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Seroprevalence of Scrub Typhus, Spotted Fever, and Murine Typhus in Vellore District, Tamil Nadu

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Abstract

Background: Rickettsial infections remain under-diagnosed and rarely reported due to limited data on seroprevalence and lack of diagnostic facilities in India. The aim of this study was to investigate the seroprevalence of *Rickettsial* infection in Vellore district, Tamilnadu. **Methodology:** A descriptive cross-sectional serosurvey was conducted from September 2017 to February 2018 in seven urban and seven rural areas of Vellore districts of Tamil Nadu. Serum samples were collected from 536 healthy individuals and tested by Enzyme-linked immunosorbent assay (ELISA) for Scrub typhus (ST), Spotted fever (SF), Murine typhus(MT) antibodies at the Christian Medical College (CMC), Vellore. **Results**: Of the 464 participants, 178 (33.2%) were males and 356 (67%) were female. The median age of participants was 42 years (IQR 31-54 years). The majority were either farmers or housewives. Exposure to rickettsial infection was observed in more than 1/3rd of the population surveyed. ST was the commonest among the three diseases surveyed. The seroprevalence of ST, SF, and MT was higher in rural areas than in urban areas. Seroprevalence of dual infection was also calculated and the commonest combination being ST and SFG infections 2.2% (12/536) followed by MT and SF infections 0.3% (2/536). **Conclusion**: Rickettsial infections are common in the Vellore district and prevalence is higher in rural than urban areas.

Keywords
Vellore; Scrub typhus; Spotted fever group rickettsiae; Typhus group rickettsiae

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INTRODUCTION:

Rickettsial infections are caused by bacterial organisms (Rickettsiae), which are found throughout the world (1). They are caused by obligate intracellular Gram-negative bacteria of genera *Rickettsiae* and *Orientia*. The infections are transmitted to humans through bites of infected arthropod vectors, such as fleas, mites, ticks, lice and chiggers (2). The genus *Rickettsia* comprises more than 30 species and traditionally characterized into two main groups, the spotted fever group (SFG) and the typhus group (TG), which are the main established human pathogens (3).

R.rickettsii, *R.conorii*, *R.prowazekii* and *R.typhi* whic h cause Rocky Mountain spotted fever (RMSF), Mediterranean spotted fever (MSF), epidemic and endemic typhus respectively are the important members of the genus *Rickettsiae* (4). The *Orientia* genus comprises two species; *O.tsutsuga mushi* and *O.chuto* together forming the (STG) scrub typhus group (5). SFG and TG rickettsiae infections have a worldwide distribution and are a significant cause of morbidity in Southeast Asia (6). STG was originally thought to be confined to be the Asia-Pacific region but now has been reported from the Middle East, Africa, and South America(1). Rickettsioses are both emerging and re-emerging

infections (7).In India, the rickettsial disease has been demonstrated from various parts of the country (1). The Vellore is in a tropical savanna climate at an average elevation of 288meter {148-909 meter} (8). The district covers an area of 6,075km2 and a housing population of 3,936,331 as reported by the 2011 census (9). The average minimum temperature ranges from 18.2°C (64.8 °F) to 26.5°C (79.7°F) whereas the average maximum temperature range from 28.9°C(84°F)to 38.2°C(100.8°F). The humidity ranges from 40%–63% during summer and 67%–86% during winter. This data was obtained from http://www.imdchennai.gov.in/. This study was performed to determine the seroprevalence associated with scrub typhus, spotted fever, murine typhus in Vellore district in Tamil Nadu.

METHODS:

Study population

The descriptive cross-sectional serosurvey was carried out from September 2017 to February 2018. Seven (rural) villages and seven Urban (town) are in the Vellore district were selected for serosurvey. Study areas were randomly selected from a list that was derived based on 13 years (2005-2017) of scrub typhus data. The selected urban and rural areas are as depicted in **Fig 1**. In each area, one individual from one household was enrolled after obtaining informed consent. Clotted blood samples were collected in red-capped serum tube, (BD Vacutainer, Franklin Lakes, NJ, USA) from eligible, consenting adults (> 18 years old) who had no history of fever in the past 3 months.

Laboratory analysis.

The serum was separated by centrifuging in a refrigerated centrifuge (Eppendorf Centrifuge 5804 R, Eppendorf, Hamburg, Germany) at 3000 rpm for 10minutes at 4^{o}C. IgG antibodies to *O.tsutsugamushi* (56kDa antigens from Karp, Kato, Gilliam, and TA716 strains) were detected in serum using the scrub typhus IgG ELISA (InBios International Inc., Seattle, WA). Murine typhus IgG antibodies were detected using R.typhi IgG ELISA (Fuller Laboratory, CA) which covers the species-specific protein rOmp B. For spotted fever IgG, we used the *Rickettsia conorii* IgG ELISA (Vircell, Granada, Spain) as per the manufacturer's instructions (14). All sera were tested at 1:100 dilution and an OD cutoff value 1.5 was considered positive as described previously (10).

Statistics: Data was entered into an Excel sheet (Excel 2016, Microsoft Corporation, Redmond, WA, USA). Median, percentage, and IQR using the Excel2016. Population data of the areas surveyed was obtained by accessing the Vellore census data (9). Seroprevalence was calculated using the online calculator developed and maintained by the Clinical and Translational Science Institute, UCSF (11), and uses the Reiczigel algorithm to calculate true prevalence (12).

RESULTS:

Serum samples of 536 adults from the Vellore district, 219 urban and 317 rural were subjected to IgG ELISA for detecting antibodies to Scrub typhus, murine typhus, and spotted fever. The median age of participants was 49 years (IQR 31-54 years), 178 were (33.2%) males, and 356 (67%) females. The most common occupation was a farmer (37%) followed by housewives (35%). Seroprevalence of ST, SF, and MT is as shown in **Fig 2, 3 and 4**.There was a significant difference in the prevalence of these three diseases between the rural and urban populations studied as shown in Table 2. Prevalence in rural areas varies from 4%-56%(ST), 3%-39%(SF), 1%-10%(MT) and in urban areas it was 3%-40%(ST), 2%-11%(SF), 2%-5%(MT).

Table 1: Results of the sero-survey with cases expected in the study areas.

Area	Population	n	Sero-positive			Expected cases in each area		
			ST	SF	MT	ST	SF	MT
Kaniyambadi *	9597	31	1	2	0	310	619	310
Latteri *	8522	34	8	4	2	2005	1003	501
Serkadu *	3455	61	24	10	0	1359	566	57
Sirukanchi *	1163	81	22	3	5	316	349	72
Thimiri *	4939	33	14	1	0	2095	150	150
Ussoor *	3248	29	2	0	0	224	112	112
Vadakadapanthangal *	1111	48	12	2	4	278	46	93
Rural	**30924**	**317**	**83**	**20**	**11**	**6309**	**2799**	**1295**
Alamelumangapuram	7900	36	2	1	0	439	219	219
Bagayam	9898	31	6	2	0	1916	319	319
K.V.Kuppam	5321	23	7	1	0	1619	231	231
Kaspa	4020	33	0	0	0	122	122	122

Thottapalayam	6000	32	3	0	1	563	188	188
Thuthipattu	2880	34	10	3	0	847	254	85
Arcot	5955	30	1	0	0	199	199	199
Urban	**41974**	**219**	**29**	**7**	**1**	**5705**	**1532**	**1363**
Total (Rural & Urban)	**74009**	**536**	**112**	**29**	**12**	**15465**	**4004**	**1657**

Legend: *Rural areas, others are all urban areas

Table 2: Prevalence of rickettsioses in Vellore district

Area	ST Prevalence		SF Prevalence		MT Prevalence	
	Raw	True (95% CI)	Raw	True (95% CI)	Raw	True (95% CI)
Kaniyambadi *	3.23	3.95 (2.34-5.12)	6.45	7.36 (5.73-8.60)	3.23	3.01 (1.43-4.13)
Latteri *	23.53	30.44 (28.11-32.73)	11.77	14.55 (12.75-16.07)	5.88	6.59 (4.96-7.82)
Serkadu *	39.33	51.8 (48.06-55.72)	16.38	20.79 (18.43-23.02)	1.65	0.88 (0.00-2.08)
Sirukanchi *	27.17	35.37 (31.37-39.58)	30.01	39.2 (35.03-43.62)	6.19	7.01 (4.70-9.26)
Thimiri *	42.42	55.97 (52.38-59.92)	3.04	2.75 (1.12-3.97)	3.04	2.75 (1.22-3.97)
Ussoor *	6.90	7.97 (6.08-9.56)	3.45	3.31 (1.60-4.65)	3.45	3.31 (1.60-4.65)
Vadakadapanthangal*	25.02	32.46 (28.54-36.58)	4.14	4.24 (2.16-6.26)	8.37	9.96 (7.38-12.53)
Rural	**20.56**	**26.43 (24.49-28.26)**	**8.88**	**10.65 (9.09-11.83)**	**4.04**	**4.11 (2.57-5.16)**
Alamelumangapuram	5.56	6.16 (4.52-7.39)	2.77	2.39 (0.80-3.52)	2.77	2.39 (0.80-3.52)
Bagayam	19.36	24.81 (22.72-26.76)	3.22	3 (1.42-4.12)	3.22	3 (1.42-4.12)
K.V.Kuppam	30.43	39.77 (36.89-42.76)	4.34	4.52 (2.85-5.78)	4.34	4.52 (2.85-5.78)
Kaspa	3.04	2.75 (1.09-4.01)	3.04	2.75 (1.09-4.01)	3.04	2.75 (1.09-4.01)
Thottapalayam	9.38	11.33 (9.52-12.82)	3.13	2.88 (1.26-4.06)	3.13	2.88 (1.26-4.06)
Thuthipattu	29.41	38.39 (35.20-41.71)	8.82	10.53 (8.49-12.32)	2.95	2.58 (0.88-3.92)
Arcot	3.34	3.16 (1.54-4.36)	3.34	3.16 (1.54-4.36)	3.34	3.16 (1.54-4.36)
Urban	**13.59**	**17.02 (15.36-18.39)**	**3.65**	**3.58 (2.05-4.62)**	**3.25**	**3.04 (1.50-4.07)**
Total (rural & urban)	**20.90**	**26.89 (24.47-28.67)**	**5.41**	**5.96 (4.45-7.01)**	2.24	**1.67 (0.12-2.69)**

Legend: * Rural areas, others are all urban areas

Figure 1: Map of Tamil Nadu showing the Vellore district with the location of urban and rural primary sample collected.

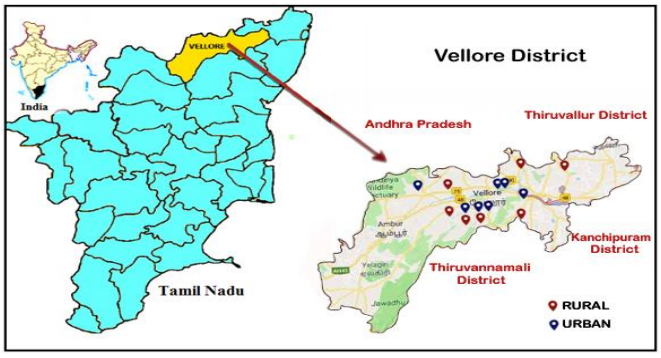
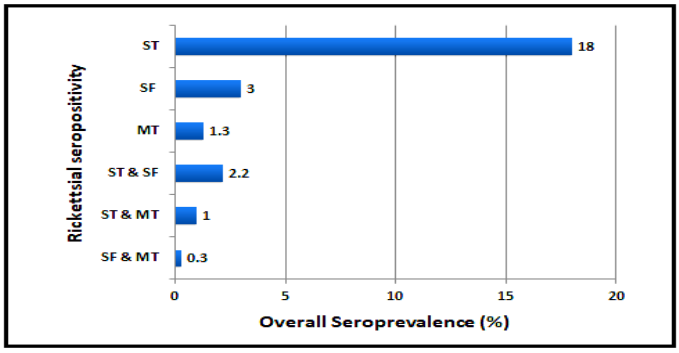


Figure 2: Overall seroprevalence of rickettsial infections in Vellore district, Tamil Nadu



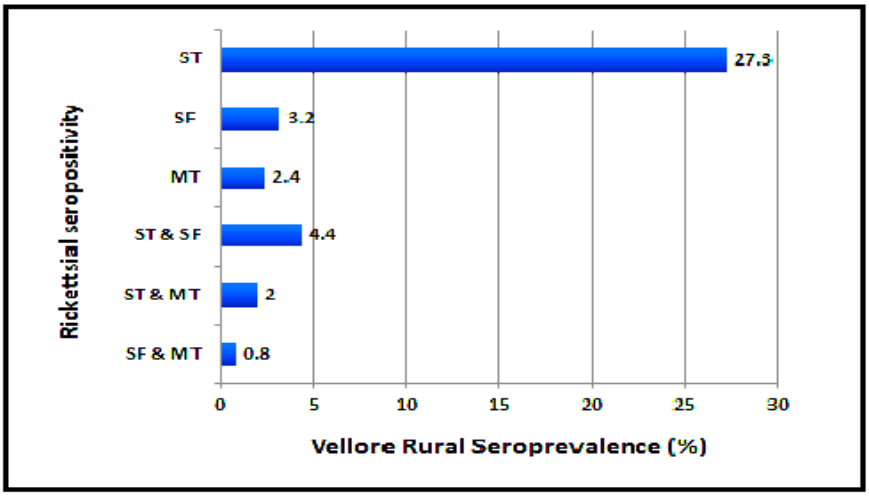


Figure 3: Prevalence of rickettsial seropositivity in Vellore district of Tamil Nadu. (Rural)

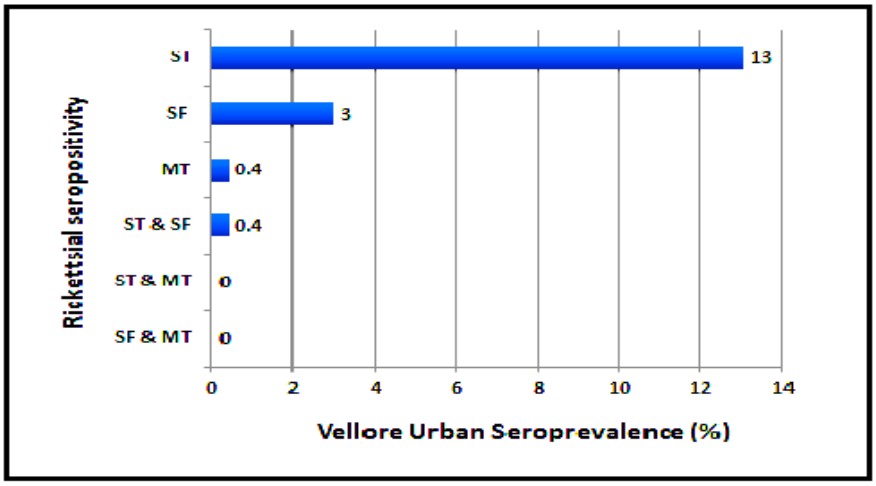


Figure 4: Prevalence of rickettsial seropositivity in Vellore district of Tamil Nadu. (Urban)

DISCUSSION:

Rickettsial infections are commonly recognized as an important cause of acute undifferentiated fever throughout India (10). The epidemiology, ecology, and clinical characteristics of the rickettsioses are highly variable and depend on geography (13).

This study demonstrated that almost 30% of the population of the Vellore district had previous exposure to rickettsial agents. Scrub typhus prevalence was highest followed by spotted fever and Murine typhus. Dual infections were also observed. Evidence of past exposure to two rickettsial agents was seen in 6.2% of the participants of which the majority were for scrub typhus and spotted fever (80%). Cross-reactions between antibodies within the rickettsia species, especially between SF and MT, are known to occur. This is most likely due to multiple infections in an endemic setting or could be possible cross-reactions (14).

A study done in Vellore stated that the prevalence of scrub typhus is 20.3 in healthy individuals in rural Vellore (15) and 15% among blood donors (16). The study by Devamani et al also reported the highest incidence of scrub typhus(20.4%) followed by spotted fever(10.4%) and murine typhus(5.4%) among the south Indian population(10). Comparable results have been demonstrated in NE India by Khan et al(17) who reported seropositivity of 30.8%, 13.8%, and 4.2% among ST, SF, and MT group respectively, while by Mane et al (18) reported IgG seropositivity of 36.7% and 15.3% for SF and MT respectively from Gorakhpur, Uttar Pradesh.

In south-east Asia, murine typhus was reported more in urban dwellers, while STG and SFG were more

prevalent in rural dwellers (19). ST and SF were definitely more common in rural regions whereas murine typhus was marginally more common in rural areas of the Vellore district. The variation in prevalence among rural and urban areas may be due to the topographical differences of these areas. Limitations of this study were: Children were not included; longitudinal sampling was undertaken based on known hot spots of ST (derived from occurrence of 2 cases every year for the last 5 years). Future cohort studies that involve active fever surveillance will be more useful in assessing the prevalence.

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