Clinical characteristics of and antibody response to spotted fever group rickettsial infections in South India: case series and serological cohort study

Wolf-Peter Schmidt[1,2], Carol S. Devamani[3], Divyaa Elangovan[4], Neal Alexander[5], Winsley Rose[6], John A. J. Prakash[4]

[1] *Department of Emergency Medicine, Christian Medical College, Vellore, Tamil Nadu, India*

[2] *Department for Disease Control, London School of Hygiene and Tropical Medicine, London, UK*

[3] *Rural Unit for Health & Social Affairs, Christian Medical College, Vellore, India*

[4]*Department of Clinical Microbiology, Christian Medical College, Vellore, India*

[5]*MRC International Statistics and Epidemiology Group, London School of Hygiene and Tropical Medicine, London, UK*

[6] *Department of Pediatrics and Pediatric Infectious Diseases, Christian Medical College, Vellore, India*

Sustainable Development Goal: Good health and wellbeing

Abstract

Objective: The clinical and serological characteristics of spotted fever group rickettsial (SFGR) infections in South Asia are poorly understood. We studied the clinical presentation and the IgM/IgG response in cases enrolled at two health care centres in South India.

Method: We enrolled 77 patients. 57 of these were recruited at a tertiary care centre, the remaining 20 at a community hospital (secondary care level). Diagnostic tests included IgM and IgG ELISA, and PCR. Over a period of one year, 41 cases were followed up for repeated sero-analysis.

Results: Median age was 9 years (range 1 to 79). A rash was present in 74% of cases (57/77). In cases aged <15 years rash was present in 94% (44/47) vs 43% (13/30) in cases aged ≥15 years. An eschar was found in two cases (3%). Severe infection or complications occurred in 10 cases (13%). These included central nervous system infection (6/77, 8%), kidney injury (3/77, 4%), shock (3/77, 4%), lung involvement (2/77, 3%) and peripheral gangrene (2/77, 3%). IgM antibody levels increased faster after fever onset than IgG antibodies, peaking at 50 and 60 days respectively. After the peak, IgM and IgG levels showed a slow decline over one year with less than 50% of cases showing persistent IgG antibody levels.

Conclusion: SFGR infections in South India may be under-diagnosed, as many cases may not develop a rash. The proportion of cases developing severe infection seems lower than for scrub typhus in this region. IgG sero-prevalence may substantially under-estimate the proportion in a population with past SFGR infection.

Keywords: Spotted fever, *Rickettsiae*, antibody, cohort

INTRODUCTION

Spotted fever group rickettsial infections (SFGR) are caused by a diverse group of intracellular bacterial species

belonging to the genus *Rickettsia* (order: Rickettsiales, family: *Rickettsiaceae*). Within the family of *Rickettsiacea*, SFGR infections are commonly distinguished from two other main groups of rickettsioses, i.e. the typhus group (*Rickettsia typhi*) and the scrub typhus group (Genus *Orientia*) [1, 2]. SFGR are mainly transmitted by ticks, mostly hard ticks that feed on animals and humans. They comprise over 30 different *Rickettsia* species, of which at least 21 are pathogenic to humans. Pathogenicity may however depend on the species, which differ in their geographic range [1, 2]. Severe SFGR infection involves the central nervous system (meningitis, meningo-encephalitis) [3, 4], the lungs such as acute respiratory distress syndrome (ARDS) and the kidneys [5, 6]. Rocky Mountain spotted fever (*R. rickettsii*) appears to be among the more pathogenic rickettsioses [5, 6], while African tick bite fever (*R. africae*) [7] may be associated with a lower risk of severe infection [8]. Severe infections, in particular neurological manifestations have been observed in Mediterranean spotted fever (*R. conorii*) in adults [9], but rarely in children [10]. In Asia and elsewhere [11], SFGR infections are thought to represent under-reported, neglected infections of likely public health relevance with a wide distribution and great species diversity [2]. Studies of fever cases at health care centres on Sri Lanka and in India suggested SFGR infection to be common alongside other rickettsial infections [12-15]. A serological survey In Tamil Nadu (South India), showed evidence of widespread previous SFGR infections in villages in plains and hill areas [16]. However, such cross-sectional serological data are difficult to interpret as the antibody responses over time following SFGR infection are unknown and may be subject to cross-reactivity.

While case reports have shown that SFGR infection in South Asia can lead to severe disease [14, 17-21], the risk of developing severe SFGR infection in this region is unclear. The Indian Tick Typhus strain of *R. conorii* is regarded as a leading causative agent of SFGR infection in the Indian subcontinent, with most SFGR infections in the region assumed to follow a mild course [22]. However, the recent isolation of a new SFGR species, *candidatus* R. kellyi, Tamil Nadu [23], raises the possibility of considerable species diversity, similar to other parts of Asia [2]. Further, a report from Sri Lanka suggested a risk of complicated SFGR infection of up to 24% [15], similar to what is observed for scrub typhus in the region [24, 25].

The present study was done to determine the clinical characteristics of SFGR infections diagnosed at two health care centres in one district of Tamil Nadu, South India. Further, we aimed at studying the antibody response to SFGR infections to better understand immunogenicity and help with the interpretation of cross sectional sero-surveys.

METHODS

The study was conducted at the Christian Medical College Vellore (CMC), a tertiary care centre in Vellore district in the South Indian state of Tamil Nadu. The study prospectively enrolled patients from two different sources:
1) Patients of all ages with an acute undifferentiated febrile illness seeking care at CMC's emergency departments ("CMC cohort"). In this cohort, testing for SFGR infection was usually done after negative scrub typhus and dengue tests with strong suspicion of rickettsial infection, or if the clinician suspected SFGR infection based on history and skin manifestations. 2) Patients of all ages clinically suspected of rickettsial infection at the Rural Unit for Health and Social Affairs (RUHSA) community hospital, a secondary care centre affiliated with CMC about 40km away from the main CMC hospital ("RUHSA cohort"). For this cohort, all patients with acute undifferentiated fever of at least 4 days

duration were tested for both scrub typhus and SFGR infection independent of skin manifestations.

Enrolment and follow up occurred between January 2018 and March 2020, using similar methods as a larger, parallel serological study on scrub typhus described previously [24]. After initial enrolment, patients were followed up preferably at 1, 3, 6, 9 and 12 months, with most samples taken during the first three months after fever onset, although the actual times were usually irregular. At initial enrolment and at each visit a venous blood sample was taken.

Blood testing

After collection, blood samples were brought to CMC on the same day. Serum was separated from blood cells, divided into 3 aliquots and stored at -70°C until testing. All samples were tested for SFGR and scrub typhus. We used enzyme-linked immunosorbent assays (ELISA) to detect IgM and IgG antibodies for rickettsial infection. For spotted fever, we used the *Rickettsia conorii* ELISA IgM/IgG (Vircell, Granada, Spain) following the manufacturer's specifications. A test was considered to be valid if the OD of the negative control was less than 0.2 and the OD of the positive control (both from the manufacturer and in-house) was more than 1.0. The sensitivity of this ELISA in our setting was 71% based on data from a small study on 21 PCR positive cases [26]. Specificity in the same study was derived from 48 cases with rash and fever of other etiologies and found to be 81% (9 false positives out of 48, 5 of which were due to scrub typhus). For scrub typhus, we used ELISA assays targeting IgM and IgG antibodies to *Orientia tsutsugamushi* (Scrub Typhus Detect, InBios International, Inc., Seattle, WA, USA). Scrub Typhus Detect uses Karp, Kato, Gilliam and TA716 recombinant proteins of the 56-kD outer membrane protein. This ELISA has been shown to have a sensitivity and specificity of over 90% in a study from Thailand [27], and 92% sensitivity and 94% specificity in a study from South India [28].

For both ELISAs we applied an OD cut-off of 1.0 for IgM to suggest acute SFGR or scrub typhus infection [29]. In a subset of 57 patients, PCR was done on whole blood (buffy coat) and/or eschar samples by amplifying the OmpA gene, as previously described [30]. DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocol.

A patient was identified as a case of SFGR infection if at least one of the following criteria was fulfilled: 1) positive PCR for SFGR (n= 22 cases), 2) increase in IgG ELISA OD from an acute to a convalescent sample of ≥1.0, or sero-conversion from an OD below 1.0 to ≥1.0 (n= 14 cases with a median OD increase of 1.3, range 0.4 to 1.8), 3) both IgM and IgG ELISA OD ≥1.0 in any acute or convalescent sample and a negative scrub typhus IgM ELISA, (n= 28 cases), 4) single IgM ELISA OD value of 1.0 or higher within one month of fever, a negative scrub typhus IgM ELISA, and defervescence within 72 hours after initiating doxycycline or azithromycin in the absence of an alternative, plausible cause of fever (n= 65 cases, 28 enrolled based on this criterion alone). For the sensitivity analysis, we excluded cases that did not meet the first two criteria (a positive PCR or a rise in OD≥ 1.0 / sero-conversion).

Based on these definitions, 57 were enrolled as the CMC cohort (the total number of screened cases could not be verified). For the RUHSA cohort, we screened 151 patients with suspected rickettsial infection for enrolment, 81 of which were diagnosed as scrub typhus and 20 as SFGR infection. These 20 RUHSA cases were enrolled in the

study, resulting in a total of 77 cases.

Collection of clinical data

Clinical data were extracted from existing clinical records. Type of rash and presence of an eschar was documented by two of the authors (DE, CD). Severe SFGR infection was defined as follows [31]: Lung involvement – any patient with oxygen saturation below 92% and tachypnea at any time during admission; shock – any patient with documented hypotension at presentation or during treatment, or any documented use of inotropes; kidney injury – any creatinine of 3.0 mg/dl or higher in the absence of a known, pre-existing chronic kidney disease; CNS – any focal neurological deficit, or any elevated white blood cell counts in a cerebrospinal fluid sample, or any focal or generalised seizure in an adult, or any focal or generalised seizure in a child not diagnosed as simple febrile seizure. Simple febrile seizure in children less than 6 years of age was assumed if there was no more than one generalised seizure lasting less than 15 minutes. Rash was categorised as diffuse poorly demarcated erythematous rash, well demarcated macular or maculo-papular rash, petechial rash and purpura fulminans.

Statistical analysis

We used quantile regression for modelling ELISA OD values over time. As these calculations were done with the individual sample as unit of analysis, we adjusted confidence intervals for repeated measurements in the same patient using robust standard errors, following the methods proposed by Parente and Silva [32]. Models were done separately for the 25^{th}, 50^{th} (median), and 75^{th} percentiles. ODs were modelled as a function of time using restricted cubic splines, with knots chosen following Harrell [33]. Missing data were rare and ignored in the analysis. All analyses were done in STATA.

Ethics

The study was approved by CMC's Institutional Review Board (CMC IRB Ref: 11726) and LSHTM's Research Ethics Committee (LSHTM Ethics Ref: 16573). Written consent was obtained from all adult participants. Written assent was obtained from minors, alongside written consent from their parents/guardians.

RESULTS

We enrolled a total of 77 cases of SFGR infection. Clinical and demographic characteristics are shown in Table 1. There was a male predominance among the cases. 61% of cases were younger than 15 years. Maculo-papular rash was the most common skin manifestation (53%, Figure 1A), followed by diffuse erythematous rash and petechial rash. Rash was more common in those aged under 15 years (44/47, 94%) than in those aged 15 years or older (13/30, 43%). Rash was more common in the CMC cohort (51/57, 89%) than in the RUHSA cohort (6/20, 30%). After skin manifestations, ankle and pedal oedema were the next most common clinical finding, occurring in 21% (Figure 1A). Only two patients were found to have an eschar (Figure 1 B and C). Central nervous system involvement was the most common feature of severe infection (Table 2). Two of these were meningitis, one case presented with multiple

seizures. One case had extra-pyramidal signs including a mask-like face and rigidity of neck and limbs. One case had myoclonus of the extremities. A further case had acute psychosis as the only CNS manifestation. The psychosis was marked by inappropriate mood swings, flight of ideas and paranoid delusions. Magnetic Resonance Imaging suggested encephalitis. All cases with CNS involvement recovered within 4 weeks without sequelae.

Other organ involvements, including kidney injury (n= 3), shock (n= 3), lung involvement (n= 2), peripheral gangrene (n= 2), liver failure (n= 2) and myocarditis (n= 1), were rare (Table 2). One of the two cases of peripheral gangrene required middle phalanx amputation of 8 fingers, and bilateral metatarsal amputation. In the other case no amputation was needed. No patient died.

The antibody response following SFGR infection is shown in Figure 2A. The fitted median IgM OD values increased earlier than IgG OD values and peaked at approximately 50 days, while IgG peaked at around 60 days after the estimated start of fever. After the peak, both IgM and IgG fitted OD medians showed a similar decline over the next 12 months. For illustration, median IgM and IgG values over time from the separate study on scrub typhus cases (see methods section) are shown in Figure 2B, suggesting a relatively weaker IgG and stronger IgM response in SFGR compared to scrub typhus infection. Restricting the analysis to cases meeting the first two criteria (see methods) resulted in a similar IgM and IgG changes in OD over time compared to including all cases (Figure S1). The changes in IgM and IgG OD over time at individual level for study participants with more than one sample are shown in Figure S2.

Figure 3A and B show the 25th, 50th (median) and 75th quantiles for IgM and IgG. The 50th and 75th quantiles for IgG diverge considerably, suggesting the presence of a subgroup of cases with IgG antibodies persisting for more than one year, while the majority of cases appear to revert to OD values below 1.0 by that time.

Among cases presenting within 10 days of fever onset, IgG levels were mostly at very low levels with more than 50% showing OD values below 0.5 (Figure 4A). Again for illustration, the corresponding scrub typhus IgG values from the separate study on scrub typhus cases (see methods section) are shown in Figure 4B, suggesting that compared to scrub typhus cases, fewer cases of SFGR infection may already have pre-existing specific IgG antibodies at the time of the present infection.

DISCUSSION

This case series of SFGR infections and serological cohort study showed that while the majority of SFGR infections in this part of South India follow a mild clinical course, severe infections in particular affecting the CNS are not uncommon. While two patients developed multi-organ failure, no deaths occurred, and the only sequelae were due to gangrene requiring amputation in one case. The IgM and IgG responses to SFGR infection were quite similar, although there appeared to be a subgroup of cases with a more pronounced, long-lasting IgG response.

The rickettsial species causing SFGR infection in South India which includes the Indian tick typhus strain of *R. conorii* may be less pathogenic than *R. rickettsii* (Rocky Mountain Spotted Fever) [5, 6], *R. australis* (Queensland tick typhus) [34] or *R. japonica* (Japanese Spotted Fever) [35]. It may however be more pathogenic than the *R. conorii* strain causing Mediterranean spotted fever. A paediatric cases series from Italy reported by Colomba and colleagues

found only one case of severe infection (meningo-encephalitis) among 415 children diagnosed with the infection [10]. However, figures on clinical characteristics and the risk of severe infection from these hospital-based studies are likely to be highly influenced by the criteria and case-definitions used to enrol cases. For example, in the present case series, the presence of a rash may often have been the trigger for the clinicians to test for SFGR infection. In the subgroup of cases from the RUHSA cohort, where testing for SFGR depended less on specific clinical characteristics of patients, only a minority of cases presented with a rash. Rash was generally less common among adults, a finding that has also been described in Rocky Mountain spotted fever [36]. This study also confirms the low prevalence of an eschar in SFGR infection in studies from Sri Lanka [37] and Australia [34], which is in contrast to reports from Japanese spotted fever (up to 90%) [35] and African tick-borne spotted fever (*R. africae,* 53% to 100%) [7]. The two eschars identified in the present case series were both untypical, the first one lacking a peri-lesional erythema (Figure 1B), the second one presenting as an oval papule (0.9mm x 0.5mm) without subsequent formation of a central necrosis (Figure 1C), as sometimes observed in early scrub typhus infection. The latter patient presented on day two of fever and was given doxycycline immediately, possibly preventing development of a necrotic lesion.

Ankle and pedal oedema, often but not always accompanied by arthritic ankle pain, were a common finding in the present case series. In contrast to a report from Sri Lanka [13], the ankle was often the only affected joint in the present study and in a case series from Central India [14]. Two cases with central nervous system involvement had extra-pyramidal signs (myoclonus and Parkinson-like signs) which was also found as a CNS manifestation in a case series from Sri Lanka [38]

Apart from being less pathogenic than scrub typhus, SFGR infection also appear to be less common in this setting. In the RUHSA cohort, SFGR infection accounted for 20% (20/101) of patients diagnosed as rickettsial infection, with the remaining 80% being diagnosed as scrub typhus.

We identified marked differences in the antibody response between SFGR infection and scrub typhus with the IgM response being more pronounced in SFGR infection while the IgG response appeared weaker [24]. The direct comparison of the OD dynamics between two infections needs to be treated with caution as the association between ELISA OD values and actual antibody concentrations may differ between the two tests. Nevertheless, the differences are remarkable given the close relatedness of the *Rickettsia* and *Orientia* genera, both belonging to the family of *Rickettsiaceae*. The differences may be related to the more complex genome structure of *Orientia* compared to *Rickettsia*, which may help *Orientiae* evading the host's immune response [39]. The clinical significance of these findings is unclear. Scrub typhus infection with one strain of *Orientia tsutsugamushi* (the most commonly isolated *Orientia* species) is thought to protect against infection with the same but not with another strain [24, 40]. For SFGR, we were unable to find epidemiological data on strain-specific, species-specific or even cross-species immunity.

A considerable proportion of scrub typhus cases have high IgG antibody levels at initial presentation, presumably from earlier scrub typhus infections (Figure 4B, [24]), while initially high IgG levels appear to be rare for SFGR (Figure 4A). In scrub typhus, IgG antibodies appear to accumulate during life, presumably due to repeated infection [24], leading to a strong increase in IgG sero-positivity with age [29, 41]. No such age-dependency of IgG

sero-positivity was found for SFGR in a cross sectional sero-survey conducted by us in Tamil Nadu (geographically overlapping with the present study) [16]. This may be due to the lack of IgG persistence as found in the present study, or perhaps due to immunity to repeated SFGR infection unrelated to IgG antibodies. Given the inconsistent IgG response following SFGR infection, sero-prevalence studies may underestimate the prevalence of past SFGR infection in a population. In the sero-prevalence study mentioned above, we used an IgG OD cut-off of 1.5 to define sero-positivity and found a sero-prevalence of SFGR of 10.4%. Using an OD cut-off of 1.0, as might be inferred from the present study (Figure 3B), would have increased the estimated sero-prevalence to 24.2%. Since less than half of cases appear to have persistent high IgG OD values following infection (Figure 3B), it could be argued that the true prevalence of ever having been infected with SFGR (symptomatically or asymptomatically) could be as high as 50% in this setting.

Major limitations of the study include the small sample size, the method of case enrolment and case ascertainment. The number of convalescent samples obtainable at follow up was lower than expected, which led to relatively wide confidence intervals in our estimates (Figure 2A) compared to the larger concurrent scrub typhus cohort (Figure 2B). Enrolment of cases into the study was done in two private, non-profit health care centres. Because health care in India involves private and government providers, the cases treated at the study centres may not be fully representative of the whole population in the area. Case identification for enrolment often depended on clinical characteristics of the patient such as a rash, prompting testing for SFGR. This is likely to have biased the sample towards cases with rash, and towards children who more often presented with rash. Tests for other infections such as dengue and leptospirosis were not done systematically for all patients.

Laboratory confirmation of SFGR is difficult, as serological tests are thought to have a relatively low specificity. The cut-off of 1.0 in this study was pragmatically chosen as the same used for scrub typhus in this setting [31]. Additional validation studies are needed to confirm the usefulness of this cut-off value. Unlike for scrub typhus, ELISA kits based on recombinant proteins are not yet routinely available for SFGR. PCR has a high specificity but low sensitivity, with only 22 cases of 51 undergoing PCR testing positive. Serial serology (from acute and convalescent samples), preferably using indirect immunefluorescence assays (IFA) can improve specificity. IFA however was not available to us in this study. For serial serology, we used an increase in ELISA OD of 1.0 to define an increase in IgG levels, which applied to 14 cases. In 12 out of 22 PCR confirmed cases a second sample was available. Four of these showed an OD increase between 0.2 and 0.3, and a further four an OD increase of 0.5 or more. The remaining four showed now increase in OD at all, highlighting the difficulties in diagnosing SFGR based on serology alone in this setting. Possibly, the IgG response may generally be low in SFGR. Conversely, the commercial ELISA test used in this study may miss cases due to poor cross-reactivity across strains, which would be surprising since most SFGR cases in South Asia are thought to be due to the Indian Tick Typhus strain of *R. conorii* [42]. More recently, a new *Rickettsia* species (*Candidatus* R. kellyi) has been isolated from a SFGR case in the same area where the present study was conducted [23], the presence of which may have limited the utility of this ELISA in our setting.

All cases in this series responded to doxycycline or azithromycin treatment within 72 hours, often rapidly, but this criterion is not very specific, as many febrile illnesses are self-limiting. There is also the possibility of cross-

reactivity with *Rickettsia typhi* (murine typhus). While we found evidence for cross-reactivity of SFGR and *R. typhi* IgG antibodies being rare [16], IgM antibodies may be more prone to cross-reactivity. Future studies should employ more rigorous and systematic enrolment strategies and aim at obtaining acute and convalescent samples from all cases and use IFA to improve the specificity of serological testing. Case confirmation may be strengthened by conducting PCR in all patients, where possible including PCR from biopsy of skin lesions. To explore cross-reactivity, samples could also be tested for *R. typhi* alongside SFGR (which is currently not done routinely in our laboratory).

To conclude, this study confirmed SFGR as causing less severe forms of acute undifferentiated fever than scrub typhus in this setting, although the risk of severe infection (in particular those involving the CNS) is still considerable. This study suggests that under-diagnosis of SFGR may be substantial, especially in older patients presenting without rash. The antibody response to SFGR appears to differ strongly from those to scrub typhus, although the clinical relevance of these differences remains to be studied further. IgG sero-prevalence on its own may be an incomplete marker of past SFGR infection, by underestimating the prevalence of past infection in a population.

ACKNOWLEDGEMENTS

We thank all participants for providing samples for this study.

The study was supported by internal funds at the Christian Medical College, Vellore (grant ID 22 Z 399). NA receives salary support from the MRC UK and DFID-MRC Grant Reference MR/K012126/1: This award is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 programme supported by the European Union.

REFERENCES

1. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier PE *et al*: **Update on tick-borne rickettsioses around the world: a geographic approach**. *Clin Microbiol Rev* 2013, **26**(4):657-702.
2. Satjanadumrong J, Robinson MT, Hughes T, Blacksell SD: **Distribution and Ecological Drivers of Spotted Fever Group Rickettsia in Asia**. *Ecohealth* 2019, **16**(4):611-626.
3. Sekeyova Z, Danchenko M, Filipcik P, Fournier PE: **Rickettsial infections of the central nervous system**. *PLoS Negl Trop Dis* 2019, **13**(8):e0007469.
4. Bradshaw MJ, Carpenter Byrge K, Ivey KS, Pruthi S, Bloch KC: **Meningoencephalitis due to spotted fever rickettsioses, including Rocky Mountain spotted fever**. *Clin Infect Dis* 2019.
5. Gottlieb M, Long B, Koyfman A: **The Evaluation and Management of Rocky Mountain Spotted Fever in the Emergency Department: a Review of the Literature**. *J Emerg Med* 2018, **55**(1):42-50.
6. Biggs HM, Behravesh CB, Bradley KK, Dahlgren FS, Drexler NA, Dumler JS, Folk SM, Kato CY, Lash RR, Levin ML *et al*: **Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever and Other Spotted Fever Group Rickettsioses, Ehrlichioses, and Anaplasmosis - United States**. *MMWR Recomm Rep* 2016, **65**(2):1-44.

7. Jensenius M, Fournier PE, Vene S, Hoel T, Hasle G, Henriksen AZ, Hellum KB, Raoult D, Myrvang B, Norwegian African Tick Bite Fever Study G: **African tick bite fever in travelers to rural sub-Equatorial Africa**. *Clin Infect Dis* 2003, **36**(11):1411-1417.
8. Espejo E, Andres M, Garcia MC, Fajardo A, Mauri M, Perez J, Bella F: **Mediterranean spotted fever in the elderly: a prospective cohort study**. *Eur J Clin Microbiol Infect Dis* 2019, **38**(7):1333-1337.
9. Alioua Z, Bourazza A, Lamsyah H, Erragragui Y, Boudi O, Karouach K, Ghfir M, Mossedaq R, Sedrati O: **[Neurological feature of Mediterranean spotted fever: a study of four cases]**. *Rev Med Interne* 2003, **24**(12):824-829.
10. Colomba C, Saporito L, Polara VF, Rubino R, Titone L: **Mediterranean spotted fever: clinical and laboratory characteristics of 415 Sicilian children**. *BMC Infect Dis* 2006, **6**:60.
11. Salje J, Weitzel T, Newton PN, Varghese GM, Day N: **Rickettsial infections: A blind spot in our view of neglected tropical diseases**. *PLoS Negl Trop Dis* 2021, **15**(5):e0009353.
12. Kularatne SA, Edirisingha JS, Gawarammana IB, Urakami H, Chenchittikul M, Kaiho I: **Emerging rickettsial infections in Sri Lanka: the pattern in the hilly Central Province**. *Trop Med Int Health* 2003, **8**(9):803-811.
13. Weerakoon KG, Kularatne SAM, Rajapakse J, Adikari S, Udayawarna K: **Revisiting clinico-epidemiological pattern of human rickettsial infections in the central region of Sri Lanka: a hospital based descriptive study**. *BMC Res Notes* 2017, **10**(1):400.
14. Rathi NB, Rathi AN, Goodman MH, Aghai ZH: **Rickettsial diseases in central India: proposed clinical scoring system for early detection of spotted fever**. *Indian Pediatr* 2011, **48**(11):867-872.
15. Kularatne SA, Rajapakse RP, Wickramasinghe WM, Nanayakkara DM, Budagoda SS, Weerakoon KG, Edirisinghe JS, Premaratna R: **Rickettsioses in the central hills of Sri Lanka: serological evidence of increasing burden of spotted fever group**. *Int J Infect Dis* 2013, **17**(11):e988-992.
16. 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.
17. Dincy PC, Susanne PA, Leni G, T S, Meera T, Aj PJ: **Clinicopathological study on rickettsial spotted fever from south India**. *Trop Doct* 2018, **48**(4):325-329.
18. Gopinath KG, Chrispal A, Boorugu H, Chandy S, Prakash JJ, Abraham AM, Abraham OC, Thomas K: **Clinico-epidemiological profile of seven adults with spotted fever from a tertiary care hospital in South India**. *Trop Doct* 2014, **44**(2):89-91.
19. Kalal BS, Puranik P, Nagaraj S, Rego S, Shet A: **Scrub typhus and spotted fever among hospitalised children in South India: Clinical profile and serological epidemiology**. *Indian J Med Microbiol* 2016, **34**(3):293-298.
20. Prakash JA, Sohan Lal T, Rosemol V, Verghese VP, Pulimood SA, Reller M, Dumler JS: **Molecular detection and analysis of spotted fever group Rickettsia in patients with fever and rash at a tertiary care centre in Tamil Nadu, India**. *Pathog Glob Health* 2012, **106**(1):40-45.
21. Stephen S, Ambroise S, Gunasekaran D, Hanifah M, Sangeetha B, Pradeep J, Sarangapani K: **Serological evidence**

of spotted fever group rickettsiosis in and around Puducherry, south India-A three years study. *J Vector Borne Dis* 2018, **55**(2):144-150.

22. Parola P, Fenollar F, Badiaga S, Brouqui P, Raoult D: **First documentation of Rickettsia conorii infection (strain Indian tick typhus) in a Traveler**. *Emerg Infect Dis* 2001, **7**(5):909-910.
23. Rolain JM, Mathai E, Lepidi H, Somashekar HR, Mathew LG, Prakash JA, Raoult D: **"Candidatus Rickettsia kellyi," India**. *Emerg Infect Dis* 2006, **12**(3):483-485.
24. Schmidt WP, Devamani CS, Rose W, Alexander N, Prakash JAJ: **Antibody response following scrub typhus infection: clinical cohort study**. *Trop Med Int Health* 2019, **24**(12):1455-1464.
25. Abhilash KP, Jeevan JA, Mitra S, Paul N, Murugan TP, Rangaraj A, David S, Hansdak SG, Prakash JA, Abraham AM *et al*: **Acute Undifferentiated Febrile Illness in Patients Presenting to a Tertiary Care Hospital in South India: Clinical Spectrum and Outcome**. *J Glob Infect Dis* 2016, **8**(4):147-154.
26. Elangovan D, Perumalla S, Gunasekaran K, Rose W, Varghese VP, Abhilash KP, Prakash JA, Dumler JS: **Spotted fever diagnosis: experience from a South Indian center**. *Pathog Glob Health* 2021, **in press**.
27. Blacksell SD, Tanganuchitcharnchai A, Nawtaisong P, Kantipong P, Laongnualpanich A, Day NP, Paris DH: **Diagnostic Accuracy of the InBios Scrub Typhus Detect Enzyme-Linked Immunoassay for the Detection of IgM Antibodies in Northern Thailand**. *Clin Vaccine Immunol* 2015, **23**(2):148-154.
28. Kannan K, John R, Kundu D, Dayanand D, Abhilash KPP, Mathuram AJ, Zachariah A, Sathyendra S, Hansdak SG, Abraham OC *et al*: **Performance of molecular and serologic tests for the diagnosis of scrub typhus**. *PLoS Negl Trop Dis* 2020, **14**(11):e0008747.
29. Devamani CS, Prakash JAJ, Alexander N, Suzuki M, Schmidt WP: **Hospitalisations and outpatient visits for undifferentiated fever attributable to scrub typhus in rural South India: Retrospective cohort and nested case-control study**. *PLoS Negl Trop Dis* 2019, **13**(2):e0007160.
30. Prakash JA, Reller ME, Barat N, Dumler JS: **Assessment of a quantitative multiplex 5' nuclease real-time PCR for spotted fever and typhus group rickettsioses and Orientia tsutsugamushi**. *Clin Microbiol Infect* 2009, **15 Suppl 2**:292-293.
31. Devamani CS, Prakash JAJ, Alexander N, Stone W, Gunasekaran K, Rose W, Schmidt WP: **High initial IgG antibody levels against Orientia tsutsugamushi are associated with an increased risk of severe scrub typhus infection**. *PLoS Negl Trop Dis* 2021, **15**(3):e0009283.
32. Parente P, Silva JS: **Quantile Regression with Clustered Data**. *Journal of Econometric Methods* 2016, **5**:15.
33. Harrell FEj: **Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis**. New York: Springer; 2001.
34. Stewart AGA, Smith S, Binotto E, McBride WJH, Hanson J: **The epidemiology and clinical features of rickettsial diseases in North Queensland, Australia: Implications for patient identification and management**. *PLoS Negl Trop Dis* 2019, **13**(7):e0007583.
35. Sando E, Suzuki M, Katoh S, Fujita H, Taira M, Yaegashi M, Ariyoshi K: **Distinguishing Japanese Spotted Fever and Scrub Typhus, Central Japan, 2004- 2015**. *Emerg Infect Dis* 2018, **24**(9):1633-1641.

36. Helmick CG, Bernard KW, D'Angelo LJ: **Rocky Mountain spotted fever: clinical, laboratory, and epidemiological features of 262 cases**. *J Infect Dis* 1984, **150**(4):480-488.
37. Liyanapathirana VC, Thevanesam V: **Seroepidemiology of rickettsioses in Sri Lanka: a patient based study**. *BMC Infect Dis* 2011, **11**:328.
38. Kularatne SA, Weerakoon KG, Rajapakse RP, Madagedara SC, Nanayakkara D, Premaratna R: **A case series of spotted fever rickettsiosis with neurological manifestations in Sri Lanka**. *Int J Infect Dis* 2012, **16**(7):e514-517.
39. Batty EM, Chaemchuen S, Blacksell S, Richards AL, Paris D, Bowden R, Chan C, Lachumanan R, Day N, Donnelly P *et al*: **Long-read whole genome sequencing and comparative analysis of six strains of the human pathogen Orientia tsutsugamushi**. *PLoS Negl Trop Dis* 2018, **12**(6):e0006566.
40. Koralur MC, Singh R, Varma M, Stenos J, Bairy I: **Scrub typhus reinfection**. *Trop Doct* 2018, **48**(1):69-72.
41. Trowbridge P, P D, Premkumar PS, Varghese GM: **Prevalence and risk factors for scrub typhus in South India**. *Trop Med Int Health* 2017.
42. Fournier PE, Roux V, Raoult D: **Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA**. *Int J Syst Bacteriol* 1998, **48 Pt 3**:839-849.

Correspondence: Wolf-Peter Schmidt, Department for Disease Control, London School of Hygiene and Tropical Medicine, Keppel Street, WC1E 7HT, London, UK. Email: wolf-peter.schmidt@lshtm.ac.uk

Table 1. Characteristics of cases

	n/N	% or mean (SD, range)
Total	77/77	100
Female gender	30/77	39
Age group		
0-4	21/77	27
5-14	26/77	34
15-44	17/77	22
≥45	13/77	17
Duration of fever prior to first ELISA test (days)	-	7.9 (3.4, 2 - 15)
Eschar present	2/77	3
Skin manifestations		
Diffuse erythematous rash	7/77	9
Macular rash	3/77	4
Maculo-papular rash	41/77	53
Petechial rash	6/77	8
Purpura fulminans	2/77	3
Pedal/ankle oedema	16/77	21
Organ involvement / complications		
Lungs	2/77	3
Shock	3/77	4
CNS	6/77	8
Kidney	3/77	4
Liver failure	2/77	3
Myocarditis	1/77	1
Peripheral gangrene	2/77	3
Any organ involvement	10/77	13
Died during admission	0/77	0
Number of samples per patient		
1	36/77	47
2	26/77	34
3	6/77	8
4	8/77	10
5	1/77	1

CNS – central nervous system

Table 2. Characteristics of complicated SFGR infections (n= 10)

ID	Age / sex	Rash	CNS	Lungs	Shock	Kidney injury	Liver failure	gangrene	comments
1	13 M	Macular	Meningitis						
2	42 M	Maculo-papular	Meningitis			X			
3	11 F	Maculo-papular	Multiple seizures						CSF not tested
4	45 F	Maculo-papular, petechial	Acute psychosis						Mood swings, flight of ideas, delusions, CSF normal, MRI suggestive of encephalitis
5	40 F	-	Myoclonus		X	X			CSF not not tested
6	79 M	-	Parkinson-like						Mask-like face, rigidity, akinesia, CSF normal
7	3 M	Diffuse erythe-matous		X	X	X	X		Intubated (ARDS)
8	62 M	-		X					Moderate respiratory distress, oxygen supplementation via mask
9	35 F	Purpura fulminans			X		X	X	Intubated, myocarditis, bilateral middle phalanx and metatarsal amputation
10	67 F	-						X	Gangrene conservatively

managed

CSF- cerebrospinal fluid; CNS – central nervous system; ARDS – acute respiratory distress syndrome; MRI – magnetic resonance imaging

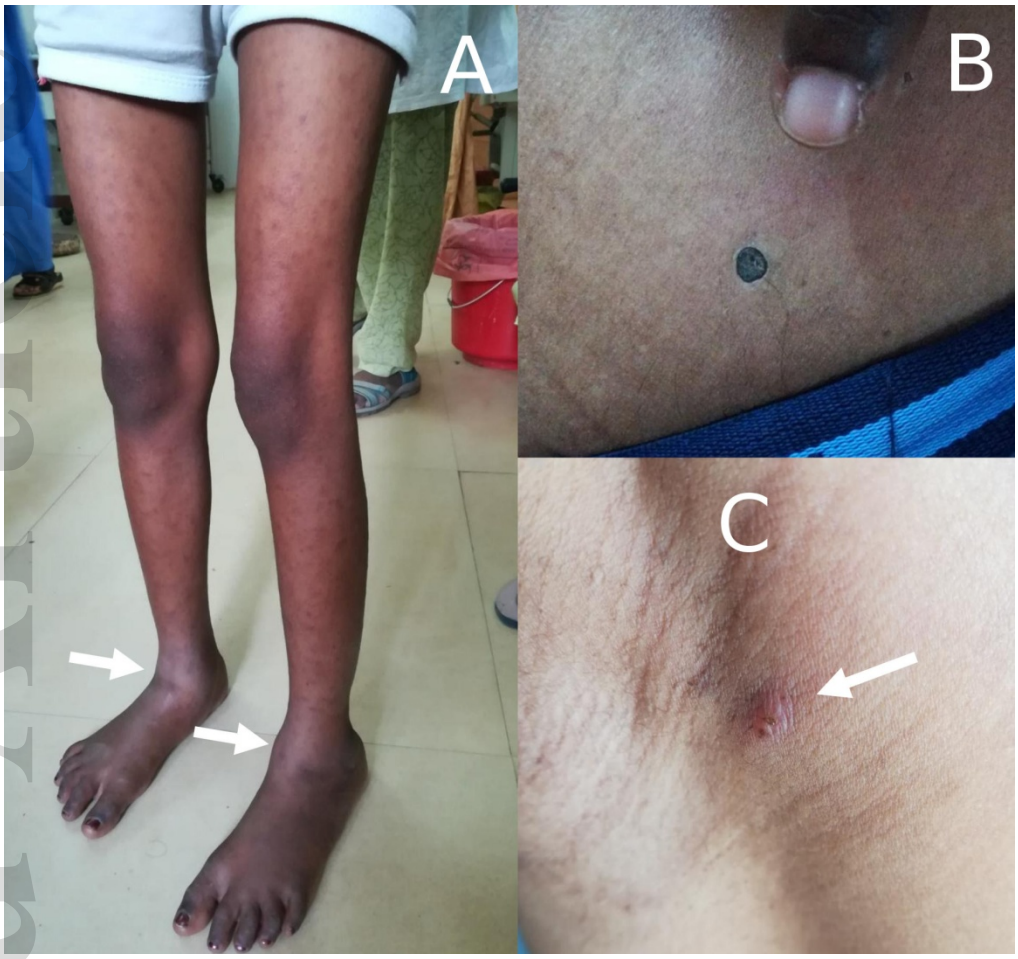

FIGURE 1. A) Typical presentation of SFGR infection in South India in a 7 year old female: maculo-papular rash with ankle / pedal oedema (arrows) and gait problems due to arthritic pain. B) Atypical circular-shaped eschar in lumbar region of 57 year old male, without per-lesional erythema. PCR from eschar biopsy was positive for OmpA. C) Oval-shaped eschar in axillary region of 13 year old male presenting on second day of fever. The lesion had formed 2 days before fever onset and regressed within two weeks of starting treatment. A biopsy was not done.

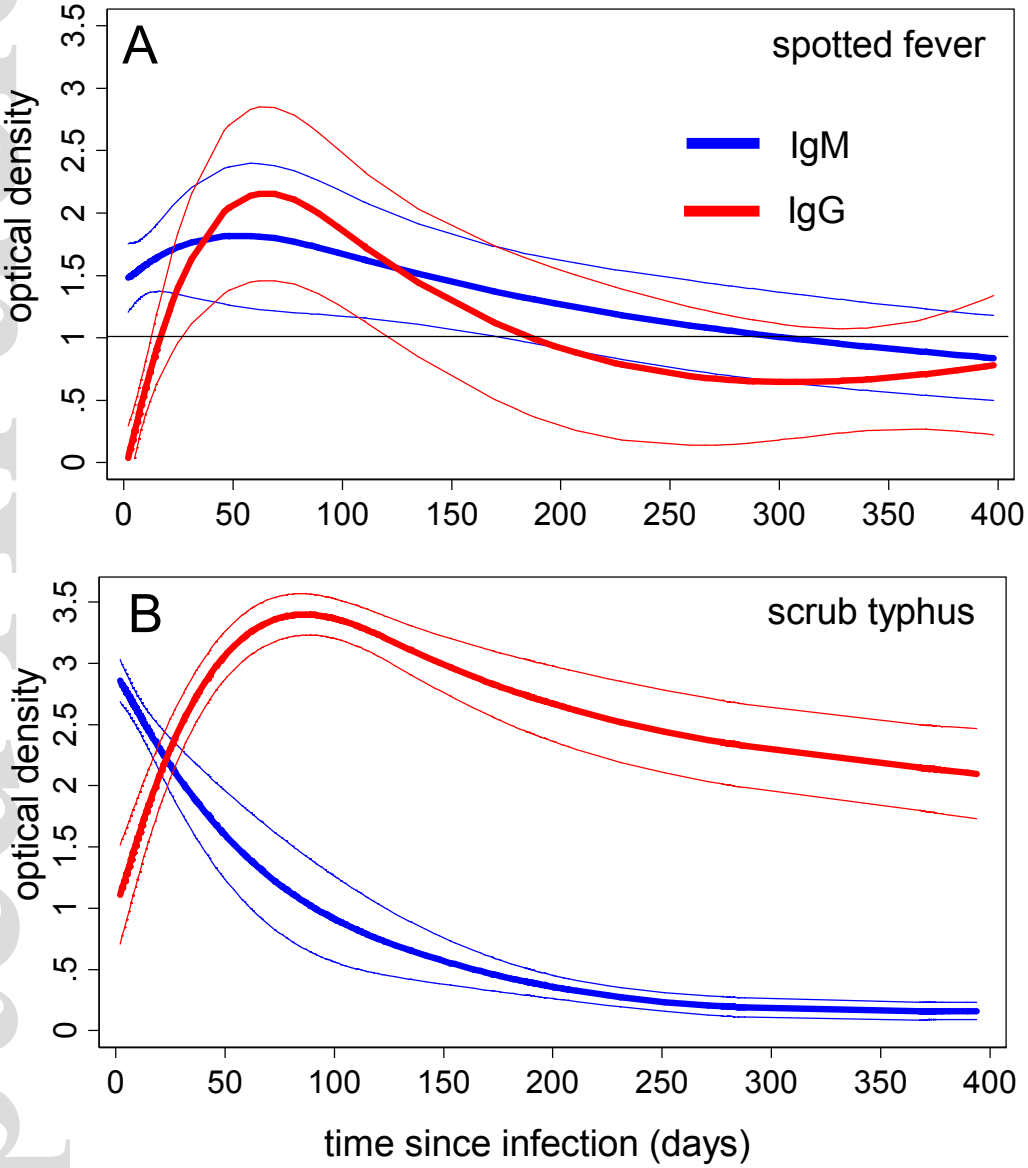

Figure 2. A) Median IgG and IgM optical densities following SFGR infection fitted using quantile regression. B) Corresponding figures for scrub typhus IgM and IgG from [24].

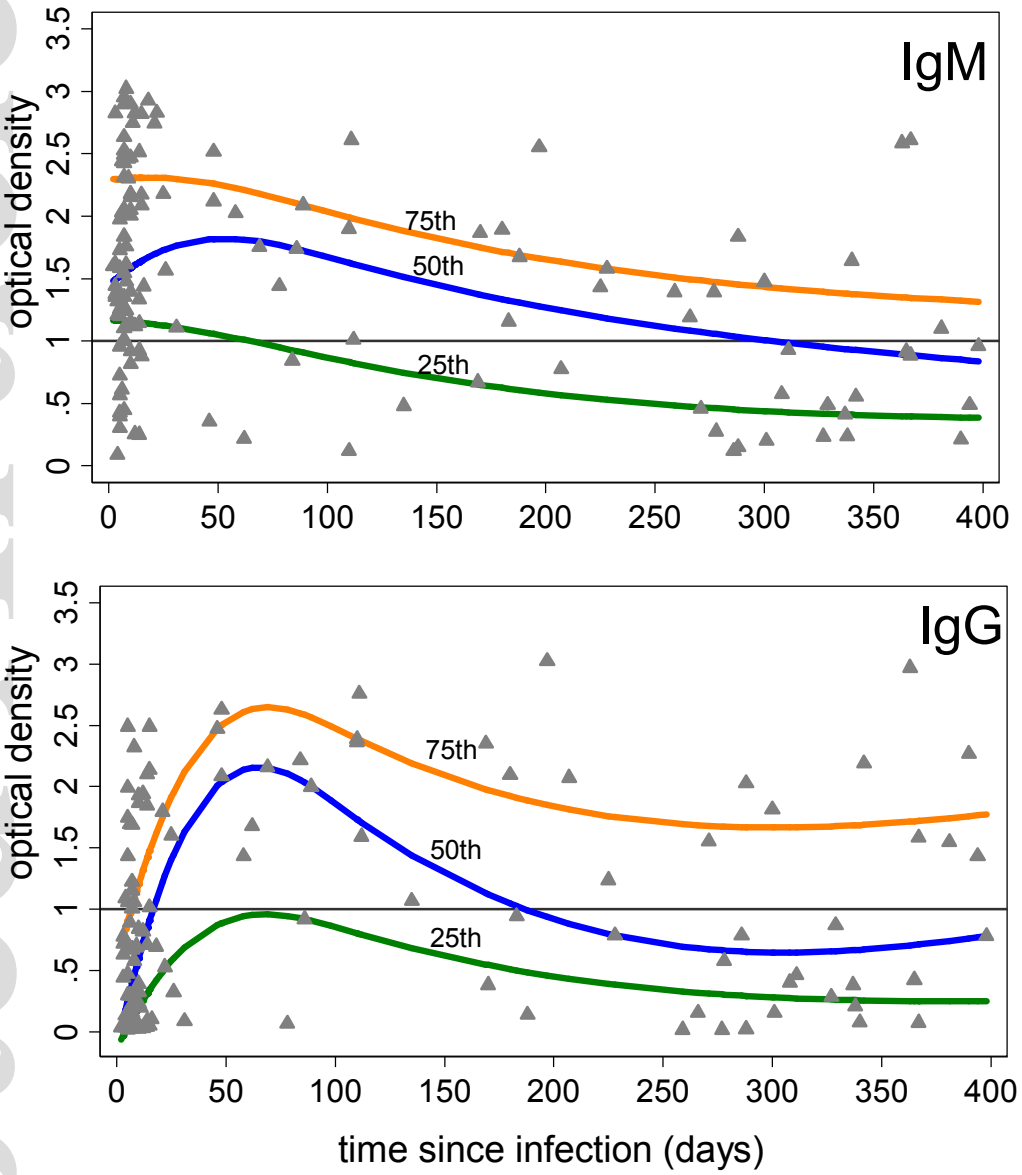

FIGURE 3. IgM (A) and IgG (B) optical densities following SFGR infection. Shown are 25th, 50th (as in Figure 2A) and 75th percentiles fitted using quantile regression. Triangles represent individual values.

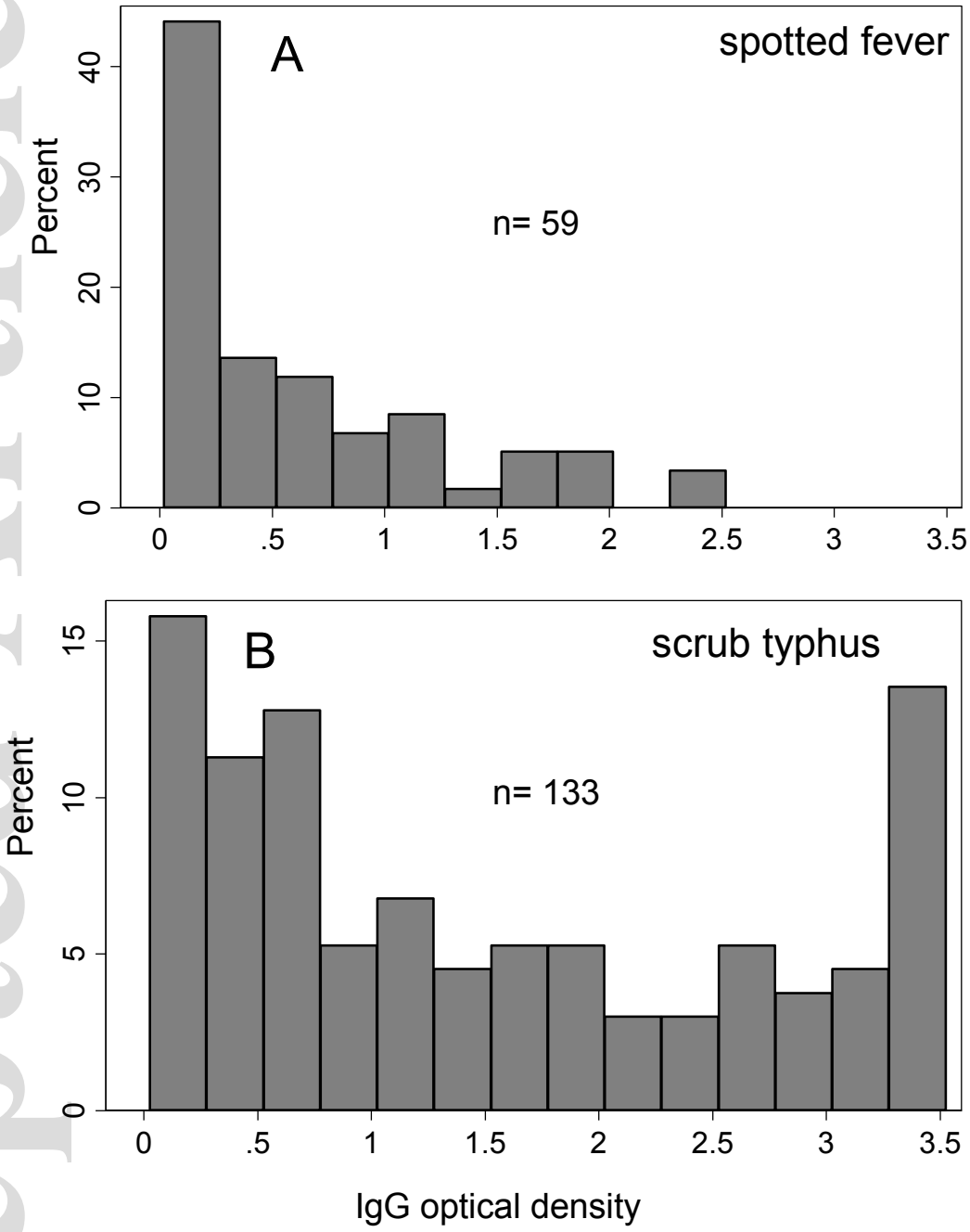

FIGURE 4. A) Distribution of IgG optical densities at presentation, restricted to cases tested within 10 days of fever onset. B) Corresponding histogram for scrub typhus (from [24])