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Leptospirosis in central & eastern Uttar Pradesh, an underreported disease: A prospective cross-sectional study

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***Background & objective*: Leptospirosis is a zoonotic disease associated with potentially fatal consequences and a grossly underreported disease in Uttar Pradesh. However, only a few studies are available which report the prevalence of leptospirosis in this State. Hence, this study was undertaken to know the status of the disease in central and eastern Uttar Pradesh.**

***Methods*: A total of 143 serum and urine samples were collected from patients with acute febrile illness from July 2017 to March 2019. All the serum samples were tested for *Leptospira* by rapid IgM antibody card and IgM ELISA and urine samples were tested by real-time polymerase chain reaction (RT-PCR) to detect *Leptospira* DNA. All positive and 10 per cent negative sera from ELISA and RT-PCR (all rapid test positive were also ELISA positive) were sent to the ICMR-Regional Medical Research Centre, Port Blair for microscopic agglutination test (MAT).**

***Results*: Thirty eight (26.6%) out of 143 samples were positive for leptospirosis either by ELISA or RT-PCR. Positive results were eight (6%) by Rapid card, 32 (22%) by IgM ELISA, 10 (7%) by MAT, 10 (7%) by RT-PCR. In MAT, the most common serovar was Lai followed by Hebdomadis, Bangkinang and Pomona.**

***Interpretation & conclusions*: Leptospirosis was found to be one of the important causes for acute febrile illness in the central and eastern parts of Uttar Pradesh. The results of the present study suggest that it is necessary to increase diagnostic facility and awareness in clinicians for the screening of leptospirosis in acutely febrile patients to decrease morbidity and mortality associated with this disease.**

Key words IgM ELISA - leptospirosis - microscopic agglutination test - rapid test - real-time polymerase chain reaction - Uttar Pradesh

Leptospirosis is a worldwide zoonosis and has been documented in India since 1931[1]. In the recent years, its incidence has been widely reported from southern, central, eastern and western India due to heavy monsoon, animal rearing practices and unplanned urbanization[1,2]. Furthermore, leptospirosis has long been recognized as one of the leading causes of acute febrile illness in these parts of the country[3]. Central and eastern Uttar Pradesh (UP) encounters similar conditions and animal tending practices, but there are hardly any reports on the prevalence of this disease in this region[4-6]. Lack of awareness, clinical suspicion and active surveillance about the infliction of leptospirosis could be the probable reasons of

under-reporting. There is a wide spectrum of clinical presentations for leptospirosis. Most of the patients with *Leptospira* infection present only with mild fever or flu-like symptoms and recover without any complications and a small proportion develops various complications due to involvement of multiple organ systems. Clinical manifestations and complications can affect most organ systems, including the liver, kidneys, lungs, and central nervous system. Timely diagnosis and specific therapy can reduce the severity of illness and, in turn, mortality[7]. The accuracy of a clinical diagnosis of leptospirosis is poor because the clinical features are similar to those of a range of other common infectious diseases, which in the tropical setting includes rickettsial infection, dengue and malaria, typhoid and viral hepatitis[8]. The diagnosis is confirmed by laboratory tests, but only a few tertiary care hospitals have leptospirosis diagnostic facilities and no facility is present at primary and secondary care hospitals in UP, so leptospirosis is neglected and under-diagnosed in UP.

As leptospirosis is under-reported from all over India especially from central and eastern UP, this study was undertaken to estimate the prevalence of the disease in these regions. Furthermore, this study was also the first in this region for the detection of *Leptospira* by real-time polymerase chain reaction (RT-PCR) in urine samples.

Material & Methods

This prospective cross-sectional study was conducted in a tertiary care institute in central Uttar Pradesh over a period of two years from July 2017 to June 2019 after approval from the Institutional Ethics Committee. A written informed consent was taken from all the patients or their family members included in the present study.

A total of 143 patients attending in and outpatients of various departments of the institute were enrolled for the study.

Inclusion criteria: The inclusion criteria were the patients with acute febrile illness with any symptoms including headache and body aches associated with severe muscle tenderness, haemorrhages including sub-conjunctival haemorrhage, jaundice, cough, breathlessness and haemoptysis, oliguria or signs of meningeal irritation. Scoring was given to each presentation according to the modified Faine scoring including clinical and epidemiological parameters[9] as shown in Table I. Only those patients who had a score >15 with suspected leptospirosis were included in the study.

Table I. Scoring system using the modified Faine's criteria (with amendment) 2012 for the diagnosis of leptospirosis[9]

Study parameter	Score
Part A: Clinical	
Headache	2
Fever	2
Fever >39°C	2
Conjunctival suffusion	4
Meningism	4
Myalgia	4
Conjuctival suffusion + meningism + myalgia	10
Jaundice	1
Albuminuria/nitrogen retention	2
Haemoptysis/dyspnoea	2
Part B: Epidemiological	
Rainfall	5
Contact with contaminated environment	4
Animal contact	1
Part C: Bacteriological and laboratory findings	
Isolation of *Leptospira* spp. in culture-diagnosis certain	
PCR	25
Positive serology	
ELISA IgM positive[b]	15
SAT positive[b]	15
Other rapid tests[a,b]	15
MAT-single positive in high titer[b]	15
MAT-rising titer/seroconversion (paired sera)	25

Presumptive diagnosis of leptospirosis: Part A or Part A and Part B score: 26 or more; Part A, B, C (total): 25 or more; Possible diagnosis of leptospirosis: Score between 20 and 25. [a]Other rapid tests-Latex agglutination test/leptodipstick/ lepto tek lateral flow/lepto tek dri dot test; [b]Any one of the tests only should be scored. PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; SAT, slide agglutination test; MAT, microscopic agglutination test

Sample processing: Two blood samples of about 3-5 ml were collected one-week apart from all the participants[10], and universal precautions for sample collections with aseptic barriers were followed. Blood samples obtained by vein puncture were allowed to clot at room temperature (20 - 25°C) and then centrifuged. The serum was separated as soon as possible. Furthermore, 10 ml of urine sample was

collected in a sterile universal screw-capped urine container of the same study participants on day one. The separated sera and urine samples were stored at a temperature of −20°C. Serum samples were used for various serological tests and urine samples were used for molecular tests.

Serological testing for leptospirosis:

Rapid test for IgM antibodies to *leptospira*: Rapid test for detection of IgM antibodies for *Leptospira* was performed using a commercial kit (Leptocheck WB; Tulip Diagnostic Pvt. Ltd., India) according to the manufacturer's instructions.

ELISA for IgM antibodies to *leptospira*: ELISA tested serum samples for specific anti-Leptospira IgM antibodies for *Leptospira* was also detected using a commercial kit (PanBio Leptospira IgM; Alare, Australia) and interpreted according to the manufacturer's instructions.

Microscopic agglutination test (MAT): In-house testing facility for MAT, which is considered as a gold standard was not available. Hence, all samples which tested positive on either of the tests (ELISA and RT-PCR as all rapid card positive tests were also positive by ELISA) and 10 per cent negative samples from all tests as negative control were sent for MAT evaluation to Regional Medical Research Centre, Indian Council of Medical Research, Port Blair, India. The result of MAT was obtained with the panel of eleven serovars used. *Leptospira interrogans* serogroups Australis serovar australis, Autumnalis serovar bankinang, Canicola serovar canicola, Grippotyphosa serovar grippotyphosa strain CK31 and Moskva V, Hebdomadis serovar hebdomadis, Icterohaemorrhagiae serovar icterohaemorrhagiae, serovar lai like, Pomona serovar pomona, Pyrogenes serovar pyrogenes, Sejroe serovar hardjo.

Molecular testing for leptospirosis: Isolate of *Leptospira interogens* serogroup Pomona serovar pomona from ICAR-Indian veterinary Research Institute, Bareilly, were obtained for standardization of RT-PCR.

DNA Extraction and RT-PCR: DNA was extracted from urine using HiPurATM Urine Bacterial Genomic DNA Purification Kit (HiMedia Laboratories, Mumbai, India), as per manufacturer's instructions with minor modification of sample preparation (three times wash of urine sample at 12000 rpm for 10 min at room temperature with the same protocol). RT-PCR was performed using primers and fluorescent labelled probe (Eurofins Scientific, Bengaluru, India) as described previously[11].

The RT-PCR detection kit [GoTaq® Probe qPCR Master Mix (Promega biotech India Pvt. Ltd., Mumbai, India)] was used as per standard protocol with requisite modification in DNA quantity for better yields of the test. The final volume of the reagents in 20 µl reaction mixture was as, master mix 10 µl; primers 1 µl; hydrolysis probe 1 µl; template DNA 5 µl; nuclease-free water 2 µl. An initial denaturation at 95°C for 3 min, followed by 40 cycles of amplification was done. Each cycle consisted of denaturation at 95°C for 3 sec, annealing at 60°C for 30 sec, and extension at 60°C for 30 sec.

Statistical analysis: All the statistical analyses was performed using the SPSS for Windows, Version 20.0 (Statistical Package for the Social Science software, IBM Corp., Armonk, NY, USA) and Microsoft Office Excel 2010. The data analysis was summarized as mean±standard deviation presented as frequency and proportion. Categorical groups were associated using Chi-square test. Using MAT as the gold standard, the sensitivity, specificity, positive and negative predictive values was calculated for each sample by Chi square test. P <0.05 was considered statistically significant.

Results

Out of 143 suspected cases of leptospirosis, 38 (26.6%) were found to be positive for *Leptospira* spp. by any of the test (rapid card, ELISA or RT-PCR). In serology testing, only eight (6%) patients detected positive for leptospirosis by rapid IgM antibody card test and these eight samples were also positive by ELISA while, 32 (22.4%) participants tested positive using IgM ELISA. On molecular testing using RT-PCR in urine samples, 10 (7%) samples were found positive. By MAT only 10 (7 %) samples were positive. In this study, ELISA showed maximum positivity for leptospirosis (n=32) as compared to PCR (n=10) and rapid IgM card test (n=8). Although 90 per cent of positive cases by RT-PCR from urine samples, were also positive by ELISA, only 31.3 per cent of ELISA-positive cases showed RT-PCR positivity. Majority of PCR-positive cases (80 %) having fever of greater than or equal to seven days duration signify leptospiriuric phase of illness.

Table II. Comparison of serological and molecular tests with microscopic agglutination test (gold standard)

Test	TP	FP	95% CI				
			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
Rapid (n=8)	0	8	0.00	100.00	0	93.01	93.01
ELISA (n=32)	8	24	80.00	81.95	25.00	98.20	81.82
PCR (n=10)	2	8	20.00	93.98	20.00	93.98	88.81

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; TP, true positive; FP, false positive

ELISA showed 80 per cent sensitivity and PCR showed 20 per cent sensitivity with MAT as a gold standard as shown in Table II.

The serovars of *Leptospira interrogans* most commonly associated with leptospirosis were found to be Lai (80%), followed by Hebdomadis (20%) and Bangkinang (10%), and Pomona (10%) each as tested by MAT. More than one serovar were found in two patients with the same titers for both serovars.

In this study, out of all patients from 26 districts of east and central Uttar Pradesh, all positive patients were from 14 districts and maximum seropositivity (9; 23.7%) found in patients from Lucknow followed by four (10.5%) from Barabanki, three each (7.9%) from Bahraich, Ayodhya (Faizabad), Basti, Sultanpur and Gorakhpur, two each (5.3%) from Unnao, Sitapur, Raebareli and Gonda, one each (2.6%) from Santkabirnagar, Ambedkar nagar and Maharajganj, as shown in the Figure.

Clinical presentations in all the positive leptospirosis patients were fever associated with headache and muscle pain. Unexplained breathlessness, bleeding tendency including sub-conjunctival haemorrhage and albuminuria was present in 29 (76.3%), 23 (60.5%) and 20 (52.6%) of patients, respectively. Deranged liver function tests and the sign of meningeal irritation were present in 22 (57.9%) and 18 (47.4%) patients. Albuminuria, bleeding tendency including sub-conjunctival haemorrhage and deranged liver function tests were found to be statistically significant as shown in Table III.

Major epidemiological factors associated with occurrence (>80%) of leptospirosis were heavy rainfall, contaminated environment and animal contacts as shown in Table III. The subset analysis demonstrated that positive cases were common between July to October in both 2017 and 2018 (monsoon and immediately thereafter) and it was found to be significantly high in September in both years.

According to the demographic data of the participants, most of the patients (64%) were found to be in their 2nd, 3rd and 4th decades of life. The mean age of all the participants was 42 yr including 40 yr for males and 44 yr for females. Out of the 143 cases included in the study, leptospirosis was found positive in 66 per cent males and 34 per cent female patients (male:female ratio of 2:1).

The geographical distribution of the participants was 76 (53%) urban and 67 (47%) from the rural area. It was found that positive cases 23 (61%) were 1.5-fold more from urban dwellings as compared to rural areas 15 (39%).

Discussion

Leptospirosis is a zoonotic disease with a widespread distribution including in Southeast Asia. Its occurrence is reported from various regions of India with higher incidence in tropical and coastal regions. In clinical practices, testing for leptospirosis is not routinely done unlike other diseases presenting with similar complaints such as dengue, malaria, and typhoid. The reason may be a lack of awareness among the physicians about the prevalence of this disease. There are only a few studies reported from this region on the prevalence of leptospirosis[4-6].

In this study, an attempt has been made to evaluate the prevalence of leptospirosis in the region of eastern and central UP on patients who presented with acute febrile illness. This study revealed that the overall prevalence of leptospirosis was 26.6 per cent. This indicates that the increasing trends of leptospirosis in Uttar Pradesh as in a prospective study in the year 2004; a seroprevalence of seven per cent was reported in a study conducted in Lucknow[4]. A seroprevalence of 14 per cent was reported in patients with prolonged febrile illness in north UP in a study conducted at Aligarh (2018)[5]. Chaurasia *et al*[6] reported a sero-prevalence of 24 per cent in a study conducted at Lucknow. The ascendance in prevalence may be due to an increased incidence of waterlogging in this area, which enhanced

Figure. Distribution of positive patients in central and eastern Uttar Pradesh. Outline of map depicting districts from census 2011 adapted with permission from *http//www.indiagrowing.com*

direct and indirect contact with animals (mice, rabbits, rodents, *etc.*). Indirect contact with contaminated water during recreational exposure and deteriorating environmental conditions also contribute to facilitating the spread of serovars.

In the present study, ELISA was found to be the most sensitive test, easy to perform with minimum infrastructure so this test should be recommended in all hospital settings. MAT is gold standard but has some limitations that require two samples and less sensitive in the early phase of the disease, as microscopic agglutinating antibodies usually appear in the blood at a detectable level during the end of the first week or early second week of the infection. It is labour intensive and complicated procedure also as there is a need to maintain *Leptospira* strain for preparing live antigen[7].

In this study, we found *Leptospira interrogans* serovar Lai as the most common serovar followed by Hebdomadis, Bangkinang, and Pomona in MAT positive cases. These results were different from serovar prevalent in south India[12]. When antibodies to multiple serovars were detected in the same patient, the serovar with the higher antibody titre was considered as the infecting serovar[13]. Multiple serovars in the same patients have also been reported from previous studies[14]. In this study also, two samples had two serovars with the same titres (Table III).

Vaccines currently in use confer serovar-specific immunity. Presumptive information on local circulating serovars should guide the formulation of effective vaccines[12]. There is not a single study in Uttar Pradesh for determining the prevalent serovars in the State, so our study is a first to provide knowledge of the prevalent serovar in the State for the formulation of a better vaccine for the same.

In this study, maximum seropositivity was found in adult males. These findings correlate with the study published by Sethi *et al*[15], who observed that majority of the patients of leptospirosis were males in the age group 26-40. The occurrence of leptospirosis more commonly in adult males in the present study (age group 30-40) may be because of their occupational

Table III. Epidemiological and clinical characteristics of patients

History and symptoms	Total cases (n=143), n (%)	Positive (n=38), n (%)	χ^2	*P*
Epidemiological parameters				
H/O rainfall	128 (90)	32 (84.2)	0.875	0.350
H/O contact with contaminated environment	123 (86)	34 (89.5)	0.198	0.657
H/O animal contact	119 (83)	30 (78.9)	0.323	0.570
Clinical parameters				
Headache	143 (100)	38 (100)	0.002	1.000
Fever	143 (100)	38 (100)	0.002	1.000
Muscle pain	143 (100)	38 (100)	0.002	1.000
Cough with haemoptysis	39 (27)	3 (7.9)	0.003	0.832
Unexplained breathlessness	107 (75)	29 (76.3)	0.001	0.977
Bleeding tendency including sub-conjunctival haemorrhage	54 (38)	23 (60.5)	10.130	0.002**
Deranged liver function tests	47 (33)	22 (57.9)	4.078	0.044*
Sign of meningeal irritation	61 (43)	18 (47.4)	0.244	0.621
Albuminuria	100 (70)	20 (52.6)	7.365	0.007**

*P**<0.05, ***<0.01

exposure (animal handling, agriculture, fisheries) also and due to exposure during outdoor and recreational activities.

In this study, seasonal variation of leptospirosis infection was also found. Maximum positivity was found during the months of September followed by October *i.e*. monsoon and immediate post-monsoon periods which was most probably due to waterlogging and stagnant infected water with an increased chance of contact during this period. These findings correlate with the study published by Pawar *et al*[16].

It has also been reported in various studies that rural areas have relatively more positive cases for leptospirosis as compared to the urban, however; the findings of our study suggested otherwise. This may be due to more patients from the urban setting included in the study. The higher positivity in general in the urban population may also be multifactorial such as increased awareness and health consciousness leading to early reporting to speciality clinics. Rapid urbanization, contact with contaminated water, consumption of street food and beverages facilitate easy contact with serovars. There are case reports on the spread of leptospirosis from cold drinks and beverage cans contaminated with rat's urine[17]. Urban construction, which is spreading with rampant pace, provides easy shelter for rodents that are carriers of *Leptospira*. An urban concentration in slums and hutments along the culverts/pool waters bodies used both by animals and human is the most possible cause of higher infection in this population. Moreover, lesser positivity in rural areas may also be attributed to the non-reporting of patients to hospitals, lack of awareness, paucity of speciality clinics and laboratory testing facilities.

In this study, non-specific flu-like symptoms such as fever, headache and myalgia were observed in almost all patients. Bleeding tendency, the sign of meningeal irritation, unexplained breathlessness jaundice and albuminuria were more commonly associated with positive patients. Association of leptospirosis with bleeding tendency including sub-conjunctival haemorrhage, Jaundice and albuminuria was observed to be statistically significant (P <0.5).

In this study, epidemiological factors associated with positive cases included rainfall, contact with contaminated environment as well as animals. All of which are in favour of the transmission of leptospires.

The study was not without some limitations, the main one being small sample size. Also this was hospital based study, so significant samples from each district of eastern and central UP could not be included.

Overall, leptospirosis was found to be an important and common cause of acute febrile illness in the central and eastern part of UP. It is therefore necessary to add screening of leptospirosis as a differential diagnosis in acutely febrile patients along with other routine testing to decrease morbidity and mortality associated

with this disease. Diagnostic facilities for leptospirosis must be available at the primary or secondary health centres of rural and urban areas of districts of central and eastern UP.

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