Indian J Med Res 150, August 2019, pp 203-205
DOI: 10.4103/ijmr.IJMR_332_19

Quick Response Code:

Correspondence

Seroprevalence of typhus group rickettsial infections in the north-east region of India

Sir,

Rickettsial diseases are considered amongst re-emerging diseases in India[1]. Among the major rickettsial diseases, scrub typhus (ST) is the most frequently reported, followed by spotted fever group rickettsiae (SFGR) infections[2,3]. In comparison, reports on typhus group rickettsiae (TGR) infections in India are scarce, with only three reports from Kashmir, Tamil Nadu and Gorakhpur[4-6]. TGR consists of two groups of rickettsiae, *viz.* epidemic or louse-borne rickettsiae and murine and endemic or flea-borne typhus rickettsiae[7]. Epidemic typhus is caused by *Rickettsia prowazekii* and murine typhus is caused by *R. typhi*[7]. Previous epidemiological surveys have provided evidence for the presence of TGR in two north-east (NE) States of India[8]. However, no information on clinical cases has been reported from this region. Due to poor diagnostic availability and non-specific disease presentation, this disease might have been overlooked for a long time. We present here evidence of previous typhus group rickettsial infections amongst patients in four States of NE region of India.

This study was conducted from October 2016 to October 2018 in the NE States of India. Patients were enrolled under ongoing projects aimed at identifying the contribution of rickettsial diseases towards acute encephalitis syndrome (AES) case burden and undifferentiated febrile illness. Consecutive patients suspected to have AES or with fever of unknown origin (FUO) attending 11 tertiary healthcare hospitals of seven NE States (Assam, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Meghalaya and Tripura) were included in the study. Blood samples (5 ml) were obtained from State hospitals, and all tests were performed at the Regional Medical Research Centre (RMRC), NE Region, Assam, India. This study was approved by the RMRC-Institutional Ethics Committee. Informed written consent was obtained from each participant or their next of kin.

Serum samples were subjected to screening for immunoglobulin G antibodies against TGR using an indirect group-specific ELISA assay employing purified whole cell *R. typhi* (Wilmington) antigens as described earlier[8]. Briefly, all serum samples were initially subjected to a screen ELISA at a dilution of 1:100. Samples with a net optical density (OD)/absorbance of >0.5 were considered as screen positive. The screen-positive samples were further confirmed by the more stringent titre ELISA[8]. In the titre ELISA, serum samples were tested at four-fold dilutions (1:100, 1:400, 1:1600 and 1:6400). Samples with net absorbance (sum of the OD of all the four dilutions) ≥1.000 were taken as confirmed positive. The inverse of the highest dilution with OD ≥0.200 was taken as the ELISA titre. In screen and titre ELISA, three negative controls and one positive control were evaluated in each test run. Depending on the availability of the sample volume, the ELISA positive samples were further subjected to molecular testing using a *Rickettsia* genus-specific 17 kDa gene semi-nested polymerase chain reaction (PCR) assay[9].

Overall, 2199 patients' serum samples were tested for TGR-specific antibodies: 762 AES suspected cases and 1437 patients presenting with FUO. Of these, antibodies against TGR were detected in 3.93 per cent (30/762) of the suspected AES cases and 2.7 per cent (39/1437) within the FUO group by screen ELISA. Seven of the screen-positive samples could not be processed for titration due to insufficient sample volume. Of the remaining 62 screen-positive samples, 64.5 per cent (40/62) were confirmed TGR seropositive by titration. ELISA results demonstrated titres of 100 (37.5%); 400 (15%); 1600 (22.5%) and

Table. Typhus group rickettsiae seropositivity

States	Number of samples tested		Number of positive ELISA titre				Total positive
	AES suspected	FUO	100	400	1600	6400	
Assam	188	132	0	0	0	0	0
Arunachal Pradesh	13	0	0	0	0	0	0
Nagaland	16	440	5	0	2	3	10
Meghalaya	0	611	0	0	0	0	0
Mizoram	0	254	4	1	0	0	5
Manipur	369	0	2	2	6	4	14
Tripura	176	0	4	3	1	3	11
Overall	762	1437	15	6	9	10	40

Seven screen-positive specimens could not be tested for titration due to low sample volume. AES, acute encephalitis syndrome; FUO, fever of unknown origin

6400 (25%), respectively (Table). All samples were also tested for ST and SFGR in the similar form of ELISA described elsewhere[8]. Samples were also tested for major AES aetiologies prevalent in NE region (Japanese encephalitis, West Nile, dengue, chikungunya, ST and *Leptospira*). The DHR-ICMR guidelines were also followed to rule out other febrile illness[2]. Only one sample was positive for antibodies against TGR and ST.

The presence of TGR-specific antibodies amongst AES and FUO patients was detected in four of seven study States, *viz*. Manipur, Mizoram, Nagaland and Tripura. Demographic characteristic revealed male preponderance over females in Manipur and Tripura. In Mizoram and Nagaland, both males and females were found to be equally affected. Almost all age groups were affected, with higher seropositivity frequency found amongst cases in the age group above 15 yr of age. Clinical records fetched from the hospitals showed no distinctive characteristic features for TGR-seropositive patients. There were no records of body rashes. There was no mortality. Seasonality of the disease was found to be May-July in Manipur and Tripura and March-June in Nagaland. Due to low sample volume and non-availability of whole blood samples of all the ELISA-positive cases, only 24.2 per cent (15/62) samples could be attempted for *Rickettsia*-specific PCR. However, no rickettsial DNA was detected in the tested samples.

The worldwide seroprevalence of murine typhus has been estimated to range from 3 to 36 per cent, with low mortality of 0-1 per cent[10]. In this study, an overall three per cent TGR seroprevalence was found among hospitalized cases in the NE region. Although low in number, this study showed the presence of TGR infections in four of the seven NE States of India. Thus, there is a need for expanded epidemiological studies to confirm the presence of TGR, not only in humans but also in small mammals and their ectoparasites.

The present study had some limitations. First, this study was based on one-time point collection; hence, a definitive diagnosis of current infection could not be determined in the absence of a convalescent serum sample. Second, very low number of samples could be attempted for PCR. Furthermore, no PCR positives were obtained in the processed samples. This could possibly be due to the time lag between time of infection and time of detection or delay in sample collection beyond the rickettsaemic period in the blood.

The NE region is recognized as one of the regions vulnerable to climate change and prone to vector-borne diseases. Lying in the belt of the Himalayas, the NE region is composed of both hills and plains and is in proximity to the countries that have reported murine typhus. Almost all the neighbouring countries have documented endemic murine typhus in recent years ranging from 0.4 to 40 per cent seropositivity[11-15]. Murine typhus is thought to be a disease prevalent in warmer climates and coastal regions[10]. However, its presence in the NE region is an indication of its wider geographical expansion in terms of its climatic adaptability. It is quite possible that the murine typhus has existed for decades or longer in the NE region but has remained undermined due to lack of awareness and poor diagnosis.

In India, rickettsial diseases have emerged as important vector-borne diseases. There is an increasing incidence of ST being reported across the country[16]; however, there are scarce reports on other major rickettsial diseases such as SFGR and TGR. Possible reasons for underreporting might be the lack of differential diagnosis, confusing clinical presentation and unawareness.

The present study demonstrated the presence of anti-TGR antibodies in clinically suspected patients. There is a need to gather knowledge on the seasonality and distribution of rickettsial diseases in India. Inclusion of TGR in the diagnostic agenda for unexplained febrile illness and also AES case diagnosis and implementing public health education on personal hygiene for disease prevention need to be emphasized.

***Acknowledgment*:** Authors thank Himanshu Medhi, Bulen Das, Rajib Chutia, Papu Patra, Deep Prasad Panika, Kumud Sona and Imran Sonowal for technical assistance in the laboratory and field, and acknowledge State Hospital Authorities of Assam, Nagaland, Meghalaya, Mizoram, Tripura and Manipur.

***Financial support and sponsorship*:** This work was supported by the Indian Council of Medical Research, New Delhi (Grant no. VIR/RCI/1/2016/ECD-I and ZON/14/9/2014-ECD-II).

***Conflicts of Interest*:** None.

Siraj Ahmed Khan[1,*], Trishna Bora[1], Jahnabi Saikia[1], Anisha Shah[1], Allen L. Richards[4], Suchismita Chattopadhyay[4], Sanjeeb Kakati[2], Manju Rahi[3] & Harpreet Kaur[3]

[1]Medical Entomology, Arbovirology & Rickettsial Diseases Division, ICMR- Regional Medical Research Centre, [2]Department of Medicine, Assam Medical College & Hospital, Dibrugarh 786 002, Assam, [3]Division of Epidemiology & Communicable Diseases, Indian Council of Medical Research, New Delhi 110 029, India & [4]Naval Medical Research Centre, Rickettsial Disease Research Unit, Maryland 20910-7500, USA

[*]*For correspondence*: sirajkhanicmr@gmail.com

Received February 20, 2019

References

1. Rathi N, Rathi A. Rickettsial infections: Indian perspective. *Indian Pediatr* 2010; *47* : 157-64.
2. Rahi M, Gupte MD, Bhargava A, Varghese GM, Arora R. DHR-ICMR guidelines for diagnosis & management of rickettsial diseases in India. *Indian J Med Res* 2015; *141* : 417-22.
3. Khan SA, Bora T, Laskar B, Khan AM, Dutta P. Scrub typhus leading to acute encephalitis syndrome, Assam, India. *Emerg Infect Dis* 2017; *23* : 148-50.
4. Kalra SL, Rao KN. Typhus fevers in Kashmir state. Part II. Murine typhus. *Indian J Med Res* 1951; *39* : 297-302.
5. Kamarasu K, Malathi M, Rajagopal V, Subramani K, Jagadeeshramasamy D, Mathai E. Serological evidence for wide distribution of spotted fevers & typhus fever in Tamil Nadu. *Indian J Med Res* 2007; *126* : 128-30.
6. Mane A, Kamble S, Singh MK, Ratnaparakhi M, Nirmalkar A, Gangakhedkar R. Seroprevalence of spotted fever group and typhus group rickettsiae in individuals with acute febrile illness from Gorakhpur, India. *Int J Infect Dis* 2019; *79* : 195-8.
7. Civen R, Ngo V. Murine typhus: An unrecognized suburban vectorborne disease. *Clin Infect Dis* 2008; *46* : 913-8.
8. Khan SA, Bora T, Chattopadhyay S, Jiang J, Richards AL, Dutta P, *et al*. Seroepidemiology of rickettsial infections in Northeast India. *Trans R Soc Trop Med Hyg* 2016; *110* : 487-94.
9. Jiang J, Blair PJ, Felices V, Moron C, Cespedes M, Anaya E, *et al*. Phylogenetic analysis of a novel molecular isolate of spotted fever group rickettsiae from Northern Peru: *Candidatus rickettsia* andeanae. *Ann N Y Acad Sci* 2005; *1063* : 337-42.
10. Peniche Lara G, Dzul-Rosado KR, Zavala Velázquez JE, Zavala-Castro J. Murine typhus: Clinical and epidemiological aspects. *Colomb Med (Cali)* 2012; *43* : 175-80.
11. Kingston HW, Hossain M, Leopold S, Anantatat T, Tanganuchitcharnchai A, Sinha I, *et al*. Rickettsial illnesses as important causes of febrile illness in Chittagong, Bangladesh. *Emerg Infect Dis* 2018; *24* : 638-45.
12. Tshokey T, Stenos J, Durrheim DN, Eastwood K, Nguyen C, Vincent G, *et al*. Rickettsial infections and Q fever amongst febrile patients in Bhutan. *Trop Med Infect Dis* 2018; *3* . pii: E12.
13. Yang WH, Dong T, Zhang HL, Wang SW, Yu HL, Zhang YZ, *et al*. Murine typhus in drug detoxification facility, Yunnan Province, China, 2010. *Emerg Infect Dis* 2012; *18* : 1388-90.
14. Parola P, Miller RS, McDaniel P, Telford SR 3rd, Rolain JM, Wongsrichanalai C, *et al*. Emerging rickettsioses of the Thai-Myanmar border. *Emerg Infect Dis* 2003; *9* : 592-5.
15. Zimmerman MD, Murdoch DR, Rozmajzl PJ, Basnyat B, Woods CW, Richards AL, *et al*. Murine typhus and febrile illness, Nepal. *Emerg Infect* Dis 2008; *14* : 1656-9.
16. Murhekar MV. Acute encephalitis syndrome and scrub typhus in India. *Emerg Infect Dis* 2017; *23* : 1434.