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A Study on Rickettsial Infection in the Acute Fever Cases of South Gujarat's Surat Region, India Indicates Higher Incidences

Amruta R. Patil[1]* and Jignaben P. Naik[2]

[1]Research Scholar, Department of Microbiology & MLT,
Shri AN Shah Science College Kholwad, Kamrej Char Rasta, Kholwad, Surat (Gujarat), India.
[2] Head (MLT) and Assistant Professor, Department of Microbiology & MLT,
Shri AN Shah Science College, Kholwad, Kamrej Char Rasta, Surat (Gujarat), India.

(Corresponding author: Amruta R. Patil)*
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ABSTRACT: Rickettsial Infection (RI) is zoonoses disease caused by intracellular bacteria where humans were accidently involved in chain of transmission between insect and animals. Factors which serve as emerging vector borne infection are deforestation, urbanization, changing land use patterns, water control projects, loss of biodiversity, agriculture, and increased global travel, human migration and trade. Because of these factors there are close contact between vector and humans. RI occurs worldwide and clinically manifests as non-specific acute febrile illness (AFI). RI is always misdiagnosed and underreported in India because of outburst of viral fever, symptoms similarities with other common causes of AFI. RI reported from almost all parts of India apart from Gujarat, so our aim is to find out the prevalence of RI in our region by serological Weil-Felix test. Patients Serum samples (> 500) with AFIs were obtained and were tested for RI and other common causes i.e., malaria, dengue, typhoid and chikungunya. In our study for RIs screening were positive in 61.98% samples. RI detected were scrub typhus 101 followed by typhus group 92 and spotted fever group 26 during 2 years of period. High positivity was found in females and age groups of 21-40 years age. This research adds to the limited information available in the literature pertaining to RI in south Gujarat region of India. Our result provides knowledge of RI seroprevalence in our region. This in order allows for more swot of RI mainly region specific.

Keywords: RI, scrub typhus, Typhus fever, spotted fever, prevalence, Weil-Felix test.

INTRODUCTION

Zoonotic RIs occur in both urban and rural communities worldwide. RI is one of the old age diseases and recently it is recognized as re-emerging vector borne infection (Sharma *et al.,* 2020). It is classically divided into 3 major groups-Spotted Fever Group (SFG), Typhus Group Fever (TG) and Scrub Typhus Group Fever (STG) (Rungrojn *et al.*, 2021). Scrub typhus is commonest in India (Kelly *et al.,* 2009). RIs are caused by gram negative obligate intracellular alphaproteobacteria in the genus *Rickettsiae. Rickettsia, Orientia, Ehrlichia, Neorickettsia, Neoehrlichia, anaplasma* and transmitted via vectors such as ticks, fleas and lice (Rahi *et al.*, 2015). These diseases vary in severity from self-limit diseases, mild to life threatening diseases if there are many complications (van Eekeren *et al.,* 2018). Mortality due to these infections is reported to occur in 1% to 30% of untreated cases (Rathi *et al.*, 2010; Asraf Ali *et al.,* 2010).

The infection clinically manifests as non-specific AFI, which is accompanied by headache, myalgia, occasional rash, often accompanied by gastrointestinal, respiratory, or central nervous system (CNS) symptoms, which may lead to severe multi-organ dysfunction in untreated cases (Rahi *et al.,* 2015). RIs are often viewed as mild or are misdiagnosed as common ailments such as the common cold and flu (Walker, 2003). Rickettsial diseases are a serious threat to public health if not diagnosed or misdiagnosed (D'Cruz, *et al*., 2022)

In cases of AFI there are many serological, molecular and cultural diagnostics tests available. RIs are diagnosed by serological methods in developing countries like us (Mittal *et al*., 2012). Weil-Felix test is an extensively used old serological test for the diagnosis of RI. In this test some Proteus strains OXK, OX19, OXK antigen suspension were used to detect RIs ST, TG and SFG (Mittal *et al*., 2012).

Prevalence of this disease is worldwide except Antarctica (Mahajan, 2012). Rickettsial infections are re-emerging or newly emerging infections that arise especially in the tropics but are also increasingly recognised in temperate climates due to global warming (Premaratna, 2022). In India it has been documented since 1930 with scrub typhus during the Second World War (Mahajan, 2012). This disease is not so popular till last decade but in last few years there are many cases for

rickettsial disease found in the part of India from Jammu Kashmir, Himalayan, Uttarakhand, Haryana, Delhi, Assam, Odisha, Karnataka, Tamil Nadu, Kerala, Maharashtra, Madhya Pradesh, Rajasthan and West Bengal (Sharma *et al*., 2020; Rathi *et al*., 2010; Sood *et al*., 2013). Rickettsial fever has been reported to be endemic in many parts of India but data on the same is limited in Gujarat. Even though no official reports of these debilitating fever infections have been reported in Gujarat in the literature thus far, the potential of it happening cannot be disregarded

RIs are re-emerging infections with various clinical appearances and are difficult to diagnose. While the clinical presentation of RI is similar, the causative species and epidemiology can vary depending on the region. For diagnosis and treatment it is important to know the distinctive symptoms and the epidemiology of a region, as they can be associated with significant morbidity and mortality (Khamesipour *et al.*, 2018).

The main aim of this study is to reduce the gap in our knowledge of RIs in our South Gujarat region- Surat, India by following the objective to determine the seroprevalence of RIs by Weil-Felix test.

MATERIAL AND METHODS

This prospective clinical laboratory based cross-sectional study was conducted from October 2020 to December 2022 at the South Gujarat's Surat region of India. The human population used for this study were anonymous specimens collected using an ongoing testing for diagnosis of febrile illnesses. Our study did not affect the routine clinical practice of the clinic, laboratory or hospital.

Blood samples of the patients having acute fever and suspected common causes of AFI submitted to clinical laboratories of Surat region and Microbiology Department, Surat Municipal Medical College, were taken for serological testing. Samples from the patient with fever history for more than one-week, high WBC count/high ESR/High CRP were taken to be processed. Patient's age and sex related data were also collected.

2 ml of peripheral venous blood was drawn into pyrogen-free, vacuum blood collection tube without any anticoagulant and carried to the Department of Microbiology and MLT, Shri A N Shah Science College, Kholwad, Surat. To obtain serum plain vial samples were centrifuged for 5 min at 3000 rpm. Serum was separated to blood clot and collected in multiple aliquots by calibrated micropipette into separate sterile vials and stored at 2-8º C till testing was complete. All serum samples were stored at -20ºC for future use.

Total 505 samples were included in the present study for rickettsial diseases as one of the differential diagnoses. After ruling out for other diseases like typhoid (performed by Widal test and RDT), chikungunya (conducted by RDT), dengue (tested by IgM ELISA) and Malaria (confirmed by PBS and RDT) samples were obtained and analyzed for RI. Any blood samples which were grossly hemolyzed, lipemic, icteric or sera microbiologically contaminated were not included in the study.

RI can be grouped into 3 antigenically defined groups: those causing ST, SFG and TG in our study Weil-Felix heterophile antibody test which is the oldest, simple, economical test was aided for initial investigation. Samples were analyzed by Weil-Felix test using the standard protocol and manufacturer's instruction, for initial screening followed by further dilutions to achieve end titre. The antigen sets which is used in this test were Progen antigen set-1 (Tulip Diagnostics (P) Ltd., Goa, India), CRI antigen set-2 (Central Research Institute, Kasauli, India) and antigen set-3 an in-house prepared according to procedure given by Mathai *et al.,* 2001. All the sample were screened through slide agglutination test. All the serologically screening positive samples were subjected to Weil-Felix tube test for titer determination with doubling dilution of 1:20 to 1: 320. Titres of more than 1:80 for OX2 and OX19 and more than 1:160 for OXK were considered diagnostically significant Cut-off titre according to manufacturer's instructions. For the negative control 25 healthy person blood samples from volunteers and PROGEN™ polyspecific positive control (Tulip Diagnostics (P) Ltd., Goa, India) were also included in the study.

RESULTS AND DISCUSSION

In our country there are large number of possible causes of febrile cases (Crump *et al.,* 2017). Most of the time treatment will cure the infection but some time fever becomes prolong, and at that time people seek medical attention. One of the important yet poorly diagnosed causes of febrile illness is the RI (Singhi *et al.,* 2017; Beresford and Gosbell 2016).

Samples Tested for Rickettsia Antibodies. In the year October 2020 to December 2022 total 505 AFI samples of patients with more than one week fever history and high WBC count/high ESR/High CRP were obtained and analyzed with multiple serological testing (Table 1). Of the suspected 505 AFI samples 313 samples were screening positive for rickettsial antibodies.

Table 1: Distribution of samples tested for rickettsiosis by year and by analysis type.

Rickettsial Infection (RI) By Year	Sample Analysis Type							
	Malaria		Typhoid		Dengue		Chikungunya	
	RIP	RIN	RIP	RIN	RIP	RIN	RIP	RIN
2020-2021	12	71	10	120	6	47	3	11
2021-2022	20	80	23	200	10	44	0	0
Total	32	151	33	320	16	91	3	11

RIP= RI Positive; RIN=RI Negative

Gender and Age Distribution of Patients with Rickettsial Antibodies. Total 505 samples analyzed, 249 were from male patients 256 were female patients. Screening Positive 313 samples represent patients with sero-positive RI. In our study we found higher positivity of RI found in females (66.79%) than the male (58.67%). Male were highly affected by RI (Sudhindra *et al*., 2017; Udayan *et al.,* 2014; Ajantha *et al.,* 2013; ML *et al.,* 2019; Nimboor *et al.,* 2018) which is contradict to our result. High positivity was found in female than the male in the study of Mittal *et al.* (2012) and Al Amin *et al.* (2021) which is comparable to our result.

Age group which is highly affected by RI was 21-40 years (66.28%) followed by 41-60 (62.70%). Lowest positivity found in the age group > 60 years. The median age was 37 years (ranging from 4 months to 86 years). Highest positivity found in age group 11-60 (Mittal et al., 2012). In one study by Sudhindra *et al.* (2017); they found positivity in the age group of 20-29 and by Nimboor *et al.* (2018) the age group which was highly affected was 20-50 by RI.

Seroprevalence of Rickettsial species: Weil-Felix is a nonspecific agglutination test which detects anti-rickettsial antibodies in a patient's serum. The Weil-Felix test is based on cross-reactions which occur between antibodies produced in acute RIs with antigens of OX (OX 19, OX 2, and OXK) strains of Proteus species. In this RIs study of multiple OX antigen sets serology determination, we found out ≈ 313 samples were RI screening positive shown in Fig. 1. The Weil-Felix test contains three antigen sets. In total RIs samples antigen OX2 for determining antibodies against (SFG) RI tested were positive ≈86; Antigen OX19 for (TG) RI were positive to ≈ 190 and Antigen OXK suggested (ST) RI were positive to ≈ 221.

Fig. 1. Multiset Weil-Felix test Positivity results: Set 1-Tulip diagnostic; Set 2- CRI, Kasuali; Set 3-Inhouse prepared antigen.

RI seropositive sample distribution in major RI groups is as shown in Table 2. Retrospectively, RI screening positive samples were serologically tested for common causes of AFI. There were such samples who gave positive test results with two or more than two antigen sets and serological test positive with other AFI profile tests. RI and coinfection of diseases according to serology detection in AFI were as shown in Table 2

Table 2: Distribution of RIs seroprevalence samples according to serology detection.

Screening Test	Positivity
OX2 (SFG) only	12
OX19 (TG) only	66
OXK (ST) only	104
OX2 (SFG),OX19 (TG) combine	12
OX19(TG) and OXK(ST) combine	57
OXK(ST) and OX2(SFG) combine	11
OX2(SFG), OX19(TG) and OXK(ST)	51
OX2 (SFG) and malaria combine	01
OX19 (TG) and dengue combine	01
OXK(ST) and dengue combine	02
OX2(SFG) and typhoid combine	03
OX19(TG) and typhoid combine	07
OXK(ST) and typhoid combine	35
OX19(TG) and chikungunya combine	01

We found co-infection with other common causes of acute febrile illness. In our study we found typhoid samples which has highest positivity with RIs in total and of RIs scrub typhus positivity is highest. In one study they found coinfection of scrub typhus with typhoid (Seow *et al*., 2017). We also found scrub typhus with dengue which was also found by Sapkota *et al.* (2017). One study carried out by Bhattarai *et al.,* (2022) in which they found coinfection of scrub typhus dengue and typhoid.

RI screening positive samples and cut-off significant titre positive samples proportional is as shown in figure 2. 20% samples positive for scrub typhus, 18.21% samples were positive for typhus group fever and 4.75% samples were positive for spotted fever group infection by tube agglutination test. In India RI is an emerging

group of zoonosis infection particularly scrub typhus and Indian tick rickettsial fever (Varghese *et al.,* 2013). One study carried out by Sudhindra *et al.* (2017) in which they found 19.3 % samples were positive for scrub typhus followed by typhus group fever (7.33%) and spotted group fever (6.66%) which is similar to our study. In one study they found 38.84% were reactive to OX2 (spotted fever group), 25% were reactive to OXK (Scrub typhus) & 5% showed significant titers to OX19 (typhus fever) (Al Amin *et al*., 2021). Rickettsial diseases were detected in 26.35% samples in the Karnataka region by Mita *et al.* (2016) which is lower than our study.

Maximum number of RI were found rainy season in the month of July to November and pre winter season and least RI found in of winter and summer season as shown in Table 3 which is similar to report published previously (Kala *et al.,* 2016; Palanivel *et al.,* 2012; Mathai *et al*., 2003; Khan *et al.,* 2022).

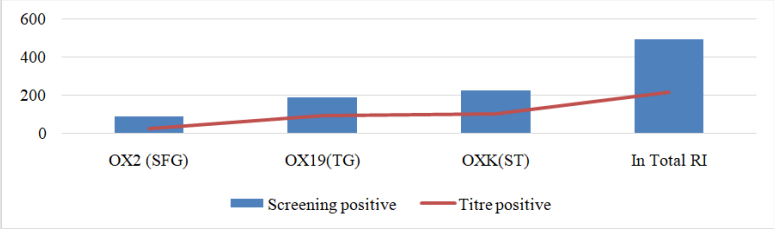

Fig. 2. Proportional Rickettsial species in seropositive RI samples.

Table 3: Seasonal distribution of RIs positive samples.

Season (In Month)	RIs	OX2	OX19	OXK
Monsoon (July to October)	330	46	118	166
Winter (November to February)	71	17	27	27
Summer (March to June)	92	23	41	28
Total	493	86	186	221

CONCLUSIONS

The RIs seroprevalence is high in our region of study. Of RIs we found that scrub typhus and typhus group fever are more prevalent. Several tests are available for differential diagnosis of AFI with high sensitivity and specificity, but they are not routinely used because of cost and technical reasons in developing countries. Serological tests are the mainstay in AFI differential diagnosis. Suspected RIs is an emergency, and the person must see a health practitioner quickly. Examination of patients' samples ensures a quick diagnosis and helps them receive the correct treatment early. Failure in diagnosis and treatment put patients at great risk. The burden suspected is high in our region so this infection should include in differential diagnosis of AFI.

FUTURE SCOPE

Generally, RIs are significantly neglected and under-recognized in the Asia Pacific region including our South Gujarat region of India, while causing a significant burden of disease. RIs are not widely studied because of the limitation of diagnostic techniques, lack of awareness and information but the distribution of this rickettsial group appears to be spreading wider. Since RIs have become more globally documented, the growing importance of RIs studies i.e. Rickettsia agents, its vector populations, diagnostic modality for accumulated data information which allows for control and prevention strategies to be identified, prioritized and implemented.

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Conflict of Interest. None.

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