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Leptospirosis: An Emerging Zoonosis in Pakistan

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Abstract.- Leptospirosis is an important zoonotic disease caused by *Leptospira* species. Many domestic and wild animals act as reservoirs and ultimate source of contamination to human population. Since it is an emerging infectious disease that is under reported in developing countries, this report would provide baseline study for clinicians and researchers. To study the serosurveillance of human leptospirosis, 100 human (78 males; 22 females) blood samples were collected from Lahore city and its peri-urban areas and processed by cELISA Serion ELISA *classic* microtiter plate. The results of this study revealed 44% prevalence of human leptospirosis. Among 78 males and 22 females, 38 males (49%) and 06 females (27%) were found positive. Age wise serosurveillance demonstrate 47% prevalence in adults and 35% in young ones. Season wise 42%, 40%, 26% and 47% were observed in Summer, Fall, Winter and Spring, respectively. It is concluded that highest prevalence was in male adults while spring and summer were more susceptible seasons having leptospirosis infection. This is the first report of serosurveillance of leptospirosis in humans in Pakistan.

Key words: Human leptospirosis, serosurveillance, zoonosis

INTRODUCTION

Leptospirosis is a worldwide zoonosis caused by various species of *Leptospira*. This disease has many domestic and wild animals as reservoirs which spread infection through their urine and ultimately contaminate soil and water which eventually causes infection to humans (Kawaguchi *et al*., 2008). In human leptospirosis, multi-organ dysfunctions have been cited like acute renal failure, myocarditis, refractory hypotension, respiratory distress syndrome (Myint *et al*., 2010).

Leptospirosis is considered as an occupation related disease of persons engaged in animal slaughtering, agriculture, pet shop owners, veterinarians, meat handlers, farm workers, and sewerage workers (Sharma *et al.*, 2006; Green-McKenzie and Kulkarni, 2010). Accurate and early diagnosis is very essential for early and effective treatment of leptospirosis. Leptospirosis has been recognized as an emerging infectious disease in most of the developing countries. This disease is of tropical countries and often it is endemic (Goncalves *et al*., 2010), although in Pakistan its prevalence in humans is completely unknown and there is no documented evidence on its incidence. Being an ignored disease, this project was intended to monitor the sero-surveillance of leptospirosis in humans in Pakistan. The findings of this study would provide basis for instituting the diagnosis, prevention and control strategies for leptospirosis in Pakistan.

MATERIALS AND METHODS

One hundred serum samples from the human population who were at high risk *viz.*, veterinarians, pet-owners and livestock holders were collected from December 2010 to November 2011 to evaluate the risk factors associated with leptospirosis. The data related to these samples were collected in a data capture form. The entries in data capture form included location, age, sex, season and previous disease history. Prevalence was calculated as per formula described by Thrusfield (2002). The collected blood samples were processed at Medicine and Microbiology Laboratories of the University of Veterinary and Animal Sciences, Lahore by using ELISA kit (cELISA SERION ELISA *classic* micro-titer plate, Germany**).** Before running the test, patient samples were diluted in dilution buffer by adding 10 µl of patient's sample into 1000 µl of dilution buffer. After dilution and before pipetting

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into the micro-titer plate, the samples were mixed thoroughly to prepare a homogenous solution. Control and standard sera were ready for use and no further dilution was required. After harvesting serum, the micro-titer plate was incubated for 60 minutes at 37°C. Then each well was aspirated and washed by repeating the process four times. After this 100 µl of pipette conjugate solution APC was added to each well and incubated for 30 minutes at 37°C. Plates were washed four times and then substrate solution pNPP was added to each well and incubated for 30 minutes at 37°C. After that 100 µl of stop solution was added to each well to stop the reaction. Optical density of each well was determined by using a micro plate reader set at 405 nm. The OD value of the substrate blank was taken < 0.25 for validity of results. By use of quantitative serion ELISA *classic* tests the mean OD value (after subtraction of the substrate blank) of the standard serum taken within the validity range, which is provided in the lot specific quality control certificate. The calculations of the results were made as per formula given in the Kit manual (cELISA serion ELISA *classic* microtiter plate, Germany).

Statistical analysis

The data thus obtained were analyzed through Chi-square by using Statistical software package STATA 9.1 college station T×77845, USA.

RESULTS

Forty four samples out of 100 were found positive for *Leptospira* antibodies. Prevalence of leptospirosis during different months of the year revealed highest number of cases in March, April and August and recorded as 67%, 67% and 60%, respectively (Table I). While there were moderate numbers of cases during the rest of months of the year except in the month of November in which no case was recorded. No significant difference ($p<0.05$) in the sero-prevalence of leptospirosis in human during the different months of the year was found. Chi-square analysis showed non-significant difference in month wise serosurveillance of leptospirosis. Chi Square ($\chi 2$) = 13.6, df 11, p-value 0.2559.

Sex wise seroprevalence

Prevalence rate of 49% (38/78) and 27% (06/22) for leptospirosis was recorded in male and female, respectively (Table II). It was concluded from the results that at $p<0.05$ there was significant difference (χ^2 = 3.203, *P*-Value= 0.03676) in the seroprevalence of leptospirosis between both sexes *i.e.* male and female.

Age wise seroprevalence

Age wise seroprevalence revealed 47% (35/74) and 35% (09/26) prevalence in adults (age ≥ 15 years) and young ones (age <15 years), respectively (Table II). There is no significant difference (χ^2 = 1.256, *P*-Value= 0.1317) in the prevalence of leptospirosis among the different ages of humans like young and adults.

Season wise seroprevalence

Season wise seroprevalence of disease showed 42% (13/31) prevalence in summer, 40% (06/15) in fall and 26% (07/27) in winter, while 47% (18/27) was recorded in spring season of the year (Table III). Marked significant difference (χ^2 = 9.361, P-Value= 0.02486) of seroprevalence in humans between different seasons of the year was also found.

DISCUSSION

Leptospirosis has been widely studied in many Asian countries except Pakistan. Therefore limited literature on seroprevalence studies is available in this area. Present seroprevalence study declared significant prevalence (44%) which should be worth attention for clinicians and researchers. Previously, Vitale *et al.* (2004) and Cinco *et al.* (2004) have already successfully used ELISA for diagnosis of leptospirosis. Sharma *et al.* (2006) and Jalii *et al.* (2000) reported 52.7% and 16.7% prevalence in India which have almost the same socio-economic and climate condition as in Pakistan which supports findings of current study. Carina *et al.* (2011) reported more number of cases in the month of July (14.3%). Similarly Vijayachari (2008) showed two peak seasons (summer and spring) of leptospirosis. In present study, highest prevalence was also observed in these seasons. These findings

Table I.- Month wise sero-prevalence of disease in human under different climatic conditions

Month	Temperature (° C)		Av. fain fall (mm)	No. of samples tested	Positive samples	Prevalence (%)
	Mean Min.	Mean Max.				
Dec. 2010	7.9	21.2	15.0	6	2	33.33
Jan. 2011	6.9	16.6	-1.0	10	3	30.00
Feb. 2011	11.1	21.2	28.1	9	2	45.00
Mar. 2011	16.3	28.0	7.0	12	8	66.66
Apr. 2011	20.2	32.9	16.0	15	10	66.66
May 2011	27.5	39.9	6.0	8	4	50.00
June 2011	27.0	37.2	147.6	8	4	50.00
July 2011	26.7	34.2	244.0	10	2	20.00
Aug. 2011	26.4	33.3	253.9	5	3	60.00
Sept. 2011	25.7	33.2	154.3	6	3	50.00
Oct. 2011	21.2	32.5	-1.0	9	3	33.33
Nov. 2011	16.0	28.0	-1.0	02	0	00.00
Total				100	44	44.00

Metrological data collected from regional metrological center, Lahore.

Table II.- Age and sex wise serosurveillance of leptospirosis in humans.

Age	No. of samples examined	Male (n = 78)	Female (n = 22)	Total Seroprevalence
Adults (< 15 years)	74	31 (40%)	04 (18%)	35 (47%)
young (≥15 years)	26	07 (9%)	02 (9%)	09 (35%)
Total	**100**	**38 (49%)**	**06 (27%)**	**44 (44%)**

Table III.- Season wise serosurveillance of leptospirosis in humans.

Season	Sample tested	Positive samples	Sero-prevalence
Spring (March- April)	27	18	47%
Summer (May-August)	31	13	42%
Fall (September-October)	15	06	40%
Winter (November-February)	27	07	26%
Total	**100**	**44**	

coincide with the rainy season and most of people are exposed to wet and humid environment during this period (Myint *et al*., 2010). Highest prevalence is also observed in the month of March, although this month is not considered as rainy season in Pakistan. This indicates that disease can be prevalent in areas of moderate climate. These findings are rarely observed in previous studies and correlates with the findings of Green-McKenzie and Kulkarni (2010) who illustrated that although leptospirosis is normally linked with tropical countries and heavy rainfall, but most cases actually occur in temperate climates, perhaps because of underreporting in some countries.

According to the serosurveillance information collected in the present study males were more affected compared to females. As subjects under study were mostly farmers, veterinarians and the pet owners who were directly or indirectly involved with animals and since these occupations are male dominating in Pakistan, the males were more at risk with leptospiral infection. Jalii *et al. (*2000), Yimer (2004) and Yanagihara (2007) also demonstrated that males who have outdoor occupations are at more risk of contracting leptospiral infection compared to females.

Young adults are considered more prone to leptospirosis as compared to young ones and middle age is taken at its highest risk of developing leptospiral infection (Collares-Pereira *et al.,* 2008;

Sharma *et al.*, 2006). Socio-demographic data represented in this study declared more adult seropositive patients as contrast to young ones. Findings of more frequent antibodies in adults were suggestive of change in social roles and more exposure to risk factors like occupation related to animal handling, working in agricultural fields where this infection may be harboring and can lead to transmission of leptospirosis to workers or employees (Zhang *et al*., 2012).

CONCLUSIONS

This study sufficiently establishes the existence of leptospirosis in Lahore and adjoining areas. However, it would be essential to carry out wider scale study to know the magnitude of the problem so that suitable diagnosis, treatment and preventive strategies could be adopted to control its incidence.

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