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RESEARCH ARTICLE

Sero-Epidemiology of Bovine Leptospirosis and Associated Risk Factors in a Flood Affected Zone of Pakistan

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ABSTRACT

Bovine leptospirosis is an emerging zoonotic disease that compromises farm economy. Keeping in view limited studies on bovine leptospirosis, this project was designed to estimate its sero-prevalence in flood-hit zone of Pakistan, along with its associated risk factors. A total of 385 serum samples were randomly collected from tehsils Rajanpur, Jampur and Rojhan of district Rajanpur with necessary information captured in data form for risk factors analysis. Chi-square test, univariable and multivariable logistic regression analyses were applied to check association of hypothesized risk factors with disease. The study showed 23.12% of the bovine samples positive for Leptospira antibodies with non-significant difference (P>0.05) between cattle (25.52%) and buffaloes (20.72%). The non-significant difference (P>0.05) of bovine leptospirosis among tehsils of study area presented 26.56, 21.88 and 20.93% in tehsils Jampur, Rajanpur and Rohan, respectively. Chi-square test analysis depicted body temperature and socio-economic status of worker positively associated (P<0.05) with the disease. The univariable analysis based on Mantel Haentzel test and Wald statistics found early age, confined system, fodder type, jaundice status, and socio-economic status of the animal's owner as significant candidates (Odds ratio OR>1) while, multivariable logistic regression showed bovine specie, gender of animal, fodder type, jaundice status, and socio-economic status of the animal's owner as key risk factors for leptospirosis dynamics (Odds Ratio>1). The study concluded that, the flood hit area is having considerable prevalence of bovine leptospirosis and is positively associated with presence or absence of certain animal, management and environmental factors.

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INTRODUCTION

The floodwater becomes stagnant and eventually, appears as potential bacterial contamination source (Christopher and Portier, 2009) that has been reported as a call for disease outbreaks (Anonymous, 2015). Apart from diseases to livestock the fodder shortages put animals to the verge of selling at cheaper rates to other areas, which may also become an additional source of zoonosis to the areas where they are sold (Anonymous, 2015). Among various zoonotic diseases leptospirosis is known as disease strongly linked with floodwater (Sharma *et al.*, 2006) due to bacterial shedding through urine of animal (Adler and Moctezuma, 2010). The infected animals are mostly silent shedders of bacteria, which become a danger to the humans exposed to urine directly or urine-contaminated water indirectly (Levett, 2010).

Leptospira, a spirochete, has vast specie vulnerability having 20 species with more than 200 serovar (Vinetz, 2001) jeopardizing smallholder's entrepreneurship with especial context to bovine leptospirosis. The disease is important both in terms of losses to the wealth and health of animals and humans. In Pakistan 2-10% prevalence of bovine leptospirosis has been reported in early nineties (Mahmood, 1992) that tuned to 85% at the end of 20th century (Bharti *et al.*, 2003). Few studies have been conducted on bovine leptospirosis in Pakistan. The rural areas are prone to this disease because of dearth lack of quarantine and precautionary measures especially during and after raining/flood seasons. The developed countries

are known to observe this disease due to outdoor recreational activities involving water (Sharma *et al.*, 2006). The identification of leptospirosis involves actual isolation of organism through microbiological growth and serological examination. The former technique is laborious, time consuming and associated with growth constraints. However, later technique is quick and globally obliged technique.

The district Rajanpur is located across Indus River that finds union of all five rivers of Pakistan in its jurisdiction resultantly observing maximum water pressure. The district faces flood every year due to hill torrents from Suleiman mountain ranges producing flash floods and riverine of Indus River. The rise in level of water in Indus River is also fostered by rain water and snow melted from mountains. The flash flood (water coming from hills) situation aggravates due to over flooded Naalas (local terms for lower water source channels) hence accounting for 80% damage to the area while disturbing 60% population in flood hit area (Anonymous, 2015). Tehsil Jampur is affected both by flash and riverine floods while tehsil Rajanpur and tehsil Rojhan is usually affected by riverine flood only (Anonymous, 2015). The leptospiral infection is arising as common malady in developing countries due to favorable transmission circumstances (Ahmad, 2000). The infection goes directly thorough contact with infected urine, uterine discharges, infected placenta, and sexual contact as direct sources of infection. The more important transmission mean is the indirect way that involves contact with contaminated environment infective of farm animals (Anon, 2003).

So far, only one study has been conducted on bovine leptospirosis in Pakistan (Ijaz *et al*., 2018). However, status of bovine leptospirosis is still not explored in district Rajanpur which is among the major flood hit regions of the country. Hence the objective of this study was to investigate cases positive for bovine anti-leptospira antibodies in this flood-affected areas.

MATERIALS AND METHODS

Study area: The sampling for current study was executed from flood affected Rajanpur district situated in south Punjab at 29.1018°N, 70.3245°E comprising of three tehsils (Rajanpur, Jampur and Rojhan). The district coincides with Dera Ghazi Khan in north, Muzafar Garh and Raim Yar Khan districts in east in such a way that it spreads across River Indus, and Jaccobabad of Sindh province in south, while Dera Bugti and Barkan of Balochistan in west occupying 12,318 Km^2 with 90 persons per Km^2 population density.

Sampling plan: There are no previous records on sero-prevalence of leptospirosis in Rajanpur district of Punjab, thus 50% prevalence was assumed at 95% confidence interval which gave value of 384 serum samples using information given in table described by Thrushfield (2005). The samples were randomly collected from each tehsil with further randomization in such a way that four union councils were selected from each tehsil and two villages from each union council. The animals were considered as primary sampling units, villages as secondary sampling units while union council as tertiary sampling unit in this study. A 16 bovine (n=8 cattle, n=8 buffalo) samples from each village were collected aseptically by drawing blood from jugular vein in such a way that it added up to 32 samples from union council level (n=16 cattle, n=16 buffalo), and 128 at tehsil level (n=64 cattle, n=64=buffalo). One additional buffalo sample was collected from Rojhan.

Sample collection: The blood samples were aseptically drawn from jugular vein into non-EDTA vaccutainer that were left at room temperature for half an hour to be centrifuged eventually getting serum decanted aseptically in 1.5ml Eppendorf's tube. The samples from field were shifted to Medicine Laboratory, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain in iceboxes. The serum samples were preserved at -20°C till further processing. The information pertinent to animal, farm management and farmer workers' involvement with animal care were captured in piloted questionnaire after formal and informal testing in order to find regression between disease response and its predictors.

Samples processing: The serum samples were put to estimation of bovine anti-leptospiral antibody prevalence following indirect ELISA protocol using "RecomBac Bovine Anti-Leptospira LipL32 IgG kit" (Alpha Diagnostic, international, USA). The plates were read using ELISA reader. The cut off values were adjusted for each experimental run.

Statistical analysis: Sero-prevalence was estimated for one-year period without peculiarity between former and new cases as "No. of existing diseased cases/No. of overall animal examined'' as per Thrushfield (2005) formula. The data recorded on data capture form was uploaded to excel sheet and was analyzed using chi-square test, univariable and multivariable logistic regression. The P-value less than "0.05" and odds ratio (OR) greater than "1" was considered significant.

RESULTS

Sero-prevalence of bovine leptospirosis: The overall prevalence of bovine leptospirosis in district Rajanpur was found 23.12% with non-significant difference (P>0.05) of bovine leptospirosis among different tehsils (Table 1). Tehsil Jampur presented highest bovine prevalence with 26.56% bovine leptospirosis followed by Rajanpur and Rojhan revealing 21.88 and 20.93% prevalence, respectively. The study showed non-significant difference between bovine species for leptospirosis however, there was 25.52% of leptospirosis in cattle while 20.72% in buffaloes from study area (Table 2). Similar non-significant (P>0.05) response in case of gender was noticed with 24.44% of females and 17.57% of males positive for leptospirosis.

Table 1: Sero-prevalence of bovine leptospirosis in different tehsils of district Rajanpur

Tehsil	Samples tested	Positive	Prevalence	P-value
Rajan Pur	128	28	21.88	
Jam Pur	128	34	26.56	0.535
Rojhan	129	27	20.93	
Total	385	89	23.12	

Table 2: Chi square analyses of data on prevalence of leptospirosis in district Rajanpur

Variables	Variable levels	Positive/ Total	Prevalence	*P-value
Age	1-2 years	13/058	22.41	
	3-5 years	57/248	22.98	0.972
	>5 years	19/079	24.05	
Bovine species	Cattle	49/192	25.52	0.160
	Buffalo	40/193	20.72	
Breed	Nili Ravi	25/116	21.55	
	Kundi	15/77	19.48	
	Sahiwal	08/28	28.57	
	Dhajal	19/78	24.36	0.520
	Cholistan	00/02	00.00	
	Fresian	00/04	00.00	
	Dhani	00/03	00.00	
	Cross	21/73	28.77	
	Non-descript	01/04	25.00	
Gender	Female	76/311	24.44	0.208
	Male	13/74	17.57	
Rearing system	Open	50/235	21.28	
	Confined	09/043	20.93	0.364
	Both	30/107	28.04	
Grazing status	No	04/014	28.57	
	Grazing alone	25/118	21.19	0.22
	With ovine	46/170	27.06	
	With equine	14/083	16.87	
Water Source	Pond/Lake	11/044	25.00	
	Canal	13/060	21.67	0.924
	Fresh/tap	65/281	23.13	
Fodder type	Fresh	72/307	23.45	0.75
	Mixed with previous	17/078	21.80	
Body condition	Good	82/354	23.16	
	Thin	03/016	18.75	0.98
	Emaciated	04/015	26.67	
Body temperature	Normal	74/341	21.70	0.049
	High	15/044	34.09	
Immunization status	Yes	13/079	16.46	0.11
	No	76/306	24.84	
Jaundice status	Yes	85/365	23.29	0.73
	No	04/020	20.00	
Urine color	Normal	66/285	23.16	0.97
	Blood in Urine	23/100	23.00	
Socio-economic status	Literate	37/147	25.17	
	Primary	19/087	21.84	0.029
	Higher Secondary	24/113	21.24	
	Graduate/More	09/038	23.68	
Call for Vet. Professionals	Only Emergency	38/155	24.52	
	Few days' post treatment	27/113	23.89	0.48
	Always on call	08/024	33.33	
	Self-treatment	04/024	16.67	
	Always veterinarian	12/069	17.39	
Veterinary Professional Consultancy	Only vet Assistant	38/158	24.05	
	Only vet officer	24/106	22.64	0.93
	Both	27/121	22.31	

Risk factors: The chi-square analysis of different assumed determinants with bovine leptospirosis showed significant association of body temperature and socio-economic status of worker with this disease (Table 2). The factors including age, bovine species, breed, gender, rearing system, grazing status, water source, fodder type, body condition, immunization status, jaundice status, urine color, call for veterinary professionals and veterinary professional consultancy presented non-significant association (P>0.05) with bovine leptospirosis.

The univariable analysis of assumed risk factors based on Mantel Haentzel test and Wald statistics (Table 3) explored the potential risk factors for leptospirosis in bovine herds of Rajanpur district based on odd ratios (OR>1). The age was found to be a potential risk factor OR=1.48 (CI=0.54-4.07) for leptospirosis sero-prevalence. Bovine specie although non-significantly associated on chi-square bases (P>0.05) was found a potential risk factor upon univariate analysis OR=1.31 (CI=0.81-2.109). In rearing system the confined system proved to be a potential risk factor for leptospirosis OR=2.37 (CI=0.23-24.48). Among grazing system, no grazing and grazing with ovine showed higher effects on disease dynamics [OR=1.70 (CI=-0.062-47.0); OR=2.01 (CI=0.038-105.6)]. The fodder type proved to be a potential risk factor OR=1.09 (CI=0.60-2.00) affecting the disease dynamics. Another factor associated with leptospirosis was the jaundice status OR=1.21 (CI=0.39-3.73). Urine color was also found positively associated OR=1.00 (CI=0.58-1.73) with sero-prevalence of leptospirosis. Mastitis status of farm animals also proved to be a potential risk factor for the sero-prevalence OR= 7.22 (CI=0.08-73.53). Beside these disposal of aborted fetus, services per conception and socio-economic status of the animal's owner all proved to be potential risk factors (Odd ratios >1) affecting the disease dynamics.

Multivariable models (Table 4) were later on developed using the elimination technique of backward manual stepwise process removing the variables with greater P-value respectively. The variables, which were found no longer statistically associated after adjustment for the other variables, were removed (P-value=0.05). Variables were eliminated or retained form the model considering the P-value of 0.05 based on Wald statistic. The confounding presence in data was countered by monitoring the calculated coefficient values and by checking that the values did not changes by >10% after dropping the non-significant variables from the model. The bovine specie, gender of animal, fodder type, jaundice status and socio-economic status of the animal's owner were found key risk factors for leptospirosis sero-prevalence dynamics (OR>1).

DISCUSSION

Seroprevalence of bovine leptospirosis: The prevalence of current study was in agreement with findings of Gamage *et al.* (2011) and Subharat *et al.* (2011) from Sri Lanka and Australia who reported 20.3 and 27.4% of bovine leptospirosis in their countries, respectively. The bovine leptospirosis slightly higher to the finding of this study was noted as 30.3, 31.3, and 31.3% in studies conducted by Schoonman and Swai (2010), Tabatabaeizadeh (2011) and Dos *et al.* (2012) from Iran, Tanzania and Brazil, respectively. Contrary to the results of current study, very high prevalence of leptospirosis was previously recorded in Bangladesh (Parvez *et al*., 2015), India (Natarajaseenivasan *et al.,* 2011), Poland (Czopowicz *et al.*, 2011) and Mexico (Joel *et al.*, 2011) with 47.27, 87.0, 89.9 and 82.2% of prevalence, respectively. The discrepancies might have appeared because of variation in geographical areas, climatic conditions and management aspects in conjunction with resistance showed by different breeds. It has been noticed that infections by *Leptospira spp.* remains usually silent with respect to clinical examination while serological assays give positive reports. This could have resulted in misdiagnosis on application of other than serological or molecular techniques and might have allowed animals to

Table 3: Univariable analysis based on Mantel Haentzel test and Wald statistics

Variables	Categories	Positive/ Total	Odd ratio (95% C.I)	*P-Value
Tehsil	Rajan Pur	28/128	0.95 (0.47-1.91)	0.535
	Jam Pur	34/128	0.56 (0.09-3.2)	
	Rojhan	27/129	1	
Age	1-2 years	13/058	1.48 (0.54-4.07)	0.956
	3-5 years	57/248	1.10 (0.53-2.28)	
	>5 years	19/079	1	
Bovine species	Cattle	49/192	1.31 (0.81-2.109)	0.160
	Buffalo	40/193	1	
Gender	Female	76/311	1.51 (0.79-2.91)	0.208
	Male	13/074	1	
Rearing sys	Open	50/235	0.97 (0.18-5.08)	0.76
	Confined	09/043	2.37 (0.23-24.48)	
	Both	30/107	1	
Grazing status	No	04/014	1.70 (-0.062-47.0)	0.22
	Grazing alone	25/118	0.85 (0.01-42.2)	
	With ovine	46/170	2.01 (0.038-105.6)	
	With equine	14/083	1	
Water Source	Pond/Lake	11/044	0.52 (0.026-10.43)	0.67
	Canal	13/060	0.684 (0.10-4.32)	
	Fresh/tap	65/281	1	
Fodder type	Fresh	72/307	1.09 (0.60-2.00)	0.75
	Mixed previous	17/78	1	
Body condition	Good	82/354	0.881 (0.14-5.22)	0.98
	Thin	03/016	0.789 (0.05-10.81)	
	Emaciated	04/015	1	
Body temperature	Normal	74/341	0.53 (0.27-1.05)	0.06
	High	15/044	1	
Immunization status	Yes	13/079	0.59 (0.31-1.14)	0.11
	No	76/306	1	
Jaundice status	Yes	85/365	1.21 (0.39-3.73)	0.73
	No	04/020	1	
Urine color	Normal	66/285	1.00 (0.58-1.73)	0.97
	Blood in Urine	23/100	1	
Mastitis status	No	49/176	1.47 (0.05-41.52)	0.04
	Acute	14/081	2.24 (0.075-67.54)	
	Sub-clinical	04/012	7.22 (0.08-73.53)	
	Chronic	08/040	2.3 (0.072-66.31)	
	NA	14/076	1	
Breeding system	AI	71/296	0.28 (0.005-16.95)	0.66
	Natural	03/014	0.62 (0.01-32.84)	
	NA	01/005	4.10 (0.067-50.39)	
	No	14/070	1	
Abortions	Yes	16/086	0.069 (NA)	0.16
History	No	60/226	3.65 (NA)	
	NA	13/073	1	
Disposal of	Buried	02/034	5.10 (NA)	0.03
Aborted Fetus	Thrown in water	05/012	1.27 (NA)	
	Thrown in field	09/041	6.6 (NA)	
	NA	73/298	1	
History of	Yes	03/013	0.157 (0.004-6.48)	0.48
stillbirth	No	73/299	0.537 (0.026-10.90)	
	NA	13/073	1	
Services Per	1	34/145	6.19 (0.35-107.5)	0.82
Conception	2	26/113	5.86 (0.30-111.7)	
	3	09/031	5.18 (0.27-96.90)	
	4	05/018	5.16 (0.25-105.33)	
	NA	15/078	1	
Socio-economic	Literate	37/147	8.50 (0.74-97.0)	0.029
	Primary	19/087	3.47 (0.15-76.44)	
status	Higher secondary	24/113	14.84 (1.32-166.78)	
	Graduate/More	09/038	1	
Call for Vet.	Only emergency	38/155	0.07 (0.006-0.92)	0.48
Professionals	Few days' post treatment	27/113	0.073 (0.009-0.603)	
	Always o call	08/024	0.203 (0.016-2.58)	
	Self-treatment	04/024	0.034 (0.001-1.070)	
	Always veterinarian	12/069	1	
Veterinary	Only vet	38/158	0.941 (0.114-7.76)	0.93
Professional	assistant			
Consultancy	Only vet officer	24/106	0.08 (0.007-1.14)	
	Both	27/121	1	

Table 4: Multinomial logistic regression

Variables	Categories	B	SE (b)	Odd ratio (95% C.I)	*P-Value
Age	1-2 years	-	-	1	-
	3-5 years	-.28	0.43	0.75 (0.32-1.75)	0.50
	>5 years	-.23	0.31	0.78 (0.42-1.48)	0.45
Bovine	Cattle	-	-	1	-
species	Buffalo	0.30	1.32	1.36 (0.80-2.30)	0.25
Gender	Female	-	-	1	-
	Male	15.52	1.56	5.51E6 (2.5E5-1.1E8)	0.000
Fodder	Fresh	-	-	1	-
type	Mixed	0.133	0.320	1.14 (0.61-2.14)	0.67
Jaundice	Yes	-	-	1	-
status	No	0.405	0.601	1.50 (0.46-4.87)	0.500
Socio-economic	Literate	-	-	1	-
	Primary	0.12	0.43	1.13 (0.48-2.65)	0.77
status	Higher secondary	-0.08	0.460	0.91 (0.37-2.65)	0.85
	Graduate/ More	-0.11	0.468	0.88 (0.35-2.22)	0.79

spread infection due to reporting less than exact prevalence estimation of this disease (Hassanpour *et al.*, 2004). The conventional techniques used in previous studies do have limitations in diagnosis of leptospirosis (Bharti *et al.*, 2003; Parvez *et al.*, 2015). The conventional techniques do require specific cells/mL (10^9*leptospires*/ mL) for Leptospira to be diagnosed under dark field microscopy (Bharti *et al.*, 2003) in addition to recommendation of early diagnosis in blood. Such limitations produce false estimation of prevalence, which is the result of discrepancies in findings of various studies.

Risk factors: The current study was in line with findings of (Bahaman *et al.*, 1987) with respect to positive association of breeds with leptospirosis occurrence but Parvez *et al.* (2015) found contrary to the finding of current study. The specie of animal was a found a significant risk factor based on univariate and multivariate logistic regression in the current study. However, there was a non-significant difference in the prevalence between cattle and buffalo based on chi-square test. These findings are in line with the study conducted by Suwancharoen *et al.* (2013) who also found bovine species as a significant effector of the disease dynamics. In this study age was associated positively with sero-prevalence of leptospirosis. These findings are supported by the study conducted by Mazeri *et al.* (2013) exploring the age as a significant risk factor based on logistic regression. In the current study confined system of animal rearing proved as a risk for the spread of leptospirosis. The possible reason could be the retention of animals in infected environment as the confinement can provide a microenvironment for the infection maintenance and transmission as stated by (Bharti *et al.*, 2003). Fodder type also proved to be a key risk factor affecting the disease dynamics. No study have yet been explored this aspect however, the possibility could be harboring of pathogens in the feed over time and if provided mixed with fresh fodder to reduce the feed costs can cause losses in terms of disease transmission. The higher education was considered as predictor of bovine leptospirosis by Parvez *et al.* (2015) which is in contradiction to the findings of current study while in agreement with findings of (Dias *et al.*, 2007). However, lower education status was in positive association of spread of this disease. This could be justified with the fact that lack of knowledge for

disease control and farm management can result in disease spread (Parvez *et al.,* 2015).

Conclusions: The study showed higher and uniform sero-prevalence of bovine leptospirosis in different tehsils of flood affected zone. The cattle and buffaloes were equally susceptible to leptospirosis while females were more prone to this disease. Confined rearing system, jaundice status, fodder type, socioeconomic status of farmer and flooded areas were found as key risk factors for spread of this disease. The study concluded this flood-hit area is having considerable prevalence of bovine leptospirosis with assumed determinants appearing as potential risk factors for leptospirosis. The study demands regular prevalence estimation of this disease and development of comprehensive preventive measures accordingly.

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