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Original Research Article

Prevalent Serogroups and Serovars of Leptospira Causing Leptospirosis in Clinically Suspected Patientsin Tertiary Care Hospital in South Kerala

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Abstract

Introduction: In Kerala, Leptospirosis, which started as an isolated public health problem of some of the water logged areas of Alappuzha and Kottayam districts in the 1990s, has now become a public health problem in all districts. This has been causing the highest number of deaths consistently for the last few years with the younglabour class getting affected posing a serious concern. Preliminary analysis of Leptospirosis deaths shows that delay in definitive diagnosis and effective treatment cause high case fatalities. According to statistical data of DHS Kerala during 2011-2012, Eranakulam and Thiruvananthapuram districts reported the largest numbers of confirmed cases, respectively. This study attempts to find out the prevalent serogroups and serovars causing the disease in Thiruvananthapuram and their association with clinical features and complications.

Objectives:

1. To estimate IgM Leptospira antibodies in 1:100 and 1:200 dilution of serum of clinically suspected patients of Leptospirosis.
2. To estimate the predominant serogroups and serovarsof Leptospira causing Leptospirosis in clinically suspected cases
3. To estimate the complications due to serogroups and serovars of Leptospira.

Conclusions: Out of the 400 suspected cases, 96 were IgM Lepto ELISA positive showing 24% (95% CI,19.8- 28.8) prevalence of Leptospirosis in the study population. The present study shows 34.78% *L. icterohaemorrhagiae*, 15.21% *L. hebdomadis*, 13% *L. autamnalis*, 8.6% L. australis, 6.5% *L. grippotyphosa*, 6.5% *L. pomona*, 6.5% *L. tarassovi*, 4.34% *L. canicola* and 4.34% *L. bataviaserovars* causing leptospirosis by MAT.

Keywords: Leptospirosis, Serogroups, Serovars, Seroprevalence.

Introduction

Leptospirosis is a zoonosis found across the globe, caused by infection of pathogenic Spirochaetes of the genus *Leptospira.* The organisms are maintained in nature by chronic renal infection of carrier mammals, which excrete the organism in their urine [1]. Humans become infected through direct exposure to infected animals or their urine or through indirect contact via contaminated water or soil [2]. Since it is a zoonotic disease with large variety of animals acting as carriers, it is difficult to eliminate and even to control it in tropical developing countries. Since the bacteria are adapted to the environment of the tropical regions with plenty of rainfall, it is difficult to avoid exposure of the people to animals or contaminated environment. Because of this, early detection, prompt treatment and creating awareness about the disease among people and public health professionals are the steps that could reduce the magnitude of the problem [3].

Leptospirosis has long been considered a rare zoonotic disease with only sporadic cases being recorded in India. [4,5] But, since the 1980s, the disease has been reported from various states during monsoon months in mini epidemic proportions. This is endemic in Kerala, Tamil Nadu, Gujarat, Andaman, Karnataka and Maharashtra. It is also reported from Andhra Pradesh, Odisha, West Bengal, Uttar Pradesh, Delhi and Puducherry [5,6].

In Kerala, Leptospirosis, which started as an isolated public health problem of some of the water logged areas of Alappuzha and Kottayam districts in the 1990s has now become a public health problem in all the districts. This has been causing the highest number of deaths consistently for the last few years in the state. The young labour class getting affected is a serious concern. Analysis of the available statistics shows that the disease which was mainly reported during monsoon seasons in the past, is now being reported throughout the year. Yet, the morbidity and mortalityare high during monsoons that extend from June to September. Preliminary analysis of mortality caused by Leptospirosis shows that delay in definitive diagnosis and effective treatment cause high case fatalities. According to statistical data of Directorate of Health Services (DHS Kerala) for the year 2011-12 Eranakulam and Thiruvananthapuram districts reported the largest numbers of confirmed cases respectively. [7] This study attempts to find out the prevalent serogroups and serovars causing the disease in Thiruvananthapuram and their association with clinical features and complications.

Objectives:

1. To estimate IgM Leptospira antibodies in 1:100 and 1:200 dilution of serum of clinically suspected patients of Leptospirosis.
2. To estimate the predominant serogroups and serovars of Leptospira causing Leptospirosis in clinically suspected cases
3. To estimate the complications due to serogroups and serovars of Leptospira.

Materials and Methods

Type of study: Cross sectional study.

Study setting: Department of Microbiology and Department of Medicine, Government Medical College, Thiruvananthapuram, India.

Study period: From October 2015 to June 2016.

Sample size: 400

Inclusion criteria: Adult patients with fever of less than 8 days of duration with clinical features suggestive of Leptospirosis as per CDC clinical criteria.

Exclusion criteria: Children, Immunosuppression, Malignancy, Chronic Liver disease, Chronic Renal Disease.

Blood samples were collected from suspected cases of Leptospirosis according to CDC case definition. Serum samples were used to detect Leptospira IgM antibodies in 1:100 and 1:200 dilutions of serum by using SD ELISA Kit and procedures were carried out as per the kit insert. Using suitable micro titre wells or test tubes, the negative control (N), positive control (P) and patient sample (S) was diluted 1:100 and 1:200 with sample diluent respectively. Calculate the mean absorbance of the negative controls, and cut off value was calculated as per the kit insert. Based on the criteria of the test, samples are classified as follows

A(sample) < cutoff = Anti Leptospira IgM negative. A (sample) > cut off = Anti Leptospira IgM positive.

Negative result: No detectable Anti-Leptospira IgM antibody. The result does not rule out leptospirosis infection. An additional sample should be tested in 7-14 days if early infection is suspected.

Positive result: Presence of detectable level of Anti-leptospiral IgM antibody. Other leptospira serology assays shall be performed to confirm the diagnosis. All serum samples were stored and were subjected to Microscopic Agglutination Tests (MAT).

The most commonly used serological technique (Gold Standard) for confirmation of diagnosis is MAT. It was performed as per the guidelines of Regional Medical Research Centres (Indian Council of Medical Research, Department of Health Research, Ministry of Health and Family welfare, Govt. of India and WHO Collaborating Centre for Diagnosis, Reference, Research and Training in Leptospirosis, Port Blair, Andaman and Nicobar Island, India).

MAT was standardized using the known positive serum sample and Panel of standardLeptospiral live cultures prevalent in Kerala procured from Government Veterinary college, Mannuthy, Thrissur. The serovars were namely *L. australis, L. autamnalis, L. Icterohemorrhagiae, L. grippotyphosa, L. Canicola, L. javanica, L. hebdomadis, L. pomona, L. tarassovi, L. bataviae, L. pyogenes*. The cultures were maintained in EMJH semisolid media by serial subcultures. The reference serovar strains were grown in Ellinghausen-Mc Cullough-Johnson-Harris (EMJH) liquid media. The cultures were incubated at 30^0 C for 5-7 days and examined for the growth of the organisms. The live cultures with density of 1.5 x 10^8 were used as antigens.

The serum antigen mixture was incubated in microtiter plate in various dilutions according to the standard procedures. The serum-antigen mixtures were examined under a dark field microscope for agglutination. For observation, one drop of mixture is transferred with a platinum loop or pipette from a well to microscopic slide and examined under dark field microscope with 20X objective without cover slip.

Thetitreis the dilution that gives 50% agglutination, leaving 50% of cells free. This was compared with a control suspension of leptospires diluted in PBS without serum. Agglutination was measured indirectly by establishing the reduction of leptospiral density of 50% in comparison with the density of free leptospires in the control. Seroconversion or four-fold rise in antibody titre in paired sera was taken as MAT positive. The significant titre in the case of single serum samples vary from one geographical area to other. A titre of 1:160 or above was considered as cut off for endemic settings and titre of 1:80 for non-endemic settings. In this study, a titre of 1:160 was taken as cut off titre.

Results:

Out of the total 400 samples collected from suspected cases of leptospirosis according to CDC clinical criteria, 96 were IgM Leptospira positive, 110 IgM Dengue positive, 61 IgM

Scrub Typhus Positive and 133 short febrile illness (Table 1).

Table 1: Distribution of cases among study population

Disease	Numbers	Percentage
Dengue	110	27.5
Leptospira	96	24
Scrub Typhus	61	15.25
Short Febrile Illness	133	33.25
Total	**400**	**100**

Month wise distribution of cases (Table 2) shows increase in number of cases in October, November, December, May and June that receive heavy rainfall in the study area.

Table 2: Month wise distribution of cases

Month	No.of cases	Percentage
October	13	13.5
November	16	16.6
December	11	11.45
January	4	4.16
February	5	5.20
March	3	3.12
April	7	7.29
May	11	11.45
June	26	27
Total	**96**	**100**

Age wise distribution of cases (Table 3) shows that the highest number of cases occur in the age group 51-60 years followed by 41-50 years. This shows that those in the working age group are the most affected.

Table 3: Age wise distribution of cases

Age	No. of cases	Percentage
20years	2	2
21-30	9	9
31-40	14	14.5
61-70	14	14.5
41-50	25	26
51-60	32	33.3

Out of the total (96) IgM positive cases, males (77%) were more affected than females (23%), showing visible gender disparity in the morbidity, possibly due to the difference in occupational exposure.

Occupational distribution of cases (Table 4) shows that Manual laborers are the worst affected followed by Farmers and Others.

Table 4: Distribution of cases by occupation

Occupation	No. of Cases	Percentage
Manual laborer	41	42.7
Farmer	20	20.8
Housewife	15	15.6
Others*	20	20.8

Distribution of clinical features of cases (Table 5) shows that all the patients of Leptospirosis have presented with Fever (100%), 92.7% myalgia, 85.4% headache, 65.62% conjunctival suffusion, 64.58% Icterus and 60.4% decreased urine output.

Table 5: Clinical features of Leptospirosis Cases

Symptoms	No. of Cases	Percentage
Fever	96	100
Myalgia	89	92.7
Headache	82	85.4
Conjunctival Suffusion	63	65.62
Icterus	62	64.58
Decreased urine output	58	60.4

Distribution of laboratory findings (Table 6) shows that 92.7% cases have raised total count, 92.7% cases shows neutrophilia, 41.6% thrombocytopenia, 68.75% raised LFT and 80.20% raised RFT.

Table 6: Laboratory findings of cases.

Laboratory findings	No of cases	Percentage
Raised total count	89	92.7
Neutrophilia	89	92.7
Thrombocytopenia	40	41.6
Raised LFT	66	68.75
Raised RFT	77	80.20

Distribution of Hepatorenal and Neurological involvement of cases (Table 7) shows that 14.5% have Hepatic, 54.1% Hepatorenal, 5.2% Neurological and 26.04% Renal involvements.

Table 7: Hepatorenal and neurological involvement in Leptospirosis

Organ involved	No. of cases	Percentage
Hepatic	14	14.5
Hepato Renal	52	54.1
Neurological	5	5.2
Renal	25	26.04

Distribution of complications of leptospirosis cases (Table 8) reveals that 64.5% suffered acute kidney injury, 14.9% ARDS, 7.46% Myocarditis, 5.9% multi organ failure and 7.46% death.

Table 8: Complications of Leptospirosis

Complications	No. of cases	Percentage
Acute kidney injury	43	64.5
ARDS	10	14.9
Myocarditis	5	7.46
Multi Organ Failure	4	5.9
Death	6	7.46

Distribution of serovars causing leptospirosis by MAT (Table 9) displays that 34.78% *L. icterohemrrhagiae, 15.21% L. hebdomadis, 13% L. autamnalis, 8.6% L. australis, 6.5% L. grippotyphosa, 6.5% L. pomona, 6.5% L. tarassovi, 4.34% L. canicola* and *4.34% L. batavia*.

Table 9: Serovars detected by Microagglutination test

Serovars	No. of patients	Percentage
L. icterohaemorrhagiae	16	34.78
L. hebdomadis	7	15.21
L. autamnalis	6	13
L. australis	4	8.6
L. grippotyphosa	3	6.5
L. pomona	3	6.5
L. tarassovi	3	6.5
L. canicola	2	4.34
L. Batavia	2	4.34
Total	46	100

Distribution of serovars causing death (Table 10) shows that 50% were caused by *L. icterohemorrhagiae*, 16.6% by *L. hebdomadis*, 16.6% *L. autamnalis* and 16.6% *L. pomona*.

Table 10: Serovars causing Death

Serovars	No of deaths	Percentage
Icterohemorrhagiae	3	50
Hebdomadis	1	16.6
Autamnalis	1	16.6
Pomona	1	16.6

Table 11. shows the complications associated with *L. hemonhagiae*. The majority (62.5%) of patients showed Acute Kidney injury, 18.75% Neurological complications, 18.75% ARDS, 12.5% myocarditis, 12.5% Multi organ failure and 18.75% Death. Patients who succumbed to death had multiple complications. Myocarditis, ARDS and Multiorgan failure were the causes of death.

Table 11: Complications associated with *L. icterohemorrhagiae*

Complications	No. of Patients	Percentage
Myocarditis	2	12.5
Multi organ failure	2	12.5
ARDS	3	18.75
Neurological complication	3	18.75
Death	3	18.75
Acute kidney injury	10	62.5

Table 12 displays the complications associated with *L. hebdomadis*. The majority (85.7%) had acute kidney injury, 28.5% ARDS, 28.5% myocarditis, 14.2% multi-organ failure, 14.2% neurological and 14.2% death. ARDS, myocarditis and multi-organ failure were causes of death

Table 12: Complications associated with L. hebdomadis

Complications	No. of patients	Percentage
Acute Kidney injury	6	85.7
ARDS	2	28.5
Myocarditis	2	28.5
Multi organ failure	1	14.2
Neurological	1	14.2
Death	1	14.2

Table 13. shows the complications associated with *L. autamnalis*. 100% acute kidney injury, 16.6% ARDS, 16.6% myocarditis, 16.6% multiorgan failure, 16.6% neurological and 16.6% death. ARDS, myocarditis and multi organ failure were the causes of death.

Table 13: Complications associated with *L. autamnalis*

Complications	No. of patients	Percentage
Acute kidney injury	6	100
ARDS	1	16.6
Myocarditis	1	16.6
Multi organ failure	1	16.6
Neurological	1	16.6
Death	1	16.6

As the complications associated with *L. australis* is concerned, 50% of the patients had acute renal failure and 25% had ARDS. Among other serovars, *L. grippotyphosa* caused 66.6% acute renal failure and 66.6% ARDS; *L. pomona* resulted in 33.3% acute renal failure and 33.3% death; *L. tarassovi* showed 33.3% acute renal failure whereas *L. bataviae* showed 50% acute renal failure.

Out of the 96 IgM leptospira positive cases that were tested for MAT, 46 samples were paired serum and 50 were single. Out of the total (46) paired samples, 43 were MAT positive and showed rise in titre. Out of 50 single samples, 3 were MAT positive above titre 200.

Table 14. shows correlation of MAT positive and IgM Leptospirosis at 100 dilution. Result

shows Sensitivity 100%, Specificity 85.88%, Positive likelihood Ratio7.08, Negative likelihood Ratio 0, Positive Predictive value 47.92, Negative predictive value100, Disease prevalence 11.50%, Accuracy of test 87.5%.

Table 14: Correlation of MAT with Ig M ELISA 100 dilution of serum

	MAT Positive	MAT Negative
IgM Positive (100 dilution)	46	50
IgM negative (100 dilution)	0	304

Table 15 shows correlation of MAT positive and IgM Leptospirosis 200 dilution sensitivity 91.3%, specificity 96.61%, positive likelihood ratio 27, negative likelihood ratio 0.09, positive predictive value 77.79%, negative predictive value 98.84%, disease prevalence 11.50%, accuracy of test 96%.

Table 15: Correlation of MAT with IgM ELISA 200 dilution of serum.

	MAT Positive	MAT Negative
IgM Positive (200 dilution)	42	12
IgMNegative (200 dilution)	4	342

Discussion

In this study, 400 suspected cases were tested for IgM Leptospira antibodies at 100dilution and 200 dilution of serum using SD ELISA Kitand Micro Agglutination Test (MAT) was performed using live cultures of eleven serovars of Leptospira.

All the cases have presented with five or more days of fever, and did not exceed 9 days. Out of 400 serum samples collected during the study period from October 2015 to June 2016, 96 were IgM Lepto ELISA positive, thus 24% (95%CI 19.7-28.7) showed prevalence of Leptospirosis. Out of 400 cases, 27.5% had Dengue, 15.25% scrub typhus and 33.25% acute short febrile illness. Whereas the study by Kanimozhi et al. 2016, (April 2014 to June 2014) in Madras Medical College shows 32% positivity. [8]

The study conducted by Sahira et al.2014 showed that out of 1924 patients presented with acute febrile illness, 11.4% were leptospirosis positive.[9] Another study by Mohammed Mushin P V et al. 2015 showed 9.52 % Leptospiosis positive among 252 suspected cases. [10] A multi centric study in India showed a prevalence rate of 12.5% in hospital cases. According to Shivakumar et al.the prevalence of leptospirosis in Calicut, Kerala was 38.1%, Chennai 31% and in Andaman and Nicober island it was 52.7%.[11]

The present study shows increase in number of leptospirosis cases during the month of November, December, May and June, which correlates with the study of Sahira et al. 2014, that showed an increase in the number of cases in November, December, May June, July and August thus corroborating the association of infection with rainfall.[8] The study by Loganathan et al. 2007 in Coimbatore showed rise in the number of cases in September to December.[12] Another study in northern India during 2004-2008 by Sethi S et al. 2010 showed increase in the number of cases from July to October.[13] Levett 2001 has described a disease seasonality with a peak incidence occurring during rainy seasons tropical regions.[1]

The present study shows rise in the number of cases between 41-50 years (26%) and 51-60 years (33.3%) thus confirming the results

of other studies like Mohammed Mushin P V et al. 2015, Sahira et al. 2014 showing rise in number of cases in the age group 31-45, 46-65 and 41-50, 51-60 respectively.[8,9] The study by Loganathan N et al. 2012 also shows rise of cases in the young and middle age groups below 60 years.[12] The study by SethiS et al. 2010 also showed that among the cases, the more affected were the young adults in their 2nd, 3rd and 4th decades of life. It also correlates with the study of Vimala et al. 2014 thus indicating a higher probability of occupational exposure of these age groups.[14]

The present study shows preponderance of infection to males (77%) compared to females (23%) which confirms to other studies; Loganathan N et al. 2007. Mohammed Mushin P V et al. 2015[8], Sahira et al. 2014[9], Antony et al. 2012 Kolenchery Kerala, Kar et al. 2013 in Mumbai[9] and Sethi S, et al. 2010.[13] This is due to their high frequency of outdoor activities compared to females. Whereas the study by Swapna R N et al. 2012 Calicut, showed higher prevalence in females that could be gender bias as majority of the participants in the study were hospital sanitary workers and female fisher folks.[14]

The present study shows more cases among manual labourers (44.4%) followed by farmers and others like painters, carpenters, students, factory workers and postmen (20% each) and home makers (15.6%). This correlates with the study of Loganathan N et al. 2012which showed the majority of the affected cases among labourers (78.8%) followed by farmers (7.7%), students (7.7%) and homemakers (5.5%) where as in the study by Muthusethupathi et al. 49% were out door workers.[12,14] and in the study by Sethu S et al. 2010 more cases were agricultural workers (32.6%), followed by homemakers (22.1%), students (12.8%) and manual labourers (11.6%).[13] The study by Swapna R N et al. 2012 in Calicut Kerala during the year 2005 showed the highest seroprevalence among hospital sanitary workers (56.2%), fisher folk and fisherman (52.8%), construction workers (40%), agricultural workers (30%), sewage workers (28.2%), veterinarians (13.3%) and laboratory staff (3.3%).[14] All these studies show correlation between outdoor activities and infection.

In the present study, all cases have presented with fever, 92.7% myalgia, 82.4% headache, 65.62% conjunctival suffusion, 64.58% with icterus and 60.4% with decreased urine output. In the case of other studies from Thiruvananthapuram, Mohammed Mushin P V et al. (2015) [9] found that 95.83% of thecases had fever with myalgia, 87.5% had icterus and 58.33% had conjunctival congestion [10] and Sahira et al. 2014showed 100% of patient presenting with fever, 85.5% had headache, 84.1% jaundice and 56.3% myalgia and 46.8% oliguria. In the study of Kanimozhi R et al. 2016, 99% of patients showed fever, myalgia and headache, 3.17% showed jaundice and renal involvement and 2.8% conjunctival suffusion.[8] A study by Sethi S et al. 2010 showed that 100% patients presented with fever, 37.2% with headache, 30.2% had myalgia, 73.3% jaundice, 29.1% oliguria and 18.6% conjunctival suffusion.[13] All these studies show that the symptoms that are not of Leptospirosis alone delay the diagnosis and treatment.

In the present study, the laboratory parameters showed that 92.7% patients had raised total count and neutrophilia, 41.6% thrombocytopenia, 68.75% and 80.20% of them had raised LFT and RFT respectively. The study by Sethi S et al. 2010 [13] showed that 61.6% patients presented with leucocytosis, 57% anaemia, 18.6% thrombocytopenia and 81.4% raised LFT. [13]The study of Saba Mohammed Monsoon et al. 2015[15] in Mangalore, showed anaemia (62.03%) raised liver enzymes (43.51%), leukocytosis (39.81%) increased serum bilirubin (37.96%)

azotemia (32.55%) and thrombocytopenia (37.96%). The study of Sahira et al. 2014showed that 84.1% leptospira patients showed increased bilirubin, 28.6% showed elevated urea and creatinine and 68% decreased platelet count.[9]

The present study shows that 54.1% hepatorenal,26.04% renal involvement, 14.5% hepatic and 5.2% neurological manifestations. It also shows 64.5% acute kidney injury, 14.9% ARDS, 7.46% myocarditis, 5.9% multi organ failure and 7.46% death as complications of leptospirosis which is correlating with previous study by Mohammed Mushin P V et al. 2015;25% renal failure, 8.33% hepatic encephalopathy, 12.5% ARDS, 4.16% myocarditis and 4.16% death.[10] Sahira et al. 2014showed 28.6% renal failure, 21.8% hepatic failure, 3.1% ARDS and 1.3% myocarditis as complications of Leptospirosis developed in some patients.[9] Mohammed Monsoon et al. 2015in Mangalore showed 12.03% Acute Renal Failure, 10.18 % ARDS and 3.07%Acute Myocarditis.[15] Parmar G et al. in their study also found Acute Renal Failure (66.3%) and ARDS (33%) as the most common complications. Renal failure was also found as the most common complication by Margarita et al.[15,16]

The present study shows 34.78% *L. icterohaemorrhagiae*, 15.21% *L. hebdomadis*, 13% *L. autamnalis*, 8.6% *L. australis*, 6.5%, *L. grippotyphosa*, 6.5% *L. pomona*, 6.5% *L. tarassovi*, 4.34% *L. canicola* and 4.34% *L. bataviaserovars* causing leptospirosis by MAT. The study by Mohammed Mushin P V et al. 2015 in Thiruvananthapuram showed *L. icerohemorrhagiae, L. Lia, L. canicola, L. hebdomadis, L. pomona, L. australis, L. grippotyphosa*.[10] A study in midland of Kerala by Kuriakose M et al. 2008 showaed all serogroups tested for except pomona were identified at least in one sample. L. autumnalis, *L. louisiana, L. grippotyphosa* and *L. australis* were the commonest serogroups, but in their previous study, *L. australis* and *L. autumnalis* were the serogroups found by MAT in the majority of patients with severe infections; *L. icterohaemorrhagiae* was less commonly found.[17] *L. grippotyphosa* was the most frequently identified serogroup among patients with milder disease.[17] In the study by Jayakumar M et al. 2006, [18] the serovars noted were *L. autmnalis, L. icterohemorrhagica, L. pomana* and *L. australis*. The most common species noted by them was L. autmnalis (62%) in contrast to their previous observation oficterohemorrhagiae as the commonest serovar.A study by Suresh Babu R et al. 2012 in 1998 at Kozhikode showed 3 serovars namely *L. icterohaemorrhagiae, L. autamnalis* and unknown.[19]

Present study shows that, acute renal failure (62.5%), neurological complications (18.75%), ARDS (18.75%), myocarditis (12.5%), multi organ failure (12.5%) and death (18.75%) are the complications associated with *Leptospiraictero hemorrhagiae*. The patients who succumbed to death had more than one complications. Myocarditis, ARDS and Mutli-organ failure were the causes of death. Whereas acute renal failure (85.7%), ARDS (28.5%), myocarditis (28.5%), multi organ failure (14.2%), neurological (14.2%) and death (14.2%) were the complications associated with *L. hebdomadis*. ARDS, myocarditis and multi organ failure were again the causes of death among these patients. Complications associated with *L. autamnalis* were acute kidney injury (100%), ARDS (16.6%), myocarditis (16.6%), multi-organ failure (16.6%), neurological (16.6%) and death (16.6%). ARDS, Myocarditis and multi organ failure were causes of death among them. The complications associated with *L. australis* and *L. grippotyphosa* were acute renal failure (50% and 66.6%, respectively) and ARDS (25% and 66.6%, respectively).

Whereas complications with *L. pomona* were Acute renal failure (33.3%) and death (33.3%). Complications with *L. tarassovi* and *L. bataviae*were acute renal failure (33.3% and 50% respectively). Thus the present study shows that there is no association between complications and serotypes. But serovars like *L. grippotyphosa, L. australis, L. tarassovi* and *L. bataviae* did not cause death in the present study.

In the present study correlation of IgM Leptospirosis 100 dilution with MAT showed sensitivity of 100%, specificity 85.88%, positive likelihood ratio 7.08, negative likelihood ratio 0, positive predictive value 47.92, negative predictive value 100, disease prevalence 11.50%, accuracy of test 87.5% whereas correlation of IgM Letospirosis 200 dilution of serum with MAT showed sensitivity of 91.3%, specificity 96.61%, positive likelihood Ratio 27, negative likelihood ratio 0.09, positive predictive value 77.79%, negative predictive value 98.84%, disease prevalence 11.50%, accuracy of test 96% thus showing that specificity, positive predictive value and accuracy of the test can be improved by testing the serum with 200 dilution.

The study by Kannan A et al. 2012 showed sensitivity and specificity of IgM Microlisa to be 60% and 55% respectively in comparison to gold standard MAT.[20] Winslow et al. 1997 have reported sensitivity and specificity of IgM Elisa with Panbio kit to be 100% and 98% respectively. [21] In the study by Swapna R N et al. 2012 the sensitivity and specificity of IgM ELISA as compared to MAT were 25.6% and 83.3% respectively with positive and negative predictive values of 87.5% and 18% respectively.[14] In the study by Sethi S, et al. 2010 [13] used Pan Bio IgM ELISA to screen for leptospirosis in acute and convalescent blood samples, and performed MAT on a few samples, a large number of leptospira cases were missed since sensitivity of PanBio IgM ELISA as used in this study has been reported to be 76 - 90%.

Conclusion

Out of 400 suspected cases, 96 samples were IgM Lepto ELISA positive at 100 dilution of serum, thus showing the prevalence of Leptospirosis to be 24% (95%CI 19.7-28.2) in the study population and increase in number of cases during the month of November, December, May and June. A rise in number of cases between 41-50 years (26%) and 51-60 years (33.3%) was observed, indicating a higher probability of occupational exposure in these age groups. It shows male preponderance of infection than females with 77% and 23% respectively correlating with their engagement in outdoor activities. The study also shows 64.5% acute kidney injury, 14.9% ARDS, 7.46% myocarditis, 5.9% multi organ failure and 7.46% death as complications of leptospirosis. It is also observed that 34.78% *L. icterohaemorrhagiae*, 15.21% *L. hebdomadis*, 13% *L. autamnalis*, 8.6% *L. australis*, 6.5% *L. grippotyphosa*, 6.5% *L. pomona*, 6.5% *L. tarassovi*, 4.34% *L. canicola* and 4.34% *L. bataviaserovars* were causing leptospirosis by MAT. It is found that there is no association between complications and serotypes. But serovars like *L. grippotyphosa, L. australis, L. tarassovi* and *L. bataviae* did not cause death in the present study.

Correlation of IgM ELISA at 100 dilutions of serum with MAT and IgM ELISA at 200 dilutions of serum with MAT showed specificity and positive predictive value, and accuracy of test can be improved by performing ELISA at 200 dilutions of sera.

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