



Short Communication

Brucellosis is Significantly Associated with Reproductive Disorders in Dairy Cattle of Punjab, Pakistan

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ABSTRACT

Brucellosis is worldwide zoonotic infection. A total of 384 serum samples were collected from cross bred dairy cattle at three different private farms located in Gujranwala, Pakistan. The samples were analysed by Rose Bengal Plate Test (RBPT) and by indirect-Enzyme Linked Immunosorbent Assay (iELISA). The overall seroprevalence was found to be 28.90 and 27.86% by RBPT and iELISA, respectively. Previous history of animal reproductive disorders was found to be significantly associated ($P=0.0056$) with seropositivity of the infection. Age, sex, parity, lactation and pregnancy status of the animal were not found significantly associated. Routine herd screening, consumption of pasteurized milk and public awareness programmes would be helpful for control and prevention of zoonotic transmission.

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Authors' Contributions

QU and TJ wrote the manuscript. QU, SB, Qudratullah, IW and MAS analyzed the samples and data. HJ, LAL and ZIQ reviewed the manuscript. SU, MZ and IK helped in planning and execution of the experiment.

Key words

Association, Reproductive disorders, Brucellosis, Dairy cattle, Pakistan.

Brucellosis is an important abortive bacterial zoonotic disease in animals. It is caused by bacteria from genus *Brucella* which are Gram-negative, non-motile, non-spore forming and facultative intracellular coccobacilli. Primarily, *B. abortus* causes infection in bovines, *B. melitensis* in small ruminants (sheep and goats), *B. canis* in dogs, *B. ovis* in rams and *B. suis* in pigs, however cross-species transmission is possible. *B. suis*, *B. melitensis*, *B. abortus* and *B. canis* have potential zoonotic importance (Aparicio, 2013; Ali *et al.*, 2017). Clinically, brucellosis manifests abortion in last trimester and retention of fetal membranes in female animals, whereas in males, it causes orchitis and epididymitis resulting in overall infertility (Corbel, 2006). Infected

animals heavily shed bacteria through milk, semen and vaginal secretions. Transmission to animals and humans occurs directly either through ingestion or contact with infected materials (*i.e.* aborted fetus, vaginal discharge or contaminated milk) or indirectly through contaminated surroundings (Aparicio, 2013). Diagnosis is usually based on serology where disease is endemic *e.g.* Rose Bengal Plate Agglutination Test (RBPT), Serum Agglutination Test (SAT), Milk Ring Test (MRT), Complement Fixation Test (CFT) and Enzyme Linked Immunosorbent Assay (ELISA). Polymerase Chain Reaction (PCR) provides better and reliable diagnosis however, isolation and identification of the agent remains the gold standard.

Brucellosis is considered as an endemic infection in ruminants in Pakistan. It has been associated with previous history of abortions (Ali *et al.*, 2016, 2017). Present study was designed to estimate the sero-prevalence of brucellosis and its association with reproductive factors among cross

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bred cattle maintained at private dairy farms in Gujranwala Division, Punjab, Pakistan.

Materials and methods

For this study, samples were collected from three different private dairy farms of crossbred cattle (Sahiwal×Friesian), in Gujranwala Division, Punjab province. A total of 384 blood samples, calculated by using formula for unknown prevalence with 95% confidence interval as described by Thrushfield *et al.* (2007), were collected through random sampling method at the farms. Information regarding animal age, sex, parity, lactation, pregnancy status and reproductive disorders were recorded on a structured questionnaire. Approximately 5 ml of blood was drawn from jugular vein of each animal in a syringe, allowed to clot, serum separated in an 1.5 ml Eppendorf tube and stored at -20 °C till further analysis.

Serum samples were screened initially by RBPT and then were evaluated by indirect-ELISA (iELISA) using SVANOVIR® *Brucella*-Ab iELISA kit (Uppsala, Sweden) as per manufacturer recommendations. Samples with Percent Positive (PP)≥40 were considered positive.

The data on different variables, *e.g.* age, sex, parity, lactation and pregnancy sero-prevalence were evaluated by Chi-square test. To determine the level of significance, *P* value ≤ 0.05 was considered as cut off.

Table I.- Association of brucellosis sero-positivity with history of reproductive disorders.

Reproductive disorders	Sero-positive (both RBPT and ELISA)	<i>P</i> -value
Abortion	12/25 (48%)	0.0056*
Stillbirth	2/13 (15.38%)	
Repeat breeder	1/18 (5.55%)	
Retained fetal membrane	4/24 (16.66%)	

**P*-value <0.05 considered as significant association.

Results

A total of 384 bovine sera were tested by RBPT and iELISA for brucellosis and were found 111 (28.90%) and 107 (27.86%) sero-positive, respectively. The two tests did not show any statistical significant association (*P*>0.05) for detection of the sero-prevalence.

Abortion was the most frequently found 12/25 (48%) reproductive disorder among seropositive animals. Overall, the reproductive disorders associated statistically significant (*P*=0.0056) with seropositivity for brucellosis (Table I).

Individual livestock parameters such as age, parity, sex, lactation status and pregnancy status did not show any statistical significant (*P*>0.05) association with the

seropositivity of the disease (Table II).

Table II.- Overall sero-prevalence of brucellosis in cross bred cattle in relation to various risk factors.

Factors	Occurrence	<i>P</i> -value
Age (years)	≤5 5-10 >10	35/135 (25.92%) 44/151 (29.14%) 28/98 (28.57%)
Parity	≤3 3-6 >6	47/179 (26.26%) 35/107 (32.71%) 11/49 (22.45%)
Sex	Female Male	93/335 (27.76%) 14/49 (28.57%)
Lactation status	Lactating Non-lactating	63/201 (31.34%) 30/134 (22.39%)
Pregnancy status	Pregnant Non-pregnant	63/248 (25.4%) 30/87 (34.48%)

**P*-value <0.05 considered as significant association.

Discussion

Brucellosis is considered endemic in ruminant animals in Pakistan (Farooq *et al.*, 2011; Ali *et al.*, 2014). Previous studies have indicated seroprevalence of 3-6% depending on the area and animals whereas *B. abortus* found as main etiology of brucellosis in the country (Ali *et al.*, 2014, 2015, 2017; Abro *et al.*, 2017; Ahmad *et al.*, 2017). The two diagnostic tests did not reveal a significant difference in seropositive animals, may be because of relatively low number of samples. Reproductive disorders, especially repeat breeding and previous abortion history have been found significantly associated with bovine brucellosis (Al-Hassan *et al.*, 2014; Lindahl *et al.*, 2014; Patel *et al.*, 2014; Chaulagain and Bowen, 2016; Geresu *et al.*, 2016; Pathak *et al.*, 2016). However, to the best of our knowledge, only one study in India did not find significant association (Asmare *et al.*, 2013). While breed was found to have significant association with brucellosis, cross bred cattle are considered more prone to the infection (Asmare *et al.*, 2013; Patel *et al.*, 2014; Chaulagain and Bowen, 2016; Kaleem *et al.*, 2016; Ali *et al.*, 2017). Sex, age and lactation status did not show any significant association in our study, however previous reports do associate sex and lactation status of the animal with the infection (Ibrahim *et al.*, 2010; Ali *et al.*, 2017).

Further studies in different areas of the country coupled with confirmatory diagnosis *e.g.* isolation and identification of the etiology could help in better understanding the epidemiology of the disease. The isolates

could be then analysed further for antibiotic resistance, gene characterization and molecular epidemiology. Vaccination could provide a potential solution. So far, there is no drug of choice against brucellosis in animals, hence culling remains the only choice. Animal movement should be regulated with strict health certificate programmes. Pasturised milk is recommended to avoid transmission to humans. Nationwide public awareness especially in herd's people is very necessary as they are at the first risk of infection (Ullah *et al.*, 2015). Routine screening of the herd is essential for prevention and control of the infection in the herd.

Conclusion

The study concludes the seroprevalence of the infection in the area and a potential threat in animals at any age, sex and lactation status. Preventive measures and public awareness remain the only hope to control and prevent the infection in animals as well as in humans.

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Statement of conflict of interest

The authors declare no conflict of interest.

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