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SHORT COMMUNICATION

Serosurvey and Potential Risk Factors of Brucellosis in Dairy Cattle in Peri-Urban Production System in Punjab, Pakistan

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ABSTRACT

Brucellosis is a well-known contagious and zoonotic infection of different animals which influences the health status of animal and economy of underdeveloped/ developing countries. Therefore, this study was conducted to estimate the sero-prevalence and risk factors of *Brucella* infection in dairy cattle through reliable diagnostic techniques. A total of 300 cattle was included for blood sampling kept at peri-urban areas of Sargodha, Sahiwal and Chiniot districts of Punjab. All the serum samples were tested through RBPT antigen, i-ELISA and RT-PCR. Results showed that 12.66% animals were seropositive for *Brucella* infection and all seropositive samples were positive for *Brucella* genus specific RT-PCR. Abortion history, gender, repeat breeding, lactating animals and pregnancy status were key factors associated with seropositivity of animals. Results on logistic regression analysis indicated significant association of different potential risk factors such as history of abortion, repeat breeding and lactating stage with brucellosis in animals. From the results of this study, it is suggested that there is a need of continuous screening of dairy animals to control brucellosis and to minimize the risk of human brucellosis.

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INTRODUCTION

Being an agricultural country, Pakistan has a variety of livestock species including cattle, buffaloes, sheep, goat and camels. These livestock species are kept at different ecological conditions of Pakistan and contribute substantially (11.8%) in gross domestic product (GDP) of the country. These animals strengthen the economy of the country by providing milk, meat, animal fat, fibers, butter, hides, manure and other products of human use. Livestock is also a major source of food and income for rural community of world (Rehman *et al*., 2017; Sotnikov *et al*., 2019).

Brucellosis is a neglected zoonosis, which affects a wide range of hosts including multiple species of animals and human beings. Livestock reproductive health and production faces serious consequences associated with *Brucella* infection (Akhtar *et al*., 2019). The reproductive health of animals is mainly affected in term of retained placenta, week calving, repeat breeding, spontaneous abortions, metritis and infertility in female while epididymitis, orchitis and sterility are common abnormalities in male. The reduction in milk production is a major loss associated with brucellosis (Imtiaz *et al*., 2018; Saeed *et al*., 2019). The epidemiology of *Brucella* infection in human cases is mostly associated with the presence of *Brucella* infections wildlife and livestock (Dadar *et al*., 2019). Persons including livestock farmers, milkers, shepherds, veterinary doctors and abattoir workers are considered as the main target of occupational associated human brucellosis (Acharya *et al*., 2018). Many developed countries have controlled brucellosis

from livestock but animal brucellosis in developing countries including Pakistan is still a big source of economic loses (Ali *et al*., 2019). Brucellosis cases of human are also well documented in Pakistan but no prevention and control strategies adapted yet (Saddique *et al*., 2019). Rose Bengal plate test and serum agglutination test are first choice for serological screening of *Brucella* infected cases. However, Enzyme linked immunosorbent assay is a reliable and useful test for the screening of *Brucella* antibodies. Dairy animals are kept for milk production based on four types of systems including peri-urban, smallholder subsistence, rural commercial and smallholder market oriented in Pakistan (Ali *et al*., 2019; Khan *et al*., 2020). Therefore, this study was conducted to estimate the prevalence of *Brucella* antibodies and to determine risk factors linked with occurrence of brucellosis in cattle.

MATERIALS AND METHODS

Study Population: The study was conducted in Chiniot, Sargodha and Sahiwal districts of Punjab, Pakistan. These areas are well-known for production of agriculture commodities and livestock. Peri-urban dairy production systems are commonly practiced in these areas. These dairy farms are located at the peripheries of main urban areas and function as commercial scale units. The herd size of these dairy farms ranges from 10 to 200 animals.

Data and Blood Collection: Data related to location (district), gender, lactation/pregnancy status, history of weak calves, abortion, retained placenta, repeat breeding, infertility and locality were collected using standard questionnaires. A total of 300 dairy cattle were selected for blood collection. Blood samples were directly obtained from jugular vein of each animal in a non-EDTA tube and transported to the Laboratory in an Ice box (4°C). Sera were harvested by centrifugation (3000 rpm for 6 minutes), separated in test tubes (1.5mL) and kept at -20°C for further investigation.

Serology: All sera were initially screened for detection of *Brucella abortus* antibodies using RBPT antigen of ID.vet France following to the manufacturer's guidelines. Then, sera were also analyzed through indirect ELISA kit (ID.vet France). The optical density (OD) was measured at 450 nm using the ELISA reader. A sample to positive ratio (S/P) was calculated. Performance of test and interpretation of results were done as instructions of the manufacturer.

DNA Extraction and Real-time PCR: Serum samples in serology (n=38) were subjected to DNA extraction using FavorPrep^{TM} DNA extraction kit as per instruction of manufacturer (Favorgen Biotech Corp, Taiwan). The primers and probes for *Brucella* genus specific PCR for BCSP-31 gene were used as described earlier (Probert *et al*., 2004). The RT-PCR was performed using MJ Bio-RAD PCR machine and results were interpreted as per standard procedure.

Data analysis: The animals were declared seropositive based on parallel interpretation on both serological tests. Chi-Square analysis was applied to find out any significant difference among epidemiological parameters. The level of significant was P-values ≤0.05. Likelihood ratio test was performed where expected frequency was <5. Logistic regression was applied to find out risk factors for brucellosis. All tests were performed using SPSS version-19 software.

RESULTS

A total of 38 (12.7%) serum samples were seropositive for *Brucella* infection from three districts. The association of seropositivity of infection with different factors is presented in Table 1. The results showed high prevalence of brucellosis in all three districts. However, a non-significant association was observed between seropositivity for brucellosis and study locations. Animal with history of abortion were more often seropositive (30%) for brucellosis. Overall, abortion history was significantly (P=0.001) associated with seropositivity for brucellosis. The seroprevalence was higher (13.6%) in female animals than males. The seroprevalence of brucellosis was more often (19.3%; n=11) in animals with retained fetal membranes and the results were not significant (P=0.094) different in other animals. A significant association (P=0.014) for prevalence of brucellosis was observed between repeat breeders and normally breeding animals. Births of weak calves were most frequently (n=9; 14.5%) observed in *Brucella* positive animals but the association was statistically non-significant (P=0.623). The seroprevalence was more in infertile cattle (15%) when compared to fertile cattle (12.8%). 25.4% lactating animals were positive for brucellosis when compared to non-lactating animals (n=5; 3.3%). The data analyzed for lactating and non-lactating animal was significant (p=0.000). 8.47 pregnant animals were seropositive and a significant association (P=0.024) was observed between pregnancy status of animal and prevalence of brucellosis. Based on logistic regression analysis abortion history, repeat breeding and lactation status were identified as potential risk factors for brucellosis in cattle. All seropositive samples (100%) were positive for *Brucella* genus specific RT-PCR.

DISCUSSION

Bovine brucellosis is endemic in Pakistan. In Present study, 12.7% cattle were found positive for *Brucella* antibodies, which is relatively higher than previous findings (8.3%) in Pakistan (Ahmad *et al*., 2017). Higher prevalence of brucellosis in this study might be an ecological trend of brucellosis in study areas. Variation in prevalence of brucellosis was not significant. The possible reason may be the similar herd management system in study areas. In present study, abortion history was most frequently associated with seropositive for *Brucella* antibodies. The association history of abortion with higher seropositivity for brucellosis in dairy animals has also been reported in Pakistan (Ali *et al*., 2017), Nigeria and India (Behera *et al*., 2020). The possible reason of strong association of brucellosis incidence in animals with history of abortion might be due to poor herd management

Table 1: Chi-square analysis procedure for Association of different Factors for prevalence of *Brucella abortus* antibodies in cattle

Variable	Categories	Sample tested	Positive (%)	Chi-square/ Likelihood Ratio	p-Value
Area					
	Chiniot	100	12 (12)	0.241	0.886
	Sargodha	100	12 (12)		
	Sahiwal	100	14 (14)		
Abortion History					
	Yes	40	12 (30)	12.535	0.00*1
	No	260	26 (10)		
Sex					
	Male	20	0 (0)	5.621*	0.018*
	Female	280	38 (13.6)		
Retained Fetal Membrane					
	Yes	57	11 (19.3)	2.798	0.094
	No	243	27 (11.1)		
Repeat Breeding					
	Yes	189	17 (9.0)	4.561	0.014*
	No	111	21 (18.9)		
Weak calves					
	Yes	62	9 (14.5)	0.242	0.623
	No	238	31 (13)		
Infertility (n=280)					
	Yes	100	15 (15)	0.271	0.603
	No	180	23 (12.8)		
Lactation status (n=280)					
	Lactating	130	33 (25.4)	36.861	0.000*
	Dry	150	5 (3.3)		
Pregnancy status (n=280)					
	Pregnant	59	5 (8.47)	7.439	0.024*
	Non-pregnant	221	33 (14.9)		

*Likelihood ratio test used for expected frequency less than 5.

practices. Therefore, the presence of *Brucella* positive animals in a herd is continuous threat for other healthy animals. The present study reported significantly higher presence of brucellosis in females compared to males. These findings are similar to previous published literature in Pakistan (Khan *et al*., 2020). Similarly, high prevalence of seropositive female reactors animals had also been reported in indigenous cattle in North and Adamawa areas of Cameroon (Awah-Ndukum *et al*., 2018). Moreover, longer reproductive life of female animals and weak immune system can be probable reasons of high prevalence of brucellosis (Awah-Ndukum *et al*., 2018). Retained placental membranes had a significant association with *Brucella* positive animals in this study.

Cattle having a history of weak calving and repeat breeding were also found seropositive in present study. The poor conception rate of animals in brucellosis positive cattle due to reproductive disorders like repeat breeding, abortion, and still birth had been previously reported (Asgedom *et al*., 2016). The increased seroprevalence of brucellosis in infertile cattle has also been recorded in India. Moreover, a significant reduction in livestock reproductive performance and production was reported due to infertility in Kenya (Lokamar *et al*., 2020). Results showed increased risk of brucellosis in lactating cattle which has also been reported (Ibrahim *et al*., 2010). The possible reason for higher seropositivity in lactating/milking animals might be due to lower disease resistance. The association of pregnancy and seropositivity for brucellosis has been previous reported in Pakistan (Khan *et al*., 2020). RT-PCR was used as confirmatory test for seropositive serum samples. Similarly, results have been reported from previous studies (Ali *et al*., 2017; Saddique *et al*., 2019; Saeed *et al*., 2019). The results of study indicate that brucellosis is a cause of multiple reproductive problems of dairy cattle. There is a need of control of brucellosis in dairy cattle to minimize the risk of human brucellosis. Moreover, training and education of livestock farmers about importance of zoonotic diseases (brucellosis) to dairy animals and consumers of dairy products and prevention strategies should be initiated.

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Ethical Approval: This study was approved by the ethical committee of College of Veterinary & Animal Sciences, Jhang, University of Veterinary & Animal Sciences, Lahore Pakistan. From each farmer prior to blood sample collection oral and written consent was taken.

Authors contribution: IK, SA and RH planned and conducted the research. IK and RH prepared manuscript. AR, MY, NK and MF reviewed the manuscript. All authors read the final manuscript.

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