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# TO STUDY THE SEROPREVALENCE OF LEPTOSPIRA INFECTION AT A TERTIARY CARE HOSPITAL

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**ABSTRACT**

**Background**

Leptospirosis, caused by spirochete bacteria *Leptospira* is an emerging zoonotic disease which commonly occurs through abraded skin and mucosal membranes due to contact with urine of infected animals. *Leptospira* have non-specific symptoms similar to those of dengue, typhoid, malaria and influenza and show co-infection with similar diseases. There are fewer cases of *Leptospira* infection in North India, possibly due to lack of knowledge and limited resource. Our study aimed to investigate the prevalence of *Leptospira* and its co-infections in Haryana region.

**Methods**

It is an observational study over 6 months from May 2023 to October 2023 in a tertiary care hospital in Haryana, India. During a six-months period, 259 blood samples from febrile patients were tested for *Leptospira* IgM antibodies with the Eiger *Leptospira* IgM ELISA kit for fever diagnosis.

**Results**

Out of total 259 specimens, 78 were tested positive for Leptospirosis. The seroprevalence was found 30% (78/259). There were more males affected by leptospirosis than females. 65% (51/78) were males and 35% (27/78) were females. The most affected age group was 20-30 years (29.7%). It was observed that LFT and KFT parameters are elevated, specially SGOT, SGPT, and Creatnine. Increased levels of SGOT, SGPT and decreased levels of Creatinine with average values of 106.91, 102.40, and 0.77 respectively. Co-infections was observed with Dengue in 10 (3.86%) of cases and 15 (5.79%) of co-infection with Typhoid.

**Conclusion**

In our study we found that some severe cases of leptospirosis were associated with co-infections with dengue, typhoid, and other infections. Ignoring it poses a risk since it can lead to several potentially fatal conditions, including kidney failure, liver failure, meningitis, hepatitis B, and multiple organ failure. Thus, we need to use caution when diagnosing and treating its symptoms.

**Keywords:** *Leptospira* infection, Human leptospirosis, Seroprevalence, ELISA, Co-infection

## INTRODUCTION

Leptospirosis is a zoonotic infection caused by pathogenic species of spirochete bacteria *Leptospira*[1]. Leptospirosis will remain subclinical in most of the infected individuals. Those who are symptomatic with the infection have non-specific symptoms similar to those of dengue, malaria, and influenza. However up to 10% of patients may also die from leptospirosis[2]. Essentially, human leptospiral infections typically stem from coming into direct or indirect contact with the urine of infected animals[3]. The primary method by which humans contract infection is through damaged skin and mucous membranes coming into contact with water or soil containing urine from rodents and other animals[4]. The illness may lead to various clinical manifestations, spanning from symptomless infections to fatal pulmonary bleeding and Weil's syndrome. The wide array of clinical symptoms, such as fever, headache, rash, and renal failure, can make diagnosis difficult. Co-infections like typhoid and malaria further complicated the process[1]. Moisture is an essential factor for the leptospires to survive in the environment. Other modes of transmission include handling of infectious animal tissues, consuming tainted food or water, and inhaling urine droplets. Several key factors have played a role in susceptibility to this infection, such as heavy rainfall, animal farming methods, urbanization without planning, and a farming way of life[3]. Leptospirosis is widespread in many regions of South East Asia. In India, it was first found in 1931. Leptospirosis recognized as a primary factor of sudden febrile sickness, has been documented in numerous instances in the Southern, Central, Eastern, and Western parts of India. There have been only a small number of reported cases of leptospirosis in North India, possibly due to lack of clinical suspicion, proactive surveillance, and public awareness[3]. Leptospirosis is mainly caused by specific type of bacteria. Diagnosis through microscopic examination and bacterial cultures of sample like urine or blood, but it is time-consuming and necessitates skilled professionals. Molecular techniques are more sensitive, precise, and able to differentiate among various species compared to microscopy. However, in many countries with low resources, the routine diagnostics using molecular techniques are limited due to their high cost and requirement of technical expertise. Diagnostics tests like MAT and ELISA are beneficial for identification in medicine. ELISA is the most favored and economical method with 95% specificity and sensitivity[1]. Leptospirosis, known as "the Great Mimicker", presents with various clinical manifestations that may result in cases going unnoticed and undetected[5]. Leptospirosis is inadvertently carried by humans. It is also referred to as "rice paddy fever" or "mud fever".

Occupational exposure is a significant factor in causing infections, especially among high-risk individuals like farmers, sewer workers, veterinarians, meat handlers, and animal handlers[5].

The goal of this study was to establish the seroprevalence of *Leptospira*. Spread of infection from patients with fever who are visiting SGT Hospital.

## AIMS AND OBJECTIVE

**Aim:** To study the Seroprevalence of *Leptospira* infection at a tertiary care hospital.

**Objectives:**

- To determine the Seroprevalence of Leptospirosis infection from febrile patients of visiting SGT Hospital.
- To study the co-infection of Leptospirosis with etiologies of similar clinical features such as Dengue, Malaria, Chikungunya, Enteric Fever, Scrub Typhus.

## MATERILS AND METHODS

The current research was conducted at the Microbiology Department of SGT University, Hospital and Research Institute in Gurugram, Haryana. The research took place between May 2023 to October 2023. Within this timeframe, a total of 259 blood samples were collected from patienst visiting the Medicine OPD at SGT Hospital. Patients clinically suspected of leptospirosis and with a history of fever exceeding 5 days were seen. This study included individuals showing various clinical symptoms such as headache, fever with chills, abdominal pain, vomiting and nausea. A well-organized clinical form was used to analyze the patient's information including their clinical history and hospital laboratory data. The sample were tested for *Leptospira* IgM antibodies with the Eiger *Leptospira* IgM ELISA kit. The testing was carried out as per the instructions supplied in the kit insert provided by the manufacturer.

The results was subsequently assessed according to the guidelines outlined in the manufacturer's manual. An OD value ranging from 0.0 to 0.15 shows a negative result, an OD value between 0.15 to 1.00 indicates a weakly-positive result, and an OD greater than 1 indicates a positive result. Results showing no reactivity, with OD readings between 0.0-0.15 units, suggests the asence of antibodies in the sample. Confirming the laboratory diagnosis 2-3 weeks later is necessary as antibodies may not be present during the early stages of the disease. Specimen showing weak

reactivity should be carefully analyzed. In typical populations, samples that are weakly reactive (0.15 to 1.00 OD) are rarely seen. Samples showing high reactivity (>1.0 OD or +++) could suggest the existence of a particular antibody. Testing for antibodies alone is not sufficient to diagnose an acute infection as antibodies can remain in circulation for an extended period after prior exposure. Both positive and negative test controls were included in every run, in addition to the samples[6].

In this research, we also examined co-infections that displays similar clinical signs as leptospirosis. The diseases being specifically sought out are dengue, typhoid, chikungunya, malaria, and scrub typhus.

## STATISTICAL ANALYSIS

The comparison of categorical data will be done by Chi square test while continuous data will be analyzed by student's t test. p value <0.05 will be taken as statistically significant.

Master chart will be prepared on MS excel.

## RESULTS

A total of 259 patients were included in the present study. Out of these 259 patients, 30% (78/259) were seropositive for the IgM antibody of *Leptospira* [Figure 1]. Among the 78 patients who tested positive for IgM, 65% (51/78) were males and 35% (27/78) were females [Figure 2]. The results showed that males are comparatively more affected by leptospirosis than females. Among all the positive patients, the highest seropositivity was observed in the age group of 20-30 years followed by above 40, less than 10 years, and 30-40 years. In the present study, it was observed that the seroprevalence of *Leptospira* in Haryana region was highest in the monsoon season between July to October.

In the present study we observed increased levels of SGOT, SGPT and decreased levels of Creatinine with average values of 106.91, 102.40, and 0.77 respectively. It was found that r-value for SGOT was calculated as 0.31 which is moderate positive correlation and for Creatinine, it was calculated as -0.25 which is moderate negative correlation. Pearson's correlation coefficient test was applied and out of all the values, these two values of SGOT and Creatinine are statistically significant as these values are >0.5 and rest all the values of correlation are statistically insignificant [Figure3,4 and Table 1]

In the present study, we also looked for co-infections with Dengue and Typhoid. We reported co-infection with Dengue in 10 (3.86%) of cases and 15 (5.79%) of co-infection with Typhoid in patients with *Leptospira* [Figure5].

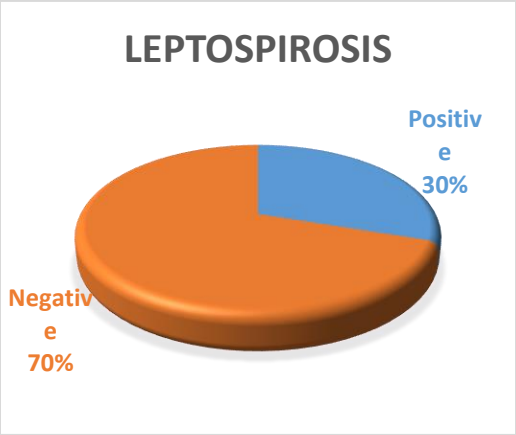


**Figure 1 - Shows the seroprevalence of leptospirosis of the study group.**

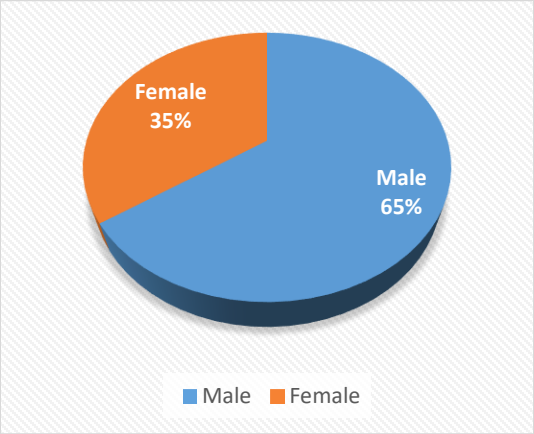


**Figure 2 – Gender wise Distribution of study participants showing positive results for *Leptospira***

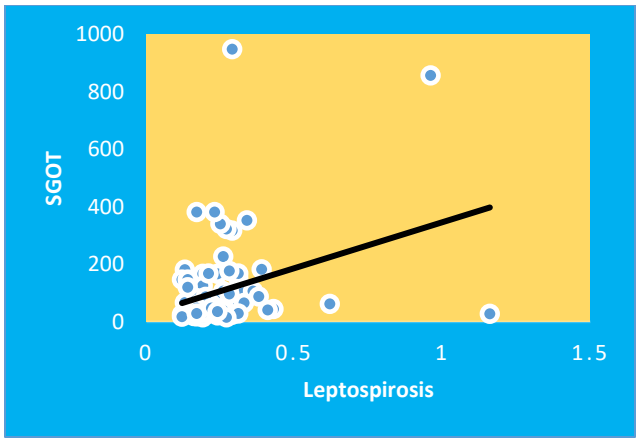


**Figure 3 - Shows the correlation between leptospirosis and SGOT.**

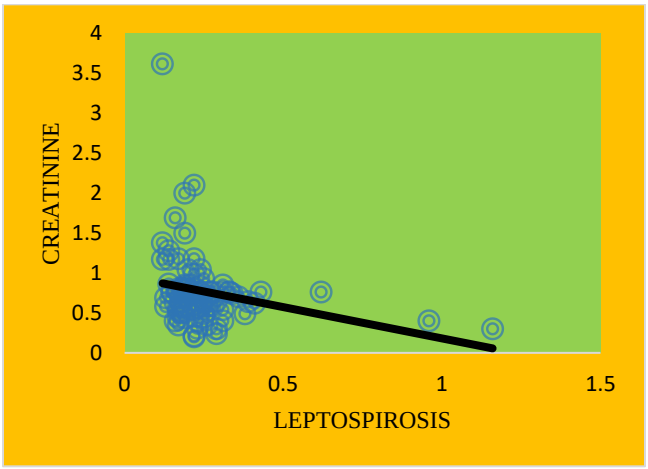


**Figure 4 - Shows the correlation between leptospirosis and creatinine.**

| | | SGOT | CREATNINE |
|---|---|---|---|
| **Leptospira** | Pearson Correlation | 0.317 | -0.254 |
| | P-value | 0.003 | 0.018 |

**Table 1- Correlation of *Leptospira* among SGOT and CREATININE**

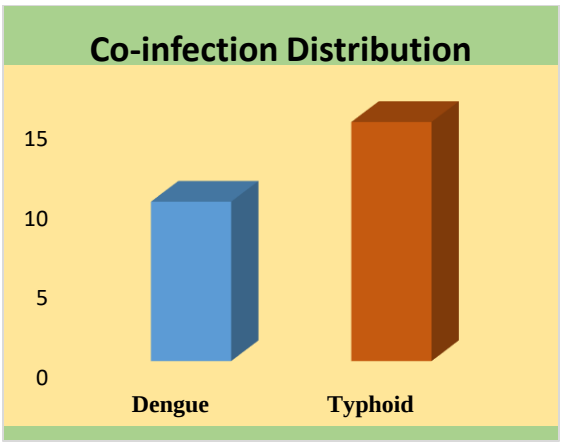


**Figure 5 – Distribution of Co-infection**

## DISCUSSION

Leptospirosis is a widespread zoonotic disease and humans are accidental host. It is also known as 'Great Mimicker' as it is often indistinguishable from other acute febrile illness including dengue, typhoid, and others. Severe leptospirosis infections may lead to multiple organ failure such as renal failure, hepatic dysfunction, cardiac and pulmonary complications[5]. Leptospirosis was first recognized as an illness by Adolf Weil in the year 1886. *Leptospira* was separately demonstrated as a pathogen the later part of 20th century by Inada and Ido in Japan and Uhlenhuth and Fromme in Germany. In India, cases of leptospirosis have been reported since 1931. Various studies from different regions of the country have reported varying seroprevalence rates, ranging between 6.4% and 30.9%. In India, the estimated yearly morbidity rate is 19.7 cases per 100,000 people[7]. The purpose of the current research was to investigate the occurrence of *Leptospira* infections in Shree Guru Gobind Singh Tricentenary University, Hospital and Research Institute located in Gurugram, Haryana.

The prevalence of *Leptospira* was detected by IgM ELISA test. The presence of *Leptospira* was identified through IgM ELISA testing. In the current study, it was discovered that 30% of individuals had leptospirosis. Our results are consistent with previous research, as a sero-epidemiological retrospective study from AIIMS, New Delhi found a 26.9% seroprevalence rate that closely matched our own findings[8]. The study conducted in Pudukkottai, India reported 38.5% seroprevalence[9]. The study conducted in Uruguay reported 46.3% prevalence in their study[10]. Another study from Western part of Nepal reported 21% of febrile patients had serological

evidence of acute leptospirosis[11]. As compare to our findings, majority of studies showing less seroprevalence of leptospirosis infections due to the fact that in our region, the main source of income of population among Haryana is farming and both males and females are involving in farming activities.

In our study, we confirmed that the common symptoms of leptospirosis are fever with a duration of about five to ten days with headache, fever with chills, abdominal pain, vomiting, and nausea. These findings correlate with the study from Turkey which confirmed that abdominal pain and vomiting were common symptoms[12]. A study looking back found that the main symptoms in patients were fever (95.6%), headache (93.8%), feeling unwell (87.5%), and muscle pain (85.6%)[13]. The Kasturba Medical College in Mangalore conducted a retrospective study which found that most patients admitted had fever (92.1%), weakness, and vomiting (32.7%). They noted increased levels of SGOT and Creatinine, consistent with our own findings[14].

In the present study, we use IgM ELISA as a diagnostic method. ELISA is able to identify both present and recent infection IgM antibody in the initial stage of the illness. ELISA is an inexpensive, accurate, and precise test that can serve as the primary diagnostic tool for early detection of *Leptospira* infection, particularly in areas with limited resources. Unlike our research, a prospective study carried out at JNMCH, AMU, Aligarh, compared serological tests such as ELISA and MAT. In their study they reported 14.9% prevalence by IgM ELISA and 13.3% prevalence by MAT[15]. In a prospective cross-sectional and diagnostic based study in Mangalore, Karnataka reported 24.8% prevalence and they also compare the seropositivity rate of serological tests including Leptocheck-WB IgM, IgM ELISA and MAT. They reported 15.6%, 20.7% and 24.8% seropositivity by Leptocheck-WB IgM, IgM ELISA and MAT respectively.

Age bracket is a significant factor in *Leptospira* infection, affecting prognosis and results. A study conducted in Brazil looked back at the impact of age on cases of leptospirosis. Among a total of 507 patients, 64 patients belonged to the elderly group (≥60 years) and 443 patients belonged to the group of young individuals (≤60 years). They stated that the average age of older group was 66 ± 6 years, while in the younger group it 34 ± 12 years, which aligns with our current study [16]. In the present study people under 20-30 years of age group (29.73%) are mainly affected by *Leptospira* infection followed by 40 years and above (21.62%) and then less than 10 and 10-20 years (16.60%) and remaining 30-40 years (15.44%). This finding correlates with previous

research. The retrospective observational study at Chettinad Hospital and Research Institute, Tamil Nadu reported 34% people under age group of 21-30 affected by *Leptospira* infection followed by 31-40 years (19%), 11-20 (14%), 41-50 years (11%) and less than 10 years (2%)[17]. Another study from Odisha reported 28.6% people belonged to the age group 41-50 and 20% were within the age group of 21-30 years[18]. In a retrospective study from Pune, India found maximum leptospirosis incidence in 21-30 years of age group followed by 31-40 years of age group[19]. A study from MGM Medical College, Kamothe, Navi Mumbai, India found the maximum number of patients were from the age group of 25-50 (64%)[20].

The number of males 51 (65%) are affected more by leptospirosis than females 27 (35%) in the present study. Our findings correlate with other studies. In contrast to the finding of the present study, more number of males affected by *Leptospira* infection have been reported from Maharashtra, India reported 62.4% were males and 37.63% were females[21]. Another study from Karnataka also reported 51.4% males and 48.6% females[22]. A study from AIIMS, New Delhi also reported 80.31% males and 19.68% females[1]. Another cross-sectional study from Mumbai, Maharashtra was reported 62.4% were males and 37.63% were females[21].

Leptospirosis is common in various countries across South East Asia, although there are fewer reports of *Leptospira* infection in North India, possibly due to low awareness and limited resources[3]. The World Health Organization and International Leptospirosis Society suggest that individuals showing symptoms such as sudden fever, body aches with muscle tenderness, headache, cough, jaundice, decreased urine output, difficulty breathing, and coughing up blood, along with signs of meningeal irritation, should be considered as potential case of *Leptospira* infection and need to undergo testing[19]. The *Leptospira* infection typically has two phases, varying from mild fever symptoms to severe organ failure, with the possibility of elevated enyme levels during this[23]. In the present study we observed increased levels of SGOT, SGPT and decreased levels of Creatinine with average values of 106.91, 102.40, and 0.77 respectively. Similarly, In the study from tertiary care teaching hospital, Tiruchirapalli, India reported elevated LFT parameters including bilirubin, SGOT and SGPT were 86.7%, 100%, and 96.7% respectively and elevated KFT parameters including creatinine and urea were 46.7% and 73.3% respectively[24]. Another study from Mumbai reported high levels of SGOT and SGPT 62% and 66% respectively[20].

There is a debate about whether antibiotics reduce the seriousness of leptospirosis. Analysis of seven randomized trials found that the evidence for or against antibiotic treatment in leptospirosis is inconclusive; patients treated with antibiotics seemed to have a shorter duration of the disease compared to those without antibiotics, yet the variations were not statistically significant. In a study looking back on past events, starting antibiotics later (2 days or longer) was linked to worsened illness. Guidelines and expert consensus recommended quick administration of antibiotics in suspected and confirmed cases. Oral doxycycline is employed for mild illness, while intravenous penicillin is utilized for severe illness, however, a study comparing ceftriaxone (1 g daily) with penicillin (1.5 million units every 6 hours) for 7 days found no notable variance in fever resolution time[25].

*Leptospira* has a tendency to co-exist with other diseases and cause co-infection in patients who already having leptospirosis which is a main cause of diagnostic and therapeutic challenge for many. This creates difficulty for clinicians and laboratory staff in diagnosis and pin-pointing the singular or particular disease and in medicine prescription to a particular patient. Dengue is often associated together with leptospirosis. Many studies show Co-infection of *Leptospira* with Dengue[26]. In the present study, we also looked for co-infections with Dengue and Typhoid. We reported co-infection with Dengue in 10 (3.86%) of cases and 15 (5.79%) of co-infection with Typhoid in patients with *Leptospira.* In a study from AIIMS, New Delhi has shown co-infections with leptospirosis with Dengue (n = 5), Typhoid (n = 7), Scrub Typhus (n = 4), and, Malaria (n = 1)[1]. Another study which correlates with our findings is conducted in Lucknow, India reported Typhoid was identified as the most common co-infection which is very similar to our study. They reported that 11 samples tested positive for Typhoid, 7 for Malaria, 4 for Dengue, 2 for Chikungunya, and only 1 for Scrub Typhus[27].

## CONCLUSION

*Leptospira* infection is not common because it is primarily found in locations that experience flooding and severe rains. Geographic circumstances are unfavorable to *Leptospira* in the region of our study. The unfavorable geographic conditions make it easy to overlook leptospirosis infection while making diagnosis of febrile patients. Every febrile patient has to have their leptospirosis checked because it can be missed during making diagnosis. In our study we found that some severe cases of leptospirosis were associated with co-infections with dengue, typhoid,

and other infections. Ignoring it poses a risk since it can lead to several potentially fatal conditions, including kidney failure, liver failure, meningitis, hepatitis B, and multiple organ failure. *Leptospira* is also referred as the "Great Mimicker" because of its propensity to mimic other illnesses. Even Nevertheless, there is still a high prevalence of *Leptospira* in this unfavorable location. Thus, we need to use caution when diagnosing and treating its symptoms.

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