



## A Serological study on Leptospirosis during post flood epidemic in Kerala

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### Abstract

*Leptospirosis is an emerging and reemerging bacterial zoonosis caused by Spirochaetes belonging to the genus Leptospira. Various serological tests are available for the confirmatory diagnosis of Leptospirosis in the laboratory. IgM ELISA is commonly done in the first week of fever in most of the laboratories. PCR has been a major advance but it is available only in a few centres in Kerala. We had an epidemic in the first week of September 2018 following the floods. A total number of 373 blood samples collected from suspected cases of Leptospirosis were subjected to serological tests in the 24 hour Serology laboratory at Government Medical college Hospital, Ernakulam. IgM ELISA for Leptospira was found to be positive in 27 cases (7.2%). Blood samples and urine samples were sent to NIV Alappuzha for PCR testing. Among the 111 serum and urine samples tested, 9 serum samples and urine sample were positive for Leptospira by PCR. Mortality rate was less than 1 % in suspected cases and 11.11% in PCR positive cases of Leptospirosis.*

**Keywords:** *Leptospirosis, ELISA, PCR.*

### Introduction

Leptospirosis is an acute febrile illness, the severity of which varies from mild to rapidly fatal. Prompt and timely diagnosis is essential since antibiotic prophylaxis and therapy provides greatest benefits and significantly reduces the mortality rate when administered early in the course of the disease. Climatic and environmental factors were responsible for epidemics seen in Kerala in the recent years.

Nowadays various diagnostic tests like ELISA, MAT, IHA are available. Besides these diagnostic approaches, PCR provides accurate molecular based detection of Leptospira during the first few

days of acute febrile illness before the development of anti -Leptospira antibodies.

### Materials and Methods

**Study Design:** Cross sectional study

**Study Setting:** Department of Microbiology and Medicine, GMC, Ernakulam

**Study Period:** Post flood epidemic phase (September 2018)

**Study Population:** Suspected cases of Leptospirosis

**Study Group (Age group):** a1 year to 70 years

### Collection of Samples

From adult patients attending Medicine OPD with acute febrile illness, 5 ml of blood was collected

from anterior cubital vein by venipuncture under sterile precautions in vacutainers (plain) for serological tests. Another 5 ml of blood was collected in EDTA vacutainers for PCR testing. Adult patients were advised to collect mid stream urine samples also in plastic screw capped wide mouthed sterile containers (SV30). Approximately 10 ml of sample was collected.

From pediatric cases 2 samples, 2 ml of blood each were collected under aseptic technique. One sample for serological tests (in plain vacutainer) and another one for PCR testing (collected in EDTA vacutainer). Urine sample (3-5ml) obtained from a few cases.

The samples were sent to the 24 hour Serology lab under department of Microbiology immediately after collection. The sample collected in the plain vacutainer was centrifuged and the serum was separated and IgM ELISA for *Leptospira* was done with the serum sample. The sample collected in the EDTA vacutainer was sent to NIV Alappuzha after perfect packing and labelling in an ice box.

**Results**

A total of 373 blood samples were collected from clinically suspected cases of Leptospirosis from Ernakulam district during the immediate post flood period in September 2018. All the samples were subjected to IgM ELISA. Out of the 373 samples tested, 27 samples were positive (7.2%). During the initial stage, 111 samples were sent to National Institute of Virology (Kerala unit) at Alappuzha for PCR testing. (9 samples were positive (8.1%)). Among the IgM ELISA positive cases, 23 (85%) were males and 4 patients (15%) were females. Age wise distribution points out that 13 cases (48%) were in the age group between 20 and 40

Analysis of samples collected from suspected cases of Leptospirosis

Total No. of cases	IgM ELISA Positive		IgM ELISA Negative	
	No.	%	No.	%
373	27	7.2	346	92.8

**Analysis of samples subjected to PCR**

Total No. of samples	PCR Positive		PCR Negative	
	No.	%	No.	%
111	9	8.1	102	91.9

**Age wise distribution of suspected cases**

Age group	No.	%
0-10	51	13.7
11-20	55	14.7
21-30	85	22.8
31-40	56	15
41-50	53	14.2
51-60	38	10.18
61-70	23	6.16
71-80	11	2.9
81-90	1	0.2
Total	373	100

**Gender distribution –PCR samples**

Male		Female		Total
No.	%	No.	%	
82	73.8	29	26.13	111

**Analysis of IgM ELISA positive cases**

**Age wise distribution**

Age group	No.	%
0-10	2	7.4
11-20	3	11.1
21-30	5	18.5
31-40	8	29.6
41-50	3	11.1
51-60	4	14.8
61-70	2	7.4
Total	27	

**Gender Distribution**

Male		Female		Total
No.	%	No.	%	
23	85.1	4	14.8	27

**IgM ELISA vs PCR**

IgM ELISA positive Total No.	PCR positive Total No.	ELISA positive PCR negative	ELISA Negative PCR positive	ELISA positive PCR positive
27	9	18	4	5

IgM ELISA was positive (85.1%) among males. IgM ELISA as well as PCR was positive in 5 cases (4.5%).

**Discussion**

In our study, the preponderance of male patients in the age group between 20 and 40 denotes that

Leptospirosis is common among men who are the working population exposed to the organism. Since males are more involved in outdoor activities they are at higher risk than females of acquiring the infection. Most of the studies have shown male preponderance. In contrast, a study from North India in sub Himalayan region by Chauhan et al in 2010 reported female preponderance of 77%. In their study, out of the 13 confirmed cases of Leptospirosis, 10 were females (76.92%) and 3 were males (24%).

All the clinically suspected cases were having fever and myalgia (100%).80% of cases had headache and only 7% of the cases had jaundice. Same finding was observed in the previous studies conducted at Vellore, Puducherry and Calicut, Kerala and in North India.

Rainfall and exposure were defined as an important correlation in patients diagnosed with Leptospirosis. Following heavy rainfall, there will be dissemination of the disease in the urban zone.

The laboratory diagnosis of Leptospirosis is based on serological tests such as MAT (Macroscopic agglutination Test) and ELISA for detection of IgM antibodies.MAT is still the cornerstone of Leptospirosis diagnosis in spite of its disadvantages. IgM ELISA has been employed as a useful alternative. It is a reliable test and when used in combination with MAT gives good results in diagnosis. PCR is an important tool for early diagnosis of Leptospirosis cases. PCR positivity peaked from the 4<sup>th</sup> day to 8<sup>th</sup> day after onset of symptoms. In early phase of the disease, the sensitivity of PCR is 71% as reported by Summaiya et al from Gujarat in 2016.The detection rate by IgM ELISA was 59% in their study. In the present study, the sensitivity rate of PCR was 80% and IgM ELISA was 72%.Fonseca et al showed that sensitivity increases to 96.5% in IgM ELISA and 93.1% in MAT along with PCR. Currently molecular based detection methods have been adopted by most of the reference laboratories because their sensitivity is more than other diagnostic tests available and they can detect very

low levels of Leptospire in blood in acute phase of illness.

Leptospirosis has been reported to be endemic in several parts of South India including the state of Kerala with a seropositivity rate of 25.6%.The seroprevalence rate during the post flood season was found to be 7.2%.It may be due to the mixing up of other causes of acute febrile illness with clinically suspected cases of Leptospirosis. A seroprevalence rate of >55% was observed among the general population of North Andamans.

The mortality rate during post flood epidemic of Leptospirosis in Ernakulam district in Kerala was less than 1%.In 2009, during an outbreak of Leptospirosis in Philippines after floods the mortality rate reported was 10.8%.It was much higher than the mortality rate reported in Kerala. The significant reduction in mortality rate is due to the involvement of the most effective health care systems. Prevention and control of the infectious disease Leptospirosis was achieved by the awareness programmes, prophylactic antibiotic administration at the primary health centre level and strict adherence to the protocol and guidelines implemented by health care providers of Health services from Government of Kerala.

### Conclusion

Leptospirosis is emerging as an important public health problem in India. Both humans and animals may be directly infected through contact with infected tissue or urine of rats or indirectly through contact with contaminated soil and water. It is an occupational hazard of agricultural workers, sewage workers, animal handlers, forestry workers and men involved in outdoor activities who work in wet conditions, Epidemics associated with high case fatality rate >15% occur after heavy rainfall. The public health importance lies in its occupational, seasonal, sex and age related incidence. The climatic conditions in tropical and sub-tropical regions provide optimal environment to support the survival of Leptospira

and infect human beings and it is a potential hazard to the community.

Leptospirosis has been under-reported in most parts of India due to non-availability of laboratory diagnostic facilities. It is a preventable disease. Timely diagnosis and early antibiotic therapy helps to reduce the mortality rate significantly which was evident in the last post flood epidemic in Kerala.

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