Introduction

Rickettsial diseases are now established as re-emerging zoonotic bacterial infections in the Indian subcontinent and are an important but often under-recognised cause of febrile illness among children. Among the wide range of rickettsial diseases, scrub typhus and spotted fever group diseases are most commonly recognised in the Asia-Pacific region. In the recent years, outbreaks have been reported in the Sub-Himalayan belt as well as in Maharashtra, Rajasthan, Punjab and Southern Indian states of Tamil Nadu, Kerala and Karnataka. In Karnataka, although scrub typhus has been reported in the various rural districts, data from the urban capital Bengaluru and surroundings are lacking.

The infection clinically manifests as non-specific febrile illness, 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. The observed varied clinical manifestations reported from India could be due to differences in the infecting strains, which are known to have a high level of antigenic variation. The low index of suspicion and non-specific symptoms coupled with the lack of a suitable diagnostic test diminish accessibility to early diagnosis and subsequent appropriate management of rickettsial illnesses. The immunofluorescence assay (IFA) is considered as ‘gold standard’ for diagnosing rickettsial infections; however, the necessary fluorescence microscope equipment is not easily available. Moreover, the test is

Abstract

**Background:** Rickettsial infections are re-emerging. In India, they are now being reported from several areas where they were previously unknown. **Objectives:** The objective of this study was to describe the epidemiology, clinical profile and outcome of serologically-confirmed scrub typhus and spotted fever among children in a tertiary care hospital in Bengaluru. **Materials and Methods:** Hospitalised children aged <18 years, with clinical features suggestive of rickettsial disease admitted between January 2010 and October 2012 were included prospectively. Diagnosis was based on scrub typhus and spotted fever-specific IgM and IgG by enzyme-linked immunosorbent assay (ELISA). **Results:** Of 103 children with clinical features suggestive of rickettsial illness, ELISA test confirmed 53 cases for scrub typhus, 23 cases for spotted fever group and 14 with mixed infection. The average age was 7.3 (±3.9) years and 44 (71.0%) children were male. Majority of cases were from Karnataka (50%), Andhra Pradesh (32.3%) and Tamil Nadu (17.7%). Common clinical features included fever (100%, average duration 11 days), nausea and vomiting (44%), rash (36%); eschar was rare. Compared to the ELISA test, Weil-Felix test (OX-K titre of 1:80) had a sensitivity and specificity of 88.7% and 43.9%, respectively. Treatment with chloramphenicol or doxycycline was given to the majority of the children. Complications included meningoencephalitis (28%), shock (10%), retinal vasculitis (10%) and purpura fulminans (7%). **Conclusions:** These findings suggest that the burden of rickettsial infection among children in India is high, with a substantially high complication rate. Rickettsial-specific ELISA tests can help in early diagnosis and early institution of appropriate treatment that may prevent life-threatening complications.

**Key words:** Children, enzyme-linked immunosorbent assay, Orientia tsutsugamushi, scrub typhus, spotted fever
expensive, requires an expert for its use, ideally needs cell culture facilities for sustaining rickettsial antigens and often can take more than a week to get the results.[14,15] The most widely used serological test for rickettsial screening is the Weil-Felix test, although its reliability is suspected due to its poor sensitivity and specificity.[5,7,16,17] Recent outbreaks are reported by detecting antigen-specific IgM or IgG antibodies by enzyme-linked immunosorbent assay (ELISA).[1,12,14,18] Scrub typhus ELISA kits use Orientia tsutsugamushi recombinant p56kD type-specific antigen of Karp, Kato, Gilliam and TA716 strains, and have more than 90% sensitivity and 90% specificity for detecting specific antibodies.[19,20] Although the presence of scrub typhus has been known for several years, the widespread availability and simplicity of the ELISA technique has probably resulted in high identification of scrub typhus in recent outbreaks in comparison to previous outbreaks. The objective of our study was to describe the clinical manifestations and outcomes of serologically confirmed scrub typhus and spotted fever group cases among children admitted in a tertiary care hospital in Bengaluru.

Materials and Methods

The prospective study was conducted between January 2010 and October 2012, on hospitalised children aged <18 years, who had either fever for ≥5 days without an identifiable infection, or fever of <5 days with any two clinical features strongly suggestive of rickettsial infection, such as rash, oedema, hepatosplenomegaly, lymphadenopathy and an eschar, with or without a history of tick exposure.[5,14,21] Children with specific non-rickettsial diagnoses such as laboratory-confirmed cases of enteric fever, malaria, dengue, leptospirosis, blood stream infection, urinary tract infection, respiratory infection and tuberculosis were excluded from the study. Informed consent was obtained from the caregivers of children who were eligible based on these criteria. Patient’s histories and clinical examinations were obtained, and routine investigations were done, which included a complete blood count and peripheral smear, microscopy of urine sample, a Widal and Weil-Felix test and blood culture when indicated. The Weil-Felix test is based on the detection of antibodies to various cross-reacting Proteus antigens with rickettsiae (Proteus vulgaris OX2 with spotted fever rickettsiae, P. vulgaris OX19 with typhus group rickettsiae and Proteus mirabilis OXK with O. tsutsugamushi).[19] The Weil-Felix test was performed on all the serum samples collected at the time of presentation at the hospital (acute sample) and after 2 weeks of treatment (convalescent sample), whenever available. The tube agglutination method was used, and a titre of 1:80 or more was considered significant for rickettsial infection.

A confirmed case of rickettsial infection was defined as one that was positive for ELISA (scrub typhus IgM antibody or Rickettsia conorii IgG antibody), with no evidence of any other infection. Rickettsial infection was confirmed with scrub typhus group ELISA Kit (InBios International, Inc., Seattle, WA, USA) to detect O. tsutsugamushi-specific IgM antibodies and R. conorii ELISA IgM/IgG kit (Vircell S. L., Granada, Spain) to detect R. conorii-specific IgM antibodies, and the tests were interpreted according to the manufacturer’s guidelines. The 96-well plates were either coated with unique scrub typhus recombinant antigen mix or with R. conorii antigen (strain Moroccan). The cut-off value to consider the test as positive for scrub typhus was set as scrub typhus group IgM ≥0.6 and/or IgG ≥0.37. Evidence of spotted fever was defined by serum positivity for R. conorii IgG antibody index value >11. The optimal cut-off value of scrub typhus IgM ELISA was initially determined using the mean OD +2 standard deviation value of 64 control sera that were positive for non-rickettsial infections (dengue [10], chikungunya [7], leptospirosis [10] and enteric fever [7]) or were non-infectious sera from normal healthy volunteers (n = 30).[5,20,22] The results were compiled in an Excel spreadsheet, and frequency distribution and Bayesian analysis were performed using SPSS v 16.0 (SPSS, Inc., Chicago, IL, USA). Ethical clearance was obtained from the Ethics Committee of our institution prior to starting the study.

Results

A total of 103 children who fulfilled the eligibility criteria were included in the analysis. The average age of presentation was 7.4 (±3.8) years and 38 (71%) children were male. There were 123 serum samples collected from the 103 children clinically diagnosed as having rickettsial infection from January 2010 to October 2012.

Serological confirmation

Of the 123 sera tested for Weil-Felix test, 78 were positive (17 paired and 61 single) and 25 were negative (3 paired and 22 single samples) from 103 patients [Figure 1]. Importantly, a total of 62 children were confirmed using rickettsial ELISA assays (scrub typhus group and spotted fever group). These included 53 (85.5%) scrub typhus and 23 (37.1%) spotted fever group. Fourteen patients (22.6%) appeared to have mixed infection or exhibited cross-reactivity as they were positive for both scrub typhus and spotted fever serology.

Epidemiological and demographic characteristics

The majority of confirmed cases were from the neighbouring districts of Karnataka 31 (50%), and the remaining were from Andhra Pradesh 20 (32.3%) and Tamil Nadu 11 (17.7%) [Figure 2]. A history of animal contact (dog or cattle such as goat, cow and oxen) or insect bite was found among 19 (30%) patients. There was a clear seasonal trend; most of the cases (53%) were seen soon after the rainy season during the months of August to November [Figure 3].
Clinical features

All children had fever with an average duration of 11 days at presentation, and ranged from one to 26 days. Nausea or vomiting was the frequent (43.5%) feature [Table 1]. Only 36% of the patients had a rash, 30% had maculo-papular type of rash and 21% had rash on palms and soles. Eschar was an infrequent feature. Hepatomegaly (71%) and tender lymphadenopathy (47%) were the commonly seen symptoms. Other features included splenomegaly (37%), tachycardia (24%) and seizures (15%). The most common complication of rickettsial diseases was meningoencephalitis (28%). Other complications were shock (10%), retinal vasculitis (10%) and purpura fulminans (7%). Children with spotted fever were more likely to have rash, vasculitis and encephalitis than scrub typhus.

Laboratory features

Laboratory evidence suggested the prevalence of anaemia (haemoglobin <11.0 g/dl) as 65.1% (67/103) and abnormal liver function in 68.0% (70/103) of the patients. Thrombocytopenia and leucocytosis were seen in 47.6% (49/103) and 44.7% (46/103) of the cases, respectively. The Weil-Felix test was positive in 80% of the patients with rickettsial diseases (sensitivity 88.7%, specificity 43.9%, positive predictive value 70.5% and negative predictive value 72%).

Treatment and outcome

Among 62 children with confirmed rickettsial fever, 57 were treated with chloramphenicol or doxycycline. Among the five children who did not receive specific anti-rickettsial treatment, all recovered without complications. There were no deaths among the children who tested positive for rickettsial infection. Among the 6 patients who were critically ill, three developed multi-organ dysfunction, but survived with supportive therapy in the Intensive Care Unit.

Discussion

The present study highlights the finding that rickettsial fevers, particularly scrub typhus and the spotted fever group, are a major cause of undiagnosed febrile illness in hospitalised children with varied clinical presentation and high degree of complications. We have shown that specific serological diagnosis of this infection using ELISA-based technology is feasible and can aid in early diagnosis and treatment of rickettsial illnesses.

Rickettsial infections are reported from South-East Asia.[2,7,12,21,23] In India, rickettsial infections constitute an emerging group of zoonosis, particularly scrub typhus and Indian tick typhus.[4,9,10] In the present study, most of the cases were seen during the months of August to November. Such post-monsoon seasonality was reported earlier.[18,24] Serological and molecular detection has confirmed the re-emergence of scrub typhus in India.[1,24,25] These reports are likely to represent an underestimate of the true burden, as many of the cases go undiagnosed due to a lack of awareness regarding the illness and a lack of specific laboratory facilities in high-burden areas.[3,4] The absence of specific diagnosis often leads to extensive

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investigations, excessive financial burden on families and varied inappropriate antibiotic use.

We observed that rickettsial infection could present with varied clinical features.\[14\] The conventionally associated features of rash and eschar were present in very few patients in our study. This is similar to the findings from recent studies from Northern\[1,7\] and Southern India.\[6,12,24\] It is likely that patients are unaware or ignore the presence of an eschar, and a detailed physical examination is rarely done specifically looking for the eschar.\[23\] Consistent with previous reports, children with scrub typhus were more likely to have thrombocytopenia, while those with spotted fever patients were more likely to manifest leucocytosis.\[8,11,12\] Rash was more commonly associated with spotted fever (56.5%) and becomes apparent after 3–5 days of the onset of symptoms.\[5,8\] Meningoencephalitis was a complication (24%) in our study population and was more common in spotted fever patients (44%), though previous studies have reported a lower level of CNS involvement; from South India of 18.8%\[12\] and 9.5%\[26\] and North India of 14.3%.\[7\] Scrub typhus can manifest with potentially life-threatening complications such as lung injury, shock and meningoencephalitis. There is also emerging evidence in India that scrub typhus has been associated with severe complications such as multi-organ dysfunction. Shock, renal failure and CNS involvement are often associated with mortality.\[11,12\] These variable clinical manifestations could be due to differences in the infecting strains that result in a high level of antigenic variation. In India, diverse strains of \textit{O. tsutsugamushi} have been reported.\[13\] Kato-like strains are predominant in the South and Northeast regions of India, whereas an equal prevalence of Karp-like and Kato-like strains was found in Northern India.\[12,13\] Although case fatality can be as high as 30–45%,\[9\] we observed no mortality among our cohort, which may be due to

\begin{table}
\centering
\caption{Clinical and laboratory data among patients with confirmed spotted fever group and scrub typhus and non-specific febrile illness} \label{tab:clinical_data}
\begin{tabular}{|l|c|c|c|c|}
\hline
Patient characteristics & Scrub typhus group & Spotted fever group & Mixed positive & Overall rickettsial positive \[n=62\] n (%) \hline
Age, years & 7.4±3.7 & 7.7±3.9 & 7.1±5.1 & 7.3±3.9 \\
Sex, male & 38 (71.7) & 16 (70.0) & 10 (71.4) & 44 (71.0) \\
Mean fever duration (range) days & 11.2 (1-26) & 11.4 (1-26) & 12.29 (1-26) & 11.0 (1-26) \\
Rash & 14 (26.4) & 13 (56.5) & 5 (35.7) & 22 (35.5) \\
Arthralgia & 4 (7.5) & 1 (4.3) & 1 (7.1) & 4 (6.5) \\
Headache & 15 (28.3) & 6 (26.1) & 6 (42.9) & 15 (24.2) \\
Oedema/facial puffiness & 13 (24.5) & 7 (30.4) & 3 (21.4) & 17 (27.4) \\
Tender lymphadenopathy & 26 (49.1) & 15 (65.2) & 12 (85.7) & 29 (46.8) \\
Hepatomegaly & 36 (67.9) & 19 (82.6) & 11 (78.6) & 44 (71) \\
Splenomegaly & 17 (32.1) & 11 (47.8) & 5 (35.7) & 23 (37.1) \\
Seizures & 3 & 2 & 1 & 4 \\
Vasculitis & 12 (22.6) & 9 (39.1) & 5 (35.7) & 16 (25.8) \\
Meningo-encephalitis & 9 (17.0) & 9 (39.1) & 1 (7.1) & 15 (24.2) \\
Anaemia (haemoglobin <11.0 g/dl) & 37 (69.8) & 15 (65.2) & 11 (78.6) & 41 (66.1) \\
Leucopenia (WBC count <5000 cells/mm$^3$) & 8 (15.1) & 2 (8.6) & 1 (7.1) & 9 (14.5) \\
Leucocytosis (WBC count >11,000/mm$^3$) & 13 (33.3) & 5 (55.6) & 9 (64.3) & 27 (43.5) \\
Platelets <150,000/mm$^3$ & 26 (66.7) & 3 (33.3) & 8 (57.1) & 37 (60.0) \\
Hyponatraemia (Na <125 mmol/L) & 3 (17) & 0 (n=17) & 0 (n=12) & 3 (n=39) \\
Low albumin <3.5 g/dl & 27 (69.2) & 6 (66.7) & 11 (78.6) & 44 (71) \\
Raised (AST >37 U/L)/(ALT >64 U/L) & 43 (81.1) & 16 (69.6) & 11 (78.6) & 48 (76.2) \\
Positive Well–Felix test & 33 (84.6) & 4 (44.4) & 13 (92.9) & 50 (80.6) \\
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\end{tabular}
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WBC: White blood cell, AST: Aspartate aminotransferase, ALT: Alanine transaminase, mixed positive - positive for both scrub typhus and spotted fever group
timely initiation of the empirical and specific therapy. Previous Indian studies have shown varied mortality of 2–17.2%.⁷,¹¹,¹⁴ although mortality has been decreasing in the recent years.¹¹

There is an urgent need for reliable alternative diagnostic methods for rickettsiae. The available tests such as Weil-Felix test, IFA, polymerase chain reaction (PCR), culture and ELISA have their own limitations. The Weil-Felix test is inexpensive and can give results overnight, but has low sensitivity and specificity due to cross-reactivity of Proteus antigens used.⁹,²⁴ It is known that Weil-Felix test results may be negative during the early stages of the disease because agglutinating antibodies are detectable only during the 2nd week of illness. In a Sri Lankan study, researchers demonstrated low sensitivity (33%) of the Weil-Felix test in diagnosing acute rickettsial infections as well as low specificity, with a positive titre of 1:320 seen in 54% of healthy volunteers and 62% of non-rickettsial fever patients.²⁷ These false-positive Weil-Felix test results can mislead the inexperienced clinician with adverse consequences such as delay in specific treatment, which can lead to more clinical complications and lower survival rates. Indirect immunoperoxidase (IIP) test, although sensitive and specific, is not available easily and labour-intensive, with a requirement for expertise and specific laboratory equipment to run the test.⁷ Detection of DNA by PCR rapid assay for the diagnosis indicates active disease, but has varying levels of sensitivity, expensive to perform and not easily available.³ Animal culture or cell culture for isolation of the organisms is restricted to research laboratories.⁹,²² For scrub typhus serological confirmation, IFA is the gold standard;⁹,²⁵ however, this test is difficult to perform, since it requires a skilled observer, mandates inclusion of several antigenic types, subjective, often expensive and most hospitals are not equipped with a fluorescence microscope that is needed to perform this test.¹⁴ The ELISA is a rapid and objective test amenable to accurate testing of large numbers of sera, often obtained in seroepidemiologic investigations.¹⁴ It is cheaper and can be more reproducible when using an automated procedure. Scrub typhus-specific IgM ELISA has shown almost equivalent sensitivity and specificity to those of IFA, and it can be performed by most laboratories.²²,²⁸ This does not require any special equipment, and there is no need for sophisticated technical training, and it is more suitable for rural clinical sites and doctors’ offices where advanced medical support is limited. We used the scrub typhus IgM ELISA kit (InBios International, Inc., Seattle, WA, USA), which has been validated in various studies in India.¹,¹²,¹⁴,¹₈,₂₆ Jones et al. have described that the purified protein has a much greater specificity for the scrub typhus antigen than the serum-derived controls.¹⁹ Currently, most of the commercially available ELISA for IgM and IgG detection has been developed using 56-kDa type-specific antigen, an immune-dominant outer membrane protein unique to O. tsutsugamushi. This protein contains 516–541 amino acids, and it is involved in host cell invasion through the binding of fibronectin.¹³ R. conorii ELISA IgG/IgM kit used in this study has a sensitivity of 85% and specificity of 100%, as determined by the manufacturer. Scrub typhus IgM/IgG ELISA kit uses recombinant antigen that gives excellent performance since it utilises the standardised commercial available antigen when compared to IFA and IIP. These kits have more than 90% sensitivity and specificity for detecting specific antibodies,²³ which probably result in higher detection rate of scrub typhus compared to the Weil-Felix test. Rahi et al. suggested that an OD >0.5 for IgM by ELISA can be considered positive for scrub typhus and spotted fever groups, and it is similar to the OD threshold of ≥0.6 that was obtained in our study.³ Re-infection of O. tsutsugamushi is not uncommon in areas where scrub typhus is endemic. Among patients with scrub typhus, there was evidence of past exposure to spotted fever group rickettsiae. Previous reports also showed past exposure in two cases and seroreactivity of spotted fever group to O. tsutsugamushi in nine cases.²¹ The initial antibody response after re-infection is mainly the result of an increase in IgG level. Therefore, detection of IgM and IgG at the same time can increase the overall diagnostic sensitivity. There are some limitations in this study. This is a single-centre hospital-based study, and the prevalence of rickettsial infections in the community was not assessed. The study is restricted to South India, and the sample size is limited.

Conclusion

The burden of rickettsial infection in children admitted with non-specific prolonged febrile illness in Southern India is high, with varied degree of clinical manifestations and a substantial rate of severe complications. Weil-Felix test has low sensitivity and specificity and has a limited role in the diagnosis and management of this infection. Rickettsial-specific ELISA-based serological tests are simple and feasible, and can help in the early recognition of the region-specific rickettsial illnesses.

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Conflicts of interest

There are no conflicts of interest.
References


