

Incidence of Human Leptospirosis among Scrub Typhus and Chikungunya Negative Children in West Bengal, India, 2022

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Received: August 12, 2022 Published: August 26, 2022

Abstract

Leptospirosis caused by the bacterium *Leptospira*, has been classified by the World Health Organisation (WHO) as a neglected tropical disease with worldwide significance. Leptospirosis in endemic places affects adults more frequently and severely than it affects children. There is a lack of data specifically focusing on children, and it is yet unknown why this discrepancy exists. As the majority of human leptospiral infections are mild or asymptomatic in endemic areas, goal of our study was to describe the prevalence of the *Leptospira* disease spectrum and the variations in clinical manifestations among patients admitted for febrile illness in a tertiary care facility.

Keywords: Chikungunya, Children, Human Leptospirosis, India, Kolkata, Scrub Typhus

Introduction

One of the most prevalent, feared and ignored spirochaetal zoonotic infections worldwide continues to be leptospirosis resulting from the harmful bacterium *Leptospira* [1,6]. By excreting bacteria through their urine, animals with *Leptospira* infections contaminate the environment and contribute to the spread of leptospirosis [4,6]. It is currently restricted to underdeveloped regions of the world, with sporadic reports coming from industrialised nations [1,5,6]. Leptospirosis incidence fluctuates across time and place and is highly correlated with regional socioeconomic, ecological, and environmental factors [7,8,9]. In tropical, humid, and temperate locations, particularly during the rainy seasons, including urban, semi-urban and rural areas, a higher incidence is seen [10,11]. It has historically been linked to specific socioeconomic or environmental conditions that favour infectivity in animal vectors and human exposure. Leptospirosis is prevalent in many Asian countries, although under reporting makes it difficult to gauge the true incidence of the disease. The first case of leptospirosis from India was reported in 1929 from Andaman and Nicobar Islands [19]. Although numerous epidemics recorded in recent years but the paediatric cases are extremely rare [2]. The neglected leptospirosis is a serious health issue in India that is unquestionably linked to the both monsoons and unsanitary circumstances [18]. The neighbouring countries of Bangladesh and Nepal, where the illness also experience environmental factors and poor sanitary circumstances, with the extra burden of overcrowding. Our study's goal was to determine the incidence of human leptospirosis among febrile patients who were admitted for being suspected of having scrub typhus/chikungunya infection, if any. This is the first hospital-based study that attempted to explore the seroprevalence, disease manifestations, and prevalence of leptospirosis among patients who tested negative for scrub typhus and chikungunya infection in a medical setting.

Materials and Methods

Study Details and Inclusion Criteria: The study was conducted in Virus Research & Diagnostic Laboratory, Department of Microbiology, R. G. Kar Medical College & Hospital, Kolkata, India, during January 2022- June 2022 (located at 22082'N, 88080'E) (Figure: 1).

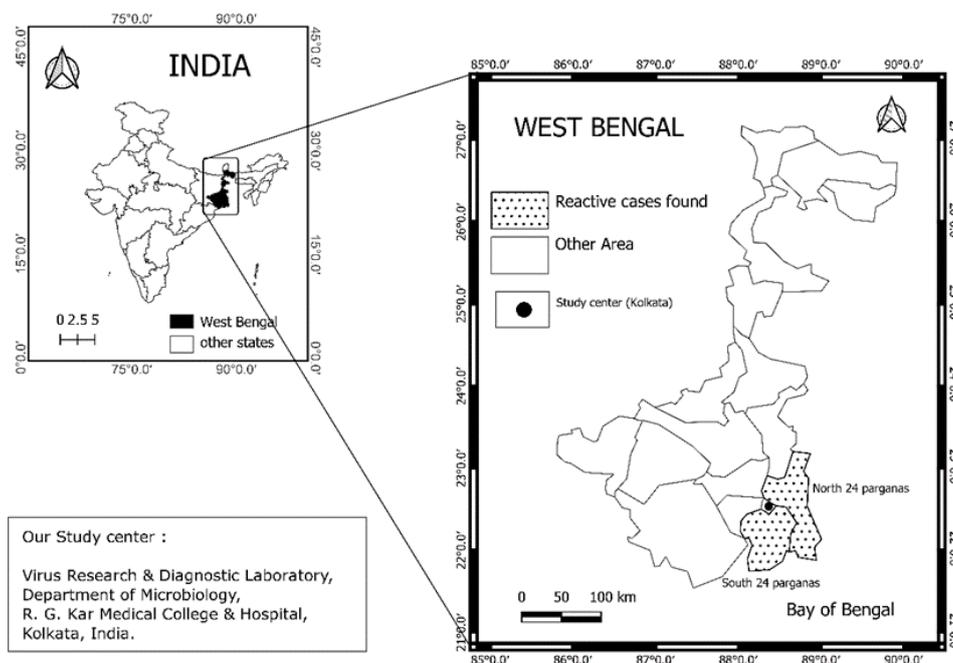


Figure 1: The geographical location of the sampling site is West Bengal, India. Reactive cases were found in north and south 24 parganas near the coastal area of Bay of Bengal (denoted as dotted patterns in map). The shape files were extracted from DIVA-GIS using the Geographical Information System (GIS). The map was developed using QGIS 3.2 for Windows™.

Our laboratory, which serves as a referral network for the Integrated Disease Surveillance Programme (IDSP), receives a large number of patient samples throughout the year and is responsible for laboratory-based confirmation of microbial infections, from Kolkata and adjoining districts of West Bengal. The inclusion criteria for reference specimens defined by the WHO were followed in this investigation. Along with fever, any three of these symptoms were believed to be indicators of chikungunya and scrub typhus infection, such as headache, body ache, abdominal pain, conjunctivitis, photosensitivity, nausea, vomiting, loss of appetite, skin rashes, arthralgia, and myalgia. During this study period 84 specimens were collected from scrub typhus/chikungunya suspected patients, and the study was planned to be non-discriminatory in terms of age or gender.

Specimen Collection and Storage: In order to identify specific IgM antibodies against scrub typhus and chikungunya, 2–3 ml of blood samples from the suspected patients were aseptically collected in two vials by the medical technologist from fever clinic unit. Preserving the cold chain one vial was sent for the required biochemical analysis, and one vial was sent to our serology laboratory to check for any possible scrub typhus/chikungunya infections. The centrifugation was used to isolate serum from the specimens at 3000g for 10 min at 4°C and kept at -20°C in aliquots.

Serological Detection: Enzyme linked immunosorbent assay (ELISA) methods were used to detect the chikungunya and scrub typhus specific IgM antibody by using chikungunya IgM capture ELISA kit (made by National Institute of Virology, Pune, India) and scrub typhus IgM Microlisa ELISA kit (made by J. Mitra & Co. Pvt. Ltd., New Delhi, India), respectively. To detect the *Leptospira* specific IgM antibody, Lepto IgM Microlisa ELISA test kit has been used (made by J. Mitra & Co. Pvt. Ltd., New Delhi, India). All these assays have been performed and OD was measured using the kit specific protocol provided by the manufacturer with the help of semi-automatic ELISA microplate reader and washer made by BeneSphera (marketed by Avantor India).

Result

Among all the 84 suspected scrub typhus/chikungunya patients, 18 patients were seropositive to scrub typhus (21.4%) and 26 were seropositive to chikungunya infection (31%) (**Figure: 2**).

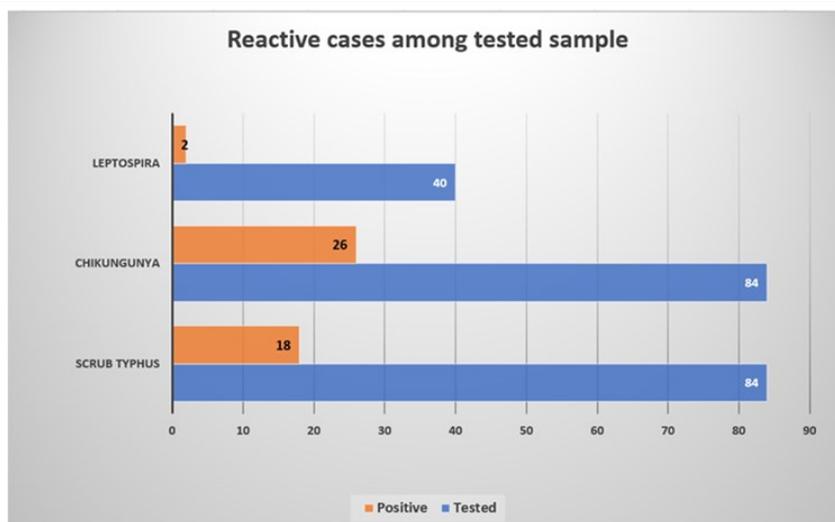


Figure 2: Distribution of seropositive cases among suspected febrile patients.

On the next day, we subjected 40 scrub typhus/chikungunya negative samples to detect IgM against *Leptospira* infection, if any, using commercially available serological test with good sensitivity (99.62%) and specificity (99.92%) for the in vitro diagnosis (IVD) of Leptospirosis. Out of a total 40 diagnosed scrub typhus/chikungunya negative samples, 2 samples were confirmed to be seropositive for *Leptospira* IgM antibodies (5%), tested in duplicate wells and immediately informed to the attending physicians for the proper treatment and prompt care of the admitted patients. In both the cases, IgM antibodies were detected between the 7th and 9th days of the onset of illness. Both the patients were admitted in the female ward with mild to moderate headache, nausea, conjunctivitis, loss of appetite, pretibial skin rashes, and myalgia. Neither renal failure nor haemorrhagic symptoms were found in any of the patients. Both the patient treated with intravenous fluids, antibiotics (cefotaxime), doxycycline was also administered. White cell count (4,500-11,000 WBCs per microliter), Hb% (10.9-15.0 g/dL) and platelets count (150,000-300,000 platelets per microliter of blood) were all within acceptable range in both the patients, except elevated serum bilirubin found in one patient (1.8 mg/dL) without any significant differences in clinical manifestation between them. Within two weeks following admission, they had made a complete recovery and was discharged. There were no incidence of coinfection between scrub typhus/chikungunya and deaths between the two reactive cases till date.

Discussion

According to our study, to account both occurrences it is documented that cases were observed in girl children, at the same time periods but from different places. As per clinical records and socio-demographical data of the reactive patients, it has been noted that, both cases involved school going children under the age of 13 and from underprivileged socioeconomic groups of north and south 24 parganas (semiurban districts) of West Bengal. Previous studies showed that virulent *Leptospira* can live and spread infection for several months in soils and are thought to be more frequently isolated from soils than from freshwater samples [16,17]. In our study, the incidence in school going children can be related to outdoor activity (plays and sports) and both the incidence are positioned near to huge subsoil deposit areas and connected to Indian Ocean via Bay of Bengal (Figure: 1). To finding the reason behind infectivity it has been noticed that both the districts are associated with the abundance of rodents, being asymptomatic carriers of leptospirosis, linked to agricultural processes, such as animal breeding and poultry farming. Previous studies also revealed that seasonal peaks are present in the majority of the *Leptospira* affected areas, such as rains, may act as "triggers" of the disease prevalence [12,13,14]. In our investigation, both reactive cases were recorded in the month of June, monsoon seasons in West Bengal, which is related. The most significant risk factors for the incidence and spread of current and future leptospirosis episodes include environmental changes in human habitat conditions, urbanisation, and climate changes [15]. Earlier epidemiological studies of Leptospirosis from several regions of India have shown that the seroprevalence ranges from 17.8% to 40.50%. However, in our study negligible circulation of Leptospirosis has been observed especially in girl children, which is a rare occurrence as claimed by previous studies [2]. As no vaccine is available, mortality remains significant due to delay in diagnosis and adequate clinical suspicion. For a proper assessment of the scenario of leptospirosis endemicity in the reported areas, additional epidemiological research and sentinel surveillance should be conducted to know the exact stretch and rate of the infection in a particular area. We didn't get samples from all of West Bengal's districts during the study period. Furthermore, due to a lack of funding for the ongoing COVID-19 pandemic, modern methods like polymerase chain reaction tests could not be carried out. The information gathered from this study might be used as a baseline in the study area and could make medical practitioners and national health policymakers more aware of *Leptospira* infections in individuals with nonspecific febrile illnesses.

Conclusion

This study proposes one of the many reasons behind the reduced number of reported *Leptospira* infection in different districts of West Bengal and states of India. Considering the presence of *Leptospira* infection among scrub typhus/chikungunya negative children, prioritized attentiveness is required to determine and roll out the exact stretch of this infection to avoid delayed diagnosis, provide adequate treatment, and to execute effective management strategies. Numerous epidemics of *Leptospira* infection in humans worldwide, increased attention should be given to human leptospirosis around the world. Prevention is largely dependent on sanitation measures that may be difficult to implement, especially in developing countries as no vaccine is available. Our study will help the community to get the proper diagnosis and treatment for the patients having undifferentiated febrile illness. For better control of the illness among the patients, strict monitoring and right state of preparedness should be given priority to encourage community participation and in order to curb the further spread of disease.

Funding

This work was financed by IDSP, Directorate of Health Services, Ministry of Health and Family Welfare, Government of West Bengal, Kolkata. [Order No. HFW-27034/15/2019-NHM SEC-Dept. of H&FW/277, Dated: 13.09.2021]

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

RRG and RPC contributed to the conception and design of the study. RPC and MC made the laboratory diagnosis and required analysis. BD made the representation of data. SS and SC made the interpretation of data. All authors drafted the report with final approval of its completed form or revising it critically for important intellectual content. All authors have read and approved the final report.

Availability of data and material

The datasets generated during and/or analysed during the current study are available in the

[**Fig share repository**] [[10.6084/m9.figshare.20166248](https://doi.org/10.6084/m9.figshare.20166248)].

Ethics approval

This study was approved by the Institutional Ethical Committee.

Consent to participate and publish

Well informed permission was acquired from discrete contributor included in this study. Written consent to publish has been received from the participant.

Acknowledgements

We express our heartfelt gratitude to the Principal, Physicians, Nurses, Medical Technologists [Mr. Niramay Maji and Mrs. Swati Ghosh], and IDSP staff member [Mr. Surajit Dutta] of R. G. Kar Medical College & Hospital, Kolkata, for their unwavering support and provision of adequate facilities for this study.

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Citation: Chatterjee RP, Sikdar S, Chatterjee S, Chowdhury M, Das B, Ghosh RR. "Incidence of Human Leptospirosis among Scrub Typhus and Chikungunya Negative Children in West Bengal, India, 2022". *SVOA Paediatrics* 2022, 1:4, 101-105. DOI: <https://doi.org/10.58624/SVOAPD.2022.01.018>

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