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SEROPREVALENCE OF BRUCELLOSIS IN CATTLE (*BOS TAURUS*) KEPT IN PERI URBAN AREAS OF PAKISTAN

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ABSTRACT

Brucellosis is a highly contagious bacterial disease of zoonotic importance that causes significant economic losses. Many factors affect the prevalence of brucellosis and variable with the diagnostic tests applied. These all necessitates to carry out seroprevalence frequently to suggest control measures, this study was thus carried out to the seroprevalence and various factors affecting its prevalence. Total of 335 cows of various age groups, breeds (Cross-bred (Sahiwal X Friesian), Friesian, Sahiwal, Cholistani and Jersey) and stages (pregnant/non-pregnant, lactating/non-lactating) were selected randomly for sampling. Blood was collected and serum was separated and subjected to Rose Bengal Precipitation Test (RBPT). RBPT positive samples were confirmed by c-ELISA for further analysis. The seroprevalence was 12.53% and 2.40% through RBPT and c-ELISA, respectively. The prevalence of the disease based on RBPT was higher in cross-bred, females, mature, good condition, pregnant and lactating animals as compared to their counterparts. It was concluded that the cross-bred cattle, pregnancy and lactation are playing important roles for the disease prevalence. Specific preventive measures be opted for control of the disease.

Keywords: Brucellosis, Cattle, Prevalence, Faisalabad, Pakistan

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INTRODUCTION

Pakistan's economy is mainly based on Agriculture. This sector directly supports the country's population and accounts for 26% of gross domestic product (GDP). Being subsector agriculture, livestock contributes approximately 56% of value addition in agriculture and nearly 11% to the GDP (Rehman et al. 2017). Livestock production makes a major contribution to agriculture value added services, however, many diseases hamper its production, e.g., brucellosis, Peste Des Petits Ruminants (PPR), foot and mouth disease (FMD), trypanosomiasis, etc. (Hussain et al. 2018, Khan et al., 2018, Imtiaz et al. 2018, Hussain et al. 2020).

Brucellosis is a highly contagious bacterial disease having significant zoonotic importance that causes abortion, low fertility, decreased milk production and cost of replacement (Shafee et al. 2011, Khan and Zahoor 2018). Serious socioeconomic issues can be posed by the disease to livestock owners (Megersa et al. 2011). Due to lack of hygienic measures, public health measures, national animal health and management strategies, the disease is more common in developing countries (Thakur et al. 2002, Farouk et al. 2017, Hassan et al. 2020). Due to rapidly increasing intercontinental tourism and animal trade, there are more chances that disease could spread in developed countries (Greenfield et al. 2002).

The way of transmission is either through infected animal discharges like milk, urine, blood, semen or contact with the placenta, fetal fluids, fetus, and vaginal discharge. The organism can also spread by equipment and clothing. The more chances of infection are in abattoir workers, laboratory technicians, farmers, veterinarians and the persons working with animal products (Mustafa et al. 2011). The entry of Brucella in the body is mainly via intact skin, mucosal layers, lungs, and digestive tract. Then it goes via the blood and lymphatic system to tissues causing a local infection (Lapaque et al. 2005).

Various tests have been developed for screening and confirmation of the disease. The samples of blood, serum, milk, nasal secretions, vaginal excretions are screened and confirmed by RBPT, complement fixation test, and polymerase chain reaction (PCR) (Gupta et al. 2010). A novel, rapid and simple method i.e., rapid vertical flow technology has been introduced for detecting brucellosis but still needs field trials (Shi et al. 2020). In farm animals, serological, bacteriological, and molecular methods are used. Some molecular and bacteriological methods, such as PCR, are economical but time-consuming. In dairy cows, RBPT and Milk Ring Test (MRT) are used for monitoring and screening of the disease (Ali et al. 2013). For diagnostic confirmation, serological tests such as Serum Agglutination Test (SAT), RBPT, and Enzyme-Linked Immunosorbent Assay (ELISA) are used (Imtiaz et al. 2018).

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Brucellosis is still uncontrolled and endemic in Asia, Middle East, Africa and Latin America (Refai 2003, Shahzad et al. 2017). The highest incidence reported in bovines around the world ranges from 0.85% to 76% (Pandeya 2013, Khan et al. 2018). Overall, the seroprevalence of brucellosis in Pakistan ranged from zero to 76% in all livestock species (Gul et al. 2014, Ahmad et al. 2017, Arif et al. 2018). The prevalence of brucellosis was affected by many factors, such as different climatic conditions, sex, age, species, geography, and diagnostic test applied (Gul et al. 2014, Gul et al. 2015), thus it necessitates to carry out seroprevalence frequently. This study was carried out on various breeds of cattle on different forms to know the present status of brucellosis so that preventive measures could be suggested.

MATERIALS AND METHODS

Ethics Statement: Before the start of the sampling, synopsis was approved by the Graduate Studies and Advanced Board, University of Agriculture, Faisalabad (UAF) for research layout and ethical work involved. All the research work was conducted according to the procedure and guidelines devised by the Bioethical Committee, UAF.

Experimental Animals*:* For this study, 335 cows from various age groups (1-5, 6-10, 11-15, and above 15 years), body condition (poor, fair, and good), and breed (cross-bred: Sahiwal X Friesian, Cholistani, Sahiwal, and Jersey) were selected randomly to investigate seroprevalence of brucellosis from two cattle colonies located in peri-urban areas of Faisalabad, Punjab, Pakistan. Approximately 5 ml blood without anticoagulant was collected from randomly selected animals. Serum after separation was stored at -20ºC till further analysis (Bertu et al. 2010).

Sero-diagnosis*:* For screening of brucellosis, serum was screened RBPT (Veterinary Research Institute, Lahore) following the standard procedure described by Aldomy et al. (2009). These samples were also subjected to c-ELISA (*Brucella* Ab C-ELISA test, kit # 10-2701-10) following the procedures described by the manufacturers (Brucellosis commercial ELISA kit, Svanova, Sweden).

Statistical Analysis*:* The data thus collected regarding various breeds, body weight, age, sex and history of abortion and parity were analyzed to find out the seroprevalence of brucellosis using software MINITAB 16.0 version.

RESULTS

The overall prevalence recorded through RBPT was 12.53% whereas by c-ELISA prevalence was 2.39%. On breed basis, the highest prevalence of the disease was recorded in cross-bred animals 15.76% followed in Jersey (11.11%), Cholistani (10.52%), Sahiwal (8.43%) and Friesian (4.54%), by RBPT. However, statistical analysis indicated non-significant difference among various breeds in terms of disease prevalence as shown in Table 1.

Through c-ELISA highest prevalence of brucellosis was recorded in the Friesian breed (4.54%) followed by cross-bred and Sahiwal breed. No animal was found positive in Cholistani and Jersey breed. This difference in prevalence was non-significant statistically (Table 1). However, the probability of the brucellosis was higher in Friesian followed by cross-bred and Sahiwal cows.

Table 1: Breed based seroprevalence of brucellosis in cattle in Faisalabad, Punjab, Pakistan

Breed	Total Animals	Positive	Percentage	P Value
RBPT				
Cholistani	19	2	10.52	0.004
Cross-Bred	184	29	15.76	0.549
Friesian	22	1	4.54	0.475
Jersey	27	3	11.11	0.772
Sahiwal	83	7	8.43	0.950
Overall	335	42	12.53	
c-ELISA				
Cholistani	19	0	0	0.999
Cross-Bred	184	6	3.26	0.999
Friesian	22	1	4.54	0.999
Jersey	27	0	0	1.000
Sahiwal	83	1	1.20	0.999
Overall	335	8	2.39	

*As the number of animals in most groups is less than five (5) so that's why the statistical analysis is not showing this data.

On the RBPT basis, seroprevalence was higher in females (12.73%) as compared to male animals (9.52%). Similarly, seroprevalence was higher in non-lactating (13.38%) than lactating animals (12.20%). Pregnant cows showed higher (9.68%) seroprevalence than non-pregnant (4.52%) cows (Table 2). P value indicated that differences among various groups based on sex, pregnancy and lactation status were non-significant in this study. While based

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on the confirmatory test (c-ELISA), more prevalence in lactating animals 4.09% as compared to non-lactating, dry and pregnant animals was found (Data not shown).

On the RBPT basis, more (P>0.05) seroprevalence was in 6-10 years group (13.63%) followed by 12.96%, 11.45% and 0% in age groups 11-15, 1-5 and >15 years, respectively (Table 3). On confirmation through c-ELISA, seroprevalence was more (P>0.05) in the age group of 11-15 years than other age groups (Table 3). The prevalence in poor condition animals was 13.33%, as compared to good body condition animals (Table 3). No animal with fair body condition was found positive for Brucella antibodies. The seroprevalence was more in poor condition animals (3.33%) as compared to good condition animals, however, the difference was non-significant.

Table 2: Seroprevalence of brucellosis in relation to sex, lactating and pregnancy status in cattle by applying RBPT

Parameters	Sex		Lactating Status		Pregnancy Status	
	Male	Female	Lactating	Non-Lactating	Pregnant	Non-Pregnant
Total	21	314	172	142	93	221
Positive	2	40	21	19	9	10
Prevalence %	9.52	12.73	12.20	13.38	9.68	4.52
	Chi sq. = 0.148; P-Value = 0.70; df = 1		Chi sq. = 0.065; P-Value = 0.798; df = 1		Chi sq. = 2.657; P-Value = 0.103; df = 1	

Table 3: Age and body condition-based prevalence of brucellosis in cattle

Test	Age (Years)				Body Condition		
	1-5	6-10	11-15	<15	Good	Poor	Fair
RBPT							
Total	96	176	54	9	295	30	10
Positive	11	24	7	0	38	4	0
Prevalence %	11.4	13.63	12.96	0	12.88	13.33	0
Chi square = 1.364; P-Value = 0.713; df = 3					Chi square = 1.292; P-Value = 0.52; df = 2		
c-ELISA							
Total	96	176	54	9	295	30	10
Positive	2	4	2	0	7	1	0
Prevalence %	2.08	2.27	3.7	0	2.37	3.33	0
Chi square = 0.639; P-Value = 0.887; df = 3					Chi square = 0.348; P-Value = 0.84; df = 2		

DISCUSSION

Brucellosis is regarded as one of the most important zoonotic diseases in the world (Abubakar et al. 2011, Zeng et al. 2019, Alkahtani et al. 2020). The economic losses arising from brucellosis in animals are mainly due to the clinical manifestation of abortions during the last trimester of gestation, the decrease in milk yield, temporary infertility and perinatal mortalities (Munir et al. 2011, Gul et al. 2015). To prevent the losses, routine monitoring of animals and early diagnosis is necessary. Controlling the disease in livestock is an important task in many countries. In Pakistan, the prevalence of brucellosis is increasing especially in large-sized dairy herds. There is no official strategy for brucellosis control and eradication. Consequently, there has not been taken any necessary action to restrict the spread of the disease in various private and government farms (Akhtar et al. 1990).

One of the rapid and sensitive techniques for screening animals against Brucella infection is RBPT (Ruiz-Mesa et al. 2005). The RBPT has low specificity, to overcome this problem an indirect ELISA (i-ELISA) is used which has high specificity (Nielson 2002). In the present study, we opted for the same pattern to screen cows against brucellosis in the study area.

In the present study, a higher prevalence of brucellosis was detected in cross-bred (Sahiwal X Friesian) cattle of more than 5 years of age. A cross-sectional type epidemiological survey carried out by Gumi et al. (2013) indicated higher seroprevalence of brucellosis in older animals as compared to the younger ones. Swiss and Schoonman (2010) also suggested that the animals of more than 6 years of age were more prone to seropositivity than animals having less than 6 years of age. That could be due to impaired immune system rendered by the disease, aging and even in pregnant animals, production of erythritol within the placenta also allows rapid multiplication of bacteria that leads to endometritis, infections of cotyledons and placentitis (Gul et al. 2007).

The presence of Brucella infection in animals having abortion history has also been supported by Dhandet et al. (2005). While Ibrahim et al. (2010) reported that the occurrence of abortion is positively correlated with the seropositivity of brucellosis. Similarly, a positive correlation was observed among seroprevalence and the occurrence of abortion as reported by Ahmad et al. (2009). In the present study, seroprevalence was higher in females as compared to males that are in accordance to the previous report by Deselegn et al. (2011) who stated that there was a significant variation in the prevalence of brucellosis based on sex with a higher prevalence in females as compared to males.

In the present study, there was higher prevalence in lactating animals as compared to non-lactating animals. Similar results were obtained by Ibrahim et al. (2010) through serological survey of brucellosis, they indicated higher seroprevalence in milking animals than non-lactating animals that could be due to the reason that lactation period

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reduces the resistance of animals, thus making them valuable to attach of *Brucella abortus* infection (Hashim et al. 2007).

Conclusion: The results of the current study indicated the risk factors considered for this study are playing an important role in the prevalence of brucellosis. Indigenous breeds like Sahiwal and Cholistani cattle less susceptible as compared to the exotic breeds. Similarly, sexually matured animals are more prone to brucellosis as compared to the younger and aged animals. A lot of other risk factors like poor management and hygienic practices observed in these farms need to be explored to control the disease.

Contribution of Authors: AK and STG conceived and designed experiments. UDK performed the experiments. AK and MKS carried out the supervision of studies. UDK performed statistical analyses of experimental data and prepared the draft of the manuscript. XXD edited the manuscript. All authors critically revised the manuscript and approved the final version.

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