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Seroprevalence and Risk factor of *Brucella Abortus* in Cattle (Indigenous and crossbred) in District Layyah

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Abstract

Background: Brucellosis is very important infectious, zoonotic disease all over the world affecting variety species, causing severe economic losses in animals and severe illness in human beings. This study was planned to find the seroprevalence and associated risk factors with *Brucella Abortus* in indigenous and crossbred cattle of District Layyah.

Methods: The blood sample were collected from 350 blood animals and screened by Rose Bengal plate test (RBPT) using anti-Brucella antibodies. The seropositive samples were confirmed through indirect ELISA tests. The information on risk factors associated with brucellosis were collected on pre-designed questionnaires.

Results: The result of the present study showed the overall prevalence of 6.97% in cattle in district Layyah. It was more prevalent in the age group of 2-5 years (14.6%) as compared to that of the age group 5-10 years (6.25%). The prevalence was significantly higher in the cattle with history of abortion (52.38%) as compared to the non-aborted cattle (3%). The crossbred animals were found to be more affected than indigenous animals.

Conclusion: There is a dire need to eliminate the seropositive animals by application of proper control programme and eradicate this zoonotic disease in developing countries especially in Pakistan.

Introduction

Pakistan is a developing and agricultural country having variation in geo-graphics and climatic condition. Livestock sector is the sub sector of agriculture and is considered the backbone of the agriculture sector. It contributes about 11.39% in national GDP and 58.3% in agriculture value during the year 2016-17 [1]. The Cattle is the main breed of the country which plays an important role in contribution of milk production and earning of foreign exchange of the country. Cattle produce milk 16.741 million tons on daily basis [2]. Bovine brucellosis remains a persistent in ruminants caused severe economic losses, has spread worldwide and affect a wide range of animals and humans [3]. It caused by bacteria of genus *Brucella* that remains live for longer time in high humid condition, low temperature, without sun light, wet soil and for several month in water, aborted fetus and manure under suitable conditions [4]. It is a facultative intracellular, gram negative, non-motile, non-capsulated, and non-flagellated and non-spore forming coccobacilli [5]. Brucellosis clinically characterized by reduced milk yield, abortion, neonatal mortality, hygroma, infertility, epididymitis and orchitis. Abortion in the last trimester and retention of fetal membranes are the typical signs in female while the orchitis and epididymitis are common in male, however infection may occur asymptomatic and infected animals may remain undiagnosed [6]. Brucellosis spread through aborted fetuses and reproductive discharge. The recovered animals might be shed the organism through milk, urine and reproductive discharges [7]. The disease also occurs in new-born calves through mother. In humans it spreads through consumption of raw milk and uncooked meat [8]. Brucellosis is also prevalent in many advanced countries in Europe like Australia, Canada and also remained uncontrolled in developing countries like Africa, Mediterranean, Middle East, part of Asia and Latin America [9]. Brucellosis is a second most zoonotic disease in the world [10]. Brucellosis is an unnoticed infection in Pakistan and no proper control measures for Brucellosis control and eradication. Brucellosis is more common in Pakistan and India and have been reported several times. Brucellosis is unnoticed infection and has no authorized scheme for Brucellosis control and extinction until now. Enlargement of testis, epididymis, seminal vesicle and ampulla has been realized in male and produce low class semen.

In developing countries, like Pakistan, India and Bangladesh, livestock especially cattle are the basis of livelihood of rural people. They get food, skin, fiber, manure and used it for draught purposes. Although brucellosis can be diagnosed by using culture and

isolation, but it takes long time for diagnosis. Presently, the most important and confirmatory techniques are serological tests which are reliable, easy to perform and less time consuming. Keeping in view the economic importance of animal brucellosis and its zoonosis factor, this study was planned to investigate the seroprevalence of brucellosis in cattle and risk factors associated with it in densely livestock populated district Layyah of Punjab, Pakistan.

Methods

Description of study area

The present study was designed to find the seroprevalence of Brucellosis in cross and indigenous cattle breed in District Layyah as being the largest numbers of animals. Geographically Layyah is situated of 70°56' East longitude and 30°58' North latitude with an altitude of 143 meters. It has highest temperature of 53°C, hot climate and minimum rainfall. It makes 0.8% area of Pakistan and 3% area of Punjab and has a covers area of 6291 square kilometer. The District Layyah has three Tehsils, 44 Union Councils and 720 revenue units or Mauzas. The Choubara Tehsil is the biggest one that covers 44% area of the district and is mostly barren and having brackish water but other two Tehsils are agriculturally well developed. The socioeconomic activities people mainly depend on the cattle.

Sample collection

A cross-sectional study was conducted on indigenous and cross breed cattle during the period from July 2018 to June 2019 over a period of one year using stratified sampling technique. A predesigned questionnaire proforma was administered to owners to collect the information with respect to age, zone, malady history, conceptive issues, for example, premature birth history and regenerative infections to assess the risk factors. A total 350 blood samples were randomly collected from three Tehsils, Layyah, Karor Lal- Esson and Choubara (125, 172 and 53) respectively. Equal number of samples (175) were collected from indigenous (Sahiwal and Cholistani) and cross breed (H-Friesian and Jersey) cattle breeds. Approximately 5ml of blood sample was collected from the jugular vein of each selected animals and mark of identification of each cattle was branded on corresponding vacutainer. After collection, samples were transported in ice boxes to District Diagnostic Lab for performing serological tests. The Serum was separated from each sample and stored at -20 C° in Lab till further process.

Serological tests

RBPT test and indirect Enzyme Linked Immunosorbent Assay (i-ELISA) were performed to find the

seroprevalence of brucellosis in cattle and to evaluate the diagnostic performance of RBPT.

Rose Bengal plate test

RBPT test was performed according to the standard procedure of our lab. Briefly, serum and antigen are brought to room temperature before use. *Brucella* positive and negative reference for tests was utilized on each plate. One drop (30 µL) of serum was put on a glass slide by micropipette and one drop (30 µL) of Rose Bengal antigen was added after shaking the antigen bottle. The antigen and serum were mixed thoroughly using stick applicator and the outcome was examined promptly after 4 - 5 min. An outcome was viewed as positive when there was any level of agglutination occurrence, and the nonappearance of agglutination was considered as negative.

Procedure of Indirect Enzyme Linked Immunosorbent Assay

The samples that were positive through rose RBPT, were also confirmed through i-ELISA in provincial diagnostic lab, Lahore using commercially indirect multispecies ELISA kits as directions of manufacturers. Briefly, precoated with purified *Brucella abortus* lipopolysaccharide antigen, 96 wells plate was used and multispecies horseradish peroxidase was used as conjugate. The excess conjugate after washing was eliminated with substrate solution (TMB+DMSO+H₂O₂). The antigen-antibody conjugate peroxidase complex coloration was observed that indicated the quantity of anti-*Brucella* antibodies present in test sample. The blue color indicate the presence of antibodies and it became yellow after addition of stop solution whereas no color appear in the absence of antibodies. The plate was read through an automatic ELISA reader at 450 nm and OD value was used to determine the result as per manufacturer's instructions.

Risk factor analysis

The designed questionnaires were used to collect the information on range of risk factors from cattle handlers and owners of animals. The assessment of some factors done by the clinical examination of animals and herds.

Statistical analysis

The data were analyzed statistically through IBM-SPSS, version 21. The chi square value was used to find the significance difference among risk factors and variables. P-value ≤ 0.05 were considered statistically for all analysis.

Results

Twenty-six samples (6.97%) were found positive using RBPT in district Layyah. The seroprevalence of brucellosis was highest (12%) in tehsil Layyah followed by 5.2% in tehsil Karor and 3.7% in tehsil choubara. The seroprevalence was significantly varied from one tehsil to another (Table 1). The result showed that the prevalence was significantly higher in the age group 2-5 years (14.63%), than 5-10 years age group (6.25%) and 10-15 years (1.67%). In relation to breed of the animal, the prevalence was significantly higher in crossbred (9.71%) than the local breed (5.14%). A significantly higher prevalence (52.38%) was recorded in animal that had the abortion history as compared with the animals which had no abortion history (3.06%). On the basis of locality, the prevalence was significantly different in different areas of District Layyah. The prevalence was much higher in female (7.77%) as compared to male (4.87%). The medium body conditioned animal has more prevalence (8.74%) as compared with emaciated (6.66%) and healthy (5.73%). The seroprevalence of brucellosis was more in larger herd (21.66%) as compared with smaller herd (11.42%). The association of infection increased with increase herd size. There was no significance difference in lactating (7.78%) and non-lactating animals. The prevalence of brucellosis was more in pregnant animals (11.19%) as compared with non-pregnant (5.14%). The animals having the history of retained placenta and stillbirth had significantly higher prevalence (P < 0.05) as with animals had no history of retained placenta and still birth as depicted in Table 2. Twenty-four (6.85%) sample were found positive through iELISA based on antibody titer. The antibody titer range of positive sample was shown in table 3.

Parameter	Area	Total no blood sample collected	Positive	%age	Chi-square Value	P-Value
Tehsil	Layyah	125	15	12.00	5.9858	0.045
	Karor	172	9	5.2	-	-
	Choubara	53	2	3.7	-	-
Total		350	26	6.97	-	-

Table 1: Seroprevalence of brucellosis in Three Tehsil of District Layyah.

Discussion

Brucellosis is an infectious, zoonotic and bacterial disease, prevalent all over the world and affecting both animals and humans. It has devastating effects on the economics of the country, human health and animal industry. It causes temporary or permanent infertility, still birth, retained fetal membranes, reduced milk production that result in adverse effects on production of dairy animals. It is more prevalent in developing countries like Pakistan because unawareness of the people of these countries about this disease, the risk

Variables	Factors	Sample Examined	Positive Sample	Prevalence (%)	Chi-square Value	p-Value
Age (years)	2-5	82	12	14.63	11.007	0.000
	5-10	208	13	6.25		
	10-15	60	1	1.67		
Breed	Local breed	175	9	5.14	2.4615	
	Cross breed	175	17	9.71		
Abortion History	Abortive Exotic breed =16 Abortive Local breed =5	21	11	52.387	2.4615	
	Non- Abortive Exotic breed=159 Abortive Non-Abortive local breed=170	229	7	3.06		
Locality Based on union councils	Thaljandy	37	7	18.91	36.68	0.000
	Mondy Town	40	4	10.00		
	Jamon shah	32	4	12.5		
	Karor City	44	2	4.54		
	Karor thal Jandy	56	2	3.57		
	98 TDA	53	5	9.43		
	Choubara city	28	1	3.57		
	Nawankot	30	1	3.33		
Jamal chapery	32	2	6.25			
Sex	Male	41	2	4.87	18.615	0.000
	Female	309	24	7.77		
Body condition of the Animal	Emaciated	45	3	6.66	12.56	0.03
	medium	183	16	8.74		
	Good	122	7	5.73		
Herd size	≥ 5	60	13	21.66	3.95	0.04
	≤ 5	35	4	11.42		
Lactating Status n=309	Lactating	167	13	7.78	1.96	0.34
	Non-lactating	142	11	7.74		
Pregnancy Status	Pregnant	134	15	11.19	5.65	0.02
	Non-pregnant	175	9	5.14		
Retained Placenta	Yes	120	17	14.16	7.16	0.01
	No	199	11	5.52		
Still birth	Yes	91	13	14.29	6.76	0.01
	No	218	9	4.13		

Table 2: Risk factors associated with brucellosis in cattle in district Layyah on the basis of Chi-square Test.

Sr No.	Name	Address	Tehsils	Cell no	No of Samples	Age of animals (Year)	Anti-body titer
1	Muhammad Tariq	143/TDA	Layyah	0302-8482240	1	7	211%
2	Muhammad Tariq	143/TDA	Layyah	0302-8482240	1	6	213%
3	Muhammad Tariq	143/TDA	Layyah	0302-8482240	1	4	207%
4	Muhammad Tariq	143/TDA	Layyah	0302-8482240	1	3	226%
5	Rahim Bakhsh	150 /TDA	Layyah	0307-7845759	1	5	195%
6	Muhammad Sohail	150 A/TDA	Layyah	0300-8430950	1	8	189%
7	Ghullam Akbar	147A/TDA	Layyah	0304-1576147	1	7	189%
8	Muhammad Akmal	147A/TDA	Layyah	0300-6761483	1	7	205%
9	Ameer Muhammad	147A/TDA	Layyah	0300-6764880	1	7	197%
10	Ijaz Hussain	125/TDA	Layyah	0307-8763537	1	5	201%
11	Farukh Hussain	125/TDA	Layyah	0308-6320818	1	2	199%
12	Najeeb Hussain	125/TDA	Layyah	0303-6977574	1	4	180%
13	Muhammad Asgher	148A/TDA	Layyah	0303-7446848	1	5	213%
14	Dilawar khan	Kotla Haji	Layyah	0300-4966576	1	7	185%
15	Taheryaqob	165/TDA	Layyah	0303-6768430	1	5	200%
16	Ghullam Abas	306/TDA	Karor Lal Eson	0301-7847964	1	6	201%
17	Ghullam Abas	306/TDA	Karor Lal Eson	0301-7847964	1	7	197%
18	Ghullam Abas	306/TDA	Karor Lal Eson	0301-7847964	1	7	197%
19	Ghullam Abas	306/TDA	Karor Lal Eson	0301-7847964	1	5	189%
20	Ghullam Abas	306/TDA	Karor Lal Eson	0301-7847964	1	10	193%
21	Khalid Mehmood	111 ML	Karor Lal Eson	0345-1031080811	1	5	211%
22	Rashid Ali	111ml	Karor Lal Eson	0344-0703163	1	7	213%
23	M Hussain	89/TDA	Karor Lal Eson	0305-8238521	1	6	190%
24	Muhammad Ayoub	Baloch Nager	Karor Lal Eson	0304-2294193	1	9	194%

Table 3: Antibody Titer Determination against *Brucella Abortus* through ELISA Test.

factors of the disease and control measures. In the present study, we found the higher prevalence to be 6.97% in crossbred and indigenous cattle in district Layyah which is higher than the previous studies in Punjab, Pakistan. However, the higher prevalence (8.6%)

was recorded in Potohar Areas of Pakistan [11]. A higher prevalence of the disease (9.3%) was recorded in camels in district Faisalabad [12]. The seroprevalence of *Brucellosis* varies from region to region, country to country, specie to specie, and also within the country. In

different countries of the world like chencha District of Gamo Gofu Zone the prevalence was noted as 4 (1.04%) by [13]. In Bangladesh the overall prevalence was noted 21.36% [14]. The prevalence of Brucellosis was observed in Pakistan is lower than that of Bangladesh and chencha District of Gamo Gafu zone. The overall prevalence was documented 3% and 3.20% at slaughter house of Quetta in Baluchistan [15]. A cross sectional study was performed in Punjab province of India and observed prevalence of brucellosis 27.95% and 18.11% by applying the RBPT and MAT test respectively [16]. Now, the seroprevalence is increasing with passage of time at farm level in Pakistan. The seroprevalence of *Brucella* is increasing in Pakistan such as at large farms [17]. At dairy farms when animals becomes infertile and non-conceptive then these are sold to small dairy farmers. These animal farms became a source of spread of disease in the field. The RBPT and ELISA test are performed to diagnose at all Districts and provincial labs of province of Punjab in Pakistan. The prevalence is higher in cross breed animals than in local breed of cattle and buffaloes by using Milk Ring Test (MRT) at region Islamabad, Rawalpindi and Attock. Similar results were noted by [18-20]. The seroprevalence at herd level 42.5% that was lower in percentage as compared to the study was performed in Syria that was 53.6% [21]. These differences shows the faults in management like grazing at same pasture and contact with herd to herd results in the spreading of disease. A study conducted at government farms, private and Gawala colony in Lahore to find out seroprevalence at different locations. They observed 15.2% and 9% by using ELISA and RBPT respectively [22]. It was noted in other studies, that the prevalence was 4.34% at age above 4 years of cattle and 3.82% at age below 4 years and the prevalence increases with advanced age [23-25]. It means in older animals, prevalence is high than that of lower age groups of cattle. In relation to sex of animals, there is also some controversy in different researcher's statements. The prevalence was higher in male than female [26] and some studies found higher prevalence in female than male [15, 22, 27]. In the present study more prevalence was observed in female as compared to male. This may be due to more population of female in the area as compared with male. The result of the present study revealed that overall high prevalence of brucellosis is recorded in cattle in district Layyah of Punjab, Pakistan. Mangemental factors such as region, locality, herd size and animal related factors such age, sex, bread of animal and typical symptoms such as abortion history, retained fetal membranes and still birth are associated risk factors with brucellosis. Brucellosis is a major public health and animal health problem in Pakistan as there in no specific control

programme exist in the country. The present study also provide important information on the epidemiology of brucellosis in cattle in district Layyah, Punjab, Pakistan and highlight the need of application of strategies for control of brucellosis and introduction of education of programs regarding transmission and control of this devastating disease in the country.

Competing Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Author Contributions

Asghar Hussain and Muhammad Tarique Tunio supervised the research and helped in troubleshooting, Muhammad Kashif, Usman Waheed and Tanveer Ahmad conducted statistical analysis, and Muhammad Ikram and Atif Rahman performed the laboratory work and Asghar Abbas drafted the manuscript.

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