



Letter to the Editor

Brucellosis remains a neglected disease in District Muzaffargarh of Pakistani Punjab: A call for multidisciplinary collaboration

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Abstract

Brucellosis is a significant zoonotic infection of Pakistani ruminants. Muzaffargarh is a historically and agriculturally important district of Pakistan, and the data on brucellosis remains scarce in this district. To overcome this gap, a preliminary study was performed on a limited number of serum samples collected from randomly selected cattle to estimate the occurrence of brucellosis. Serological examination of fifty-three sera using Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) was followed by molecular diagnosis by applying the *Brucella* genus-specific real-time Polymerase Chain Reaction (RT-PCR). Only four samples (7.54%) were seropositive by RBPT and SAT, and only one sample (1.88%) was positive by RT-PCR. The results point toward a persistent zoonotic threat in cattle in the district and the potential spillover infection when close contact with other animal species. Therefore, pasteurization of milk and regular screening of animals is recommended to prevent transmission. Brucellosis is still a neglected disease in District Muzaffargarh; however, further studies are required to estimate the current situation; meanwhile, the potential risk of positive cases has to be considered.

Keywords: Muzaffargarh, Brucellosis, Serology, PCR

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Background

Brucellosis is an essential bacterial zoonosis, mainly caused by *Brucella* (*B.*) *abortus*, *B. melitensis*, and *B. canis* in domestic animals (bovines, sheep, goats, camels, and dogs). It has significant economic importance to farmers regarding abortion, fetal and milk production losses, and culling of infected animals. It causes placentitis and abortion in the last trimester leading to heavy shedding of bacteria through vaginal secretions (Jamil et al., 2021).

Brucellosis is transmitted via direct contact with infected animals or indirectly via contaminated environment/fomites. In humans, it is transmitted mainly via the ingestion of contaminated unpasteurized milk or dairy products (Hakeem and Saeed, 2019). Therefore, early diagnosis is critical for disease control and eradication. Serological testing by Serum Agglutination Test (SAT), Rose Bengal Plate Test (RBPT), Enzyme-Linked Immunosorbent Assay (ELISA), Com-

plement Fixation Test (CFT), and Milk Ring Test (MRT) are essential tools for animal testing at individual or herd levels (Jamil et al., 2021). Real-Time Polymerase Chain Reaction (RT-PCR) is applied to detect/differentiate *Brucella*-DNA in the samples. Isolation remains the gold diagnostic standard; however, it would require high biosafety levels (BSL), e.g., BSL-3, due to its biohazard potential.

Preliminary investigation of brucellosis in district Muzaffargarh

Muzaffargarh is located on the Chenab river bank in the Southwest of Punjab, Pakistan (30.069° north and 71.194167° south). This area is very fertile due to the flow of rivers Sindh on the west and Chenab on the east. It has very hot summers (~54°C) and mild winters (~-1°C). Dust storms are common, and the average rainfall is 127 millimeters. The district comprises



Figure 1: The spatial location of the area of the study.

four administrative sub-divisions (tehsils), i.e., Muzaffargarh, Kot Addu, Alipur, and Jatoi (Figure 1).

Muzaffargarh is one of the oldest districts of Punjab and is known for cotton, citrus, and mango production in the world. Despite a large bovine (cattle and buffaloes) population of around 1.77 million animals (Anonymous, 2006), studies on the occurrence of brucellosis had been neglected in the district. Few reports on animals (Abubakar et al., 2010; Ullah et al., 2015; Shahzad et al., 2017; Shabbir et al., 2020) and human brucellosis exist in the district and adjoining areas (Khan, 1970; Noor et al., 1986). Given the importance of brucellosis pertaining to the region's public health and livestock, a preliminary study was conducted to estimate the prevalence of brucellosis in bovines in this district.

In the present study, serum samples were collected from 53 randomly selected cows raised with camels in different twelve nomadic herds in Muzaffargarh. Each herd size varied between 15-85 animals with variable numbers of camels and cattle. The sera were screened parallelly by RBPT and SAT standardized antigens (Veterinary Research Institute, Lahore, Pakistan) as described by (Alton et al., 1988).

DNA was extracted from sera by QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Genus-specific PCR targeting the gene (*bcs* 31) was applied using primers described by (Probert et al., 2004). RT-PCR was performed according to Wareth et al. (2015). Briefly, 25 μ l PCR reaction mixture containing 12.5 μ l TaqMan™ Universal Master Mix (Applied Biosystems, USA), 0.25 μ l probe (0.1 μ M), 0.75 μ l of each primer (0.3 μ M), and 2 μ l of DNA template was used. The mixture was filled with HPLC grade water. *Brucella*

DNA and HPLC water were used as the positive and negative control, respectively.

The real-time PCR was performed in duplicates for each sample as follows: One cycle each for initial decontamination at 50°C for two mins and denaturation at 95°C for ten minutes. Fifty cycles each at 95°C for 25 seconds and 57°C for one minute. A cycle threshold (Ct) \leq 40 was considered as positive. Results showed that out of fifty-three serum samples, four (7.54%) were positive for RBPT and SAT (Table 1) and RT-PCR revealed only one positive sample.

A call for multidisciplinary collaboration

Despite brucellosis being endemic in Pakistan, few studies have been found in Muzaffargarh. Previous studies in this district revealed seroprevalences of 0-56.3% (Abubakar et al., 2010; Shabbir et al., 2011; Ullah et al., 2015; Shahzad et al., 2017; Shabbir et al., 2020). However, cross-reactions may be observed due to other Gram-negative bacteria, e.g., *Yersinia*, *Francisella*, and *Proteus* spp. (Abubakar et al., 2012). The current positive samples were tested by two different serological tests, i.e. RBPT and SAT, which exclude the possibility of cross-reaction with other bacteria. The low quality of the DNA extracted and low amounts of circulating DNA in the serum may impair PCR sensitivity. Real-time PCR could improve the lower analytical sensitivity in molecular diagnosis (Gwida et al., 2011).

Although PCR-based techniques are specific, their reliability strongly depends on the equipment, protocol validation, and DNA extraction method (Yu and Nielsen, 2010). Moreover, PCR technology is not always readily available and is too expensive for devel-

Table 1: Number of animals for each test and the health status.

Tests performed	Positive (Prevalence %)	Negative (Prevalence%)
Rose Bengal Plate Test (RBPT)	4 (7.54)	49 (92.45)
Serum Agglutination Test (SAT)	4 (7.54)	49 (92.45)

oping countries. Hence serology remains the mainstay in brucellosis diagnosis in Pakistan (Jamil et al., 2021). Population-based cohort studies and herd-based studies could clarify the epidemiology and potential risk factors of brucellosis, especially in mixed herds in Pakistan. However, because of its intracellular lifestyle, no 100% successful treatment regimen exists for brucellosis to date (Jamil et al., 2017).

Hence, good animal husbandry practices, vaccination, and routine testing of the animals are recommended to prevent the infection. However, culling the infected animals remains the safest choice. Moreover, pasteurization of milk for human consumption will help minimize transmission to humans. For this, awareness programs would be helpful. Brucellosis is an endemic disease in Pakistan, either among animals or humans (Jamil et al., 2021; Yousaf et al., 2021); however, investigation of brucellosis in Muzaffargarh not completed, and very few studies have been carried out, placing a significant burden on the human health and livestock industry in this district.

Based on the previous studies (Abubakar et al., 2010; Shabbir et al., 2011; Ullah et al., 2015; Shahzad et al., 2017; Shabbir et al., 2020) and our preliminary results, we recommend the following measures to control brucellosis in district Muzaffargarh of Pakistani Punjab:

- A large-scale epidemiological survey of brucellosis in cattle, sheep, goats, and camels allocated in the district Muzaffargarh using at least two different serological assays combined with molecular detection is highly recommended to assess the current situation and incidence among different animal hosts.
- If a low prevalence is proved, a test and slaughter strategy must be implemented to eradicate brucellosis in Muzaffargarh effectively.
- If the prevalence rate is high, transportation of animals between herds and from the district has to be controlled. We also recommend vaccination of cattle using *B. abortus* RB51 and *B. melitensis* Rev 1 in the case of sheep and goats, besides test and slaughter strategy.
- Implementing high biosafety and biosecurity measures within herds, raising awareness, and occupational hygiene are necessary to prevent diseases in humans and reduce dissemination among farms and herds.

- Following food-safety measures such as pasteurization of milk for direct consumption and creating derivatives such as cheese is essential in preventing transmission from animals to humans.
- Hygienic measures such as protective barriers and correct handling and disposal of afterbirths, animal carcasses, and internal organs from infected animals are highly recommended for a prevention strategy.

Article Information

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