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Scrub Typhus in South India: a Re-Emerging Infectious Disease

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Scrub typhus (ST) caused by *Orientia tsutsugamushi* and transmitted by chiggers of trombiculid mites is an important emerging infectious disease in India. Without early intervention, ST is known to cause serious complications and high morbidity; thus, awareness of this disease among physicians and availability of serological kits can contribute to the control of this rickettsiosis. A serological survey conducted in the 1970s revealed the prevalence of ST in few parts of India (1); however, outbreaks and cases of ST started to appear in several states during the last 10 years, some of which occurred in unanticipated areas (2–18). ST outbreak in 28 adults from October 2001 to February 2002 was first reported in Vellore, Tamil Nadu (3). From the Union Territory of Pondicherry (South India), 50 cases of ST (mostly adults) were recorded by Vivekanandan et al. (13) during a 2-year period (April 2006–April 2008) based on clinical features and results of the Weil–Felix (WF) test. After a gap of 4 years, we encountered 26 presumptive ST cases in adults during 5-month period (November 2011–March 2012). During the same period, we encountered 40 presumptive ST cases in children (19). Hence, there exists a possibility of the re-emergence of ST in this part of South India. This preliminary communication of 26 presumptive adult ST cases within a short period of 5 months is aimed at alerting physicians so that these cases in the Asian community are not dismissed as cases of fever of unknown origin (FUO).

During 5 relatively cooler months (November 2011–March 2012), 58 adults with FUO from Pondicherry and neighboring Tamil Nadu were screened for ST. The patients were admitted to Mahatma Gandhi Medical College & Research Institute, Pondicherry, a super specialty tertiary care teaching hospital. This work was scrutinized and approved by the institutional research and human ethical committees of Mahatma Gandhi Medical College & Research Institute.

Of the 26 ST cases positive for IgM by ELISA, 9 were aged 21–30 years, 5 were aged 31–40 years, 7 were aged 41–50 years, 2 were aged 51–60 years, and 3 were aged 61 years or more. The youngest patient was 21 years old and the eldest was 75. All the patients presented with a history of fever for 1–3 weeks and clinical features, in-

cluding headache, myalgia, hepatosplenomegaly, conjunctival suffusion, eschar, and other symptoms suggestive of ST. Eschar is an important sign of ST; however, it is difficult to be identified on dark skin. In Southeast Asia and India, the number of adults with eschar varied from none (20) to many (3,4,13,16). Only 1 of our patients exhibited umbilical eschar. However, it is likely that patients are not aware of the presence of eschar or may have ignored it and therefore did not mention it to their physicians, which again emphasizes the need for a thorough physical examination, because eschars are predominantly observed in the covered areas of the body and cultural stigma may be a hindering factor. Fever and headache/myalgia occurred in all patients, splenomegaly in 3, hepatomegaly and lymphadenitis in 2 each, and retro-orbital pain/conjunctival suffusion in 2. Informed consent was obtained from all the patients before collecting blood samples for complete blood count, liver enzyme profiles, and other tests. Before proceeding to a work-up for ST, our clinicians routinely considered common diseases prevalent in this part of India, such as tuberculosis, malaria, typhoid, dengue, and leptospirosis.

Comprehensive patient data, including salient clinical features, platelet counts, liver enzyme levels, and specific and non-specific ST serology results are presented in Table 1. Thrombocytopenia was present in 73.91% (17/23), and one or more liver enzymes were elevated in 100% (23/23) of the sera samples tested. Patients with clinical suspicion of ST were first screened by a rapid immunochromatographic test (RICT) (SD Bioline Tsutsugamushi kit; Standard Diagnostics, Seoul, Korea), which incorporated 56-kDa major surface protein present in 3 strains of *O. tsutsugamushi* (Karp, Gilliam, and Kato) to detect IgG/IgM/IgA antibodies to *O. tsutsugamushi*. The WF test was performed using colored antigens against *Proteus vulgaris* OXK (Plasmatech, South El Monte, Calif., USA). Sera positive in ST RICT were preserved at –20°C for ST IgM ELISA with the Scrub Typhus IgM Detect kit (InBios International, Seattle, Wash., USA). The ELISA plates were coated with 10 recombinant antigens of *O. tsutsugamushi*. All the tests were performed with strict adherence to the manufacturer's instructions. As a control, 12 healthy blood donors were included in all 3 tests. Thirty FUO-negative cases via ST RICT screening were also tested for the presence of *Proteus* OXK agglutinins. ST IgM ELISA could not be performed for these cases. All the 12 blood donors were serologically negative for ST by both RICT and IgM ELISA. Of the 28 patients who

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Table 1. Clinical and laboratory findings of presumptive ST patients

Sample no.	Age/sex	Fever (days) ¹⁾	AST (U/L)	ALT (U/L)	ALP (U/L)	Platelets/mm ³	Liver/spleen/LN/eschar/other infections	OXK titer
1	42/F	17	216	158	545	80,000	LN-neck, inguinal, axillary	1:320
2	58/F	10	45	32	167	74,000	Typhoid (Widal positive)	1:40
3	50/F	10	160	80	341	55,000	Hepatomegaly + eschar	1:40
4	30/F	7	375	526	615	104,000		1:40
5	32/F	5	266	140	711	150,000	Leptospirosis (IgM)	1:160
6	43/F	9	NT	NT	NT	110,000		1:80
7	21/F	14	351	222	179	45,000	Dengue (NS1 ag)	1:80
8	32/F	20	89	82	89	117,000		<1:20
9	30/F	15	21	16	111	324,000		1:320
10	25/F	7	58	31	315	109,000		1:80
11	62/F	7	44	54	292	56,000		1:40
12	37/M	4	75	54	130	114,000		1:2,560
13	21/M	7	105	79	121	84,000	Splenomegaly	<1:20
14	42/M	6	26	20	107	NT		<1:20
15	75/F	10	134	70	243	84,000		<1:20
16	45/F	15	71	42	326	112,000	Hepatosplenomegaly	1:640
17	21/M	90	21	13	139	100,000	LN + malaria (Pl)	1:20
18	23/M	7	77	47	80	172,000		<1:20
19	50/F	15	27	18	203	263,000		<1:20
20	42/F	6	34	21	141	80,000	Splenomegaly	1:40
21	38/F	6	NT	NT	278	306,000		1:20
22	56/F	22	70	45	190	NT		1:2,560
23	75/M	10	NT	NT	NT	160,000		1:40
24	32/M	15	NT	NT	NT	126,000		1:20
25	27/F	15	246	253	271	115,000		1:640
26	22/F	10	114	65	158	NT		1:320
27	85/F	7	NT	NT	NT	179,000		1:20
28	24/M	4	44	41	130	146,000		<1:20

Only acute serum samples could be tested.

Sample no. 1-26 were positive in both rapid immunochromatographic test (RICT) and IgM ELISA; no. 27-28 were positive by RICT but negative by IgM ELISA.

Indirect fluorescent antibody/micro immunofluorescence assay for scrub typhus (ST) not done due to its non-availability in India.

¹⁾: Refers to sampling date.

AST, aspartate aminotransaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LN, lymph node; NT, not tested; Pl, plasmodium vivax.

were positive by ST RICT, only 26 (92.86%) were confirmed by ST IgM ELISA. *Proteus* OXK agglutination titers of these 28 patients were 1:2,560 (2 cases), 1:640 (2), 1:320 (3), 1:160 (1), 1:80 (3), 1:40 (6), 1:20 (4), and <1:20 (7). The OXK titers of the 12 healthy blood donors were 1:40 (5), 1:20 (5), and <1:20 (2). OXK titers of 30 ST RICT-negative FUO patients were 1:80 (2), 1:40 (9), 1:20 (10), and <1:20 (9).

The WF test is a non-specific commonly used test to detect typhus, spotted fevers, and ST. In India, an OXK titer $\geq 1:80$ is considered significant (4,6,13,16), and some perform the gold standard indirect fluorescent antibody/micro immunofluorescence (IFA/MIF) assay and correlate the results with the WF test at this titer (8,12). A single OXK titer $\geq 1:320$ or a 4-fold increase in paired sera for *Proteus* OXK is accepted as significant for ST (8,10). Because all the sera samples were obtained from patients with a single acute onset of infection, we followed this criterion; and 7 of our 26 ST IgM ELISA-positive patients (26.92%) had *Proteus* OXK titers ranging from 1:320 to 1:2,560. For the serological confirmation of ST, IFA is the gold standard (3,4,8,21,22); however, this test is difficult to perform, cumbersome, requires a skilled observer, mandates in-

clusion of several antigenic types, subjective, and too expensive to be imported. The sensitivity and specificity of ST IgM ELISA are almost equivalent to those of IFA, and it can be performed by most laboratories (21,22). We employed the ST IgM ELISA kit and ST IgM/IgG ELISA (Panbio, Brisbane, Australia), which have been sufficiently validated in India (3,4,7,18). RICT for the detection of ST is a simple and rapid point-of-care test; however, there are conflicting reports regarding its sensitivity and specificity, ranging from very low (21) to moderate/good (18,23-26). In our experience, RICT has shown a good correlation (92.86%) with conventional ST IgM ELISA. However, caution should be exercised for use of this assay, and further studies involving larger sample sizes and meta-analysis studies are warranted to exclude the possibility of false-positive results in other clinical conditions. The quality of the imported kits and maintenance of appropriate storage conditions from the time of import until usage are two essential requirements for consistent and satisfactory performance of these rapid identification kits. Interpretation and clinical correlation is always essential to arrive at a correct diagnosis and initiate appropriate treatment. In the present study, patients positive for ST

by RICT and presenting with other supportive clinical/laboratory findings were first empirically treated with doxycycline/ceftriaxone/azithromycin. Of the 28 patients, 16 received doxycycline, 11 received ceftriaxone, and a pregnant patient received azithromycin. Whenever positive ST RICT results were conveyed to the clinicians, the patients receiving ceftriaxone were administered an additional course of doxycycline. All the patients responded well, and the body temperatures returned to normal limits within 1–3 days. Four ST patients had mixed infections with leptospirosis, dengue, vivax malaria, and typhoid.

In conclusion, the frequent reports on ST outbreaks from different parts of India indicate the need to identify and map “typhus islands” and “mite islands.” Although the WF test is affordable and within the reach of even small laboratories, it is non-specific and has low sensitivity; thus, it must be interpreted in the correct clinical context. Hence, ST RICT may be recommended as a point-of-care test in resource-poor settings alone, where IFA/PCR facilities are not available. ST IgM/IgG ELISA kits are suited for batch testing of samples and serological surveys in district level laboratories. The gold standard IFA/MIF and PCR for the detection of ST will be made available in India; however, these kits require standardization under Indian conditions by incorporating strains isolated from India before they can be recommended for regular use.

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Conflict of interest None to declare.

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