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Short Communication

Seroprevalence of spotted fever group and typhus group rickettsiae in individuals with acute febrile illness from Gorakhpur, India

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ABSTRACT

Background: The aim of this study was to estimate the seroprevalence of spotted fever group (SFG) and typhus group (TG) rickettsiae among individuals with acute febrile illness (AFI) in the scrub typhus endemic district of Gorakhpur in India. This district is one of the worst affected by annual seasonal acute encephalitis syndrome (AES) outbreaks.
Methods: Antibodies against SFG and TG rickettsiae and the associated risk factors were determined in 294 individuals presenting with an AFI, encountered during a community-based survey conducted during the AES outbreak period October–November 2016.
Results: Respective IgM and IgG seropositivity was 13.6% and 36.7% for SFG, and 7.1% and 15.3% for TG. SFG IgM positivity was significantly higher among females, while IgG positivity was significantly higher among individuals ≥45 years of age. IgM and IgG seropositivity for TG rickettsiae were significantly higher in individuals involved in outdoor activities and housewives, but did not differ according to age group, sex, or educational status.
Conclusion: The study results present serological evidence of SFG and TG rickettsiosis, in addition to scrub typhus, among individuals with AFI in Gorakhpur region and indicate the need to explore their roles as potential causes of AES in the region.

Introduction

Gorakhpur and the adjoining districts of the Indian state of Uttar Pradesh, have been witnessing annual seasonal acute encephalitis syndrome (AES) outbreaks with high mortality, and scrub typhus has been identified as one of the predominant aetiologies (Mittal et al., 2017; Mittal et al., 2018). Among the other rickettsial infections, spotted fever group (SFG) and typhus group (TG) rickettsiae have been reported from various parts of the country (Khan et al., 2016; Kumar et al., 2011; Mahajan et al., 2007). Both are reported aetiologies of acute febrile illness (AFI) and if left untreated may develop severe consequences with multi-organ involvement, including neurological complications (Anon, 2009). This community-based investigation was undertaken to estimate the seroprevalence of SFG and TG rickettsiae and the associated risk factors in individuals presenting with AFI during the peak period of an AES outbreak in Gorakhpur.

Methods

The study was approved by the Ethics Committee of the National AIDS Research Institute, Pune (NARI EC/2015-16/25). Written informed consent was obtained from all participants and assent was obtained from minors. This sub-study was conducted as part of a community-based cross-sectional serosurvey undertaken by the Indian Council of Medical Research, Government of India, to assess the extent of scrub typhus infection during the peak period of an AES outbreak (October–November 2016) in the district of Gorakhpur (Figure 1). Consecutive individuals aged 6 years and above with fever of more than 5 days in duration, who were encountered during the parent survey, were included in this study.

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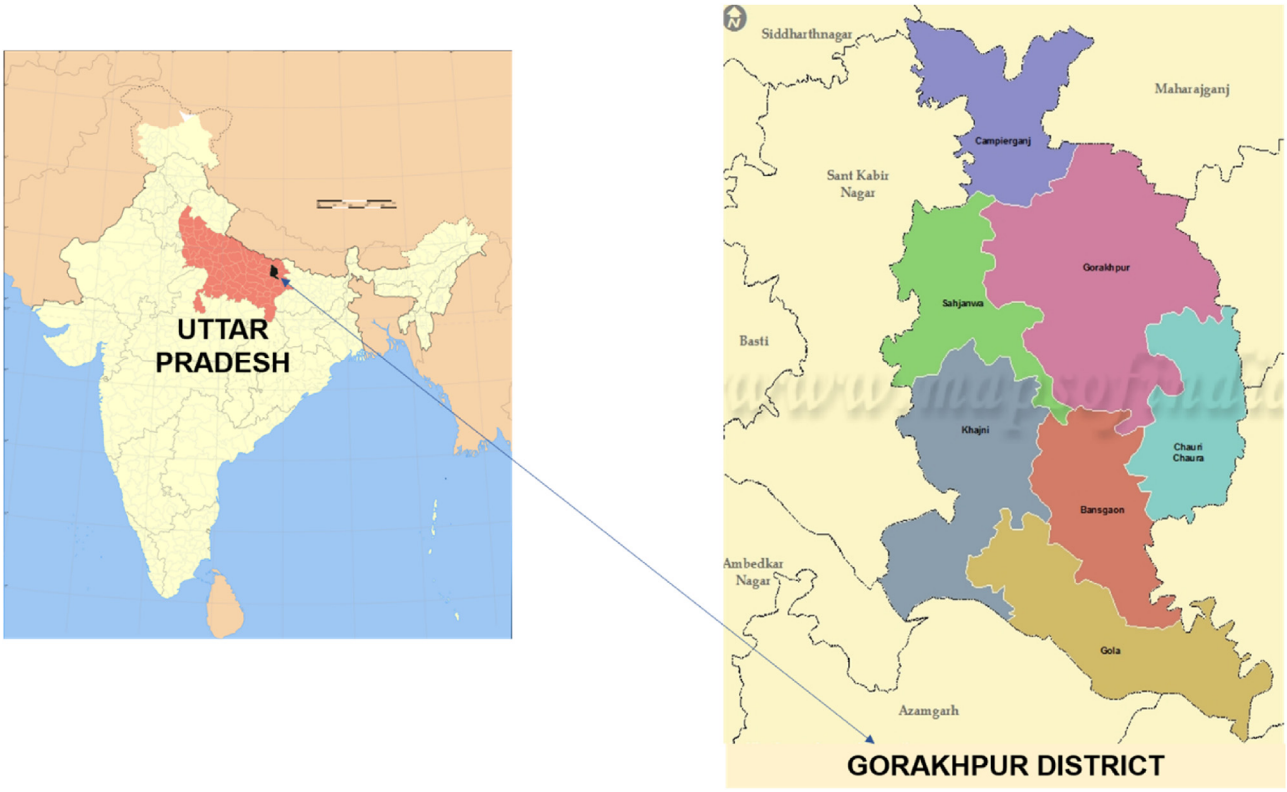

Figure 1. Geographical location of Gorakhpur district.

A blood sample was collected from every participant for the detection of scrub typhus and an aliquot from this sample was used for the detection of antibodies against SFG and TG rickettsiae.

Antibodies against SFG were detected using a *Rickettsia conorii* IgG/IgM ELISA kit (Vircell Microbiologists, Spain), which uses *R. conorii* antigens prepared from Moroccan strain (ATCC VR-141), with sensitivity of 94% and specificity of 95% for IgM detection and sensitivity of 85% and specificity of 100% for IgG detection. Antibodies against TG were detected using a *Rickettsia typhi* IgG/IgM ELISA (Fuller Laboratories, California, USA), which uses species-specific protein rOmp B purified from *R. typhi*, with sensitivity and specificity of >99% and 97% for IgG detection and >99% for IgM detection.

For the determination of current infection, other possible local endemic causes of undifferentiated febrile illness, like malaria, typhoid, leptospirosis, dengue, and Japanese encephalitis, were ruled out; sole IgM seropositivity for SFG or TG was considered. Antibody indices were calculated by dividing the optical density (OD) of the test sample by the mean OD of cut-off calibrators (provided with the kit for discrimination between positive and negative sera); indices >11 for SFG IgM and IgG, >1.1 for TG IgM, and >1.2 for TG IgG were interpreted as positive.

Results

A total of 329 individuals presented with fever during the survey period. Samples were available from 294 individuals. The median age of participants was 22 years (interquartile range 14–35 years) and 133 (45.2%) were male. Besides fever, the other presenting symptoms included headache ($n = 83$, 28.2%), vomiting ($n = 31$, 10.5%), rash ($n = 9$, 3.1%), altered mental status ($n = 5$, 1.7%), and seizures ($n = 3$, 1%).

IgM antibodies against SFG were detected in 13.6% (40/294) of individuals. IgM positivity was significantly higher among females ($p = 0.01$), but did not differ according to age group, educational status, or occupation. IgG antibodies against SFG were detected in 36.7% (108/294) of individuals. IgG positivity was significantly higher in individuals ≥45 years or age ($p = 0.005$); however it did not differ by sex, occupation, or educational status (Table 1).

IgM and IgG antibodies to TG were detected in 7.1% (21/294) and 15.3% (45/294) of individuals, respectively. Labourers, both agricultural and those involved in outdoor activities, had significantly higher IgM positivity ($p = 0.006$). In addition to labourers ($p = 0.008$), housewives ($p = 0.03$) and those involved in a self-run business or service ($p = 0.009$) had significantly higher IgG positivity. Neither IgM nor IgG positivity differed significantly according to age group, sex, or educational status (Table 1).

Four individuals (1.4%) had antibodies against both SFG and TG. IgM seropositivity for scrub typhus was 11.9% ($n = 35$) and IgG seropositivity was 50.7% ($n = 149$).

Discussion

This report represents the first serological evidence for SFG and TG rickettsioses among individuals with a febrile illness in Gorakhpur, one of the regions worst affected by AES in India. Variable seroprevalence rates for SFG and TG rickettsioses have been reported worldwide, which could be attributed to geographical differences and the diagnostic techniques employed (Khan et al., 2016; Bolaños-Rivero et al., 2011; Daniel et al., 2002; Faruque et al., 2017; Thiga et al., 2015).

The study findings are comparable to those of studies from the north-east region of India, Bangladesh, and Kenya (Khan et al., 2016; Faruque et al., 2017; Thiga et al., 2015). Among the SFG rickettsiae, Indian tick typhus caused by *R. conorii* is one of the most common rickettsial infections reported from India (Khan et al., 2016; Anon, 2009). The geographical setting, warm climate, and presence of pet and stray dogs living in close vicinity to

Table 1
Characteristics of febrile patients tested for Spotted Fever Group (SFG) and Typhus Group (TG) rickettsiae IgM and IgG antibodies, Gorakhpur, India.

Characteristic	Number of individuals (%)	SFG IgM positives (%)	OR (95%CI)	AOR (95%CI)	SFG IgG positives (%)	OR (95%CI)	AOR (95%CI)	TG IgM positives (%)	OR (95%CI)	AOR (95%CI)	TG IgG positives (%)	OR (95%CI)	AOR (95%CI)
Gender													
Male	133 (45.2)	10 (7.5)	1	1	45 (33.8)	1		11 (8.3)	1		22 (16.5)	1	
Female	161 (54.8)	30 (18.6)	**2.82** (1.32,6.00)**	**2.66* (1.23,5.74)**	63 (39.1)	1.26 (0.78,2.03)		10 (6.2)	0.73 (0.30,1.79)		23 (14.3)	0.84 (0.45,1.59)	
Age (years)													
6–14	74 (25.2)	6 (8.1)	1	1	14 (18.9)	1	1	2 (2.7)	1	1	6 (8.1)	1	1
15–24	93 (31.6)	17 (18.3)	2.54 (0.95,6.79)	2.22 (0.82,6.04)	27 (29)	1.75 (0.84,3.65)	1.42 (0.62,3.29)	11 (11.8)	**4.83* (1.04,22.52)**	2.81 (0.51,15.49)	18 (19.4)	**2.72* (1.02,7.25)**	1.46 (0.46,4.63)
25–34	45 (15.3)	10 (22.2)	**3.24* (1.09,9.64)**	2.69 (0.89,8.19)	18 (40)	**2.86* (1.24,6.57)**	1.9 (0.59,6.17)	2 (4.4)	1.67 (0.23,12.32)	0.43 (0.04,4.34)	5 (11.1)	1.42 (0.41,4.94)	0.39 (0.08,1.93)
35–44	38 (12.9)	3 (7.9)	0.97 (0.23,4.12)	0.77 (0.18,3.31)	19 (50)	**4.29* (1.81,10.14)**	2.64 (0.75,9.28)	1 (2.6)	0.97 (0.09,11.09)	0.24 (0.02,3.69)	6 (15.8)	2.13 (0.64,7.11)	0.52 (0.11,2.57)
≥ 45	44 (15)	4 (9.1)	1.13 (0.30,4.26)	1.07 (0.28,4.08)	30 (68.2)	**9.18*** (3.88, 21.72)**	**5.02* (1.47,17.13)**	5 (11.4)	4.62 (0.86,24.90)	1.14 (0.15,5.68.82)	10 (22.7)	3.33* (1.12,9.94)	0.85 (0.19,3.69)
Occupation													
Students	126 (42.9)	19 (15.1)	1		27 (21.4)	1	1	5 (4)	1	1	11 (8.7)	1	1
Housewives	82 (27.9)	16 (19.5)	1.36 (0.66,2.84)		37 (45.1)	**3.02*** (1.64,5.54)**	1.04 (0.36,3.04)	6 (7.3)	1.91 (0.56,6.48)	3.87 (0.86,17.42)	14 (17.1)	2.15 (0.93,5.00)	**3.80* (1.16,12.50)**
Service / business	25 (8.5)	-	1.07 (0.33,3.48)		10 (40)	2.44 (0.99,6.05)	1.01 (0.31,3.33)	3 (12)	3.30 (0.74,14.82)	5.37 (0.94,30.70)	7 (28)	4.07*** (1.39,11.85)	**6.01** (1.55,23.30)**
Agriculture & non-agriculture labourers	41(13.9)	4 (16)	-		26 (63.4)	**6.36*** (2.96,13.66)**	2.55 (0.93,6.98)	7 (17.1)	**4.98** (1.49,16.69)**	**7.68** (1.82,32.48)**	10 (24.4)	3.37*** (1.31,8.67)	**4.99** (1.52,16.39)**
Unemployed	20 (6.8)	1 (5)	0.29 (0.04,2.35)		8 (40)	2.44 (0.91,6.58)	1.23 (0.39,3.91)	0	-		3 (15)	1.85 (0.47,7.29)	1.92 (0.42,8.72)
Education													
Literate	216 (73.5)	34 (15.7)	1		64 (29.6)	1		15 (6.9)	1		31 (14.4)	1	
Illiterate	78 (26.5)	6 (7.7)	0.45 (0.18,1.11)		44 (56.4)	**3.07*** (1.80,5.25)**		06 (7.7)	1.12 (0.42,2.98)		14 (17.9)	1.31 (0.65,2.61)	

OR, odds ratio; CI, confidence interval.
Boldface indicates statistical significance. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

humans (which act as reservoirs for *Rhipicephalus sanguineus* ticks), favour the infection in Gorakhpur. Women are more likely to come into contact with ticks that infest pets and eventually lodge in the floors and walls of houses, which may explain the high seropositivity observed in the female participants. In line with earlier studies, higher IgG seropositivity was observed among older individuals, which may be attributed to their longer duration of exposure (Bolaños-Rivero et al., 2011; Daniel et al., 2002).

With regard to TG, although *R. typhi* and *Rickettsia prowazekii* cannot be differentiated serologically, it is posited that *R. typhi* was probably the cause of the observed seropositivity, because conditions for transmission of *R. prowazekii*, like louse infestations, have not been observed in the region. Furthermore, an ecosystem favourable for rodents, houses made up of mud with crevices, and the lack of rodent control measures in this region support the presence of murine typhus. In corroboration with earlier reports, individuals involved in outdoor activities and housewives who are at risk of contact with rodents had significantly higher *R. typhi* seropositivity (Daniel et al., 2002).

Fever and headache were the most common presenting symptoms; however rash was relatively infrequent. This finding is in agreement with reports from India, where an absence of rash in SFG rickettsioses has been noted (Kumar et al., 2011; Mahajan et al., 2007). This may be due to the presence of relatively mild and inconsistent rash, which may not be easily visible on dark skinned people (Kumar et al., 2011).

This study was limited by the availability of a single acute phase serum sample, making definitive diagnosis of current infection difficult; however this remains the only seroevidence for SFG and TG rickettsiae in Gorakhpur.

In conclusion, the study results present serological evidence and highlight the endemicity of SFG and TG rickettsiae in addition to scrub typhus in the Gorakhpur region. There is an urgent need for further prospective studies to explore the role of SFG and TG rickettsiae as potential causes of AES in the region.

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Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

AM, SK, and RG conceived the study; SK and AM designed the study protocol; MKS carried out the clinical assessment; MR and AN carried out the laboratory assays and statistical analysis of the data. AM drafted the manuscript; AM, SK, and RG critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. AM and SK are guarantors of the paper.

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