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Short Communication

Bovine and Caprine Brucellosis Detected by Milk Indirect ELISA and Milk Ring Test in Islamabad Capital Territory, Pakistan

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IK, RH and AY conceived and designed the study. MN, MS and ZN collected samples, performed the experimental work and analyzed the data. AZ, MU, ZN, QU, and MS wrote and critically reviewed the manuscript.

Key words
MRT, Milk i-ELISA, Brucella, Abortion, Prevalence

ABSTRACT

Brucella abortus and *Brucella melitensis* are important zoonotic pathogens. Brucellosis is an occupational hazard for veterinarians and animal handlers. The current work aimed to determine the prevalence of brucellosis in buffaloes and goats of Islamabad Capital Territory, Pakistan. A total of 341 milk samples from buffaloes (n=180) and goats (n=161) were screened by (Milk Ring Test) MRT and milk i-ELISA for anti-*Brucella* antibodies. Higher prevalence was found in buffaloes (5.6% and 16.1%) than in goat (4.97% and 1.9%) both through MRT and i-ELISA respectively. Commercial production and old age were important risk factors for spread of brucellosis both in goats and buffaloes. A history of abortion was significantly associated ($P<0.05$) with *Brucella* positive MRT and milk i-ELISA tests. In contrast, history of stillbirth had no significant association with anti-*Brucella* antibody titers. In conclusion, brucellosis is prevalent in Islamabad Capital Territory.

Brucellosis is a highly contagious zoonotic disease caused by intracellular, facultative and Gram-negative bacteria of the genus *Brucella (B.)*. Almost all species of animals can be infected including humans. *B. abortus* (mainly infecting bovines), *B. melitensis* (mainly infecting sheep and goats) and *B. suis* (mainly infecting pigs) have got great attention due to their potential zoonotic importance (Blasco and Molina-Flores, 2011; Godfroid *et al*., 2011). *Brucella* infection causes huge economic losses by decreased milk production, retention of fetal membranes, weak offspring, low fertility rate, metritis, arthritis, epididymitis, orchitis and abortion (Wadood *et al*., 2009; Nicoletti, 2010; Ali *et al*., 2017). Impact of brucellosis is more obvious in rural areas where sale of dairy products and production of livestock are the main sources of income and humans are more prone to infection due to direct contact with infected animals and their products. There are various predisposing factors that are directly linked with spread of the disease such as vaccination status, age of animals, parity number, insemination method or purpose of rearing (Aulakh *et al*., 2008; Abubakar *et al*., 2010; Ali *et al*., 2017; Khan *et al*., 2018).

Brucellosis can be diagnosed by different tests including Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Complement Fixation Test (CFT) and Milk Ring Test (MRT) from suspected serum and milk samples (Alton *et al*., 1988; Samartino *et al*., 1999). Enzyme Linked Immunosorbent Assays (ELISAs) have also been standardized for serum and milk samples and are used for confirmation of clinical disease (Shafee *et al*., 2011; Abubakar *et al*., 2012). Polymerase Chain Reaction (PCR) is a latest technique used for detection of *Brucella* DNA in different species. MRT is an economical assay and

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easy to perform. False-positive results are reported from colostrum samples, samples from animals vaccinated in less than four months, animals with subclinical or clinical mastitis or soon after parturition (Nielsen, 2002; OIE, 2009). In the current study, MRT and milk i-ELISA tests were used for screening of herds. The aim of the present study was to determine the seroprevalence of brucellosis and identify different risk factors related to *Brucella* infection in buffaloes and goats in Islamabad Capital Territory (ICT).

Materials and methods

The present study was carried out in the peri-urban area of ICT, Pakistan during the period February-May, 2017. Islamabad lies in a hilly area having a great diversity of fauna and flora. The area is located in north-eastern Pakistan with an elevation of 575 m between the northern part of the Punjab and the western part of Azad Kashmir.

A total of 341 milk samples (5 ml) were collected from buffaloes (Nili-Ravi) and goats (Teddy, Beetal and crossbred) according to standard procedures (OIE, 2009). Initial milk streams were discarded before the actual milk samples were collected. The samples were transported to and stored at 4°C at Veterinary Research Laboratory, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. Samples were analyzed within 6 hours by Milk Ring Test (MRT). All screened animals were reported to be unvaccinated.

In the present study, parameters like education level and main occupation of the farmer, type of husbandry, vaccination and deworming status were also documented. The samples were taken during routine visits and the owners were informed and asked for participation in the study.

For Milk Ring Test the MRT antigen was purchased from Veterinary Research Institute (VRI), Lahore, Pakistan. Milk samples were analyzed as per manufacturer's recommendations. Briefly, 30-50µl of homogenized MRT antigen was mixed with 1ml milk sample in a test tube at room temperature and was well shaken. This antigen-milk mixture was incubated at 37 °C for 1 hour and observed for presence of a violet/bluish colored ring over the milk fraction. Presence of ring indicated positive reaction of anti-*Brucella* antibodies.

For Milk Indirect ELISA the milk i-ELISA kit (ID Vet, France) was used for detection of anti-*Brucella* antibodies as per manufacturer's instructions. Briefly, all milk samples were centrifuged at 5,000 *g* for 5 mins. A volume of 100 µl skimmed milk was collected under the cream and transferred to a well of the iELISA plate and incubated at 21 °C for 45 mins. After washing a (3×2) volume of 100 µl of conjugate was added and incubated again at 21 ºC for 30mins. After (3×2) washing 100 µl of substrate was added and incubated at 21ºC for 15mins. Finally, 100 µl of stop solution was added to each well and optical density was measured at 450 nm.

Results

A total of 4.97% (8/161) goat samples and 5.6% (10/180) of buffalo milk samples were found positive by MRT. iELISA showed 1.9% (3/161) and 16.1% (29/180) positive results for goat and buffalo samples, respectively.

Table I. Comparative seroprevalence of MRT and milk iELISA in buffaloes and goats.

Animal species	No.	MRT	Milk iELISA
Goats	161	4.97% (8/161)	1.9% (3/161)
Buffalos	180	5.6% (10/180)	16.1% (29/180)
Total	341	5.27% (18/341)	9.38% (32/341)

History of abortion was found to be significantly associated with a seropositive to MRT and iELISA results (Table II). Still birth was not found significantly associated with positive results (Table III).

Table II. Association of abortion with brucellosis.

Category	No.	Positive	%age	Chi-square	P-value
MRT +ve					
Non-aborted	332	6	1.8	33.892	0.001**
Aborted	9	3	33.3		
ELISA +ve					
Non-aborted	332	4	1.2	10.731	0.000**
Aborted	9	5	55.6		

P-value<0.05 was considered significantly associate; *P*-value <0.01 was considered highly significant; *P*-value >0.01 was consider non-significant.

Table III. Association of still birth with brucellosis.

Category	No.	Positive	%age	Chi-square	P-value
MRT +ve					
Normal birth	149	11	7.4	0.15	0.619^{NS}
Stillbirth	12	1	8.3		
ELISA +ve					
Stillbirth absent	149	10	6.7	1.596	0.221^{NS}
Present	12	2	16.7		

P-value <0.05 was considered significantly associate. *P*-value <0.01 was considered highly significant. *P*-value >0.01 was consider non-significant.

Age [Young stock (goats<6months and buffaloes<24months) compared to old stock

(goats>6months and buffaloes>24months)] and purpose of rearing (domestic use or business purpose) of animals showed significant association with seropositivity by iELISA. Old stock was more prone to be seropositive than young stock. The animals kept for business purpose were more often seropositive as compared to the domestic purpose animals. Farm type (home vicinity and away from home) and unit of animals/farm (1-8, 9-15 and >15) did not show apparent influence on prevalence of anti-*Brucella* antibodies (Table IV).

Table IV. Relationship of different factors with positive results of the milk iELISA.

Factor	Category	Positive samples	Chi-square	P-value
Age	Young stock	5	6.084	0.009
	Old stock	27		
Farm type	House vicinity	6	0.471	0.321
	Away from house	26		
Animal unit	1 to 8	8	1.201	0.549
	9 to 15	14		
	>15	10		
Rearing purpose	Domestic	1	3.502	0.041
	Business	31		

P-value<0.05 was considered significantly associated.

Discussion

Milk ring test (MRT) and milk indirect ELISA are used to screen anti-*Brucella* antibodies in buffaloes and goat milk. Higher prevalence was reported for buffaloes compared to goats with both the tests. Milk iELISA was found to be highly sensitive for the diagnosis of bovine brucellosis (98.2%) (Kattar *et al*., 2007). Milk iELISA could be an effective screening test because it can detect lower titers of anti-*Brucella* antibodies especially during early infection (Guarino *et al*., 2001). A previous report describes similar prevalence of anti-*Brucella* antibodies in cattle and buffaloes using MRT and milk iELISA (Shafee *et al*., 2011).

Several studies reported that milk ELISA is more sensitive than MRT for the diagnosis of brucellosis in farm animals (Kerkhofs *et al*., 1990; Kang'ethe *et al*., 2000; Vanzini *et al*., 2001). Variation of sensitivity and specificity of serological tests may be influenced by external environment (season or temperature), disease endemicity, vaccination status of animal and herd and presence of any other gram-negative bacterium that might cross-react due to similar epitopes with *Brucella* (Greiner and Gardner, 2000; Mainar-Jaime *et al*., 2005; Matope *et al*., 2011; Khan *et al*., 2018).

Abortion and *Brucella* seropositivity showed significant association. It can be speculated that relevant number of abortions in buffaloes or goats were caused by brucellosis. Thus brucellosis is to be considered a threat for other animals as well as humans. Another study conducted in the same area also reported that abortion is risk factor for brucellosis. Relationship of stillbirth was recorded only in goats; however, there is no available data in buffaloes. Results showed non-significant association between brucella seropositivity and stillbirth in goats.

In the present study, age and rearing purpose of animals were found to be the main risk factors for brucellosis. Similar findings were recorded earlier where association of age with presence of brucella infection was observed (Mohammed *et al*., 2011; Ali *et al*., 2017; Mohamand *et al*., 2014; Khan *et al*., 2018). Higher seroprevalence of brucellosis in animals kept for commercial purposes might be due to frequent selling and buying events, exposure of purchased to infected animals in markets, no screening before sale or purchase time because of fear of business loss among people in peri-urban areas.

Conclusion

In conclusion, the overall prevalence of brucellosis was 4.97% and 1.9% in goats and 5.6% and 16.1% in buffaloes through MRT and i-ELISA respectively in Islamabad Capital Territory. Goats older than 6 months, buffaloes older than 2 years and animals kept for business purpose were at high risk for brucellosis.

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Statement of conflict of interest

The authors have declared no conflict of interests regarding the publication of this article.

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