European Journal of Medical and Health Sciences
www.ejmed.org

Prevalence of Chikungunya and Scrub Typhus Coinfection among Dengue Negative Patients in Kolkata, India-A Newly Emerging Public Health Hazard

Rajendra Prasad Chatterjee, Shilpa Chatterjee, Subhendu Sikdar, Biswajit Das, and Reena Ray Ghosh

ABSTRACT

The chikungunya virus (CHIKV) and scrub typhus infection has scattered worldwide creating human health hazards in India and Asia specific region. We aimed to identify chikungunya, scrub typhus, and their co-infection in dengue-negative samples having undifferentiated febrile illnesses.

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 and scrub typhus IgM Microlisa ELISA kit, respectively. OD value was measured with the help of BeneSphera (India) ELISA microplate reader.

Among 490 suspected patients, 57 (11.63%) samples were tested positive for chikungunya IgM antibodies, while 43 (8.77%) came positive for scrub typhus IgM antibodies, but all samples tested negative for dengue IgM antibodies. Additionally, 5% of the total positive cases were positive for both chikungunya and scrub typhus infection.

Our study offers a hypothesis regarding one of the possible causes of the decline in the frequency of scrub typhus and chikungunya cases reported in Kolkata and other districts of West Bengal. As an outcome, physicians treating undifferentiated febrile patients in endemic locations should look for chikungunya, scrub typhus, and existing coinfection between them to avoid delayed diagnosis and provide proper treatment against these infections.

Keywords: Chikungunya Virus, Coinfection, Dengue Virus, Febrile Illness, Scrub Typhus.

Submitted : November 17, 2022

Published : March 5, 2023

ISSN: 2593-8339

DOI: 10.24018/ejmed.2023.5.2.1598

R. P. Chatterjee
Virus Research & Diagnostic Laboratory, Department of Microbiology, R. G. Kar Medical College & Hospital, 1 K. B. Sarani, Kolkata, India.
(e-mail: chatterjeeraja90@gmail.com)
S. Chatterjee
Department of Biomedical Science, Chosun University College of Medicine, Gwangju, South Korea.
(e-mail: shilpachatterjee@chosun.kr)
S. Sikdar
Virus Research & Diagnostic Laboratory, Department of Microbiology, R. G. Kar Medical College & Hospital, 1 K. B. Sarani, Kolkata, India.
(e-mail: subhendu.kolkata26@gmail.com)
B. Das
Department of Microbiology, University of Kalyani, Nadia, West Bengal, India.
(e-mail: biswabiology@gmail.com)
R. R. Ghosh*
Virus Research & Diagnostic Laboratory, Department of Microbiology, R. G. Kar Medical College & Hospital, 1 K. B. Sarani, Kolkata, India.
(e-mail: ghoshreena@hotmail.com)

****Corresponding Author***

I. Introduction

Chikungunya, dengue, and scrub typhus are all prevalent causes of acute febrile illness in India [1], [2]. Dengue, chikungunya and scrub typhus are likely reasons of undifferentiated fever in tropical and subtropical climates. Chikungunya fever (CHIKF) is a viral disease transmitted by arthropods and caused by the CHIKV which has emerged as a global health concern since 2006 [3], [4]. The main vector of CHIKV is Aedes mosquitoes (Aedes aegypti and Aedes albopictus) [3]. The first case of CHIKV was discovered in West Bengal in 1963-1965, during a dengue fever outbreak. Since then, incidence of chikungunya has been documented in numerous Indian states, including West Bengal [5]. The virus then vanished from India and remained latent for about three decades [6]. After a long absence, CHIKV resurfaced in 2005-2006 and again declined in the West Bengal and from different states of India during 2010-2014 [7]. Scrub typhus or bush typhus is a bacterial disease caused by the gram-

negative intracellular parasite Orientia tsutsugamushi and transmitted to human via bites of infected mite (chigger), which serves as both a vector and a reservoir for the bacterium [8]-[10]. It is identified via the formation of eschar at the mite feeding site. In India, scrub typhus is a re-emerging infection since the prevalence of the illness has been rising recently throughout the nation [10]. Scrub typhus is a febrile illness with around one million new cases reported each year in Asia, and more than a billion people may be at risk [11]. During World War II, scrub typhus was a major cause of sickness and mortality among troops stationed in Southeast Asia [12]. Prevalence data are difficult to find since rigorous field and hospital-based surveillance has only recently started [13], and it is under reported [10], [14]. Dengue and chikungunya fever are the viral infections which can be spread to human via the bite of Aedes mosquitoes [2], [15]. Dengue virus (DENV) seems to have a 4-10 day incubation period with an occurrence of mild to moderate disease symptoms such as fever, abdominal pain, myalgia, and rashes while CHIKV has the incubation period varies from 1-12 days with mild symptoms like febrile illness, rashes, arthralgia, and fatigue that often misdiagnosed [15]-[17]. Chikungunya-related deaths do happen in individuals with severe infections and atypical presentations, particularly in the elderly, even though they are not very prevalent [18]. The case fatality rate (CFR), which may have been caused by chikungunya virus infection, was 0.1 percent during the La Réunion outbreak in 2005-2006, and it rose sharply with age. According to the Pan American Health Organization, the chikungunya virus was responsible for at least 182 fatalities out of roughly 1.2 million infections as of February 13th, 2015, in the Caribbean and other parts of the Americas (overall case fatality rate 0.02 percent) [19], [20]. Scrub typhus mortality is affected by host characteristics such as age, immunological status, and co-morbidities as well as the environment, the pathogenic strain involved, and other host factors [21]. The median fatality rates for untreated scrub typhus are 6 percent, whereas for treated cases are 1.4 percent, according to several reports [13]. Although it is recognized that scrub typhus is endemic in India, the suspicion and identification of this illness have long been disregarded. Misdiagnosis is further facilitated by other fever disorders' post-monsoon seasonal predominance and overlapping clinical symptoms [21]. Despite eschar formation, scrub typhus often underdiagnosed due to its unspecific clinical features such as severe fever, rashes, headaches, and myalgia becoming undifferentiable among other febrile illnesses [8]. Both illnesses have non-specific and frequently overlapping clinical symptoms. The clinical symptoms of the coinfections with scrub typhus and chikungunya were quite comparable [22]. Scrub typhus infection frequently alters the clinical profile, causes complications, and affects the outcome of chikungunya [22]. Therefore, nonspecific clinical manifestations of chikungunya and scrub typhus along with DENV and its differential diagnosis as an undifferentiated fever makes it challenging for appropriate prognosis and treatment. As a result, a thorough assessment of clinical characteristics and consequences, as well as relevant laboratory tests, is required. Further latency in diagnosing and differentiating such illnesses could exacerbate symptoms and lead to a greater fatality rate [23]. Although, several studies have performed previously to identify the coinfection among several febrile diseases in patients but this is the first hospital-based study where we aimed to look into the seroprevalence, clinical profiles, and incidence of coinfection associated with chikungunya and scrub typhus infection among patients showing DENV negative in a clinical setting.

II. Materials and Methods

A. Study Area

West Bengal is located between the Himalayas and the Bay of Bengal with 34,267 square mile area (88,752 km^2), with approximately 100 million people [24]. There are 1,100 people per square kilometer. Kolkata is the capital of West Bengal and the world's seventh largest metropolitan city by size and population. It is located at 22082'N, 88080'E (Fig. 1). It has a hot and humid climate, which helps to proliferate the both, Aedes mosquitoes and mites (chiggers).

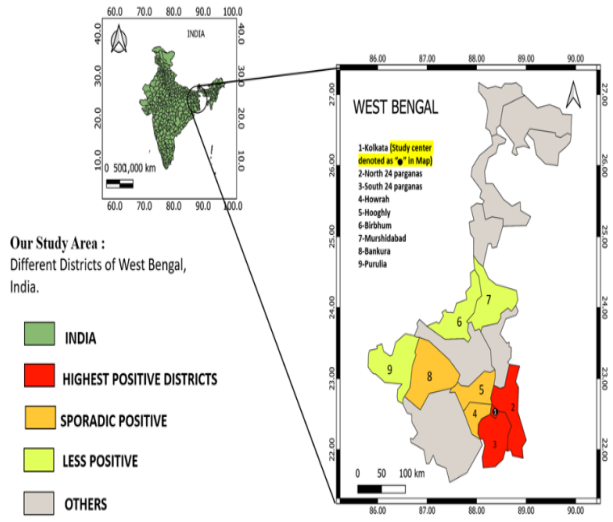

Fig. 1. The geographical location of the sampling site is West Bengal, India. The map shape files were extracted from DIVA-GIS. using the Geographical Information System (GIS). The map was developed using QGIS 3.22.7 'Białowieża for Windows™

B. Patient Information and Clinical Sample

The study was conducted in Virus Research & Diagnostic Laboratory, Department of Microbiology, R. G. Kar Medical College & Hospital, Kolkata, India, during October 2021-June 2022. Our laboratory, which serves as a referral network for the Integrated Disease Surveillance Programme (IDSP), receives many patient samples throughout the year and is in responsible of laboratory-based confirmation of viral infections, particularly febrile illnesses, from Kolkata and other West Bengal districts. The inclusion criteria for reference specimens defined by the World Health Organization (WHO) were followed in this investigation. Along with fever, any two of these symptoms were believed to be indicators of chikungunya and scrub typhus infection, such as headache, body ache, abdominal pain, conjunctivitis, photo sensitivity, nausea, vomiting, loss of appetite, skin rashes, arthralgia, and myalgia. Specimens were collected randomly from dengue suspected patients, and the study was planned to be non-discriminatory in terms of age or gender.

European Journal of Medical and Health Sciences
www.ejmed.org

C. Specimen Collection and Storage

Approximately 3-4 ml of blood samples were collected by the medical technologists from the patients along with available history of illness from fever clinic unit for the detection of suspected dengue virus infection. On a daily basis, the suspected samples were transported to our serology division maintaining the cold chain for the detection of dengue virus infection. The centrifugation was used to isolate serum from the specimens at 3000 g for 10 min at 4 °C and kept at -20 °C and -80 °C in aliquots.

D. Serological Detection

On the basis of WHO inclusion criteria, we have selected 490 dengue negative patients and employed serological methods for the diagnosis of chikungunya and scrub typhus infection, if any. 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. All the assay has 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).

E. Statistical Analysis

Statistical analysis was performed by using SPSS 16 (SPSS, Inc., Chicago, IL, USA). Mean, standard deviation (SD) and percentage positivity of the socio-demographical and clinical parameters were estimated for both the CHIKV and scrub typhus infections. The relationship between subject characteristics and CHIKV seropositivity or current infection was investigated using univariate and multivariate analysis. The odds ratios (OR) and 95 % confidence intervals (CI) were obtained using the multivariate analysis using the Enter method. Statistical significance was defined as a P value of ≤ 0.05.

III. RESULT

A. Socio-Demographic Parameters and Seroprevalence Rate Associated with Infected Study Population

In the current study, among the enrolled 490 suspected patients, 57 (11.63%) samples were tested positive for chikungunya IgM antibodies, while 43 (8.77%) samples came positive for scrub typhus IgM antibodies, but all 490 samples tested negative for dengue IgM antibodies. In addition to this, 5% of the total positive cases were positive for both chikungunya and scrub typhus infection. The month-by-month distribution pattern of chikungunya cases showed a peak in November, followed by April, while the peak for scrub typhus infection was in the month of December. During the month of December, the number of positive samples for both infections was the same, and percentage positivity fell in both infections during the months of January and February, indicating a correlation (Fig. 2A). From the area-wise distribution of positive sample it was observed that majority of percentage positivity were from south 24 parganas followed by north 24 parganas (Fig. 2B).

In this study, age wise distribution of the chikungunya and scrub typhus infected samples revealed that the most of chikungunya patients are between the ages of 31-40, while the most of scrub typhus patients are between the ages of 21-30 (Fig. 3A). From the sex distribution pattern of the positive samples, it was observed that a slightly higher percentage of female infected, 51.16 % for scrub typhus and 54.39 % for chikungunya compared to male patients (Fig. 3B). However, the difference is very negligible for scrub typhus samples to make any gender-based difference of infections, whereas in case of chikungunya it showed significant difference.

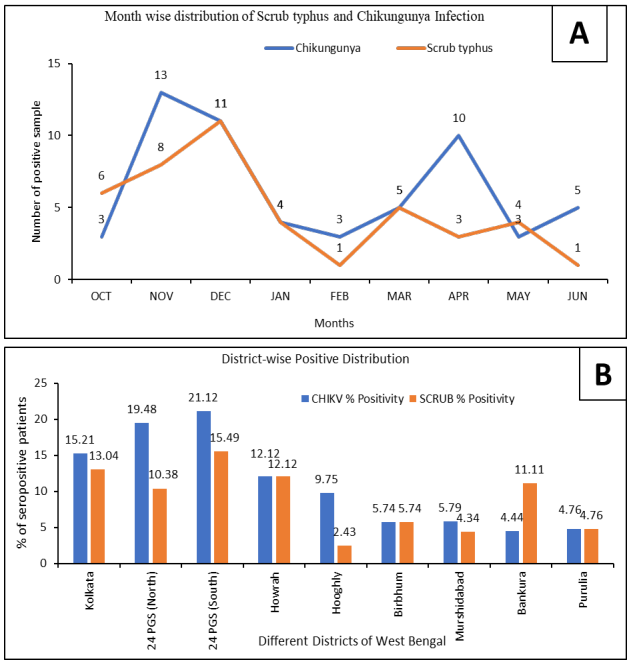

Fig. 2. Distribution of chikungunya and scrub typhus seropositive cases. 2A: Month wise distribution of scrub typhus and chikungunya infection 2B: District wise positive distribution of scrub typhus and chikungunya infection.

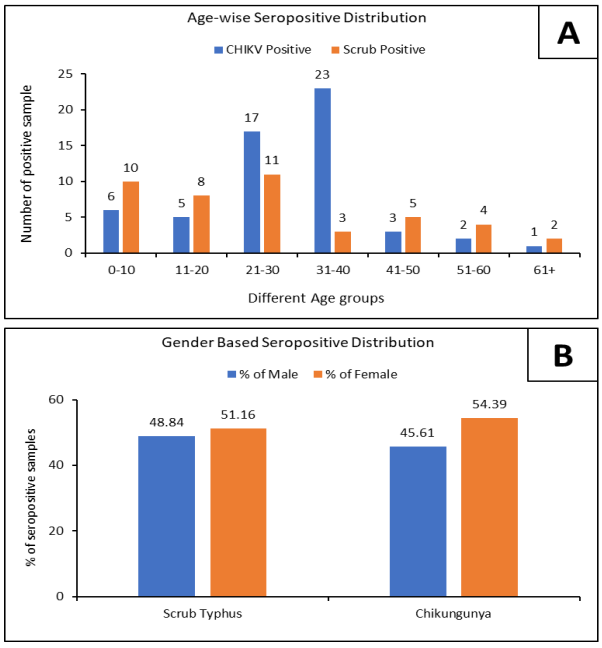

Fig. 3. Distribution of chikungunya and scrub typhus seropositive cases. 3A: Age wise distribution of scrub typhus and chikungunya infection 3B: Gender based positive distribution of scrub typhus and chikungunya infection.

B. Probable Risk Factors Associated with CHIKV and Scrub Typhus Infections

Multivariate regression analysis was performed for both the CHIKV and scrub typhus IgM seropositive groups as dependent variables and all the socio-demographic parameters were considered as independent variables. A P value of ≤ 0.05 was considered as statistically significant. In this study, we considered if the age, gender, occupation, locality, monthly income, educational status, and household conditions had any significant risk to anticipate the prevalence of scrub typhus and CHIKV infections. Age was found to be a significant risk factor for both the CHIKV and scrub typhus infections among patient with febrile illness (Table I).

In the current study, all of the studied individuals had moderate to high-grade fever. Coinfected individuals reported considerably higher joint pain, severe headache, abdominal pain, muscle pain, and maculopapular rashes, than those infected with chikungunya and scrub typhus solely (Table II).

TABLE I: MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF SOCIO-DEMOGRAPHIC FEATURES AMONG CHIKUNGUNYA AND SCRUB TYPHUS INFECTED PATIENTS

Parameters	Total Sample Tested (490)	Total CHIKV Positive (57)	Total Scrub Typhus Positive (43)	CHIKV % Positivity (11.63)	Scrub Typhus % Positivity (8.77)	Odd Ratio (95% CI)	P value
Age							
0-10	68	6	10	8.82	17.54	0.6 (0.2 - 1.6)	0.008
11-20	70	5	8	7.14	14.81	0.6 (0.2 - 1.9)	
21-30	120	17	11	14.16	10.47	1.6 (0.7 - 3.6)	
31-40	143	23	3	16.08	4.47	8.9 (2.6 - 30.5)	
41-50	47	3	5	6.38	5.26	0.6 (0.1 - 2.5)	
51-60	30	2	4	6.66	5.88	0.5 (0.1 - 2.7)	
61+	12	1	2	8.33	4.54	0.5 (0.03 - 5.8)	
Gender							
Male	233	26	21	11.15	9.01	1.3 (0.7-2.3)	NS*
Female	257	31	22	12.06	8.56	1.5 (0.8-2.6)	
Occupation							
Employed	88	6	2	6.81	2.27	3.1 (0.6 - 16.0)	NS
Farmer	102	12	11	11.76	10.78	1.1 (0.5 - 2.6)	
Business	27	3	1	11.11	3.7	3.3 (0.3 - 33.4)	
Housewife	129	17	15	13.17	11.62	1.2 (0.5 - 2.4)	
Unemployed	144	19	14	13.19	9.72	1.4 (0.7 - 2.9)	
Locality							
Urban (Kolkata)	156	16	9	10.25	5.76	1.8 (0.7 - 4.3)	NS
Rural (ROWB)	334	41	34	12.27	10.17	1.2 (0.7-2)	
Monthly Income (INR)							
Below 10000	253	34	26	13.43	10.27	1.3 (0.7 - 2.3)	NS
11000-30000	167	15	14	8.98	8.38	1.1 (0.5 - 2.3)	
31000-50000	51	6	2	11.76	3.92	3.3 (0.6 - 17.0)	
Above 51000	19	2	1	10.52	5.26	2.1 (0.2 - 25.5)	
Educational Status							
Illiterate	78	27	17	34.61	21.79	1.9 (0.9 - 3.9)	NS
School	261	23	16	8.81	6.13	1.5 (0.8 - 2.9)	
College	117	5	9	4.27	7.69	0.5 (0.2 - 1.7)	
University	34	2	1	5.88	2.94	2.1 (0.2 - 23.9)	
Household Conditions							
Hygienic	109	11	8	10.09	7.33	1.4 (0.5 - 3.6)	NS
Unhygienic	381	46	35	12.07	9.18	1.3 (0.8-2.1)	

*NS-Not significant

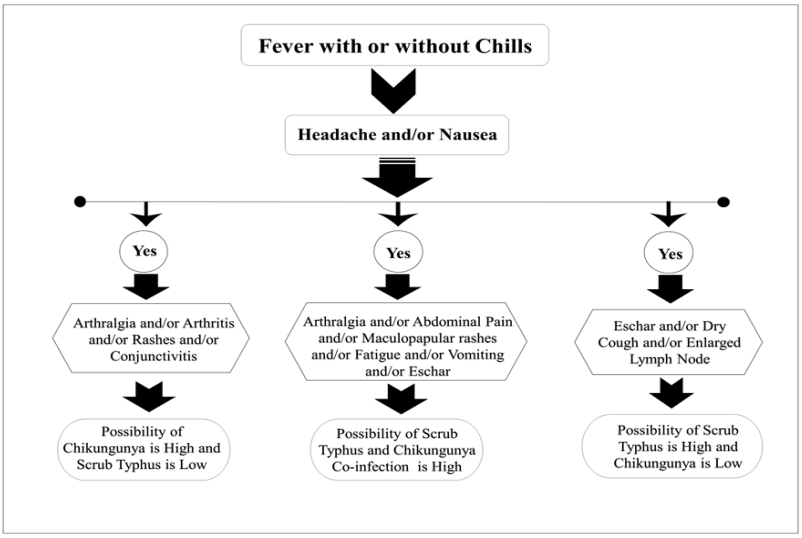

Fig. 4. Schematic diagram showing the significant clinical features and major overlapping symptoms among chikungunya and scrub typhus infected patients.

Patients with chikungunya and scrub typhus infection may experience wide spectrum of clinical feature which creates a significant suspicion to evaluate coinfection. Patients with CHIKV infection exhibited body ache in 81% of cases, acute arthritis in 56% of cases, conjunctivitis in 17% cases and 3.5% meningitis cases. Patients with scrub typhus infection exhibited vomiting in 53.5% of cases, eschar in 48.8% of cases, dry cough in 41.9% cases and 9.3% enlarged lymph node cases. The scrub typhus infected patients had enlarged lymph node and vomiting than the coinfected patients, while the coinfected patients had more severe dry cough and fatigue. Confusion, enlarged liver, enlarged spleen and coma were not seen in the chikungunya, scrub typhus, and coinfected individuals in this investigation. Headache and nausea were the most usual clinical signs among the infected individuals in both the cases. In the current investigation, the most prevalent signs of coinfected patients were headache, joint pain, nausea, dry cough, eschar, vomiting, and abdominal pain (Fig. 4).

TABLE II: COMPARISON OF CLINICAL MANIFESTATIONS AMONG THE CHIKV AND SCRUB TYPHUS INFECTED PATIENTS

Symptoms	CHIKV	Scrub Typhus	Coinfection
Fever	✓	✓	✓
Arthralgia	✓	×	✓
Headache	✓	✓	✓
Abdominal Pain	×	×	✓
Bodyache	✓	×	×
Muscle Pain	×	×	✓
Skin Rashes	✓	×	✓
Maculopapular Rash	×	×	✓
Arthritis	✓	×	×
Nausea	✓	✓	✓
Conjunctivitis	✓	×	×
Vomiting	×	✓	✓
Fatigue	✓	×	✓
Enlarged Lymph Node	×	✓	×
Eschar	×	✓	✓
Dry Cough	×	✓	✓
Enlarged Liver	×	×	×
Enlarged Spleen	×	×	×
Meningitis	✓	×	×
Confusion	×	×	×
Coma	×	×	×

✓ Present, × Absent

IV. DISCUSSION

According to earlier research, 31.3 percent of suspected CHIKV cases in West Bengal's various districts between 2015 and 2016 were positive for the virus [25]. Our analysis found a lower incidence of CHIKV cases (11.63%) in various areas of West Bengal. This may be because we only included patients who tested negative for dengue and because post-monsoon season not arrived yet. In contrast to studies conducted in western Odisha (20%) and northern West Bengal (10.05%), scrub typhus seropositivity (8.77%) in our findings was lower [26], [27]. Previous studies from West Bengal claimed that females were infected by scrub typhus, however studies from a neighbouring state showed that a higher percentage of men were affected [26], [27]. According to a CHIKV study, male patients were more affected than female patients [28]. In our study, we discovered that females had a higher percentage of seropositivity than males in both types of infection. This may be because both the vectors, Aedes and mites, are domestic and peri-domestic in nature, and because females tend to spend more time at home than males do, exposing them to more of the infection. A recent comprehensive study found that adults between the ages of 20 and 40 are more susceptible to getting chikungunya, whereas people over the age of 60 had the highest seropositivity for scrub typhus [16], [26]. Contrary to the findings of the aforementioned study, the majority of males with chikungunya and scrub typhus infections were between the ages of 31 and 40 more often than between the ages of 21 and 30 in our study. This is most likely due to the fact that individuals in this age group work in rural regions tending to agricultural fields. The majority of people in this age group are directly exposed to the vector because it breeds in stagnant water in agricultural areas. Due to their immature immune systems, females in the age range of 0-10 exhibited the highest positive in both illnesses. The highest proportion of positive for the CHIKV virus was reportedly found in Kolkata, followed by the south 24 Parganas, according to earlier studies [28]. In our investigation, it was found that the south and north 24 parganas had the highest percentages of positivity, followed by Kolkata and Howrah. The district of South and North 24 Parganas is connected to the Indian Ocean via the Bay of Bengal, Kolkata is connected to the Netaji Subhas Chandra Bose International Airport and two of the busiest railway stations in India (Sealdah and Kolkata), and Howrah is connected to one of the busiest railway junctions in the world (Howrah) and the river Hooghly, which is ranked third among Indian ports. These are all surrounding areas where there is a high danger that infected individuals will be moved through ports and railroad stations. A big number of migrating birds also act as the carriers of these infections. Prior studies found that deforestation, industrial development, and rapid urbanisation are all influencing the spread of scrub typhus, which is causing inflections to rise [29]. It is wise to screen for both pathogens in locations where they coexist in diseases that are clinically suspected. When clinicians are aware of the type of infection a patient has, they can treat them more successfully and keep track of their side effects, disease severity, comorbid conditions, and recurrence of earlier illnesses. Numerous occurrences of CHIKV, scrub typhus, and coinfections between them in various West Bengal regions suggest that the epidemiology of these viruses is changing. Our research demonstrates that the clinical indicators of chikungunya and scrub typhus coinfection can seem extremely differently from one another, even while common symptoms including fever, body pains, arthralgia, headaches, nausea, and skin rashes are present. More epidemiological study is needed to develop control methods and identify ways to decrease the effects of these illnesses. It is recommended to diagnose both illnesses due to the pattern of coinfections. To the best of our knowledge, no prior published study has compared the coinfection of scrub typhus and chikungunya among patients who tested negative for dengue.

V. Limitations

During this study period, we did not receive samples from all the districts of West Bengal. Further, advanced method such as polymerase chain reaction test could not be performed leading to a shortage of fund due to ongoing COVID-19 pandemic. The data generated from this investigation may be utilized as a baseline in the study region and may raise physicians' and the nation's health policymakers' awareness of CHIKV and scrub typhus infection and cocirculation in patients with undifferentiated febrile illnesses.

VI. Conclusion

Our study suggests on one of the potential reasons why the number of scrub typhus and chikungunya cases recorded in Kolkata and other districts of West Bengal has decreased. Due to the co-infection of CHIKV and scrub typhus and the absence of vaccines for either illness, urgent attention is required. Comorbidity should be considered because chikungunya and scrub typhus are significant aetiologies of non-malarial febrile fever in Kolkata, West Bengal. Routine surveillance of febrile illnesses is necessary to determine the prevalence of these infections in this area, to provide appropriate treatment, and to put in place effective control measures. By providing precise diagnoses and treatments for people with undifferentiated febrile illnesses as well as knowledge on the prevalence of these diseases and their precise incidence rates in a given place, our research will be helpful to the native communities. Patients can better manage their febrile diseases, encourage community involvement, and reduce the spread of the illness by prioritising the appropriate level of preparedness.

Author Contributions

RRG, and RPC contributed to the conception and design of the study. RPC made the laboratory diagnosis and required analysis. RPC and SC made the interpretation of data. RPC, SS, and BD drafted the report with final approval of its completed form. All authors have read and approved the final report.

Ethics Approval

This study was approved by the Institutional Ethical Committee.

Availability of Data and Material

Data and materials are available upon request to the corresponding author.

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 participants.

Acknowledgments

We express our heartfelt gratitude to the Principal, Doctors, Medical Technologists [Mr. Niramay Maji and Mrs. Swati Ghosh], and IDSP staff [Mr. Surajit Dutta] of R. G. Kar Medical College & Hospital, Kolkata, for their unwavering support and provision of adequate facilities for this study. We are grateful to the Indian Council of Medical Research (ICMR) - National Institute of Virology (NIV), Government of India, for preparing and supplying CHIKV IgM ELISA kits.

Funding

This work was financed by Integrated Disease Surveillance Programme (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]

Conflict of Interest

Authors declare that they do not have any conflict of interest.

References

[1] Annual report of hospital based surveillance of acute febrile illness in India in. Manipal Centre for Virus Research. [Internet] 2017. [cited 2022 January 21] Available from: https://manipal.edu/miv/research/projects/AFIstudy.html.
[2] Robinson ML, Kadam D, Khadse S, Balasubramanian U, Raichur P, Valvi C, al. Vector-borne disease is a common cause of hospitalized febrile illness in India. *Am J Trop Med Hyg*. 2018; 98(5): 1526-33.
[3] Mohan A. Chikungunya fever: clinical manifestations & management. *Indian J Med Res.* 2006; 124: 471-474.
[4] Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis*. 2007; 7: 319-327.
[5] Padbidri VS, Gnaneswar TT. Epidemiological investigations of chikungunya epidemic at Barsi, Maharashtra state, India. *J Hyg Epidemiol Microbiol Immunol.* 1979; 23(4): 445-51.
[6] Pavri K. Disappearance of Chikungunya virus from India and South East Asia. *Trans R Soc Trop Med Hyg.* 1986; 80(3): 491.
[7] Khatun T, Panja AS, Chatterjee RP, Chatterjee S. Chikungunya declining in West Bengal, India: A retrospective study (2010-2014). *Int. Res J Bio Sci.* 2016; 5(7): 1-4.
[8] Walker DH. Scrub typhus - scientific neglect, ever-widening impact. *N Engl J Med.* 2016; 375(10): 913-5.
[9] Pathak S, Chaudhary N, Dhakal P, Shakya D, Dhungel P, Neupane G, et al. Clinical profile, complications and outcome of scrub typhus in children: A hospital based observational study in central Nepal. *PLoS One*. 2019; 14(8): e0220905.
[10] Morch K, Manoharan A, Chandy S, Chacko N, Alvarez-Uria G, Patil S, et al. Acute undifferentiated fever in India: a multicentre study of aetiology and diagnostic accuracy. *BMC Infect Dis.* 2017; 17(1): 665.
[11] Xu G, Walker DH, Jupiter D, Melby PC, Arcari CM. A review of the global epidemiology of scrub typhus. *PLoS Negl Trop Dis.* 2017; 11(11): e0006062.
[12] Mahajan SK. Scrub typhus. *J Assoc Physicians India.* 2005; 53: 954-8.
[13] Bonell A, Lubell Y, Newton PN, Crump JA, Paris DH. Estimating the burden of scrub typhus: a systematic review. *PLoS Negl Trop Dis*. 2017; 11(9): e0005838.
[14] Khan SA, Bora T, Chattopadhyay S, Jiang J, Richards AL, Dutta P. Seroepidemiology of rickettsial infections in Northeast India. *Trans R Soc Trop Med Hyg.* 2016; 110(8): 487-94.
[15] Dey M, Sengupta M, Chatterjee RP, Sarkar S, Chatterjee SB. Co-Circulation of All Dengue Serotypes among Patients Attending a Tertiary Care Hospital in Kolkata. *Virology & Immunology Journal*. 2018; 2(9).
[16] Simmons CP, Farrar JJ, Nguyen V, Wills B. Dengue. *N Engl J Med.* 2012; 366(15): 1423-32.

[17] Zeller H, Van Bortel W, Sudre B. Chikungunya: its history in Africa and Asia and its spread to new regions in 2013-2014. *J Infect Dis.* 2016; 214(suppl 5): S436-40.
[18] Mavalankar D, Shastri P, Bandyopadhyay T, Parmar J, Ramani KV. Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. *Emerg Infect Dis.* 2008; 14(3): 412-415.
[19] Gogia A, Sairam B, Kakar A. Chikungunya: a mortality report. *Open Forum Infectious Diseases.* 2017; 4(Suppl 1): S518.
[20] Renault P, Josseran L, Pierre V. Chikungunya-related fatality rates, Mauritius, India, and Reunion Island. *Emerg Infect Dis.* 2008; 14(8): 1327.
[21] Chaudhry R, Thakur CK, Gupta N, Sagar T, Bahadur T, Wig N, et al. Mortality due to scrub typhus - report of five cases. *Indian J Med Res.* 2019; 149(6): 790-794.
[22] Pathak S, Chaudhary N, Dhakal P, Yadav SR, Gupta BK, Kurmi OP. Comparative Study of Chikungunya Only and Chikungunya-Scrub Typhus Coinfection in Children: Findings from a Hospital-Based Observational Study from Central Nepal. *Int J Pediatr.* 2021; 2021: 6613564.
[23] Rao PN, Van Eijk AM, Choubey S, Ali SZ, Dash A, Barla P, et al. Dengue, chikungunya, and scrub typhus are important etiologies of non-malarial febrile illness in Rourkela, Odisha, India. *BMC Infectious Diseases*. 2019; 19(1): 1-3.
[24] Annual Administrative Report 2019-20. [Internet] 2022 Available from: http://westbengalforest.gov.in//upload/publication/AnnualAdminReport2019-20.pdf.
[25] Chatterjee RP, Chatterjee A, Chakraborty N, Chatterjee S. A Sero-epidemiological insight into the current status and rapid dissemination of Chikungunya virus infection in West Bengal – a two years retrospective study. *Int J Sci Res.* 2017; 6(9).
[26] Mukherjee S, Dash A, Tiwari S. Seropositivity of scrub typhus in patients attending a tertiary care hospital in Western Odisha. *J Evolution Med Dent Sci*. 2020; 9(04): 178-181.
[27] Mallick S, Hazra S, Nandi T, Sarkar A. Scrub typhus: a hospital-based study in the northern districts of West Bengal, India. *International Journal of Research in Medical Sciences.* 2019; 7(6): 2403-2407.
[28] Sengupta S, Mukherjee S, Haldar SK, Bhattacharya N, Tripathi A (2020). Re-emergence of Chikungunya virus infection in Eastern India. *Brazilian Journal of Microbiology*. 2020; 51(1): 177-182.
[29] Park SW, Ha NY, Ryu B, Bang JH, Song H, Kim Y, et al. Urbanization of scrub typhus disease in South Korea. *PLoS Negl Trop Dis.* 2015; 9(5): e0003814.