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SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF BRUCELLOSIS IN BUFFALOES IN DISTRICT LAYYAH

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ABSTRACT

Brucellosis causes huge economic losses in the dairy industry and highly contagious and zoonotic disease lead to infertility, decreased overall milk production, and abortion in dairy animals. The aim of this study was to find the seroprevalence and associated risk factors of brucellosis in different breads of buffalo in District Layyah. For this purpose, Rose Bengal Plate Test (RBPT) and Milk Ring Test (MRT) were performed using the standard procedure. The results showed that the higher prevalence of brucellosis was found (6.6%) in tehsil Layyah compares to the other tehsils. The overall prevalence in the District Layyah was found 4.7% and Nilliravi bread was found more susceptible than the Kundi breed. The animals older than 5 years were found to be more susceptible as compared with younger animal. The prevalence of brucellosis was more in female buffaloes.as compared with the male. There was more abortion and reduction in milk in seropositive animals. It was concluded that higher prevalence of brucellosis in buffaloes and higher authorities should take measures to eliminate the seropositive animals to prevent the spread in people who are directly engaged with these animals' veterinary staff and farmers.

Keywords: *Bubalus bubalis*, buffaloes, seroprevalence, brucellosis, Layyah

INTRODUCTION

Pakistan's livestock has a huge contribution to agriculture and has a unique position in economic development. Above 8.0 million families are dependent upon livestock. Livestock contribution to agriculture is 56.3% whereas its contribution to national gross domestic production (GDP) is 11.8%. The population of buffalo in Pakistan is 34.60 million while in Punjab it's 22.46 million. In Punjab, according to breed, there are 11.95 million *Nilliravi* buffalo's 1.15 million Kundi buffalos 0.06 million Azakhali buffalos while 9.31 million buffalos of mixed breeds. Buffalo produced 31.252 billion litter of milk during the year 2014 to 2015. (Economic Survey of Pakistan. 2014 to 2015). Pakistan is the 4th largest milk producing country.

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In Pakistan milk is a chief cash crop and that will be approximately 60% high than that of the major crop of wheat and cotton (Singh *et al*., 2002).

"Bang's disease" and "contagious abortion" are also terms used for brucellosis. A bacterium *Brucella abortus* i*s* the key cause of the ailment brucellosis. It can also cause a disease in a human known as "undulant fever". Brucellosis infection in buffalo caused premature calving, abortion of newly infected animals. The abortion due to *Brucella abortus* occurred mostly during the third trimester of pregnancy. Retention of the placenta is frequently seen in brucellosis after birth is difficult to get rebred and sometimes become sterile. These are gram-negative, non-capsulated, non-motile, facultative intracellular, non-flagellated coccobacilli and non-spore-forming (Kaur and Deepti, 2017). Affected animal drops the fetus in the last trimester of pregnancy and mortality of calves in areas where culling and other preventive measures are not adopted. The long-term chronic infection causes carpal hygroma and infertility. The major sources of transmission are aborted fetuses and reproductive discharge. *Brucella* organisms also spread through the milk and reproductive excretion in animals. It also occurs in newborn calves through vertical transmission. The major source of transmission of brucellosis in humans are the feeding of raw milk and uncooked meat. It reduces the survival of newborn calves and milk production. It reduces the 20% milk production of the infected animals. But death rate in an adult is not significant (Kala *et al*., 2018).

The prevalence of brucellosis is very low advanced countries in Europe like Canada, Australia, and remains uncontrolled in developing countries like Africa, the Middle East, the Mediterranean, a portion of Latin America, and Asia (Mahajan *et al*., 2017). Brucellosis is an additional most zoonotic disease in the world (Chisi *et al*., 2017). It is a serious and difficult issue for animals in the world (Gogoi *et al*., 2018). Brucellosis is considered one of the major bacterial infections of buffaloes which causes infertility in male as well as female animals. The prevalence of brucellosis in several areas of Pakistan is not determined. Therefore, the present study was designed to observe seroprevalence of Brucellosis in Kundi and *Nilliravi* buffalo breeds in district Layyah, Punjab, Pakistan.

MATERIALS AND METHODS

Study area

This study was planned to estimate the seroprevalence of Brucellosis in buffalo breed in District Layyah as being the largest number of animals in the study area. Layyah is one of the Southern District of Punjab provinces. The district Layyah has 3 Tehsils, and 44 Union Councils and most of the areas are barren and brackish water and only few areas are well developed for agriculture.

Sample collection

A total of 180 milk samples were collected from the study area. 90 milk samples from each age group and 60 milk samples from each Tehsil. Similarly, 360 serum samples were collected from the targeted population (*Nilliravi* and Kundi). Total of 180 from each age group and 120 from each Tehsil. Data collection sheets were used to gather information of the sampled animals including abortion history, retained placenta, age of the buffalo, species of the animal, bread to find the risk

factor association with disease.

Samples examination

The milk and serum samples that were brought to the laboratory were examined under a microscope. The following tests were conducted for the interpretation of collected samples.

Milk Ring Test (MRT)

One ml of milk was taken in a test tube and a drop of *Brucella* antigens was added to it. This suspension was kept in the refrigerator at 4°C overnight. After 1 h of incubation at 37°C, the results were read. A stained cream layer over a white column of milk was found in positive reactions.

Rose Bengal Plate Test

Rose-Bengal Plate Test was performed according to the procedure of our laboratory. Briefly, one drop of Rose Bengal antigen was added with one drop of serum sample and mixed thoroughly with the help of applicator for 4 to 5 minutes. The formation of agglutination after reaction was considered positive whereas nonappearance of agglutination considered as negative.

RESULTS AND DISCUSSIONS

Tehsil wise results in different tehsil are shown in (Table 1). The percentage of disease in the Nili Ravi breed in tehsil Layyah was 6.6%, and 3.3% were in both the Karor and Choubara among the age group of 3 to 5 years while it was 10%, 3.3%, and 3.3%.

In the same tehsil respectively in the age group of more than 5 years correspondingly. The overall prevalence of brucellosis in buffalo was 4.7% in district Layyah. The 17 samples were positive out of 360. The statistical results showed that the prevalence ratio is significantly higher in Nilliravi of age for more than five years and the data is significant by using the chi-square method at $P<0.01$.

A serological test (RBPT) was also performed to confirm the disease in both the aborted and non-aborted animals (Table 2). Results obtained via history taken through datasheets interprets that the disease was more prevalent in the aborted animal.

Comparison of seroprevalence of *Brucella abortus* regarding retains placenta history in district Layyah (Table 3). The statistical results show that the prevalence ratio is significantly higher among female buffaloes than male buffaloes (Table 4). The MRT test was also performed in the laboratory using a milk sample. The prevalence of brucellosis was observed in district Layyah using MRT was shown in (Table 5). The overall prevalence of *Brucella abortus* was found 3.3% (6/174) in 3 to 5-year age and 4.97 (9/171) in more than 5 years of age by using the MRT test. The prevalence of brucellosis comparatively higher in old age buffaloes.

The present study was conducted in district Layyah to check the seroprevalence of Brucellosis in buffaloes. Out of 360 serum samples, 17 (4.72%) were found positive. Many researchers worked on the prevalence of brucellosis in various regions of the world. They conclude different results for different areas. The variations are seen among age, breed, sex, and species. In different countries in the world like the chencha District of the GamoGofu region the prevalence was noted as 4(1.04%) (Yilma *et al*., 2016). The overall prevalence in Bangladesh was calculated as 21.36% (Raies-ui-Islam *et al*., 2013). The prevalence of Brucellosis in Pakistan was seen

Table 1. Prevalence of *B. abortus* in district Layyah within the different age groups.

Tehsils	Breed	3-5 Years (n=30) (RBPT)		>5 years (n=30) (RBPT)			
		(+)	Prevalence %	(+)	Prevalence %	Chi-square	P-Value
Layyah (n=120)	Nilliravi (n=60)	2/30	6.6	3/30	10	10.05	0.00*
	Kundi (n=60)	1/30	3.3	1/30	3.3		
Karor (n=120)	Nilliravi (n=60)	1/30	3.3	3/30	10		
	Kundi (n=60)	1/30	3.3	1/30	3.3		
Choubara (n=120)	Nilliravi (n=60)	1/30	3.3	1/30	3.3		
	Kundi (n=60)	1/30	3.3	1/30	3.3		
Total	n=360	7/180	3.85	10/180	5.53		

Table 2. Comparison of Seroprevalence of *Brucella abortus* regarding abortion history in Tehsil Layyah.

Tehsil	Breed	Abortion History (n=25) (RBPT)			Non abortion History (n=25) (RBPT)		
		(+)	(-)	Prevalence (%)	(+)	(-)	Prevalence (%)
Layyah	Nilliravi (n=50)	3	22	12	1	24	4
	Kundi (n=50)	2	23	8	0	25	0
Karor	Nilliravi (n=50)	2	23	8	1	24	4
	Kundi (n=50)	1	24	4	1	24	4
Choubara	Nilliravi (n=50)	1	24	4	1	24	4
	Kundi (n=50)	1	24	4	1	24	4
Total	N=300	10	140	6.67	5	145	3.33

Table 3. Comparison of Seroprevalence of *Brucella abortus* Regarding Retain Placenta History in district Layyah.

Tehsil	Breed	No of samples	Retained Placenta (RBPT)			No Retained Placenta (RBPT)		
			P	N	Prevalence (%)	P	N	Prevalence (%)
Layyah	Nilliravi	50	3	22	12	1	24	4
	Kundi	50	2	23	8	0	25	0
Karor	Nilliravi	50	2	23	8	1	24	4
	Kundi	50	1	24	4	1	24	4
Choubara	Nilliravi	50	1	24	4	1	24	4
	Kundi	50	1	24	4	1	24	4
Total		300	10	140	6.7	5	145	3.3

Table 4. Comparison of different para meter of sex under Chi-square Test.

Parameter		No of sample	Positive	%age	Chi-square Value	P-Value
Sex	Male	60	2	3.33	19.841	0.000
	Female	300	15	5		

Table 5. Comparison of seroprevalence of *Brucella abortus* through Milk Ring Test in Tehsil Layyah.

Tehsil	Breed	3-5 years (MRT)			More than 5 years (MRT)		
		P	N	Prevalence (%)	P	N	Prevalence (%)
Layyah	Nilliravi (n=60)	1	29	3.3	3	27	10
	Kundi (n=60)	1	29	3.3	1	29	3.3
Karor	Nilliravi (n=60)	1	29	3.3	2	28	6.6
	Kundi (n=60)	1	29	3.3	1	29	3.3
Choubara	Nilliravi (n=60)	1	29	3.3	1	29	3.3
	Kundi (n=60)	1	29	3.3	1	29	3.3
Total	n=360	6	174	3.3	9	171	4.97

as lower than the chencha district of the GamoGafu region and Bangladesh. The overall prevalence was renowned 3% and 3.20% at abettor of Quetta in Baluchistan by Shafee *et al.* (2012). Zadon *et al.* (2015) described the results of their study in the Punjab province of India and found 27.95% and 18.11% the prevalence of brucellosis by applying the test RBPT and MAT accordingly.Presently the prevalence of brucellosis is increasing at the farm level in Pakistan. The disease is more prevalent at commercial dairy farms in Pakistan (Iqbal *et al*., 2013). When animals become unproductive, infertile, and non-conceptive at these commercial farms then are sold to smaller dairy farmers and are major source of spread of the disease in the field. The RBPT and ELISA test was used as a screening test for brucellosis. These tests are considered most reliable overall in all the provinces labs of Pakistan. Ali *et al.* (2013) stated that the crossbreed cattle and buffaloes were observed as more prevalent than local breed cattle and buffaloes. MRT was performed as a screening and a confirmatory test. This study was carried out in Attock, Rawalpindi, and Islamabad areas. (Mittal *et al*., 2018) got the same results. They described the herd-level prevalence (62%) which was higher than that of 42% noted by (Zahid *et al*., 2013). In the above-mentioned studies, different researchers found different results so there is a controversy present in their finding (Ali *et al*., 2013) noted different results. In his study, he found the seroprevalence 6.9% in cattle and 6.6% in buffaloes in Islamabad Capital Tertiary, Rawalpindi, and Attock areas. MRT was used as a confirmatory test in his research. In his study, Nilliravi buffaloes were more prevalent than local and Kundi breeds in the study area. In his study, he also concluded that disease was more prevalent in crossbreed cattle than a local breed of cattle. In our study, the results were the same for Nilli ravi buffaloes. The disease is more prevalent in Nilli ravi buffaloes in all three Tehsils of district Layyah. The same Findings were noted by (Ali *et al*., 2013) in his research regarding flock. He found the herd-based prevalence that was 42% and (Aslan S *et al*., 2016) presented herd-based prevalence was 42.5% in a sheep flock in District Layyah. (Rehman *et al*., 2011) stated the prevalence is high in males than females and (Trangadia *et al*., 2016) denied this statement and said the prevalence is high in females than males and presented a ratio between males (6%) in both tests and females (3 to 72% and 3.88%). Shafee *et al.* (2012) also studied and gave similar results the prevalence in males is less than that of the female. They also used both RBPT and ELISA. The prevalence in males was 6% in both tests but in females, the percentage was 3.72% by RBPT and 3.88% by ELISA. In our study, the result is the same as the findings of (Rehman *et al*., 2011); (Munir *et al*., 2011). In conclusion, overall high prevalence was observed in all Tehsils of district Layyah affecting the production and reproduction of buffalo breeds. The Nilli ravi breed of buffaloes is more affected than that of Kundi. It decreased the milk production, increase abortion rate. Therefore, this study suggested that higher authorities should take measures to control this production limiting disease.

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