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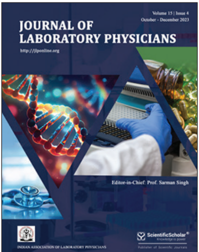
Journal of Laboratory Physicians



Letter to Editor

Laboratory diagnosis of non-scrub rickettsiosis – An Indian scenario

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Received: 13 October 2023
Accepted: 18 October 2023
EPub Ahead of Print: 15 February 2024
Published: 08 June 2024

DOI
10.25259/JLP_9_2023

Quick Response Code:

Dear Editor,

In a setup of reducing the prevalence of malaria, a recent study from Uganda[1] ascertains spotted fever and typhus fever as a cause of acute undifferentiated febrile illness in 33.8% of cases. Yet, in India, rickettsiosis apart from scrub typhus is rarely investigated. Rickettsiosis is caused by organisms of genus *Rickettsia* and Orientia that are obligate intracellular Gram-negative coccobacilli maintained in nature in arthropods. Very few studies in India have used immunofluorescent assay (IFA) and they have focused on *R. conorii* causing Indian tick typhus and *R. typhi* causing flea-borne endemic typhus, while other species remain uninvestigated.[2] Genus *Rickettsia* is classified into four groups: An ancestral group with *Rickettsia bellii* and *Rickettsia canadensis*; spotted fever group (SFG) containing *Rickettsia rickettsii*, *Rickettsia conorii (R. conorii)* etc.; typhus group (TG) having *Rickettsia prowazekii* and *Rickettsia typhi (R.typhi)*; and transitional group with *Rickettsia akari, Rickettsia australis*, and *Rickettsia felis*. Recent studies depicting seroepidemiology of the SFG and TG group rickettsial illness in the Indian subcontinent are depicted in Table 1.[3-10]

Serological tests form the mainstay of laboratory diagnosis. However, they have many limitations as follows: (1) Diagnosis is possible to serogroup level only (SFG and TG), (2) confirmed diagnosis rests on seroconversion or four-fold rise in immunoglobulin G (IgG) antibody titers of paired sera taken 7–14 days apart because of missing baseline titers as well as persistence of immunoglobulin M (IgM) antibodies after clinical cure. A single rise of IgG aids clinical diagnosis without confirming infection, (3) appropriate antigen as per prevailing species should be used for these assays, and (4) IgM antibodies may be demonstrated in patients for whom no other clinical signs of a recent rickettsiosis.

Weil-Felix test is the oldest and cheapest serological assay but has low sensitivity with fair specificity of detection and a titer of >1:80 taken for positivity in Indian studies. Enzyme-linked immunosorbent assay (ELISA) (IgM and IgG) is the most widely used qualitative assay because of good sensitivity, specificity, and ease of use. Elongation factor thermal unstable (EF-Tu), 60 KDa chaperonin (groEL), and putative outer surface protein (adr2) are non-specific antigens, while recombinant truncated outer membrane proteins A and B (omp A and omp B) antigens are 100% specific and 90% sensitive. For murine typhus, *R. typhi* IgG ELISA (Fuller Laboratory, Fullerton, CA) covers specific protein Omp B, and for spotted fever, *R. conorii* ELISA IgG/IgM (Vircell, Granada, Spain) has been extensively evaluated in India. Immunofluorescent and immunoperoxidase assays are gold-standard quantitative assays with high sensitivity and specificity and can aid in species detection among serogroups but are technically demanding. Micro-immunofluorescence assay (IFA) is a modification of IFA where multiple species can be detected at once using indirect immunofluorescence. An IFA cut-off titer of >1:128 for SFG with *R. conorii* IgG IFA test (Fuller

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Table 1: Recent studies from India showing seroepidemiology of spotted fever and typhus fever groups in India.

Area	Year	Study settings	Test used	Number of patients	Type of patients	Common age group	Sex predilection	Season	SFG	TG	Reference
North India											
Lucknow	2013–2015	Prospective	IgM/IgG ELISA IFA	432	AUFI	0–15	M (54%)>F (46%)	July–October	26.6%	-	Tripathi *et al*., 2017[3]
Chandigarh	April and May 2019	Prospective	*glt* nested PCR	200	AUFI	-	F (57%)>M (43%)	-	4%	3%	Biswal *et al*., 2020[4]
Gorakhpur	2016	Retrospective	IgM/IgG ELISA using *R. conorii* IgM/IgG *R. typhi*	294	>6 years AUFI during an AES outbreak	15–24 – IgM SFG seropositivity>45 years - IgG SFG seropositivity	F (55%)>M (45%) for SFG No sex predilection in TG	-	IgM- 13.6% IgG- 36.7	IgM- 7.1% IgG- 15.3%	Mane *et al*., 2019[5]
South India											
Puducherry	2012–2015	Prospective	ELISA by paired sera using *R. conorii* IgM/IgG	320 paired sera 195 adults (>18 years) 125 children (<18 years)	AUFI	-	-	-	Adult- 46.6% Children- 40.8%	-	Stephen *et al*., 2019[6]
Tamil Nadu	2017–2020	Prospective	IgG ELISA using *R. conorii* *IgG* ELISA *R. typhi*	2565	Healthy adults	46–60 years for SFG	-	-	6%	2.1%	D'Cruz *et al*., 2022[7]
Tamil Nadu	2020	Crosssectional	IgG ELISA using *R. conorii* *IgG* ELISA *R. typhi*	1353	Healthy adults in scrub affected area	No age predominance	SFG- No gender predominance TG-F>M (RR=1.8)	-	10.4%	5.4%	Devamani *et al*., 2020[8]
North Eastern states											
North Eastern states	2016–2018	Cross sectional	*R. typhi IgG* ELISA	762 AES and 1437 with FUO	AES and AUFI	>15 years	M>F in Manipur and Tripura M=F in Nagaland	May–July in Manipur and Tripura March-June in Nagaland	-	2.7% in AUFI 3.93% in AES	Khan *et al*., 2019[9]
Assam, Arunachal Pradesh and Nagaland	2016	Cross sectional	IgM/IgG ELISA using *R. conorii* *IgM/IgG R. typhi*	1265	Healthy people in scrub affected area	-	-	-	13.8%	4.2%	Khan *et al*., 2016[10]

SFG: Spotted fever group, TG: Typhus fever group, AUFI: Acute undifferentiated fever illness, AES: Acute Encephalitis syndrome, FUO: Fever of unknown origin, M: Male, F: Female, RR: Risk ratio, ELISA: Enzyme-linked immunosorbent, PCR: Polymerase chain reaction, IgM: Immunoglobulin M, IgG: Immunoglobulin G, *R. conorii: Rickettsia conorii, R. typhi: Rickettsia typhi*, IFA: immunofluorescent assay.

Laboratories, Fullerton, California, USA) is adopted in Indian studies. A new Dip-S-Ticks (INDX Integrated Diagnostics Inc., USA) test is available as a screening tool for rickettsia infections before performing the IFA as a confirmatory test.

Molecular tests in conventional polymerase chain reaction (PCR)/nested PCR/quantitative PCR formats in samples such as whole blood, buffy coat, serum, tissue biopsies (such as skin), eschar scrapings, swabs from a deroofed eschar, and cerebrospinal fluid are different samples to detect infection in the early stage of disease. Sensitivity of detection in the blood is reduced in view of low bacteremia in the early stages of disease. PCR with multiple targets, first to detect rickettsial genes (citrate synthase [gltA], 16Svedberg ribosomal ribonucleic acid [16S rRNA], and 17 Kilodalton [17kDa] lipoprotein outer membrane antigen genes) followed by SFG gene (omp A and omp B), improves sensitivity of detection. The omp A gene being specific for the SFG *rickettsia* is used to exclude a TG organism. The sensitivity and specificity of detection by molecular techniques vary with sample, timing of collection, and targeted primer.

Loop-mediated isothermal amplification (LAMP) assay having omp B target, a rapid molecular-based test was evaluated in China and had 100% specificity and 73% sensitivity when using whole blood samples. Recombinant polymerase amplification test based on the detection of 23S rRNA for SFG and 16S rRNA for all *Rickettsia* species, a rapid test format has been evaluated. The test products can further be biotin and fluorescein amidite (FAM) labeled and detected in a lateral flow assay has also been used for the detection of *R. rickettsii*. In the Indian setup, we are yet to validate and develop a reliable Point-of-care testing (POCT) tool for rickettsial infections apart from scrub typhus.

Antigen detection by immunohistochemistry and immunofluorescence techniques in paraffin blocks of biopsy of eschar is 100% specific and 70% sensitive for diagnosis. Culture of this organism with clinical samples processed through shell vial technique requires a BSL-3 setup (particularly for *R. rickettsii*) in cell line that has lower sensitivity and longer time to positivity and is reserved for reference laboratories.

With climatic change, there is emerging evidence of newer rickettsiosis in the Asia-Pacific region. Thus, large-scale studies should be undertaken in India to determine the actual burden. Further, antigenic, phenotypic, or genotypic variation and geographic differences in rickettsiosis apart from Scrub typhus should be ascertained.

Ethical approval

The Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent was not required as there were no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Kigozi BK, Kharod GA, Bukenya H, Shadomy SV, Haberling DL, Stoddard RA, *et al.* Investigating the etiology of acute febrile illness: A prospective clinic-based study in Uganda. BMC Infect Dis 2023;23:411.
2. Krishnamoorthi S, Goel S, Kaur J, Bisht K, Biswal M. A review of Rickettsial diseases other than scrub typhus in India. Trop Med Infect Dis 2023;8:280.
3. Tripathi CD, Singh M, Agarwal J, Kanta C, Atam V. Seroepidemiology of spotted fever rickettsiosis in Uttar Pradesh: A prospective study. J Clin Diagn Res 2017;11:C04-9.
4. Biswal M, Krishnamoorthi S, Bisht K, Sehgal A, Kaur J, Sharma N, *et al.* Rickettsial diseases: Not uncommon causes of acute febrile illness in India. Trop Med Infect Dis 2020;5:59.
5. 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.
6. Stephen S, Gunasekaran D, Pradeep J, Ambroise S, Sarangapani K. Serological diagnosis of Indian tick typhus in and around Puducherry: Application of indirect immunofluorescence assay. J Clin Diagn Res 2019;13:DC06-9.
7. D'Cruz S, Perumalla SK, Yuvaraj J, Prakash JA. Geography and prevalence of rickettsial infections in Northern Tamil Nadu, India: A cross-sectional study. Sci Rep 2022;12:20798.
8. Devamani CS, Schmidt WP, Ariyoshi K, Anitha A, Kalaimani S, Prakash JAJ. Risk factors for scrub typhus, murine typhus, and spotted fever seropositivity in urban areas, rural plains, and peri-forest hill villages in South India: A cross-sectional study. Am J Trop Med Hyg 2020;103:238-48.
9. Khan SA, Bora T, Saikia J, Shah A, Richards AL, Chattopadhyay S, *et al.* Seroprevalence of typhus group rickettsial infections in the North-East region of India. Indian J Med Res 2019;150:203-5.
10. 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;110:487-94.

How to cite this article: Rout D, Otta S. Laboratory diagnosis of non-scrub rickettsiosis – An Indian scenario. J Lab Physicians. 2024;16:238-40. doi: 10.25259/JLP_9_2023