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SEROPREVALENCE OF LEPTOSPIROSIS AND ITS ASSOCIATION WITH REPRODUCTIVE AND PRODUCTIVE PARAMETERS FROM BUFFALO POPULATION OF RAJANPUR AND MUZAFFARGARH DISTRICTS OF PAKISTAN

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ABSTRACT

Leptospirosis is one of the important infectious diseases adversely affecting the fertility and reproductive parameters of domestic animals. The current study was aimed to investigate the sero-prevalence of leptospirosis and its association with reproductive and productive parameters from buffalo population of Rajanpur and Muzaffargarh districts of Punjab, Pakistan. A total of 386 buffalo serum samples were analyzed for anti-*leptospira* antibodies through indirect ELISA using "Anti-*Leptospira* IgG kit". The information regarding disease determinants were captured in questionnaire. Univariable and multivariable logistic regression models were used to correlate the potential risk factors with the disease dynamics. The results revealed an overall 12.69% prevalence of leptospirosis in both of the study districts, with higher percentage reported in district Rajanpur followed by Muzaffargarh presenting 20.72 and 04.66% prevalence at significant difference ($p \leq 0.05$), respectively. The univariable analysis found district, estrus repetition, body condition score and history of retained placenta as significant candidates ($p \leq 0.05$) while, multivariable logistic regression presented district, gender of animal and estrus repetition as the key risk factors (Odds Ratio>1). The study concluded that leptospirosis is prevalent in study districts and there is association of assumed risk factors with disease dynamics, which demands early installment of control strategies before the disease become unleashed.

Key words- Leptospirosis, Sero-prevalence, Risk factors, Buffalos.

INTRODUCTION

Leptospirosis is one of the important infectious diseases which adversely affect the fertility and reproductive parameters of domestic animals (Saglam *et al.,* 2008). It is emerging infectious disease of livestock with zoonotic significance (Ko*et al.,* 2009). The etiological agent responsible for this disease occurrence is a pathogenic bacterium of genus *Leptospira* (Fornazari *et al*., 2012). This disease affects not only the milk production of animals but also causes several reproductive disorders like stillbirth, abortion and birth of weak calves leading to tremendous financial losses to small holder farming communities (Sanhueza *et al*., 2013; Ndengu *et al*., 2017). Due to availability of favorable environment for the transmission of *Leptospira*, this disease is prevalent in developing countries (Bharti *et al*., 2003). In bovines, this disease leads to abortion after several weeks' infection which is usually not associated with an obvious ailment (Bahari *et al*., 2011). This disease has an adverse influence on the reproductive tract of bovines leading to compromised production and ultimate economic losses (Bomfim and Koury, 2006). The exact data regarding the economic losses due to the abortions from leptospirosis is limited. It is very difficult to determine the exact extent of economic losses attributed to leptospirosis. An outbreak of this malady in Argentina for a period of 1 year resulted in losses of up to US $ 150,000 for deaths of 100 calves, treatment and vaccination of 1300 survivors (Draghi *et al*., 2011). While in France, the estimated cost of losses due to this disease in cattle herds was from US $97 to 2611 per aborted cow (Ayral, 2013). However, the cost of problems besides abortion which are common and frequent has not yet been estimated. The climatic conditions such as warm temperatures, soil pH and the high rainfall, are favorable for the existence of *Leptospira* and also for the long term preservation of its pathogenicity (Martins and Lilenbaum, 2015; Andre-Fontaine *et al*., 2015).

The definite diagnosis and identification of leptospirosis is by isolation technique but the detection by anti-leptospiral antibodies is also practiced. The isolation of bacteria is time consuming and it also demands livability of pathogen along with the ability of bacteria to grow on culture media, thus serological method is obliged for the detection purpose worldwide (Brandao *et al*., 1998). Reported prevalence of leptospirosis in various animal species across different countries of world ranges between 2.00-46.00% (Leal-Castellanos *et al*., 2003; Faria *et al*., 2007). This wide variation might be related to several factors such as climate, animal species, time of the year, and method of investigation. Due to the presence of promising transmission circumstances,

leptospiral infection is emerging health malady in developing countries (Ahmad, 2000). The transmission of infection has been reported by direct contact with uterine discharges, urine, placenta, and sexual contact with affected animals but indirect way of transmission of disease that involves the contact with contaminated environment (Bharti *et al* ., 2003).

In this context the current study was aimed to investigate the sero-prevalence of leptospirosis and its association with reproductive and productive parameters from buffalo population of Rajanpur and Muzaffargarh districts of Pakistan.

MATERIALS AND METHODS

Study design: The study area of current project comprised of two flood hit districts–Muzaffargarh and Rajanpur (Figure 1) – of south Punjab province of Pakistan. The study districts are livestock rich and animals are the main source for livelihood of more than 2/3rd population. The district Muzaffargarh is having four tehsils, namely Muzaffargarh, Kotaddu, Alipur and Jatoi, whereas, district Rajanpur has three tehsils, namely Jampur, Rajanpur and Rojhan.

A total of 386 blood samples (193 from each study district) were collected aseptically from buffalos which were not having vaccination history against leptospirosis, by convenient sampling technique. From Muzaffargarh district sampling was conducted in such a way that 6 samples were collected from each village; from on tehsil four union councils were selected and from each union council two villages were further selected for sampling which comprised 192 blood samples, one additional sample was taken from kotaddu tehsil. Similarly from Rajanpur district 8 blood samples were collected from each village, from one tehsil four union councils were selected and from each union council two villages were further selected for sampling which comprised 192 blood samples, an additional sample was taken from Rojhan tehsil. The data regarding the risk factor analysis was collected in questionnaire.

Blood sampling: All the blood samples were collected aseptically from the jugular veins of buffalos individually and transferred to non-EDTA coated vacutainers separately. The collected blood samples were then placed undisturbed at room temperature for 30 minutes to clot formation. The samples were centrifuged within 2 hours of collection in order to prevent hemolysis and serum was decanted into sterile Eppendorf tubes by sterile technique. The collected serum samples were shipped to Medicine Laboratory Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences (UVAS), Lahore maintaining the cold chain. The samples were kept at 4 ºC till further processing.

Processing of samples by serology: The serum samples were processed by indirect (Enzyme linked immunosorbent assay) ELISA using (Alpha Diagnostic, international, USA) "RecomBac Bovine Anti-Leptospira LipL32 IgG kit" following the manufacturer instructions.

Statistical analysis: The data regarding the summary of risk factors was analyzed by logistic regression analysis. Firstly, all the study variables were analyzed by univariable analysis, the variables which produced P≤0.2 were further analyzed by logistic regression analysis to find out the potential risk factors. The analysis was conducted on SPSS Version 20.00.

RESULTS

The study has detected 12.69 % of the buffalo's positive for anti-leptospiral antibodies in both of the study districts. The buffaloes of district Rajanpur presented higher prevalence (20.72%) as compared to district Muzaffargarh's (04.66%). Results have presented study districts to be statistically associated ($p \leq .05$) with leptospiral sero-prevalence, the buffaloes of Muzaffargarh district are at higher risk (OR = 8.464; CI = 3.307–21.665) towards *Leptospira* exposure than buffaloes of Rajanpur district. Gender of the animals was also found to be a key risk factor, having significant ($p \leq .05$) impact on *Leptospira* sero-prevalence. The female buffaloes are more than two times as likely (OR = 2.009; CI = 0.470–8.584) to be seropositive than those to male buffaloes. Data analysis revealed that the buffaloes having history of estrus repetition are at higher risk (OR = 8.244; CI = 1.4849–46.899) as compared to those who are not having history of estrus repetition towards anti-leptospiral antibodies.

The assumed risk factors like study district from which sampling was conducted, gender of animals, mastitis history, history regarding repetition of estrus, body condition score, history of retained placenta, previous history of any treatment given to animals, history of blood in milk and reduction in milk production, physiological and immune status of animal and abortion history were statistically analyzed (Table 1). To find out the association of the key risk factors with the seropositivity, all the risk factors were analyzed through univariable and multivariable logistic regression analysis. The gender of animal, history of mastitis, reduction in milk production, previous history of treatment given to the animals, status of animals, history of blood in milk and abortion history were not proved to be significant candidates towards anti-leptospiral antibodies.

Initially, twelve variables were included in statistical analysis (Table 1), the variables which produced $p \leq 0.2$ by univariable analysis were further analyzed by multivariable analysis to find out the potential risk factors, so six variables were included in

final multivariable analysis model (Table 2), from which three were found to be the potential risk factors towards leptospiral sero-positivity. So, multivariable logistic regression presented buffaloes from Muzaffargarh district, female and animals having history of repeat breeding to be associated with the occurrence of disease dynamics. The history of retained placenta and immune status of the animals were proved to be significant candidates on the basis of univariable analysis but non-significant on the basis of multivariable logistic regression model.

Table 1.Univariable analysis association and descriptive statistics in Buffaloes.

Variable	Categories	Positive (%)	Negative	*p*-Value
District	Muzaffargarh	09 (04.66)	184	0.001*
	Rajanpur	40 (20.72)	153	
Gender	Female	44 (12.08)	320	0.180
	Male	05 (22.73)	17	
Mastitis	Yes	06 (08.00)	69	0.358
	No	10 (13.16)	66	
	NA	33 (14.04)	202	
Estrus repetition	Yes	08 (05.13)	148	0.001*
	No	09 (09.47)	86	
	NA	32 (23.70)	103	
Body Condition Score	Normal	41 (18.06)	186	0.001*
	Thin	07 (05.26)	126	
	Emaciated	01(03.85)	25	
Reduction in milk production	Yes	27 (12.27)	193	0.273
	No	14 (17.50)	66	
	NA	08 (09.30)	78	
Previous history of treatment given	Yes	31 (13.42)	200	0.600
	No	18 (11.61)	137	
Animal status	Pregnant	31 (13.25)	203	0.913
	Non pregnant	07 (12.28)	50	
	NA	11 (11.58)	84	
Immune status against major diseases	Non Vaccinated	22 (09.91)	200	0.057
	Vaccinated	27 (16.46)	137	
History of blood in milk	Yes	06 (12.00)	44	0.717
	No	38 (12.33)	270	
	NA	05 (17.85)	23	
Abortion history	Yes	43 (12.11)	312	0.530
	No	04 (18.18)	18	
	NA	02 (22.22)	7	
History of retained placenta	Yes	02 (15.38)	11	0.001*
	No	13 (06.37)	191	
	NA	34 (20.12)	135	

*$p \leq 0.05$= significant difference

Table 2. Final Model of Multivariable logistic regression identifying the potential risk factors in the studied predictors.

Variable	Category	S. E**	Wald	P-value	Odds Ratio	Lower C.I (95%)	Upper C.I (95%)
District	Muzaffargarh	.480	19.840	.000	8.464	3.307	21.665
	Rajanpur						
Gender	Female	.741	.887	.346	2.009	.470	8.584
	Male						
Estrus repetition	Yes	.887	5.656	.017	8.244	1.449	46.899
	No	.834	1.296	.255	2.585	.504	13.263
	NA						

Body Condition Score	Normal	4.730	.030	.094	.011	.792	1.086
	Thin	.455	.500	.470	.053	4.210	1.118
	Emaciated						
Immune status	Non Vaccinated	.516	2.379	.123	.451	.164	1.241
	Vaccinated						
History of retained placenta	No	.968	.692	.406	.447	.067	2.980
	Yes	1.168	3.157	.076	.126	.013	1.239
	NA						

**Standard error

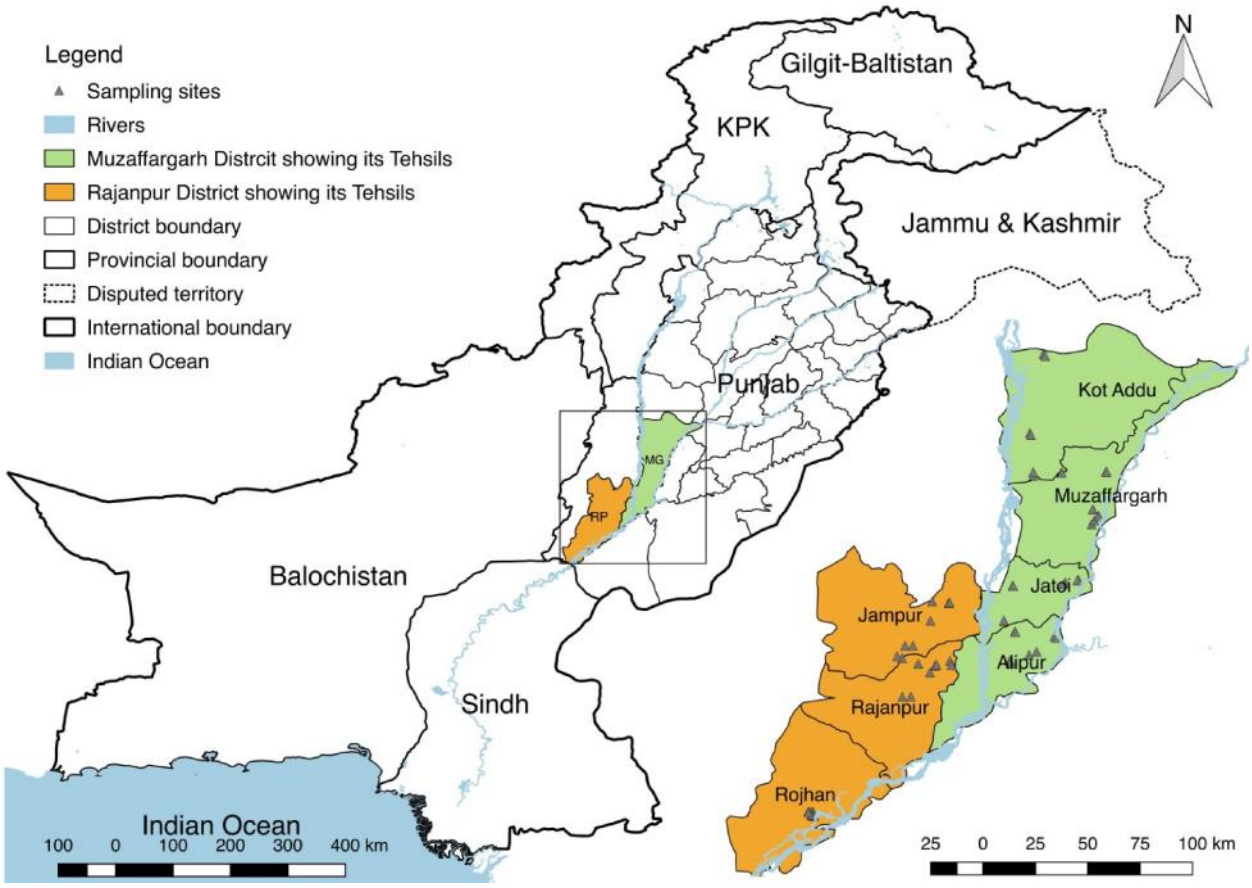

Figure 1. Map showing the study districts.

DISCUSSION

Most of the infectious diseases are proved to be serious threat to the livestock (Yilmaz *et al*., 2016; Elhaig *et al*., 2016; Qayyum *et al*., 2016). Leptospira infection frequently remains clinically silent but is found positive on serological assays (Hassanpour *et al*., 2011).

Letospira's sero-prevalence has been reported in human as well as in domestic animals of Pakistan by rapid microscopic slide agglutination test (Ahmad, 1987). There are also few studies which have reported this pathogen in dogs (Saleem *et al*., 2013a), horses (Sohail *et al*., 2016), bovine (Ijaz *et al*., 2018a, b; Islam *et al.,* 2019) and human population (Saleem *et al*., 2013b; Sohail *et al*., 2018) of Pakistan. This study shows the association of *leptospira* sero positivity with reproductive parameters which is supported by Boqvist *et al*. (2002). This study was conducted in buffaloes of flood hit zones of Pakistan, and flood water is supposed to be positively associated with the occurrence of disease. These findings are justified by Bandino *et al*. (2015) and Sohail *et al*. (2016) as they proposed floods and rain fall both strongly associated with sero-prevalence of *Leptospira*.

The current study revealed 12.69% of the buffalo's positive for anti-leptospiral antibodies. The reported sero-prevalence of *leptospira* in buffaloes of Uganda (Atherstone *et al.,* 2014) and Malaysia (Bahaman *et al.,* 1987) is 42.39% and 31%, respectively which is quite higher than that of India in which prevalence was reported 2.8% (Schoonman and Swai, 2010). The

variation in prevalence might be due to distinct geographies, different management and husbandry practices and sampling strategies.

A significant difference was observed between the *Leptospira* sero-prevalence and study districts. In this study buffaloes of district Rajanpur presented higher prevalence of anitleptospiral antibodies than the animals from Muzaffargarh district. These types of results are in agreement with the findings of Sohail *et al*. (2016) who also have reported significant difference of *Leptospira* sero-prevalence and study area in horses of Pakistan. The difference in prevalence in both of the study districts might be due to different production and rearing systems. Prevalence of leptospirosis was higher in buffaloes having history of abnormally repeating estrus. *Leptosira* infection is found to be strongly associated with estrus repetition in cattle (Libonati *et al.,* 2018). Female were found to be at higher risk of getting the disease as compared to males. These findings are also in line with findings of Sohail *et al.* (2016), who has reported higher sero-prevalence of *Leptospira* in female horses of Pakistan. It might be due to pregnancy stress or lactation stress in females.

Although non-significant in this study, but *Leptospira* infection has been found to be associated with abortions in animals (Prescott *et al*., 1988). If reproductive system's infection is involved it could result in a "storm of abortions" causing considerable losses in form of meat and milk yield reductions (Tooloei *et al*., 2008). Infertility, shown as delayed calving intervals and increased services per conception, is associated with this infection (Guitian *et al*., 1999; Dhaliwal *et al*., 1996). The exact pathogenesis is not clearly understood, however apparently the evidence for the presence of this pathogen in the oviduct and uterus of infected animals might interferes with the implantation of embryo or some other early pregnancy events (Grooms, 2006). The body condition score of animals was proved to be significant candidate on the basis of univariable analysis but was not found to be risk factor on the basis of final logistic regression model which resembled with the findings of Parvez *et al.* (2015).

The study concluded that the buffalo population of Rajanpur and Muzaffargarh districts is having considerable amount of anti-leptospiral antibodies and leptospirosis affects the animals silently. The study demands the scheduled vaccination program to control this zoonotic malady.

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