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Original Article

Microbiology Section

Seroprevalence and Clinicoepidemiological Profile of Leptospirosis in Acute Febrile Illness Cases at a Medical College in Amritsar, Punjab, India

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ABSTRACT

Introduction: Leptospirosis is an anthropozoonotic disease; it occurs worldwide but is most common in tropical and subtropical countries. It is usually underreported due to a lack of awareness, atypical presentations and lack of diagnostic facilities, especially in a resource-limited setting like India. Knowing the local prevalence of such infections is of utmost importance for appropriate control and management.

Aim: To estimate the seroprevalence of leptospirosis among suspected Acute Febrile Illness (AFI) cases and to assess the clinicoepidemiological profile of leptospirosis cases presented at Government Medical College, Amritsar, Punjab, India.

Materials and Methods: This hospital-based cross-sectional study was conducted at Government Medical College, Amritsar, Punjab, India from 1st January 2020 to 30th June 2021. A total of 360 clinically suspected AFI cases were enrolled in the study. The demographic and clinical profile of the cases were taken on a semi-structured predesigned proforma. Blood sample was collected from the patients, and an Immunoglobulin M (IgM) Enzyme-linked Immunosorbent Assay (ELISA) was done for diagnosis. Data analysis was done using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) statistical package for windows.

Results: Out of 360 AFI cases suspected of leptospirosis, 62 (17.2%) were positive for leptospirosis IgM ELISA. The majority of AFI cases and leptospirosis cases belonged to the 21-40 years age group followed by the 41-60 years age group. Seroprevalence was found to be higher in males (20.8%) as compared to females (12.7%). Maximum seroprevalence (20.1%) was seen during the monsoon season followed by the autumn, summer and winter season. The association between risk exposure to risk factors and leptospirosis seroprevalence was found to be statistically significant (p-value=0.034). Fever, myalgia, headache, nausea/vomiting, icterus and hepatomegaly were common clinical features observed in leptospirosis cases.

Conclusion: Leptospirosis has rapidly emerged to become the major cause of AFI in many parts of India. High seroprevalence of leptospirosis was reported among undifferentiated AFI cases in the present study. A programmatic approach towards the prevention, control and management of these emerging diseases in Punjab is highly recommended.

Keywords: Anthropozoonotic infection, Epidemiology, *Leptospira*

INTRODUCTION

Acute Febrile Illness (AFI) with non specific signs and symptoms is one of the most common clinical presentations to healthcare services in developing countries [1]. AFI can be caused by diverse pathogens like bacteria, viruses, parasites and fungi. The aetiological agents causing AFI vary according to geographical location [2]. Leptospirosis is an emerging anthropozoonotic infection caused by the pathogenic *Leptospira* spp. [3]. Leptospirosis was first described as a disease by Adolf Weil in 1886 [4]. Later in the 20th century, the pathogen *Leptospira* was demonstrated independently by Inada and Ido in Japan and Uhlenhuth and Fromme in Germany [5].

Leptospirosis is worldwide in distribution, however, it occurs more commonly in the tropics and subtropics, which are areas with heavy rainfall [6]. The whole region of Southeast Asia is an endemic area for leptospirosis. According to the World Health Organisation (WHO) reports the incidences range from approximately 0.1-1 per 100,000 per year in temperate climates to 10-100 per 100,000 in the humid tropics [7]. In India, leptospirosis cases are being reported since 1931. Leptospirosis is becoming an emerging public health issue of significant proportion in the country [8]. Different studies from various parts of the country have reported varying seroprevalence rates ranging from 6.4-30.9% [9,10]. The estimated annual morbidity in India is 19.7 cases per 100,000 populations [11]. Here, the disease is endemic in the southern and western states [12].

Rodents and domestic animals such as cattle, pigs and dogs, serve as major reservoir hosts. Humans are accidental hosts which are infected by direct or indirect exposure to the urine of carrier animals. Common epidemiological risk factors favouring disease spread are residing in proximity to reservoir animals; high temperature, rainfall, water logging, poor sanitation and outdoor occupations [13]. The clinical spectrum of the disease range from subclinical infections to severe fatal complications and Weil's syndrome. Clinical presentations include fever, headache, myalgia, conjunctival suffusion, rash, hepatosplenomegaly, haemorrhagic manifestations, renal failure, icterus, aseptic meningitis, Acute Respiratory Distress Syndrome (ARDS), and pulmonary haemorrhage [3]. Leptospirosis has been underreported and underdiagnosed in the Punjab region as there is a lack of awareness of the disease, insufficient epidemiological data, and a lack of competent diagnostic facilities. There is a scarcity of research work on leptospirosis in this region [10]. Hence, the present study aimed to estimate the seroprevalence of leptospirosis among suspected AFI cases and to assess the clinicoepidemiological profile of leptospirosis cases presented at Government Medical College, Amritsar, Punjab, India.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Amritsar, Punjab, India, from 1st January 2020 to 30th June 2021. The study

was approved by the Institutional Ethics Committee (IEC) (Ethics approval number: 14363/D-26/2019, dated 21st June, 2021) and informed written consent was obtained from all study participants.

Patients of any age group with an acute undiagnosed febrile illness (body temperature >38.2°C) presented to the outpatient or inpatient Department of Medicine, Paediatrics and Obstetrics and Gynaecology Department of Government Medical College, Amritsar during the study duration constituted the study population.

Inclusion criteria: A suspected case of leptospirosis (according to case definition) [14]. Patients giving consent for participation in the study were included in the study.

Exclusion criteria: Cases known to have confirmed alternate diagnoses. Patients not willing to participate in the study were excluded from the study.

Operational Case Definition

Suspected case: AFI with headache, myalgia and prostration associated with the history of exposure to an infected animal or an environment contaminated with animal urine with one or more of the following signs/symptoms: calf muscle tenderness, conjunctival suffusion, anuria or oliguria, jaundice, haemorrhagic manifestations, meningeal irritation, nausea, vomiting, abdominal pain and diarrhoea [14].

Sample size calculation: The sample size was calculated using Daniel's formula [15], $N=Z^2P(1-P)/d^2$, Where N is the sample size, Z is the statistic corresponding to the level of confidence (1.96 for the level of confidence interval of 95%), P is expected prevalence, and d is precision. Taking the prevalence of leptospirosis from a previous study [16] as 14.1% and precision as 5%, the minimum sample size required comes out to be 185. However, 360 AFI cases reported during the study duration and fulfilling the inclusion criteria were included in the study.

Data collection: Patients fulfilling the inclusion criteria was assessed for socio-demographic profile, exposure to epidemiological risk factors and clinical history using a predesigned semi-structured proforma. Clinical features and appropriate investigations were also recorded on the proforma. Multiple responses were allowed was applicable.

Specimen collection, processing and interpretation: Approximately 5 mL of blood was taken in a plain vacutainer from each subject observing strict aseptic universal precautions. Blood was allowed to clot and serum was separated. The samples were tested for the detection of IgM antibodies for leptospirosis using the PANBIO *Leptospira* IgM ELISA kit. Test was carried out as per manufacturer's instructions and result interpretation was as follows: Panbio units <9=negative, 9-11 units=equivocal, and >11 units=positive. Equivocal samples were subjected to repeat testing after one week.

STATISTICAL ANALYSIS

Data were entered in Microsoft Excel sheets and statistical analysis was done using IBM SPSS statistical package for windows. Seroprevalence among different groups were calculated by dividing leptospirosis positives cases by suspected AFI cases. Normally distributed numerical data were presented as mean and Standard Deviation (SD). Categorical data were presented as percentages and the Pearson Chi-square test was used to test the level of significance. A p-value <0.05 was considered statistically significant.

RESULTS

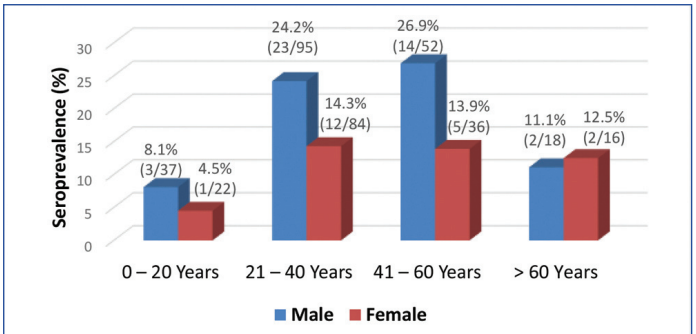
Out of 360 AFI cases suspected of leptospirosis, 62 (17.2%) were positive for leptospirosis IgM ELISA. Among 202 male and 158 female AFI cases, 42 males (20.8%) and 20 females (12.7%) were leptospirosis positive. The majority of AFI cases and leptospirosis cases belonged to the 21-40 years age group followed by the 41-60 years age group. The mean age of affected patients were 37.5±13.3 years. In the male patients, the highest seroprevalence (26.9%) was found in the 41-60 years age group. In females, the seroprevalence was found to be highest (14.3%) in the age group of 21 to 40 years. The association of seroprevalence with gender, age and occupation was found to be statistically significant (p-value <0.05) [Table/Fig-1-3].

Parameter	Suspected AFI Cases (%)	Leptospirosis (IgM ELISA) Positive (%)
Gender		
Male	202 (56.1)	42 (67.7)
Female	158 (43.9)	20 (32.3)
Age group (years)		
0-20	59 (16.4)	4 (6.5)
21-40	179 (49.7)	35 (56.5)
41-60	88 (24.4)	19 (30.5)
>60	34 (9.5)	4 (6.5)
Residence area		
Rural	195 (54.2)	33 (53.2)
Urban/Urban slums	165 (45.8)	29 (46.8)
Seasonal variation		
Summer (March to May)	76 (21.1)	12 (19.4)
Monsoon (June to September)	144 (40.0)	29 (46.8)
Autumn (October to November)	98 (27.2)	16 (25.8)
Winter (December to February)	42 (11.7)	5 (8.0)
Occupation		
Agriculture/Diary/Poultry	76 (21.1)	17 (27.5)
Service/Business/Self-employed	101 (28.1)	15 (24.2)
Construction Worker/Labourer	54 (15.0)	11 (17.7)
Sweeper/Garbage Cleaner/ Domestic servant	36 (10.0)	8 (12.9)
Student/Others#	93 (25.8)	11 (17.7)

[Table/Fig-1]: Demographic profile of AFI cases (N=360) and leptospirosis positive cases (N=62).
Others include unemployed, retired and housewives

Parameter	Seroprevalence (%)	p-value (Chi-square)
Gender		
Male	20.8	0.042
Female	12.7	
Age group		
20-60 years (54 positive out of 267)	20.2	0.010
<20 and >60 Years (8 positive out of 93)	8.6	
Residence area		
Rural	16.9	0.484
Urban/Urban slums	17.6	
Seasonal variation		
Summer (March to May)	15.8	0.602
Monsoon (June to September)	20.1	
Autumn (October to November)	16.3	
Winter (December to February)	11.9	
Occupation		
Agriculture, Diary, Poultry, Construction Worker, Labourer, Sweeper, Garbage Cleaner and Domestic Servant (36 positive out of 166)	21.7	0.037
Service/Business/Self-employed, Student and others# (26 positive out 194)	13.4	

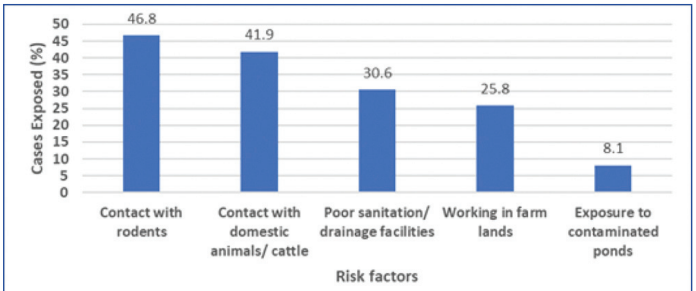
[Table/Fig-2]: Seroprevalence of leptospirosis (N=62).
Others include unemployed, retired and housewives; p-value <0.05 considered significant
Here authors have compared seroprevalence in the 20-60 years age group, which is more involved in outdoor activity with a combined age group of <20 years and >60 years. Chi-square came out to significant. If we compare four age groups then the Chi-square p-value will not be statistically significant. Also, positives in <20 and >60 years age groups are very less in number. Applying test of significance on such small numbers will not be much valid



[Table/Fig-3]: Gender wise seroprevalence of leptospirosis among different age groups (N=62).

The occupation of the majority (27.4%) of cases was related to agriculture, dairy or poultry work. The next predominant occupational group was service, business or self-employed (24.2%). Seroprevalence of leptospirosis among rural and urban residents was 16.9% and 17.6% respectively. Maximum seroprevalence (20.1%) was seen during the monsoon season followed by the autumn, summer and winter season. However, the association of seroprevalence with the area of residence and season was not found to be statistically significant (p-value >0.05) [Table/Fig-1,2].

Major epidemiological risk factor reported was contact with rodents (46.8%) followed by contact with domestic animals/cattle, poor sanitation/drainage facilities and working in farmlands [Table/Fig-4]. Association between risk exposure and seroprevalence was found to be statistically significant (p-value=0.034). Odds ratio calculation suggests that Leptospirosis positive cases are 1.81 times more likely to be exposed to risk factors than negative cases (OR=1.81) [Table/Fig-5].



[Table/Fig-4]: Epidemiological risk factors exposure in leptospirosis cases. (N=62, Multiple responses were allowed).

Exposure to risk factor	Leptospirosis positive	Leptospirosis negative	Total
Exposed	37	134	171
Not exposed	25	164	189
Total	62	298	360
Chi-square=4.4538 p-value=0.034			
Odds ratio=1.81			

[Table/Fig-5]: Association between leptospirosis and exposure to risk factors. p-value <0.05 considered significant

Fever was reported in all leptospirosis cases. Other common symptoms reported were myalgia (69.4%), headache (62.9%), nausea and vomiting (32.3%) and abdominal pain (27.4%) [Table/Fig-6]. Most common physical finding was icterus (45.2%) followed by hepatomegaly (40.3%), pallor (33.9%) and splenomegaly (32.3%) [Table/Fig-7].

Raised Serum Glutamic Oxaloacetic Transaminase (SGOT) (72.6%), raised Serum Glutamic Pyruvic Transaminase (SGPT) (54.8%), hyperbilirubinemia (51.6%) and leucocytosis (56.5%) were the most predominant altered laboratory parameters [Table/Fig-8]. Liver dysfunction was the major complication (38.7%) in leptospirosis cases followed by renal dysfunction (33.9%), respiratory distress/ pneumonitis (8.1%), neurological complications (6.4%), and multiorgan failure (4.8%).

Symptoms	Frequency	Percentage
Fever	62	100.0
Myalgia	43	69.4
Headache	39	62.9
Nausea/Vomiting	20	32.3
Abdominal pain	17	27.4
Decreased urine output	15	24.2
Arthralgia	11	17.7
Respiratory symptoms	6	9.7
Neurological symptoms	5	8.1

[Table/Fig-6]: Symptoms reported by leptospirosis positive cases. (N=62, Multiple responses were allowed).

Signs	Frequency	Percentage
Icterus	28	45.2
Hepatomegaly	25	40.3
Pallor	21	33.9
Splenomegaly	20	32.3
Oedema	11	17.7
Conjunctival suffusion	8	12.9
Lymphadenopathy	6	9.7
Hypotension/Shock	4	6.5

[Table/Fig-7]: Signs observed in leptospirosis positive cases. (N=62, Multiple responses were allowed).

Laboratory parameter	Frequency	Percentage
Haematological investigation		
Haemoglobin (<10 g/dL)	24	38.7
TLC (>11000/µL)	35	56.5
Platelet Count (<100000/µL)	19	30.6
Biochemical investigation		
S. Creatinine (>1.5 mg/dL)	23	37.1
Blood urea (>40 mg/dL)	25	40.3
S. Bilirubin (Total) (>2 mg/dL)	32	51.6
SGOT (>80 IU/mL)	45	72.6
SGPT (>80 IU/mL)	34	54.8

[Table/Fig-8]: Altered laboratory parameters in leptospirosis positive cases (N=62). TLC: Total leukocyte count; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase

DISCUSSION

There is limited information regarding the seroprevalence of leptospirosis in the Punjab region. The present study was thus undertaken to estimate the seroprevalence of leptospirosis in cases of undifferentiated AFI. The seroprevalence of leptospirosis has been found to be 17.2% in the present study. Almost similar seroprevalence was reported in studies from Chennai (17.8%) [6] and Andhra Pradesh (18%) [17]. However, Mansoor T et al., reported low seroprevalence (6.4%) in a study from the Kashmir valley [9]. This may be attributed to the climate conditions of the region.

About two-thirds of leptospirosis cases in the current study were males. Also, the seroprevalence rate among males (20.8%) was higher than females (12.7%) and was found to be statistically significant (p-value=0.042). Ahmad N et al., reported that males constituted 66.7% of total cases [16]. The male majority was also observed by Kumari P et al., (60%), Moinuddin SK and Nazeer HA (70.6%) and Banukumar S (60.4%) [6,17,18]. Male preponderance can be attributed to increased risk exposure due to outdoor activities and occupation.

The age range of the leptospirosis positive cases in the current study was 14 to 65 years with a mean age of 37.5±13.3 years. The seroprevalence of the 20-60 years age group was significantly higher

than other age groups (p-value=0.010). The majority of the affected patients were young active population involved in outdoor work and agricultural activities and hence an increased chance of exposure to infection. These findings were in concordance with other studies [6,9] as this age group is more exposed to risk factors. In a study conducted by Kumari P et al., the mean age of the patients affected with leptospirosis was 36.4 years which correlates with the present study [6].

Agriculture, dairy and poultry were major occupational groups among leptospirosis cases. More than 50% of the patients affected by leptospirosis were outdoor manual workers. In a study from a coastal town in south India, fishing (33.9%) was a major occupation followed by agriculture (19.3%) [17]. Studies by Ahmad N et al., (50%) and Srinath M et al., (32%) have shown agriculture as a predominant occupation [16,19]. This establishes the fact that persons who are more exposed to contaminated environments are at a higher risk of contracting the disease [20].

The seasonal variation was observed in this study. During the monsoon season, a higher seroprevalence rate was observed of leptospirosis, which was consistent with the previous studies [3,21]. The high incidence of infection during these months concludes that the rain and dampness promote the spread of infection and favours the survival of *Leptospira* in soil. Exposure to various risk factors were elicited from study participants. The major epidemiological risk factors observed in present study were contact with rodents (46.8%) and contact with domestic animals/cattle (41.9%). Almost similar risk factors were reported by Thalva C and Desamani KK but the major being poor drainage facilities [13].

Leptospirosis mimics many other diseases in its atypical presenting symptoms and clinical features. In the present study, fever (100%) was the universal symptom observed in all the cases followed by myalgia (69.4%), headache (62.9%), nausea/vomiting (32.3%) and abdominal pain (27.4%). A similar pattern was observed by Sethi S et al., in their study, but fever, myalgia and oliguria were reported as the most common signs and symptoms. But as mentioned earlier, the clinical spectrum of disease is variable based on the serotype infected and the age profile and immunological status of the individual [22]. Chauhan V et al., in their study in sub Himalayan regions reported jaundice, splenomegaly and breathlessness as the major features in their study [23]. Predominant clinical signs seen in the present study were icterus (45.2%), hepatomegaly (40.3%), pallor (33.9%) and splenomegaly (32.3%). However, Prakash K in his study reported pallor and icterus in 96% of cases [24]. These varying presentations may be due to the change in the serovar pattern causing the disease in a particular locality.

In comparison with laboratory parameters, in present study raised SGOT (72.6%), Raised TLC (56.5%), Raised SGPT (54.8%), and hyperbilirubinemia (51.6%), were the predominant altered parameters. Similar laboratory parameters profile in leptospirosis cases were reported by Holla R et al., [25], Sethi S et al., [22] and Thalva C and Desmani KK [13]. However, raised blood urea and anaemia were major altered laboratory parameters in a study by Agrawal SK et al., [3]. Atypical manifestations like neurological, cardiac and pulmonary leptospirosis are usually overlooked while establishing the clinical diagnosis. This leads to a delay in the initiation of appropriate therapy resulting in increased morbidity and mortality. Therefore, laboratory investigations of clinically suspected cases must be done to confirm the diagnosis and to start early treatment effectively.

Limitation(s)

A large proportion of leptospirosis infections are subclinical with mild symptoms. These cases are usually not reported in healthcare facilities. Thus, the present study would underestimate the community prevalence of leptospirosis. The gold standard test (Microscopic Agglutination Test) and molecular methods (Polymerase Chain Reaction) for the diagnosis of leptospirosis were not performed in the present study due to resource constraints. IgM ELISA is a genus specific test which cannot detect the specific serovars of *Leptospira*. However, serological tests such as ELISA are effective and useful for estimating seroprevalence, especially in resource-limited settings.

CONCLUSION(S)

Leptospirosis has rapidly emerged to become the major cause of AFI in many parts of India. Non specific and overlapping clinical features of leptospirosis and other AFIs make their clinical diagnosis challenging. In resource-limited settings, serological test like ELISA is valuable for estimating seroprevalence and establishing the diagnosis. Due to under-reporting, misdiagnosis and scarcity of research, leptospirosis is not considered as a disease of public health concern in Punjab. But high seroprevalence of leptospirosis was reported among undifferentiated AFI cases in the present study. A programmatic approach towards the prevention, control and management of these emerging diseases in Punjab is highly recommended.

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