

Original Research Article

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Seroepidemiological Study of Leptospirosis among Clinically Suspected Cases

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ABSTRACT

Keywords

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Leptospirosis is zoonosis, presenting as a febrile illness with protean manifestations. Since disease is amenable to antibiotic therapy, early diagnosis helps in better management and prevention of complications. In many parts of our country, prevalence is either underestimated or unestimated. Hence this study was conducted with the objective of determining the seroprevalence of leptospirosis among clinically suspected cases, describe the various sociodemographic parameters, occupation and risk factors among seropositive cases. This is a descriptive study, done at the department of microbiology, Hassan institute of medical sciences, Hassan, Karnataka, India, by including cases 253 cases over 2 year period based on the clinical criteria for diagnosis of leptospirosis as described in the NCDC (National Centre for Disease Control). The serum samples of these cases were tested by Ig M ELISA for leptospirosis. Overall seroprevalence among clinically suspected cases was 19.37%. Higher prevalence was noticed among males (22.29%). Commonest age group affected was 31-40 (29.63%) followed by 21-30 (21.43%). All the cases were febrile at presentation with constitutional symptoms like myalgia (83.67%), headache (79.59%) and conjunctival suffusion (53.06%). Among the seropositive cases, 8.16% cases presented with heart failure, 10.20 % with renal failure and 6.12% with hepatorenal syndrome. Seroprevalence found to be highest among sewage workers (30%), followed by construction workers (25.45%).

Introduction

Leptospirosis is a common spirochetal zoonosis worldwide that affects mammals, including human beings. Infection is endemic and occurs with greatest frequency in tropical and subtropical regions. It is emerging as an important public health problem in India (Vimala *et al.*, 2014; NCDC, 2015).

Leptospirosis is a diagnostic challenge because of its protean manifestations and elusive features. It is a disease amenable to antibiotic therapy and if left untreated, may prove fatal (WHO, ILS, 2002).

Incidence of leptospirosis ranges between 10-100/1,00,000 cases per year in developing countries. As per the estimates, India should report 0.1-1.0 million cases per year. However cases reported is considerably less, which are around 10,000 cases per year. Kerala, Tamilnadu, Maharashtra and Gujarat report majority of cases. Other states in India reporting Cases of leptospirosis are Andhra Pradesh, Assam, Goa, Delhi, Karnataka, Orissa, Pondicherry, Andaman and Uttar Pradesh (Shivakumar, 2008).

Definite diagnosis of leptospirosis is mainly by thorough laboratory investigations. Most cases of leptospirosis are diagnosed by serology. IgM-ELISA is useful for detecting leptospirosis in the first week of fever which is the genus specific test. Antibodies are detectable in the blood 5 to 7 days after the onset of symptoms (Sahira *et al.*, 2015).

Prevalence varies from region to region. Prevalence in our region is probably unestimated. Hence the present study was conducted with the objective to highlight the seroprevalence of leptospirosis in and around Hassan district, Karnataka, India, also to describe the clinical features with which they presented, probable risks factors associated, and demographic parameters contributing to this illness.

Materials and Methods

This is a descriptive study done over a period of 2 years at the department of microbiology, Hassan Institute of medical sciences, Hassan, Karnataka.

Inclusion criteria

Cases were suspected for leptospirosis based on criteria/case definition recommended by NCDC (NCDC 2015).

Exclusion criteria

All the cases whose details needed for the study were not available and cases with equivocal results were excluded.

A total of 253 patients clinically suspected to be suffering from leptospirosis were included in the study.

The socio demographic and clinical data such as age, sex, occupation, history of exposure to predisposing factors, epidemiological linkage

to confirmed cases, duration of illness and clinical features were collected from requisition forms, contacting physicians and patients.

Blood samples (5 ml) from 253 cases, were collected during the acute phase and the serum were separated. The serum samples were stored at -4⁰C in serum vial until further use. Detection of IgM antibodies to *Leptospira* species was determined using a commercially available *Leptospira* IgM ELISA (Panbio Ptv., Ltd., Queensland, Australia).

The assay was performed according to the manufacturer's instructions. Briefly, test sera, calibrator, and positive and negative control sera were diluted 1:100 in sample diluent, and 100 µL added to *Leptospira* antigen-coated microwells and incubated for 30 minutes at 37°C. After washing 6 times with phosphate-buffered saline containing 0.05% Tween 20, 100 µL of HRP conjugated anti-human IgM was added and incubated for another 30 minutes at 37°C.

After further washing, 100 µL of tetramethylbenzidine substrate was added and incubated at room temperature for 10 minutes, after which the reaction was stopped with 100 µL of 1 M phosphoric acid. The absorbance of each well was read at a wavelength of 450nm with reference wavelength of 630 nm.

Antibody levels >11 panbio units were reported positive by IgM ELSA. Samples with values between 9 to 11 panbio units considered equivocal and antibody levels < 9 pan bio units were reported negative. In case of any indeterminate/equivocal results, repeat blood sample was requested after one week and the test was repeated.

Statistical analysis of the data was done using percentage/proportion.

Results and Discussion

Seroprevalence

Seroprevalence among clinically suspected cases is represented in figure 1.

Gender distribution

Gender distribution of seropositive cases is shown in figure 2.

Age distribution of seropositive cases

Commonest age group affected was 31-40(29.63%) followed by 21-30(21.43%), 41-50(19.05%) (Table 1).

Risk factors reported among seropositive cases

Exposure to drainage (27.27%) was the commonest risk factor reported by the patient followed by exposure to rodents (26.58%) and exposure to stagnant water in the fields or after rainfall (24%) (Table 2).

Occupational data of seropositive cases

Sewage workers (30%) were the commonest victims to leptospirosis, followed by Construction workers (25.45%) which were the next common occupational group to be affected with leptospirosis (Table 3).

Clinical features at presentation

All the patients were febrile (100%) at the time of presentation; next common complaint was myalgia (83.67%), followed by head ache (79.59%) and conjunctival suffusion (53.06%) (Table 4).

Complication noticed in these patients were jaundice with deranged liver function (26.53%), renal failure (10.20%), heart failure (8.16%), hepatorenal syndrome (6.12%), hemorrhagic complications (4.08%).

Among the 253 cases included in the study, based on the NCDC case definition, 49 cases were serologically positive for leptospirosis by Ig M ELISA giving overall prevalence of 19.37%.

Table.1 Age distribution of seropositive cases

Age(years)	Suspected cases (N)	Seropositive cases (N)	Seropositive cases (%)
0-10	07	00	00
11-20	32	05	15.63
21-30	56	12	21.43
31-40	54	16	29.63
41-50	42	08	19.05
51-60	34	06	17.65
61-70	15	01	6.66
>71	13	01	7.69
Total	253	49	19.37

Table.2 Risk factors reported among seropositive cases

Risk factors(exposure)	Suspected cases (N)	Seropositive cases (N)	Seropositive cases (%)
Drainage	22	06	27.27
Stagnant water-fields/rainfall	75	18	24
Rodents	79	21	26.58
Domestic animals	30	1	3.33
Flood water, streams	22	2	9.09
No history	25	1	4
Total	253	49	19.37

Table.3 Occupational data of seropositive cases

Occupation	Suspected number of cases (N)	Seropositive cases (N)	Seropositive cases (%)
Agriculture	76	18	23.68
Construction work	55	14	25.45
Sewage work	20	06	30
Veterinarians	06	01	16.67
Hospital sanitary workers	13	02	15.38
Domestic officials	47	03	6.38
Students	36	05	13.89
Total	253	49	19.37

Table.4 Clinical features at presentation

Clinical features	Seropositive cases (out of 49 seropositive cases)	Percentage
Fever	49	100
Myalgia	41	83.67
Head ache	39	79.59
Conjunctival suffusion	26	53.06
Nausea, Vomiting	17	34.69
Pain abdomen	15	30.61
Jaundice	13	26.53
Meningism	03	6.12
Renal failure	05	10.20
Heart failure	04	8.16
Hepatorenal failure	03	6.12
Hemorrhagic	02	4.08

Fig.1 Seroprevalence among clinically suspected cases

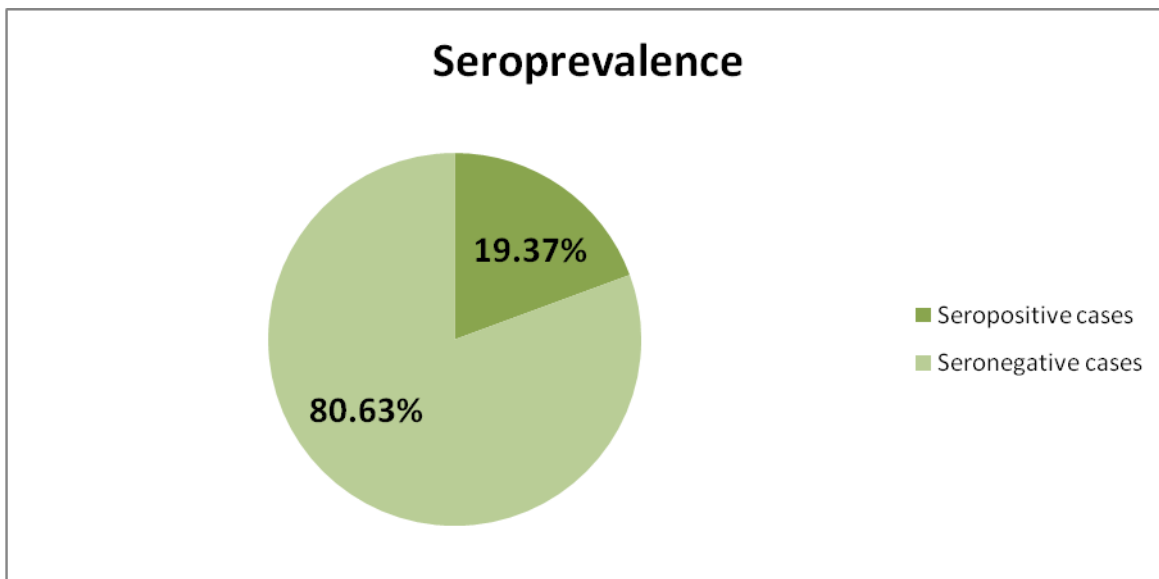
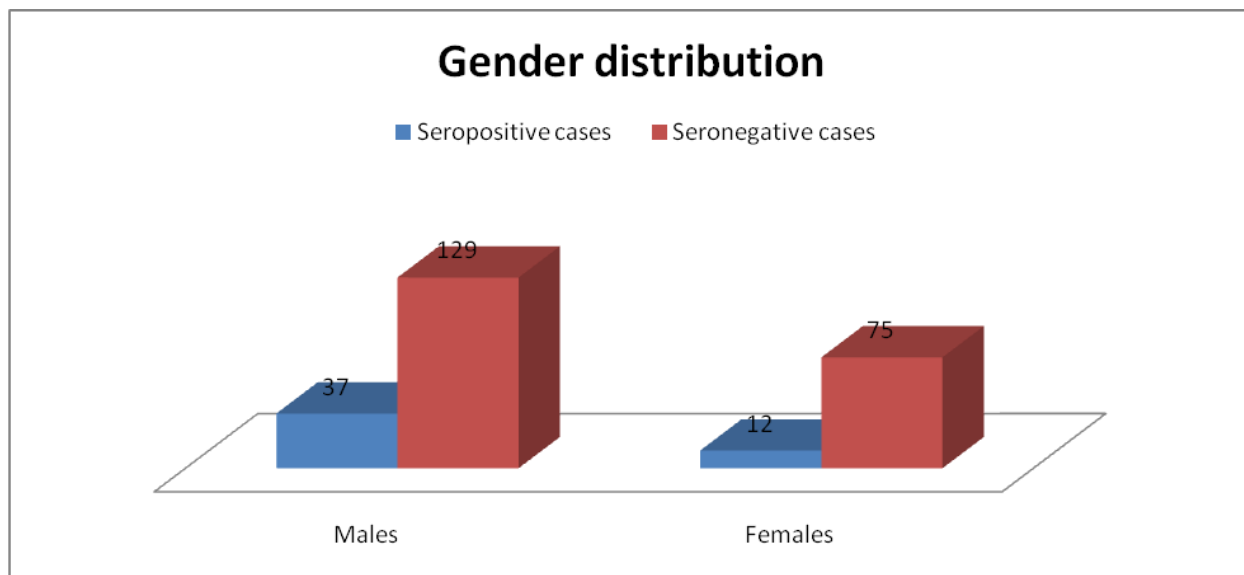


Fig.2 Gender distribution of seropositive cases



As per the national reference centre, ICMR seropositivity in different parts of south India ranged between 0- 46.8% (Smita *et al.*, 2010). In a recent study by Aravind *et al.*, seropositivity for leptospirosis (by Ig M ELISA) was 43.47 % (50/115 cases) (Aravind *et al.*, 2015). Another study reports seroprevalence to be 26.90 % (Chaudhry *et*

al., 2013). Another study from east delhi region, reports seroprevalence by similar method to be 15% (Kaur *et al.*, 2003).Hence prevalence varies from region to region.

Seropositivity was higher among males (22.29%) in comparison to females (13.79%) in the present study. This is probably because

males are mostly engaging in outdoor works and more likely to be having been exposed to the sources of infections. Almost all studies report seropositivity with male preponderance (Kaur *et al.*, 2003; Chaudhry *et al.*, 2013; Aravind *et al.*, 2015).

Commonest age group affected was 31-40 (16/54, 29.63%) followed by 21-30 (12/56, 21.43%), 41-50 (8/42, 19.05%). Probably because these age groups constitutes the majority of outdoor working population. Many studies report higher prevalence of leptospirosis in age group between 20-40 (Sahira *et al.*, 2015; Aravind *et al.*, 2015; Sunil *et al.*, 2010 and Jena *et al.*, 2004. Since this disease affects the productive age group, this has lot of economic burden both on the family and society (Souza *et al.*, 2011). Hence proper care, early recognition and treatment can prevent grave consequences faced by the individual and also the family.

Exposure to drainage (27.27%) was the commonest risk factor reported by the patient followed by exposure to rodents (26.58%) and exposure to stagnant water in the fields/after rainfall (24%). Similar risk factors have been documented by a number of studies. Studies by Kamath *et al.*, and Phraisuwan *et al.*, reports exposure to drainage water and contaminated soil and water by animals as the commonest risk factor among cases compared to the controls. (Kamath *et al.*, 2014 and Phraisuwan *et al.*, 2002).

Sewage workers (6/20, 30%) were the commonest occupational group with higher seropositivity for leptospirosis followed by construction workers (14/55, 25.45%), agriculturists (18/76, 23.68%) and veterinarians (1/6, 16.67%). Many studies documented similar results. This may be because people in these occupational groups will have more chance of coming in contact with contaminated soil and water

contaminated with animal urine (Kamath *et al.*, 2014; Samsuddin *et al.*, 2015 and Nazri *et al.*, 2012). It's proven that sewage work had significant association (p value 0.021) with seropositivity in a study involving municipal workers (Samsuddin *et al.*, 2015).

All the cases were febrile at presentation with varied constitutional symptoms. Commonest constitutional symptom was myalgia (41/49, 83.67%) followed by headache (39/49, 79.59%), conjunctival suffusion was found in 26/49 cases (53.06%). These findings correlate with similar other studies, where the constitutional symptoms at presentation were headache, myalgia, and vomiting (Vimala *et al.*, 2014).

In this study, jaundice was noticed in 26.53% seropositive cases (13/49), 8.16% of the seropositive cases presented with heart failure (4/49), 10.20 % with renal failure (5/49) and 6.12% cases with hepatorenal syndrome (3/52).

In a study (Arvind *et al.*, 2003), reported hepatic complications (74%) to be common in their study followed by renal (46%) and multiorgan involvement (36%). Another study (Muthusethupathi *et al.*, 1995) reports hepatic complications to be in 84% of seropositive cases followed by renal complications which were seen in 71.92% of cases.

All these studies report quite higher rates of complications than our study probably because these studies include a larger sample size. It can also be because of thorough evaluation of all the patients with renal, hepatic, respiratory and cardiac profile was not done in our study. Also one more reason may be all our patients have presented to the hospital within 7 days of fever as compared to a study by (Arvind *et al.*, 2003) where a larger group of patients have presented after 6 days of illness. Duration of fever in other studies

(Sahira *et al.*, 2015; Muthusethupathi *et al.*, 1995) are not available to comment on variation in the results.

Though this study reports the seroprevalence to be 19.37%, this can be an underestimate. This is because it's a hospital based study where, only those who have reported to laboratory alone have been evaluated. One more reason may be all the equivocal cases were excluded in order to decrease the false positive result in this study.

Main limitation of the present study is that, confirmation of the seropositive cases was not done using microscopic agglutination test, which is considered to be the gold standard. Confirmation with paired sera was also not done. Community based studies with better study designs, studying for serovars, comparing more than one serological tests in parallel which may increase the sensitivity would give better data regarding the current problem statement from this region.

Thus to conclude seroprevalence of leptospirosis according to this hospital based study is 19.37%. A higher degree of suspicion, evaluation for the risk factors like occupation or history of exposure to contaminated water or soil, helps in early diagnosis of such cases and preventing life threatening complications. Knowledge about the prevalence may help clinician to decide to start antibiotic early based on the clinical features and risks associated. This study may also help further more studies from this region to describe the trend in incidence of leptospirosis over a period of time.

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