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RESEARCH ARTICLE

Serological Study for the Detection of Antibodies against *leptospira* in Goats

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ABSTRACT

Leptospirosis is globally distributed disease of zoonotic significance, caused by pathogenic spirochetes of the genus *leptospira*. There is no study regarding the sero-epidemiological investigation of leptospirosis in goats of Pakistan. The study was intended to assess the sero-prevalence and risk factors of *leptospira* along with the alterations in hemato-biochemical parameters between seropositive and seronegative goats. Serum samples from 155 goats were collected aseptically from tehsil Pattoki and screened by indirect ELISA to detect anti-leptospiral antibodies. A total of 15 herds were selected and from each herd 10-15 goats were taken for sampling by convenient sampling technique. The records regarding hypothesized risk factors were analyzed by logistic regression model on SPSS. The overall sero-reactivity to *leptospira* was 21.29%. The potential risk factors contributing towards disease occurrence were; age of animals, different types of rearing systems, grazing status, call of veterinary professionals and body condition scores of animals. Hematological parameters of seropositive animals showed significant variations (p<0.05) in values of total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb) and packed cell volume (PCV) as compared to seronegative animals. The serum biochemical values for alanine transaminase (ALT) and blood urea nitrogen (BUN) in seropositive goats increased significantly (p<0.05). The study emphasizes comprehensive control strategies to minimize the losses associated with the occurrence of this zoonotic malaise.

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INTRODUCTION

Leptospirosis is a globally distributed disease of zoonotic importance caused by pathogenic spirochetes of the genus *leptospira*. The disease causes economic losses due to its increased incidence, reduced reproductive performance and reduced productivity (Lilenbaum *et al.,* 2008). Goats are source of food in many developing countries and very limited information is available regarding the status of leptospirosis in goats as compared to cattle, pigs and even in sheep (Ellis, 2015). According to agricultural census report 2018-19, Pakistan has 76.1 million goat population which is producing 940 thousand tonnes milk annually (Economic Survey of Pakistan 2018-19). Goats had conventionally resilient impact on socio-economic life of humans, particularly in less developed and rural areas of the world. Meat and milk of goat constitutes a significant source of food and proteins by modifying different natural resources of inferior quality (Dubeuf *et al.,* 2004). Although there is less milk production as compared to bovines, still goats are considered as a major source of income for poor rural families in developing countries (Lilenbaum *et al.,* 2007).

Leptospirosis, being important infectious disease, adversely affect the fertility and leads to decrease in production in goats (Santos *et al.,* 2013). *Hardjo, Shermani, Sejroe* and *Gripottyphosa* are the most prevalent serovars of *leptospira* in goats (Lilenbaum *et al*., 2009). The disease has been recognized as an emerging and potentially epidemic infection linked with

excessive rainfall in tropical regions. Evidence of *leptospira* species have been found in all mammals indicating persistence of infection (Adler and Moctezuma, 2010). Infection in small ruminants occurs through direct or indirect contact with urine or by contaminated tissues. Following infection, the pathogen localize in many organs, especially in kidneys (Grooms and Bolin, 2005). Epidemiological forms of leptospirosis varied, however its extreme influence is on closely adapted and populated regions (Lau *et al.,* 2010). In developed countries leptospirosis is usually associated with outdoor recreational activities involving water. Presence of stray dogs, pigs, domestic rats and bandicoots may contribute to the spread of pathogen. In rural areas, rice field workers, agricultural crops, cane field workers and animal husbandry staff are at high- risk of having the pathogen (Levett *et al.*, 2005).

Diagnosis or identification of leptospirosis can be done either through bacterial culture or through detection of anti-leptospiral antibodies. The first technique is arduous, time consuming and is associated with growth constraints. While, later method is quick and globally obliged (Brandão *et al.*, 1998).

Keeping the importance of leptospirosis in goats, and lack of epidemiological studies in Pakistan, the research was planned to study the sero-prevalence, hemato-biochemical changes and associated risk factors with the occurrence of leptospirosis in goats.

MATERIALS AND METHODS

Study design: The study was conducted on goats of tehsil Pattoki. It is the second largest tehsil of district Kasur (Fig I). It is located at 31°1'0N latitude, 73°50'60E longitude on world map with an altitude of 186 metres (610 ft). The district is bounded by the Ravi River in the north-west and river Sutlej in the south-east. Majority of the population is living in rural areas and livestock is the main source of income. A total of 155 goats with no vaccination against leptospirosis, regardless of their age, sex and breed were included in this study. Samples were collected randomly from tehsil Pattoki in such a way that 10-15 animals were selected for sampling from one herd by convenient sampling technique. Data was recorded in brief questionnaire during sample collection for analysis of potential risk factors associated with the occurrence of disease.

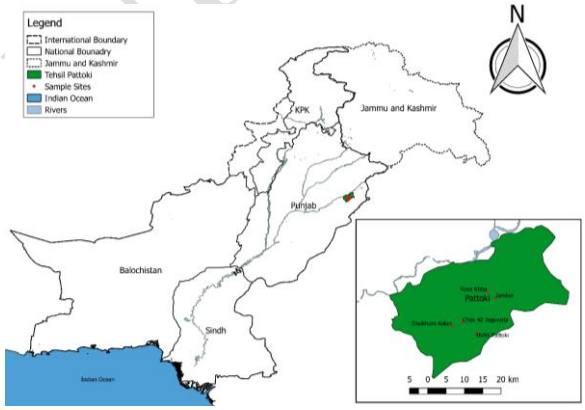

Fig. I: Study area.

Blood collection and Serological assay: For the collection of serum, blood (3mL) was collected individually and aseptically into plain vacutainers having no anti-coagulant. Centrifugation was performed, within 3-4 hours after collection of blood in order to avoid hemolysis, for the separation of serum at room temperature. Serum samples were dispensed individually to Eppendorf tubes (1.5 ml) using sterile technique. Samples were shipped to Medicine Lab, (University of Veterinary and Animal Sciences) UVAS Lahore in cold chain. The samples were processed by Indirect ELISA (Enzyme-linked immunosorbent assay) following kit protocols "Goat *leptospira* IgG, Lep IgG ELISA kit" (Nanjing Pars Biochem, China).

Hemato-biochemical analyses: For hematological analysis twenty animals (sero-negative =10, sero-positive =10) were selected and blood was collected in EDTA coated vacutainers separately. Serum samples were also used to analyze biochemical parameters. Hematological parameter includes; hemoglobin (Hb) concentration, packed cell volume (PCV), Total erythrocytes count (TEC), and Total leukocytes count (TLC). Liver Function test (LFTs) included alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and total bilirubin while renal function tests (RFTs) included creatinine and blood urea nitrogen (BUN). The complete blood count (CBC) liver functions test (LFTs) and renal function tests (RFTs) were accessed by using automated hematology and blood chemistry analyzer.

Statistical analysis: Prevalence was measured through the method provided by Thursfield, (2005). Initially chi-square and univariable analysis was used to analyze risk factors and to find out potential risk factors final logistic regression model was conducted. Student t-test was performed to analyze hematological and biochemical parameters between seropositive and seronegative animals. The data was analyzed and processed by using SPSS (version 20.00). Statistically *p*-values (<0.05) and odds ratios (>1) were considered significant.

RESULTS

Sero-prevalence of *leptospira* in goats and association of disease determinants with the occurrence of disease: The study revealed 21.29% (33/155) sero-prevalence of *leptospira* in tehsil Pattoki of district Kasur. The prevalence was statistically (P>0.05) non-significant in different breeds of goats. However, higher prevalence 23.33% (14/60) was observed in Beetle breed as compared to Teddy 17.77%, (8/45) and Non-descript breed 22%, (11/50) respectively. Sex of the animal was also found non-significant (P>0.05) candidate with disease occurrence. Age of the animal was observed as key risk factor (P<0.05) (Table 1).

Initially seventeen factors were analyzed by chi-square and univariable model. Breed, sex and age of animals, rearing systems, body health, grazing system, urine physical appearance, history of jaundice, source of water, history regarding abortion, mastitis status, still birth, kidding interval, physiological status, disposal of aborted fetus, No. of services per conception and call for veterinary professional were analyzed to find out the association (Table 1).

Table 1: Variables included in the questionnaire

Variable	Variable levels	Positive (%)	Negative	Univariable analysis	
				95% CI	*p-value*
	Beetle	14 (23.33)	46	0.440-2.648	0.868
Breed	Teddy	08 (17.77)	37	0.278-2.117	0.607
	Non-descript	11 (22.00)	39	1	
Sex	Female	28 (24.78)	85	0.873-6.807	0.069
	Male	05 (11.90)	37	1	
Age	≥ 1 year	16 (48.48)	17	0.005-0.366	0.000
	≥ 2 year	01 (01.82)	55	0.138-3.817	0.568
	≥ 3 year	14 (35.00)	26	0.009-0.634	0.002
	≥ 4 year	01 (04.00)	24	1	
Rearing system	Confined	08 (14.29)	48	0.291-2.888	0.062
	Open	19 (31.66)	41	0.914-7.110	0.00
	Both	06 (15.38)	33	1	
Body Health	Normal	15 (22.73)	51	0.169-1.508	0.228
	Thin	11 (15.71)	59	0.103-0.992	0.053
	Emaciated	07 (36.84)	12	1	
Grazing Status	With Bovine	07 (30.43)	16	0.119-1.217	0.107
	Alone	18 (23.68)	58	0.215-1.343	0.174
	Mixed species	08 (16.66)	48	1	
Urine Physical appearance	Normal	32 (21.05)	120	0.047-6.070	0.625
	Blood in urine	01 (33.33)	2	1	
Jaundice History	No	31 (20.95)	117	0.123-3.579	0.642
	Yes	02 (28.57)	5	1	
Water Source	Fresh	14 (19.44)	58	0.184-1.727	0.325
	Lake	13 (20.63)	50	0.195-1.886	0.395
	Canal	06 (30.00)	14	1	
Abortion History	No	10 (19.23)	42	0.552-5.626	0.330
	Yes	18 (29.51)	43	1.048-9.157	0.030
	N/A	05 (11.90)	37	1	
Mastitis Status	Acute	09 (29.03)	22	0.899-10.192	0.067
	Chronic	17 (32.69)	35	0.805-7.003	0.100
	Sub-clinical	02 (16.67)	10	0.249-8.797	0.673
	N/A	05 (11.90)	37	1	
History of Stillbirth	No	21 (20.00)	84	0.648-5.282	0.230
	Yes	07 (87.50)	1	0.525-2.234	0.00
	N/A	05 (11.90)	37	1	
Kidding Interval	<1 year	11 (22.92)	37	0.696-6.956	0.167
	>1 year	16 (27.12)	43	0.920-8.240	0.057
	>2 year	01 (16.67)	5	0.142-1.538	0.750
	N/A	05 (11.90)	37	1	
Physiological Status	Pregnant	04 (18.18)	18	0.393-6.874	0.499
	Non-Pregnant	14 (25.45)	41	0.830-7.695	0.089
	Lactating	10 (27.78)	26	0.870-9.308	0.075
	N/A	05 (11.90)	37	1	
Aborted fetus disposal	Burry	08 (19.51)	33	0.534-6.027	0.339
	Throw away	20 (27.78)	52	0.979-8.271	0.041
	N/A	05 (11.90)	37	1	
No. of services per conception	1	05 (18.52)	22	0.437-6.470	0.451
	2	13 (27.08)	35	0.888-8.510	0.068
	3	10 (26.32)	28	0.840-8.943	0.086
	N/A	05 (11.90)	37	1	
Call for veterinary professional	Self-treatment	09 (20.93)	34	0.185-2.063	0.437
	Always on call	10 (15.87)	53	(0.137-1.420)	0.179
	Few days post infection	08 (27.58)	21	0.253-3.121	0.854
	Only in emergency	06 (30.00)	14	1	

Table 2: Final logistic regression model

Variable	Response level	Wald Statistics	Standard Error	Significance	Odd ratios	95% C.I Lower	Upper
Body condition score	Normal	3.506	0.855	0.061	4.955	0.928	26.458
	Thin	4.764	0.843	0.029	6.297	1.207	32.866
	Emaciated	Ref					
Rearing system	Grazing	2.003	0.677	0.157	0.384	0.102	1.446
	Confined	1.654	0.869	0.198	3.056	0.557	16.776
	Both	Ref					
Call for veterinary professional	Self	0.731	0.915	0.393	2.186	.364	13.138
	Only emergency	1.466	0.862	0.226	2.839	0.525	15.362
	Few days post	0.167	0.903	0.682	1.447	0.247	8.490
	Always on call	Ref					
Grazing status	With bovine	.424	0.527	0.515	1.410	0.501	3.963
	Alone	1.627	0.728	0.202	2.531	0.608	10.544
	Mixed species	Ref					
Age	≥ 1 year	9.086	1.130	0.003	30.144	3.291	276.064
	≥ 2 year	15.629	1.153	0.000	95.244	9.949	911.783
	≥ 3 year	1.690	0.550	0.194	2.043	0.696	5.998
	≥ 4 year	Ref					

Table 3: Hematological parameters in sero-negative and seropositive caprine population

Parameter	Units	Sero-positive animal	Sero-negative animal	P-value
Hb	g/dl	05.48±0.74	09.84±1.15	0.000
TEC	× 10^6/µl	10.64±2.45	13.95±2.08	0.011
TLC	× 10^3/µl	13.60±3.64	08.26±2.43	0.004
PCV	%	18.79±2.06	24.83±3.53	0.001

Table 4: Biochemical parameters in seropositive and sero-negative caprine population

Parameter	Units	Sero-positive animals	Sero-negative animals	P-value
ALP	IU/L	121.38±30.75	114.38±35.56	0.682
ALT	IU/L	51.13±7.68	19.00±5.45	0.000
AST	IU/L	85.37±18.78	109.0±33.34	0.103
BUN	mg/dl	52.50±7.65	29.62±12.24	0.001
Creatinine	mg/dl	01.28±0.23	01.25±0.22	0.768

To identify the potential risk factors contributing towards *leptospira* in goats, final regression model was developed. Initially, this model was run on those variables which have given P<0.2 in univariable analysis (Table I). The non- significant variables were removed one by one using backward stepwise approach. The key risk factors responsible for disease dynamics were; history of abortion, aborted fetus material disposal, history of acute or chronic mastitis and history of still birth (Table 2). As far as body conditions of animals are concerned, the animals having thin (OR=4.955; CI .928-26.458) and emaciated (OR=6.297; CI 1.207-32.866) conditions were at more risk of having antileptospiral antibodies as compared to the animals having normal body condition. Similarly, the odds of getting *leptospira* antibodies were 3.056 times (CI 0.557-16.776) more in animals having confined system of rearing. The animals having ≥1 year age are at 30.11% (CI 3.219-276.064) more threat followed by 95.244 (CI 9.949-911.783) and 2.04% (CI .696-5.998) by ≥ two years and ≥ three years respectively as compared to animals having age ≥4 years. The animals which were reared with bovine were at 3.225% (CI .822-8.386) more risk of having *leptospira* antibodies as compared to animals which were reared alone. Similarly the owners which were giving self-treatment (OR=2.186; CI .364-13.138) to animals were at more risk followed by only in emergency (OR=2.839; CI .525-15.362) and few days post clinical ailment (OR=1.447; CI .247-8.490) as compared to those animals which were properly attended by veterinary staff.

Hemato-biocheimcal parameters of seropositive and seronegative animals: The values of different parameter like TEC, Hb, and PCV of seropositive animals were decreased significantly, while the value of TLC was increased in seropositive animals (Table III). Serum biochemical variables like ALT and BUN showed statistically significant change (P<0.05) in seropositive animals, whereas variations in ALP, AST and creatinine values were found non-significant (Table 4).

DISCUSSION

Sero-prevalence of *leptospira* in goats: The overall prevalence of anti-leptospiral antibodies evaluated in this study (21.29%) was in consistent with the results of (Lilenbaum *et al.,* 2008) and (Ciceroni *et al.,* 1997), they reported 20.8% and 19.7% prevalence respectively from Brazil and Bolivia. In contrast to the results of current study, higher prevalence was reported by (dos Santos *et al.,* 2012) and (Campos *et al.,* 2017) from Brazil, (Habasha and Sultan, 2010) from Iraq, (Vijayachari *et al.,* 2014) and (Krishna *et al.,* 2012) from India. The authors have reported 31.3, 34.6, 22.4, 29 and 36.36% prevalence respectively. While lower prevalence of *leptospira* was documented by (Cortizo *et al.,* 2014) in Brazil, (Ciceroni *et al.,* 2000) in Italy, (Santos *et al.,* 2013) and (Lilenbaum *et al.,* 2007) in Brazil, (Sunder *et al.,* 2014) in India and (Suepaul *et al.,* 2011) in Trinidad. The authors have reported the prevalence of 10.9, 2.1, 8.7, 11.1, 16.42 and 3.3% respectively. The reason for such discrepancies in the prevalence might be due to changes in geographical locations, sampling techniques, husbandry and management practices along with natural immunity titers and disease resistance in different breeds. Furthermore, transmission of leptospirosis is favored by tropical and subtropical conditions. The study is the first evidence of *leptospira* in goats of Pakistan. Previously authors (Ijaz *et al.,* 2018, a) have reported antibodies in bovine of flood affected areas of Pakistan.

Risk factors associated with the occurrence of disease: Age of animals was proved to be positively associated factor towards having antileptospiral antibodies, the findings of (dos Santos *et al.,* 2012) are in accordance with the current study that adult animals were more prone to *leptospira* as compared to young goats. It might be due to exposure of animals to natural infection with age and also could be due to the presence of more opportunities of contact with the source of infection. Body condition of animals was also proved significant candidate. The animals having weak body condition were at more chances of having anti-leptospiral antibodies. These findings are supported by *(*Ijaz *et al.,* 2018), the authors have reported that emaciated animals were having more prevalence of antibodies as compared to animals which were having good body condition. If the animals are weak, their immune system may be compromised and they may be at more risk of having infections. Mixed specie farming was also proved to be key risk factor towards having anti-leptospiral antibodies. Some other studies have also reported same type of findings where chances of infection were more where animals were reared with other animal species (Rashid *et al.,* 2019). The animals reared in confined system were at more risk of having antibodies. The results are in accordance with the findings of (Ijaz *et al.,* 2018). In confined system, if few animals are exposed to infection then there will be more chances of spread of disease. The current study revealed open or semi-intensive system as potential risk factor for the disease occurrence which was in accordance with the finding of (Cortizo *et al.,* 2014), but was contrary to (dos Santos *et al.,* 2012), the study found intensive system of breeding as risk factor. In Pakistan, biosecurity measures are not followed strictly in open rearing farms therefore the chances of exposure with the pathogens including *leptospira* are increased. The prevalence of anti-leptospiral antibodies were found more in female as compared to male animals. (Lilenbaum *et al.,* 2008a) has confirmed DNA of *leptospira* from the female

reproductive system, further indicating that there is an association of female reproductive system and leptospirosis. History of reproductive failures and still birth were two positively associated risk factors according to present study which was in agreement with the results of Cortizo *et al.,* (2014).

Hemato-biocheimcal parameters of seropositive and seronegative animals: The present study showed remarkable variations (P<0.05) in hematological findings including TEC, TLC, Hb and PCV (Hct) of seropositive animals as compared to those of seronegative animals. Increased value of TLC in seropositive animals was because of the neutrophilia which occurs in bacterial infections. The results of current study are in accordance with findings of Vihol *et al.* (2016). Current study outcomes are analogous to (Tonin *et al.,* 2012), they evaluated same findings in rats by inducing *leptosipra*. Similarly the findings of present study showed contradiction with Pinna *et al.* (2010). The variations in findings of present study might be because of differences in physiological status like estrus, pregnancy, age factor, stress etc and some minor pathological conditions (Vihol *et al.*, 2016).

Biochemical parameters help clinicians for diagnosis of disease. The biochemical parameters profile including ALT and BUN in both seropositive and sero-negative animals showed significant difference (P<0.05) but parameters like AST, ALP and creatinine did not show significant variations. ALP and BUN both are nonspecific indicators of liver damage in ruminants and confounded by many other factors like bile duct blockage and bone abnormalities. Analogous findings were observed in a study conducted on sheep *(*Millar *et al.,* 1977). In contrast to the findings of current study, (Tonin *et al.,* 2012) reported increase in the levels of BUN, creatinine, ALT and ALP in Wistar rats that were experimentally infected. Contrary to the current study findings, in goats (Vihol *et al.,* 2016) reported changes in all the biochemical parameters. Pinna *et al.* (2010) has reported similar type of findings in horses. However, there was a minor increase in total bilirubin, ALT and AST that might be due to slight liver damage. So, hypothetically these variations could be related and are of diagnostic importance for leptospirosis, as a few unknown etiologies like parasitism, hepatic disorder and low or poor protein level (Yang *et al.,* 2001).

Conclusions: The study is the first evidence of *leptospira* in goats of Pakistan. The study emphasizes comprehensive control strategies to minimize the losses associated with the occurrence of this zoonotic malaise.

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Authors contribution: MI designed the study. MUA, AG helped in study execution. IA, FNS and MZZ contributed in data analysis and interpretation. MI, HNG, AH, MA and MUA prepared the manuscript. MI, WS and AG reviewed the manuscript. All authors gave final approval of the manuscript.

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