwal breed. From 176 Local Breed cattle, 14 (7.95%) and 4 (2.27%) were found positive for brucellosis on bases of SPAT and MRT respectively. Similarly in Friesian cross, 7 (10.29%) and 6 (8.82%) and in Jersey cross, 3 (8.10%) and 2 (5.40%) animals were found positive on SPAT and MRT

respectively. No significant association was found among different breeds of cattle ($P > 0.05$) (Table 2).

Present study also distributed the cattle in to different age groups. In age group of 2–4 year, 5–7 year, 8–10 years and 11–16 years the incidence rate of brucellosis were found 2.6%, 20.3%, 22% and 21.4% respectively (Table 3).

From total 176 artificial inseminated (AI) cattle, 12 (6.81%) and 6 (3.40%) having *brucella* infection on the bases of SPAT and MRT respectively. Similarly in 103 naturally mated cattle with community bull, 9 (8.73%) and 4 (3.88%) were positive for brucellosis on the bases of SPAT and MRT respectively.

The rest of 23 cattle were with unknown source of breeding pattern, showed 3 (13.04%) and 2 (8.69%) samples

positive on SPAT and MRT, respectively. The incidence of brucellosis was found higher in cattle mated with unknown source followed by community bull and then artificially inseminated (Figure 1). Moreover no significant association of brucella infection in cattle with respect to breeding practices were found ($P > 0.05$).

Table 3: Age wise distribution of *brucella* infection in the cattle in Bannu and Lakki Marwat distracts, Khyber PakhtunKhwa Pakistan

| Age | Total Sample | SPAT | MRT | Total |
|-------------|--------------|------------|------------|------------|
| 2-4 years | 151 | 3 (1.98%) | 1 (0.66%) | 4 (2.6%) |
| 5-7 years | 64 | 8 (12.5%) | 5 (7.81%)* | 13 (20.3%) |
| 8-10 years | 59 | 9 (15.25%) | 4 (6.77%)* | 13 (22%) |
| 11-16 years | 28 | 4 (14.28%) | 2 (7.14%) | 6 (21.4%) |

| Feeding Practice | Total Samples | SPAT | MRT | p- value |
|------------------|---------------|------------|-----------|------------|
| Stall feeding | 246 | 18 (7.31%) | 9 (3.65%) | $P > 0.05$ |
| Grazing | 56 | 6 (10.71%) | 3 (5.35%) | |

Table 4: Prevalence of *brucella* infection in the cattle on the basis of feeding practices in Bannu and Lakki Marwat distracts, Khyber PakhtunKhwa Pakistan

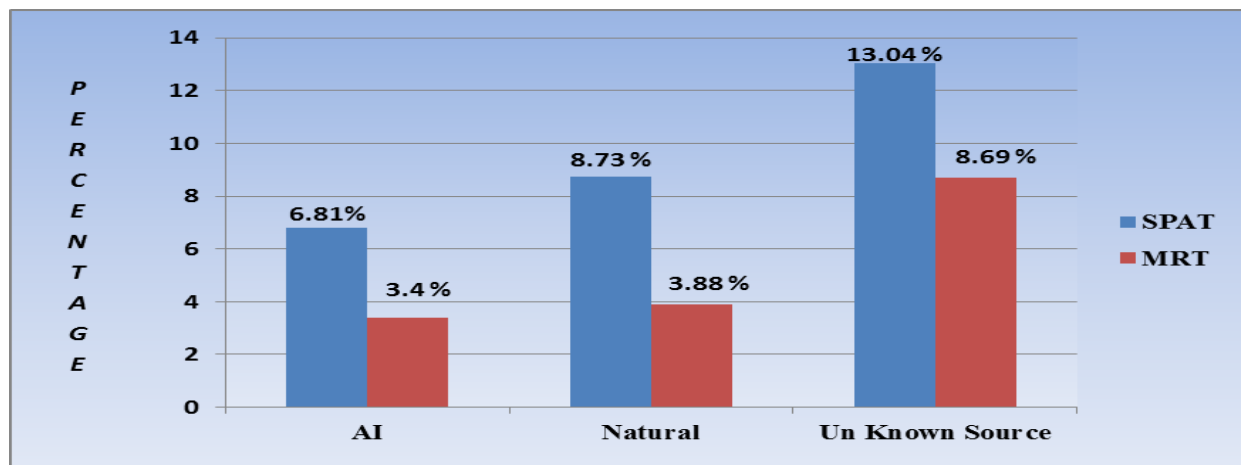


Figure 1: Prevalence of *brucella* infection in the cattle with respect to breeding practice in Bannu and Lakki Marwat distracts, Khyber PakhtunKhwa Pakistan

In the present study a total 246 cattle were found stall feeders and 56 were grazed. In case of stall feeders, 18 (7.31%) and 9 (3.65%) samples were found positive on the basis of SPAT and MRT respectively.

Similarly from total 56 samples, 6 (10.71%) and 3 (5.35%) were positive for brucellosis on SPAT and MRT, respectively. No significant association was found in them ($P > 0.05$) (Table 4).

Brucellosis is considered most important zoonotic disease. As this disease make significant economic losses to livestock industry, therefore common diagnostic techniques are essential for its efficient diagnosis. Diagnostic laboratories mostly utilize serological test and MRT for identification of *Brucella* species in the host.

In present study, sero-prevalence of brucellosis was found higher on SPAT (7.94%) as compared to MRT (3.97%). These findings are in agreement of Saleha et al., (2014), they also revealed in their study that SPAT test showed more positive results compared to MRT. This might be due to fact that with the passage of time animals are likely to be exposed to the *Brucella* infection. Radostits et al., (2000) also revealed that this disease is chronic and increases with age.

In present study overall prevalence of brucellosis in District of Bannu and Lakki Marwat was found 5.29% and 10.9% respectively on the bases of SPAT and MRT. Shafee et al., (2011), in their study reported the prevalence of 8.5% in Quetta Pakistan. Similar study was also conducted by Song et al., (2009) who estimated the sero-prevalence brucellosis 5.0% and 0.5% in cattle located in Bangladesh Agriculture University, by using antigens of *Brucella abortus* and *Brucella melitensis*. The same patterns of results were observed by Wali et al., (2005), who assessed the sero-prevalence of brucellosis in livestock at different districts of Khyber PakhtunKhwa. The variation in results might be due to sample size and different habitat of animals.

In the present study the prevalence of brucellosis in age group of > 5 showed higher prevalence compared to the other age group of Cattle (Table 3). These findings are in agreement with Ahmad et al., (2009), who reported the high prevalence of brucellosis in age group of more than five compared to the younger animals. The reason might be due to fact that with the passage of time animals are likely to be exposed to the *Brucella* infection. Radostits et al., (2000) also revealed that this disease is chronic and increases with age.

The present study also revealed that Sahiwal (Native) and local breed of Cattle showed relative low prevalence of brucellosis 0.0% and 7.95% respectively, compared to Friesian cross and Jersey cross breeds (Table 2). It might be due to fact that the native and local cross breeds have developed immunity and more resistance to *brucella* infection as compared to Friesian and Jersey cross breeds. These findings are in agreement with the result of Akbarmehr and Ghiyamirad (2011), revealed that the brucellosis was dominant in non-local breeds of Cattle compared to native and local cross breeds.

Moreover the present study also studied the breeding pattern. It was determined that naturally mated samples have high risk of brucellosis with respect to artificial insemination. It may be due to the regular use of community bull not for single cattle or herd. In the present study high prevalence of brucellosis was also reported in grazing cattle followed by stall feeder. It might be due to congregation of the animals feeding and drinking from common source.

It is concluded that brucellosis is prevalent in these districts and this disease can be diagnosed through regular testing of the animals and awareness should be created to lower the risk of this disease.

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