



## Original Research Article

# Clinical profile of leptospirosis and role of various diagnostic methods, a hospital based prospective observational study

K Prakash<sup>1,\*</sup><sup>1</sup>Dept. of Microbiology, Government Medical College (old RIMS), Prakasham, Andhra Pradesh, India

## ARTICLE INFO

## Article history:

Received 14-12-2019

Accepted 28-03-2020

Available online 20-07-2020

## Keywords:

Leptospirosis

Darkground microscopy

Culture

IgM ELISA

Polymerase chain reaction

## ABSTRACT

**Objectives:** Leptospirosis is a potentially life-threatening zoonotic disease of worldwide distribution. Accurate diagnosis and prompt treatment are essential to minimize morbidity and mortality. The current study was conducted to analyse the clinical profile of leptospirosis and the diagnostic yield of various diagnostic methods.

**Materials and Methods:** The present study was a cross-sectional study. A total of 60 patients who were suspected of leptospirosis were enrolled in the study. Direct examination of blood was done using dark-ground microscopy, the culture was done by inoculation of the blood sample into the EMJH medium, antibodies against *Leptospira* was demonstrated using Panbio IgM ELISA kit, and antigen products were demonstrated using polymerase reaction (PCR) with primers G1 and G2.

**Results:** The study population included 63% of males and 37% females. A majority of 38% of the study subjects were farmers. Pallor and icterus are the predominant clinical signs found among 96% of the study population. IgM ELISA has labelled the highest number i.e. 55 (91.66%) of subjects as positive. The number of subjects diagnosed positive by PCR, culture and Dark ground microscopy (DGM) were 33 (55.00%), 22 (36.66%) and 12 (20%) subjects respectively.

**Conclusion:** Leptospirosis proves to be an important health concern. Prominent clinical conditions were observed. IgM-ELISA proved superior to Dark ground microscopy (DGM), Culture and Polymerase chain reaction (PCR) and suitable for early diagnosis of leptospirosis.

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## 1. Introduction

Leptospirosis is one of the most widely prevalent zoonotic diseases globally. It is caused by spirochetes of the genus *Leptospira*.<sup>1</sup> The disease is acquired through contact of abraded skin with the water or soil which is contaminated with infected urine. Hence humans are accidental hosts. Once in the soil, the bacteria can survive for prolonged periods if the soil is damp.<sup>2</sup>

In India, outbreaks of leptospirosis occur during monsoon seasons due to flooding. In South-India, the peak incidence of leptospirosis occur between June and October.<sup>3</sup> Bacteraemia heralds the onset of clinical illness. Manifestations may range from subclinical infection to as

severe as multi-organ dysfunction, that is associated with a high case fatality rate. In advanced stages, liver failure, pulmonary haemorrhage, acute kidney injury and bleeding manifestations may occur. Hence a high index of suspicion and timely diagnosis is vital in preventing serious morbidity and mortality.

Despite common occurrence and the possibility of serious adverse consequences, the diagnosis is often missed by clinicians. This is due to varied manifestations and the majority of cases presenting as undifferentiated febrile illnesses.<sup>4</sup> It is often misdiagnosed as influenza, fever of unknown origin or aseptic meningitis.<sup>5</sup> Also, the misconception that it is predominantly a rural disease, contributes to delayed diagnosis. Along with a high index of suspicion, choosing an appropriate laboratory test is of paramount importance.

\* Corresponding author.

E-mail address: [drprakashk218@gmail.com](mailto:drprakashk218@gmail.com) (K. Prakash).

Lab diagnosis includes methods such as microscopy, culture, serology and molecular diagnostic tests. Dark field microscopy can visualize leptospirosis, but it requires a minimum of 104 organisms/mL to be visible in microscopy.<sup>6</sup> IgM ELISA is widely used, but it can give false-positive results.<sup>7</sup> PCR can detect leptospira DNA in the serum and urine samples of patients. But it requires a large amount of DNA in the sample to give a positive result.<sup>8</sup>

The current study was conducted to analyse the clinical profile of leptospirosis and the diagnostic yield of various diagnostic methods.

## 2. Materials and Methods

The present study was a cross-sectional study, conducted at Govt. Medical College (old RIMS), Ongole in the department of Microbiology. The study was conducted between January 2017 to December 2018. A total of 60 patients who were suspected of leptospirosis were enrolled in the study. Those who did not full fill the inclusion criteria were subjected for exclusion. The study was approved by the intuitional human ethics committee. Informed written consent was obtained in the local language from all study participants. The confidentiality of the study participants was maintained throughout the study. The study has thus been conducted in compliance with the ethical standards required by the 1964 Declaration of Helsinki and its subsequent amendments.

Direct examination of blood was done using dark-ground microscopy, the culture was done by inoculation of the blood sample into the EMJH medium, antibodies against *Leptospira* was demonstrated using Panbio IgM ELISA kit, and antigen products were demonstrated using polymerase reaction (PCR) with primers G1 and G2.

## 3. Results

The study population included a total of 60 subjects with 63% males and 37% females. Among the study population, 11 individuals (18%) belonged to the paediatric age group of fewer than 18 years, and the remaining 42 individuals (82%) were adults. The age distribution of the study subjects based on gender is given in Table 1.

**Table 1:** Age and sex distribution of subjects in the study (N=60)

Age in year	Male	Female
0-10 yrs	3(5%)	0(0%)
10-20 yrs	5(8%)	3(5%)
20-30 yrs	7(12%)	2(3%)
30-40 yrs	8(13%)	2(3%)
40-50 yrs	10(17%)	12(20%)
>50 yrs	5(8%)	3(5%)
Total	38(63%)	22(37%)

**Table 2:** Distribution of subjects based on occupation

Parameter	Number	Percentage
<b>Occupation (N=60)</b>		
Farmer	23	38%
Poultry worker	10	17%
Animal rearing	10	17%
Sewer	5	8%
Gardner	5	8%
Sedentary	5	8%
Veterinary	2	4%
<b>History of contact with the animal (N=43)</b>		
Dog	20	47%
Cattle	10	23%
Rodents	6	14%
Cat	5	12%
Hen	2	4%
<b>Source of water (N=28)</b>		
Public source	10	36%
Canal	6	21%
Pond	5	18%
Seage	5	18%
River	2	7%

A majority of 38% of the study subjects were farmers. There were 17% each working as poultry workers or in animal rearing. Among the remaining study subjects, there 8% of the subjects each reported working in sewers, gardening, and sedentary jobs. Only 4% were involved in the veterinary occupation. With regards to contact with infected animals, almost 47% of the study population had a history of contact with dogs. A little less than one-fourth, 23% had a history of contact with cattle. Only 14% had a history of contact with rodents. Among the rest, history of contact with cats and hens were present among 12% and 4% of the study population respectively.

With regards to water being the source of infection, 36% of the study population had a history of contact with a public source of water. History of contact with ponds or sewage was present among 18% of each of the study population. Almost one-fifth (21%) of the study population had a history of contact with canal water. Only 7% had a history of contact with river water (Table 2).

**Table 3:** Clinical signs among the study population. (N=60)

Clinical signs	Frequency (%)
Pallor	58(96%)
Icterus	58(96%)
Hepatomegaly	40(67%)
Hypochondrium tenderness	30(50%)
Splenomegaly	20(33%)
Lymphadenopathy	10(17%)
Edema	2(3%)
Purpura	5(8%)
Meningeal signs	2(3%)

With regards to clinical signs, fever, conjunctival congestion and nausea were present in all the study subjects. The other most common clinical symptoms were pallor and icterus seen in 58 (96%) of subjects. Hepatomegaly was present in 67% and splenomegaly was present in 33% of cases. Meningeal signs were present in 3% of subjects (Table 3).

**Table 4:** Comparison of positive results of dark-ground microscopy, blood culture, IgM ELISA and PCR. (N=60)

Test	No of positives	Percentage
IgM ELISA	55	91.66%
PCR	33	55.00%
Culture	22	36.66%
Dark ground microscopy (DGM)	12	20.0%

IgM ELISA has labelled the highest number i.e. 55 (91.66%) of subjects as positive. The number of subjects diagnosed positive by PCR, culture and Dark ground microscopy (DGM) were 33 (55.00%), 22 (36.66%) and 12 (20%) subjects respectively.

**Table 5:** Comparison of dark-ground microscopy (DGM), blood culture, IgM ELISA with PCR

Parameters	PCR		Total
	Positive (n=33)	Negative (n=28)	
<b>DGM</b>			
Positive	12 (37.5%)	0 (0%)	12
Negative	20 (62.5%)	28 (100%)	48
<b>Blood Culture</b>			
Positive	22 (68.7%)	0 (0%)	22
Negative	10 (31.2%)	28 (100%)	38
<b>IgM ELISA</b>			
Positive	30 (93.7%)	25 (89.2%)	55
Negative	2 (6.25%)	3 (10.7%)	5

Among the 33 PCR positive *Leptospira* cases, 30 (93.7%) were identified by IgM Elisa as positive. Blood culture and Diagnostic microscopy (DGM) had diagnosed 22 (68.7%) and 12 (37.5%) respectively. Among the 28 cases diagnosed as negative by PCR all of them were labelled as negative by DGM and Blood culture, but only 3 (10.7%) were labelled as negative by IgM ELISA.

#### 4. Discussion

Leptospirosis is a worldwide public health problem. The magnitude of the problem in tropical and subtropical regions can be largely attributed to climatic and environmental conditions. Despite this knowledge, the information about the existing status of the disease in the country is lacking and we do not have an accurate estimate of disease burden in the country. Probably the disease is under reported in humans. All available evidences suggest that the Leptospirosis is now

emerging in India as important public health problem.<sup>9–11</sup>

The source of infection among the study population is either in direct contact with animals and poultry or direct contact such as farmers. Among the occupational group, farmers were majorly affected. A study by Patil VC et al.<sup>12</sup> had of total 23 patients among which 18 (78.26%) were farmers. This is concurrence with the review by Levett PN et al.,<sup>13</sup> where the authors mention that farmers, veterinarians and abattoir workers are at risk for infection with leptospirosis through direct contact. Indirect modes of transmission can occur in sewage workers or canal workers. Also, similar to the present study, other studies,<sup>14,15</sup> have reported outbreaks of leptospirosis after recreational exposure to water such as swimming, exposure to public water sources, ponds, and canals.

With regards to the clinical features of the patients, 96% had icterus in the present study, and all had a fever and conjunctival suffusion. This proportion of icterus in concurrence with the study by Edwards CN et al.,<sup>16</sup> where 95% had jaundice. But a lesser proportion of the study population had a fever (76%) and conjunctival suffusion (54%) when compared to the present study. In a study by Holla R,<sup>17</sup> majority of the patients presented with fever (92.1%). In a prospective study, the most common organs involved were liver (27, 71.05%).<sup>18</sup> In a study done by Ibrahim SK et al.<sup>19</sup> Hepatomegaly was found in 88% of the total population. The extent of respiratory involvement is different among various studies on leptospirosis. In the current study around 8% of the study, the population had respiratory symptoms. In the study by Yersin C et al.,<sup>20</sup> 12% of the study population had hemoptysis, and pulmonary infiltrates on chest X-ray which is higher compared to the present study.

The present study revealed that IgM ELISA showed the highest number of positive subjects. Similarly in a study done by Niloofa R et al.<sup>21</sup> IgM-ELISA positivity was 45.8% which was greater than MAT and Leptocheck-WB. Khan F et al.<sup>22</sup> in their results found that thirty-one (14.9%) patients were found positive for specific anti-leptospira IgM antibodies by ELISA.

#### 5. Conclusion

Leptospirosis remains a significant public health issue that mainly affects the population of the productive age group. Current study results depict the role of occupation and development of leptospirosis. Also, the source of water had a significant part to play. Febrile and hepatic conditions were most common. As this disease is of endemic nature leading to a fatal outcome, it should raise a high index of suspicion among the medical practitioners when they come across a person suffering with fever and jaundice. A well planned multicentric study done at different geographical locations should be carried out to bring out better insight to the epidemiology of leptospirosis.

## 6. Acknowledgements

We acknowledge the technical support in data entry, analysis and manuscript editing by “Evidencian Research Associates.”

## 7. Source of Funding

Self-funded.

## 8. Conflict of Interest

None.

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## Author biography

**K Prakash** Associate Professor

**Cite this article:** Prakash K. Clinical profile of leptospirosis and role of various diagnostic methods, a hospital based prospective observational study. *Indian J Microbiol Res* 2020;7(2):195-198.