

Study on Incidence of Leptospirosis by Serodiagnosis in Rural Population in the District of Kanchipuram, Tamilnadu, India.

Shanthi Banukumar^{1*}, Kannan I², Premavathi R. K³, Shantha S⁴

Department of Microbiology, Tagore Medical College and Hospital, Rathinamangalam, Chennai – India

Available Online: 29th February, 2016

ABSTRACT

Background and Objectives: Leptospirosis, a zoonotic disease caused by occupational or recreational contact with contaminated fluids. The aim of the present study is to know the incidence of the disease and to know the seroprevalence in the district of Kanchipuram, Tamilnadu, India. This helps to maintain adequate epidemiological data which indirectly favors the preventive and control measures to be carried out at State level and National level. **Materials and Methods:** The patients who attended our medicine outpatient department from Jan 2011 to Dec 2014 are screened for evidence of signs and symptoms of leptospirosis. A detailed history relevant to leptospirosis was elicited. Blood and serum of patients with history of fever more than a week was subjected to darkfield microscopy (DFM) and Dridot – Panbio respectively. 477 blood samples were analysed by (DFM), IgM ELISA and subjected to microscopic agglutination test to our referral lab. Sera having MAT titre more than 1:80 to any serovar with signs and symptoms of leptospirosis were considered to have leptospiral infection and were defined as seropositive cases. **Result:** From 477 samples 105 serum samples were seropositive and the incidence is 22.1%. A high incidence of 40.91 % in male between 21- 30 years and 38.46% in female between 11-20 years are reported. It also shows seasonal variations, more common in months of September, October and November.

Keywords: Leptospirosis, DFM, Microscopic agglutination test, ELISA, Dridot – Pan bio, Kanchipuram district, serovar prevalence.

INTRODUCTION

Leptospirosis, one of the zoonotic diseases is having a worldwide distribution and spread directly to human beings without any intermediate vertebrate or invertebrate or inanimate vectors¹. The common reservoir is rat, human acquire the disease through accidental, occupational, or recreational contact with contaminated water or through contact with urine, fluids or tissues of infected animals². The reservoir status differs in different animals for leptospira and has been demonstrated. This is an important factor in the study of epidemiology of the human leptospirosis and their persistence in the infection of human community³. Median global incidence of endemic human leptospirosis excluding cases due to outbreaks is 5 per 1,00,000. But in some areas as high as 975 cases per 1,00,000 has been reported. Mean global incidence of epidemic Leptospirosis reported in outbreaks is 14 per 1,00,000⁴. This disease is caused by pathogenic spirochaetes of genus *Leptospira* and recognised as an important emerging disease in the 1980 and in 1990 in Andamans, Tamilnadu and Kerala⁵. Most of the outbreaks of leptospirosis are reported from coastal regions of the states of India like Gujarat, Maharashtra, West Bengal, Orissa, Kerala, Tamil Nadu, Karnataka, and Andaman Islands⁶. Leptospirosis leads to multiple organ involvement associated with atypical clinical presentations. High mortality and morbidity rates have

been reported in outbreaks followed by natural calamities such as cyclone and flood. Several epidemics have been reported in different parts of the country. The major risk groups identified include agricultural workers, veterinarians, fisherman, workers in animal farms, poultry, butchers, lab staffs, miners and those who participate in recreational and leisure activities⁷. In Mumbai, in 1967 out of 150 Infective Hepatitis positive patients one was positive for leptospirosis. In suspected leptospira cases out of 17, 5 patients were positive for leptospirosis. Sera from 11 workers of animal farm showed positivity⁸. In Pondicherry in 1995 patients with fever and jaundice showed a prevalence rate of 12%. The area chosen for our study has a dense population of farmers and they have the chance of being infected through contact with environment contaminated by urine of shredder host – Rodent⁹. The disease is widespread in farm and domestic animals in many parts of India¹⁰. It is confused with malaria, influenza, dengue fever, viral hepatitis, rickettsial infections, typhoid fever, melioidosis and others¹¹. To reduce the risk of more serious infection and mortality, adequate knowledge to differentiate it from other diseases and early diagnosis are important¹². The disease typically occurs as an epidemic lasting a few weeks during monsoon season¹³. There is no data regarding the seroprevalence rate in Kancheepuram district of Tamilnadu, India which has people with agriculture as their main occupation. Thus in

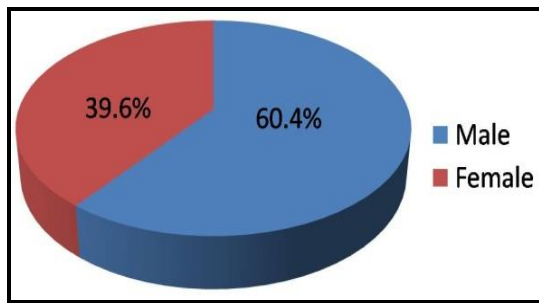


Figure 1: Sex wise distribution of patients.

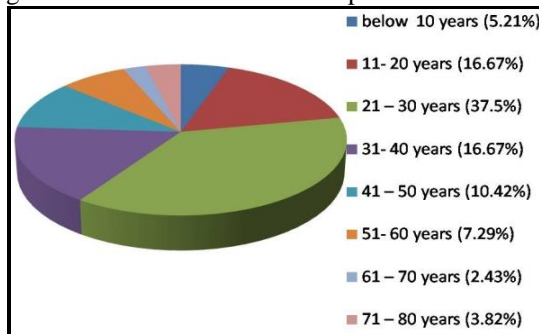


Figure 2: Age wise distribution of male patients.

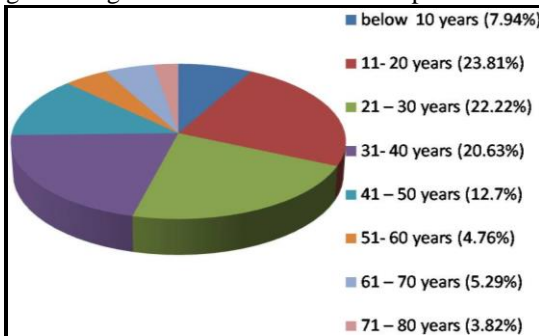


Figure 3: Age wise distribution of female patients.

the present study an attempt has been made to know the incidence of the disease and to diagnose the seroprevalence in this particular area. This aids to maintain adequate epidemiological data which indirectly favors the preventive and control measures to be carried out at State level and National level.

MATERIALS AND METHODS

A three year study was performed for the patients who attended outpatient department of Tagore Medical College from Jan 2011 to Dec 2013. This included all age groups, different occupational groups, agricultural workers, manual laborers, house wives. Those patients who complained of fever, head ache, prostration, myalgia, anorexia, high colored urine, conjunctiva suffusion and decreased urine output were included for the study to assess the seroprevalence of leptospirosis among the various high risk groups. Four hundred and seventy seven patients were identified as respondents who were between the age group 10 – 80 years. From each patient urine sample and 5ml of paired blood sample with an interval of one week was collected by venipuncture in a sterile tube and serum was separated. The serum samples were stored at -20°C until use. All the blood samples and urine samples were analysed by dark field microscopy (DFM).

The samples were further subjected to serological tests like Lepto tek Dri – dot (Rapid test) and leptospira IgM enzyme linked immunosorbent assay (ELISA) by Panbio Leptospira IgM ELISA kit. The samples that were positive by both DFM and Leptospira IgM ELISA were sent to Madras Medical College, Chennai, India, a Regional Referral Center for microscopic agglutination tests (MAT). The serovar panel included are icterohaemorrhagiae, australis, autumnalis, griphotyphosa, hebdomadis, pomona, patoc, javanica and sejroe. Those samples within an initial titer of more than or equal to 1:80 or fourfold rise in titer of MAT was considered significant.

RESULTS AND DISCUSSION

Out of 477 samples screened for the study in a period of three years i.e., 2011 to 2014, 288 (60.4%) were men and 189 (39.6%) were women (Figure 1). On age wise analysis, out of 288 men screened, about 37.5% belonged to the age group of 21-30 years followed by other age groups (Figure 2). On age wise analysis, out of 189 women screened, about 23.81% belonged to the age group of 11-20 years followed by other age groups (Figure 3). From all these patients a detailed history pertaining to leptospirosis was elicited, including their nature of job, duration of illness, animal contact, barefoot walking, sanitation facilities, water supply, rodents present in and around their residence, their routine activities etc were taken. 105 serum samples were seropositive out of 477 samples screened and the incidence is 22.1%. Those samples with an initial titer of more than or equal to 1:80 or fourfold rise in titer of MAT was considered significant. The Table 1 shows the seropositivity among the male and female. In the male patients, the seroprevalence is found to be high (40.91%) in the age group of 21 to 30 years. In the female the seroprevalence is found to be high (38.46%) in the age group of 11 to 20 years. The Table 2 depicts the seasonal seroprevalence of leptospirosis. From the table it is understood that the peak incidence of leptospirosis is high during the months of September, October and November which is the monsoon season of that area. The high incidence of infection during these months concludes that the rain and dampness promotes the spread of infection and favors the survival of leptospira in soil. These 105 positive seropositive samples were sent to Madras medical college, Chennai Regional Referral Center for Leptospirosis for speciation. The Table 3 shows the identified serovars. The most common serovar is australis (40 %).

DISCUSSION

The specificity of MAT is more when compared to ELISA and it is a good reference test accepted by World Health Organisation. The most common serovar identified in our study was australis. None of the serum samples were positive for sejroe, javanica and patoc. The present epidemiological survey revealed that australis was predominant in 40%, followed by Pomona 22.86 %, autumnalis and hebdomadis each 11.43 %, griphotyphosa 8.57 % and icterohaemorrhagiae 5.71 %. Further, the study revealed that leptospirosis is common both in males (22.9%) and females (20.63%) especially in active age

Table 1: Seropositive among male and female.

Age and Sex Wise Distribution Age Group	Male			Female		
	Total sample	Seropositive	% seropositive	Total sample	Seropositive	% seropositive
Below 10 years	15	3	9.09	15	3	7.69
11 to 20 years	48	0	0	45	15	38.46
21 to 30 years	108	27	40.91	42	6	15.38
31 to 40 years	48	18	27.27	39	9	23.08
41 to 50 years	30	12	18.18	24	6	15.38
51 to 60 years	21	6	9.09	9	0	0
61 to 70 years	7	0	0	10	0	0
71 to 80 years	11	0	0	5	0	0
Total	288	66	100	189	39	100

Table 2: Seasonal seroprevalence

Month	2011		2012		2013		Total positive	Percentage positive
	Total cases	Positives	Total cases	Positives	Total cases	Positives		
January	12	2	9	2	5	2	6	5.71
February	5	1	6	2	4	1	4	3.80
March	4	1	4	1	3	0	2	1.90
April	2	0	2	0	4	1	1	0.95
May	2	0	0	0	3	1	1	0.95
June	0	1	2	0	2	0	1	0.95
July	1	1	3	1	1	1	3	2.86
August	14	4	10	5	8	3	12	11.43
September	18	8	18	7	14	5	20	19.05
October	19	9	17	7	18	6	22	20.95
November	16	7	14	9	17	7	23	21.90
December	13	5	12	3	6	2	10	9.52
Total	106	37	97	38	85	30	105	100

Table 3: Epidemiological seroprevalence.

S. No	Species	Number of Seropositive samples	% Seropositive
1.	Icterohaemorrhagiae	6	5.71
2.	Australis	42	40.00
3.	Autumnalis	12	11.43
4.	Griphotyphosa	9	8.57
5.	Hebdomadis	12	11.43
6.	Pomona	24	22.86
7.	Patoc	0	0
8.	Javanica	0	0
9.	Sejroe	0	0

groups between 21- 30 years and 11- 20 years respectively. Leptospirosis shows seasonal variations, being more

common during the month of September, October and November. These months, the farmers seem to be active in their fields barefooted which promotes the high percentage of incidence. This proves that *Leptospira* multiplies in those areas where water is stagnant for 2- 3 days especially after the rains. Most of the farmers who walk bare footed in the poverty ridden areas had little protection against this disease. This study also infers that most commonly occurring serovar in this area are in high risk groups are *australis*, *pomona*, *autumnalis*, *hebdomadis*, *griphotyphosa*

and *icterohaemorrhagiae*. Development of a vaccine incorporating these serovars will be of advantage in prevention of the disease, apart from other routine preventive measures in this particular area.

ACKNOWLEDGMENTS

We sincerely thank the heads and the whole team of Medical and paramedical staffs of Institute of Microbiology of Madras Medical College, Chennai. Tamil Nadu for providing all the facilities to carry out this present study.

REFERENCES

1. Leptospirosis worldwide 1999. Weekly epidemiol Rec. 1999;74:237- 42.
2. Murhekar MV, SugunAP, Vijayachri PSharma S, SehgalSC. Risk factor in the transmission of leptospiral infection. Indian J Med Res 1998; 107:218-23.
3. Faine S, Adler B, BoeinC, Perolat P. *Leptospira* and Leptospirosis in; Sources, Transmission and spread of leptospirosis Faine S, editors. 2nd ed. MedSci 2000 p134- 135.
4. Murugan S, Ghatala MZ, Sankar AS, Krishnan J. Atypical Presentation of Multiorgan Failure Leptospirosis (Weil's Disease) without Fever Indian journal of Clinical practice. 2014; 24:948-950.

5. John J. Reemerging bacterial pathogens. India. J. Med. Rev. 1996; 104:4-18.
6. Himani D, Suman MK, Mane BG Epidemiology of Leptospirosis: An indian prospective. Journal of food borne and zoonotic Diseases 2013; 1:6- 13C
7. Human Leptospirosis: Guidance for diagnosis, surveillance and control. WHO Library Cataloguing-in-Publication Data World Health Organization 2003.
8. Patil D, Dahake R, Roy S, Mukherjee S, Chowdry A, Deshmukh R. Prevalence of leptospirosis among dogs and rodents and their possible role in human leptospirosis from Mumbai, India. Indian J. Med. Microbiol. 2014; 32:64-67.
9. Shekatkar SB, Harish BN, Menezes GA, Parija SC. Clinical and Serological evaluation of leptospirosis in Puducherry, India. J. Infect. Dev. Ctries 2010; 4(3):139- 43
10. Karuniawati A, Yasmon A, Ningsih I. Optimizing real time PCR method to detect *Leptospira* sp. in human blood and urine specimens. Med J Indones. 2012; 12:13-17.
11. Nabity SA, Ribeiro GS, Aquino CL, Takahashi D, Damiao AO, Goncalves AH, et al. Accuracy of dual path platform (DPP) assay for the rapid point of care diagnosis of human leptospirosis. PLOS Neg Trop Dis 2012; 6: e 1878.
12. Pappachan MJ, Mathew S, Aravindan KP, Khader A, Bharghavan PV, Kareem M.M. et al. Risk factors for mortality in patients with leptospirosis during an epidemic in northern Kerala. Nat Med. J. India 2004; 17:240-42
13. Silva MV, Camargo ED, Batista L, Van AJ, Brando AP, Nakamura PM, et al. Behaviour of specific IgM, IgG and IgA class antibodies in human Leptospirosis during the acute phase of the disease and during convalescence. J Trop Med Hyg 1995; 98:268-72.