

Seroprevalence Trends of Leptospirosis at a Tertiary Care Centre of North India from 2016-2019 Determined by Two Commercial Elisa Kits

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Abstract

Leptospirosis is a worldwide zoonotic disease which remains under diagnosed in most of the regions of our country due to the lack of proper diagnostic facility and set up. Diagnosis usually depends on Microscopy, culture and serological tests like ELISA, MAT (Microscopic agglutination test). MAT is the gold standard diagnostic test but it requires live *leptospire*s and it can only be done in a reference laboratory. Hence, ELISA is the most preferred cost-effective serological method with both sensitivity and specificity of 95%. The Aim of our present study was to evaluate the trends of seroprevalence of leptospirosis from January 2016 to December 2019 and to compare two ELISA kits for the serodiagnosis of Leptospirosis. The study was conducted between January 2016 to December 2019 and 1611 blood samples were collected from clinically suspected cases of leptospirosis. Detection of IgM antibodies to leptospira was done by DRG and Recombilisa (CTK) ELISA kits. Results by both the kits were analysed. Seventy samples were taken as controls from different patients on random basis to prevent any type of biasing. Samples were tested for other co-infections and Demographic parameters were studied. Seroprevalence was estimated to be 4.8% by DRG KIT and 5.5% by Recombilisa CTK kit. Average seroprevalence was noted to be 5.1%. The diagnosis of presumptive and possible Leptospirosis was made in 96(78%) and 27(22%) respectively, by modified Faine's criteria. The seroprevalence between 2016 to 2019 varied from 0.57 – 1.8 %. The months of July to November of year 2017 noted the highest prevalence. Out of these patients, majority (96%) showed significant improvement during the follow-up period after 2-3 months with empirical treatment. Typhoid was seen as most common co-infection in these patients.

Keywords: Leptospira, ELISA, Microscopic agglutination test (MAT), seroprevalence, serological methods

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(Received: February 26, 2020; accepted: May 09, 2020)

Citation: Patel SS, Singh S, Sahu C, Verma H. Seroprevalence Trends of Leptospirosis at a Tertiary Care Centre of North India from 2016-2019 Determined by Two Commercial Elisa Kits. *J Pure Appl Microbiol.* 2020;14(2):1263-1268. doi: 10.22207/JPAM.14.2.21

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INTRODUCTION

Leptospirosis is a worldwide zoonotic disease caused by a pathogenic spirochaete belonging to the genus, *Leptospira*¹. Rats are the most common reservoirs of infection and are involved in transmission, either directly or indirectly by contaminating water and soil with their urine. The clinical manifestations of Leptospirosis range from mild catarrh-like illness to icteric disease with severe liver and kidney involvement^{2,3}. Organism enters the host through skin abrasions, mucosal surfaces or the eye. Incubation period ranges from 3 to 30 days; usually 10- 12 days.

The disease remains underdiagnosed in most of the regions of India due to the lack of proper diagnostic facility and set up⁴. Diagnosis usually depends on Microscopy, culture and serological tests like ELISA, MAT (Microscopic agglutination test). Microscopic agglutination test (MAT) is the gold standard test and is serogroup specific. However, it requires live *leptospire*s and also complex in performance, so this test always requires a reference laboratory. IgM ELISA is a good alternative serological test and is genus specific⁵. It allows the detection of antibody in the 1st week of illness with sensitivity ranging from 68% to 100% and specificity up to 97%. ELISA is the most preferred cost-effective serological method with both sensitivity and specificity of 95%⁵.

The aim of our present study was to evaluate the trends of seroprevalence of leptospirosis from January 2016 to December 2019 and to compare the results of two commercial ELISA kits.

MATERIALS AND METHODS

Clinical samples

The study was conducted at the department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow between January 2016 to December 2019. The study was approved by the Institute's ethical committee. A total of 1611 blood samples were collected from clinically suspected cases of leptospirosis attending either outpatient departments or were admitted in the Gastroenterology, Neurology, Paediatrics, surgery or Gynaecology wards, etc. and were analysed retrospectively.

Diagnosis of presumptive and possible Leptospirosis was done by modified FAINE'S Criteria^{6,7}. Inclusion criteria was all Patients who were suspected clinically of leptospirosis and presented with a history of fever for >7 days accompanied with any of the following features i.e., severe headache, myalgia, arthralgia, conjunctival suffusion, uveitis, rash, hepatosplenomegaly, hemorrhagic manifestations, renal failure, aseptic meningitis, Acute respiratory distress syndrome⁷.

Serological testing protocol

Five ml of venous blood was collected in a plain vial without anticoagulant and serum was separated after centrifugation at 3000 rpm for 5 minutes. This serum was used for further testing. Detection of IgM antibodies to leptospira was simultaneously done by DRG ELISA kit (Inc. USA) and Recombilisa ELISA kit (CTK Biotech, Inc. California). They are qualitative enzyme immunoassays for the detection of antibodies to *Leptospira biflexa* in samples of human serum or plasma. Seventy samples were taken as controls from different patients randomly and were tested by both the ELISA kits.

Interpretation of ELISA Results

DRG Kit

Non-reactive = 0.0 -0.15 OD units;

Reactive > 1.0 OD.

Sample OD between 0.15-1.00 = No interpretation as per the Kit. These samples were taken as Equivocal in our study.

Recombilisa CTK Kit

Cut-off value = 0.244+Negative control OD.

Specimen OD Ratio = Specimen OD/Cut-off value.

Negative < 1.00 specimen OD ratio

Positive \geq 1.00 specimen OD ratio

Testing for other co-infections

Samples were tested for other infections in the following manner:

Malaria by SD Bioline rapid test kit for *P. falciparum* and *P. vivax*

Typhoid by Widaltest for TO, TH, AH, BH titres

Dengue by Panbio ELISA kit

Chikungunya by SD and NIV ELISA kit.

Scrub Typhus by InBios ELISA kit for IgM detection.

Statistical Analysis

Data was analyzed using the SPSS (Statistical Package for Social Sciences) software for descriptive statistics, and the results were expressed in proportions, mean, and standard deviation. Categorical data were described using numbers and percentages. Statistical significance was calculated by *P* value.

RESULTS

A total of 1611 patients were enrolled in this study of over 4 years time period. All the serum samples were simultaneously tested by both the ELISA kits for comparison.

Out Of 1611 samples , 77 were positive for anti-leptospira IgM antibodies with OD values > 1.00; whereas 46 samples tested as Equivocal with OD values between 0.540 - 8.50 by DRG ELISA kit. Rest of the samples were negative with OD values below 0.15. Seroprevalence was calculated as 4.8% by DRG kit.

Out of 1611 samples, 89 tested positive with specimen OD ratio >1.00. Rest of the samples were found to be negative with specimen OD ratio <1.00. Seroprevalence was calculated as 5.5 % by CTK RECOMBILISA kit.(Fig. 1)

All the 70 control samples were tested as negative by both the ELISA kits with OD values between 0.12-0.22. These samples were

Table 1. Distribution of Signs & Symptoms in the patients

Signs & Symptoms	Patients (%)
Fever	100
Jaundice	89
Myalgia	88
Headache	76
Chills & rigors	52
Arthritis & Arthralgia	42
Hepatomegaly	36
Oliguria	34
Abdominal pain	33
Splenomegaly	25
Cough	23
Conjunctival suffusion	19
Renal involvement	14

also tested for other infections like Malaria, Chikungunya, Typhoid, Scrub typhus and Dengue. Among these; 11 samples tested positive for Typhoid, 7 for malaria, 4 for dengue, 2 for chikengunya and only 1 for scrub typhus.(Fig.2).

The diagnosis of presumptive and possible Leptospirosis was made in 96(78%) and 27(22%) respectively, by modified Faine’s criteria.

The seroprevalence between 2016 to 2019 varied from 0.57 – 1.8 %. The months of July to November of year 2017 noted the highest prevalence; confirming seasonal prevalence of

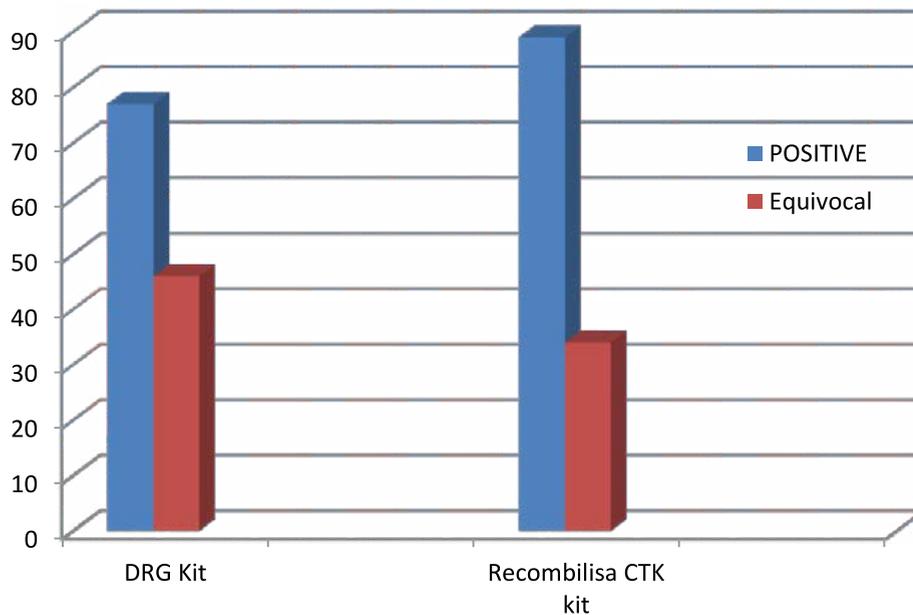


Fig. 1. Comparison of DRG and Recombilisa CTK ELISA kits.

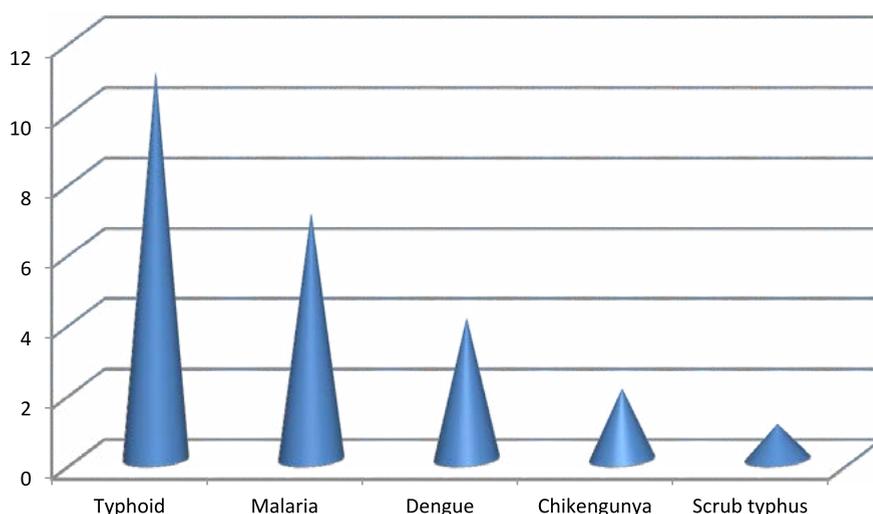


Fig. 2. Co-infections observed in patients of Leptospirosis.

leptospirosis in these rainy seasons. Among the 123 positive samples, 95 (77%) were males and 28 (23%) females; with male: female ratio of 3.39:1. Age of the patients ranged from 8 to 82 years. The most common presenting features were fever, myalgia and jaundice and was seen in almost 88% of the patients (Table 1).

Follow-up of the Patients

Out of these 84 patients, 79 were given empirical treatment for leptospirosis and showed significant improvement during the follow-up period after 2-3 months. Out of the remaining 5 cases, 2 required dialysis; whereas, 3 suffered mortality due to renal failure which was attributable to other comorbid conditions in these patients.

DISCUSSION

The present study evaluated the seroprevalence of leptospirosis along with the associated epidemiological risk factors and compared two ELISA kits for serodiagnosis of leptospirosis.

Seroprevalence was estimated to be 5.1% in our study. The seropositivity observed in our hospital was much lower as compared to other similar studies which was 18-38%^{8,9}. However, our study seroprevalence rates correlated well with a similar study done at AIIMS, New Delhi with a seroprevalence rate of 6.4%¹⁰. This decrease in seroprevalence compared to other studies may be attributed to several factors such as increase

in awareness, improved hygienic practices, use of safe drinking water, and better socioeconomic condition. The DRG kit appeared to produce false negative results with twelve sera. Four were from confirmed cases of leptospirosis with no further samples included in this study. Three were from convalescent samples taken five months after onset, an earlier sample was positive by CTK KIT. These twelve sera were positive by CTK recombilisa kit. The occasional false negative results seen with DRG kit indicates that paired serum samples must be examined from suspected clinical cases before a diagnosis of leptospirosis is made. The sensitivity and specificity of the kits as mentioned in their literature was: DRG KIT 90%/94% and CTK Recombilisa kit 94%/97% respectively.

Seasonal prevalence of leptospirosis in the rainy seasons was also noted in our study as the peak of seropositivity was observed between months of July to November in 2017. Rainy season leads to flooding and infestation with rodents, rats leading to transmission and perpetuation of the rodent-human infection cycle^{12, 13}. Males (77%) showed higher positivity as compared to females (23%) in our study owing to the more outdoor activities by men as compared to women. This observation correlates well with other similar studies^{11,12}.

The most common presenting feature was fever with myalgia and headache. This finding was consistent with other similar studies from India¹⁴.

However, one such study also reported headache with fever as the most common symptom¹⁵. Out of the diagnosed patients, majority were treated for leptospirosis and showed significant improvement during the follow-up period. Typhoid was identified as the most common co-infection in these patients^{16,17}. The higher rates of typhoid in these patients may be due to the common symptoms like fever, jaundice, liver enlargement which may also be suspected in leptospirosis. These infections sharing the same presenting features in the patient pose a challenge to the clinicians and require a high index of suspicion on his part^{17,18}.

CONCLUSION

In our study we used ELISA to study the seroprevalence of leptospirosis in our hospital. ELISA can prove to be a very useful tool for diagnosis in resource-limited settings. We also compared two different ELISA kits to further strengthen our study. Both the ELISA kits showed comparable seroprevalence of 5.1%. The present study gives a good idea on the seropositivity rate of Leptospirosis in this region of North India. Improvement in the socio-economic conditions, hygiene and sanitation has led to the decrease in seroprevalence rates of Leptospirosis. This neglected tropical disease must be dealt with high index of suspicion both in diagnosis and treatment as it may give rise to life-threatening complications.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SS performed literature search, data analysis, and first draft of the manuscript and figures. SSP and CS carried out the subsequent revisions of the manuscript, data analysis and final draft of the manuscript. HV contributed with laboratory work and investigations.

FUNDING

None.

ETHICS STATEMENT

Ethical clearance was taken from the institute's ethics committee.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Tables.

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