**Incidence of Scrub Typhus in Rural South India: Cohort Study**

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**Abstract**

Background: Hospital studies suggest that scrub typhus is a leading cause of severe undifferentiated fever in endemic regions across Asia, but the population-based incidence of infection and illness has not been systematically studied.

Methods: This was a cohort study of 32,279 individuals of all ages from 7,619 households living in 37 villages, selected purposively for high endemicity in Tamil Nadu, India. Participants were visited every 6-8 weeks over two years for acute febrile illnesses. A venous blood sample was taken in those reporting a fever since the last visit. A sub-cohort of 3554 participants underwent annual blood sampling. Blood samples were tested for scrub typhus using ELISA, indirect immunofluorescence assays and PCR.

Results: During 54,588 person-years, we observed 6175 fever episodes, and a blood sample was taken in 72% of these episodes (4474 episodes). Of these, 328 (7.3%) met the case definition. The incidence of scrub typhus was 6.0/1000 person-years (95% CI 4.8, 7.5). Seventy-one cases (21.6%) were hospitalised (incidence rate 1.3/1000 person-years, 95% CI 1.0, 1.7). Twenty-nine cases (8.8%) were severe, with an incidence of 0.5/1000 person-years (95% CI 0.3, 0.8). The incidence of sero-conversion in the sub-cohort was 81.2/1000 person-years (95% CI 70.8, 91.6). Older age groups and women showed a higher incidence, but the risk of severe infection was higher in men. Baseline IgG sero-positivity did not protect against clinical illness but reduced severity.

Conclusion: This study demonstrates a high burden of scrub typhus in endemic areas and provides important epidemiological parameters to estimate disease burden across settings. (Study registration number NCT04506944 (clinicaltrials.gov).

## Introduction

Scrub typhus is an acute febrile infection, endemic to large parts of South Asia, East Asia and Southeast Asia,[1] with cases also reported from Chile[2] and East Africa.[3] Severe illness is characterised by acute respiratory distress syndrome (ARDS), shock, renal failure and meningo-encephalitis [4]. Scrub typhus is caused by intracellular bacteria of the genus *Orientia*, with *O. tsutsugamushi* being the dominant species in Asia.[5] Strain diversity of *O. tsutsugamushi* limits immunity against re-infection.[6] *Orientia* spp. are transmitted by the bite of trombiculid mite larvae (chiggers), leaving a characteristic eschar at the inoculation site.[7] Hospital-based studies indicate scrub typhus is a common or even predominant cause of severe undifferentiated fever throughout Asia.[4,8-12] Although doxycycline and azithromycin, alone or in combination,[13] are highly effective against *O. tsutsugamushi,* lack of awareness often leads to failure to initiate treatment,[14] and hampers efforts to estimate the burden of infection.[9,15] The population-based incidence of infection and illness has not been systematically studied. A Malaysian study from 1976 reported an annual incidence of clinical infection of 12/1000 among plantation workers.[16] Population-based studies found incidence rates of serological infection ranging from 5 to 20 per 1000 person-years.[17-19] However, the proportion of infections leading to clinical illness is unclear, limiting the applicability of serological data to calculate disease burden. There is also a lack of data on age and sex distribution by illness severity and on the immunogenicity of infection. We enrolled a large population-based cohort in an endemic setting in South India to estimate these important epidemiological and clinical parameters.

## Methods

*Enrolment and follow-up of participants*

This was a population-based cohort study done over two years in 37 rural villages of two districts (Vellore and Ranipet) in the South Indian State of Tamil Nadu, where scrub typhus mainly occurs from August to February, with a peak from October to January.[20] Villages were eligible for enrolment if: 1) earlier studies had indicated a sero-prevalence of *O. tsutsugamushi* infection of at least 15% (n= 28 villages),[10,21] or 2) at least two scrub typhus cases from a village were admitted to the study institution (Christian Medical College, Vellore) between 2016 to 2019 (n= 9). All residents who expected to reside for at

least 6 months in selected villages were requested to take part in the study. To estimate the incidence of serological infection, one participant aged 10+ years per household present at the time of enrolment was randomly selected and asked to take part in a sub-cohort (sero-cohort). Enrolment was conducted from February to July 2020. This was an open cohort with previously unenrolled households or new members within already enrolled households, being eligible for enrolment throughout follow-up.

Written consent for enrolment was obtained at the household level from the household head. Individual consent/assent was obtained prior to obtaining a blood sample. After enrolment, households were visited every 6 to 8 weeks for approximately two years, from August 2020 to July 2022. At each visit, participants were asked whether they had fever since the last visit or in the previous 2 months, whichever was shorter. For those not present, other household members were asked about their fever history. If a whole household was not present, an attempt was made to contact the household by phone. A venous blood sample was taken from all those reporting a fever, and a brief questionnaire on clinical details, including self-reported comorbidities was administered. Excluded from blood sampling were PCR-positive SARS-CoV-2 cases and cases with a local cause of fever (e.g., cellulitis). Whenever appropriate, a study nurse examined cases for the presence of an eschar. If feasible, a venous blood sample was collected from an asymptomatic control within the case or a neighbouring household. Acute fever cases were visited by a study nurse on the same day and, if indicated, advised to seek treatment from a health care provider of their choice. For ongoing fevers, we aimed to take a second blood sample 4-6 weeks later (convalescent sample).

Participants enrolled into the sero-cohort underwent blood sampling at enrolment and between March and June of each year. If any participant died during the study, a study nurse administered a brief verbal autopsy questionnaire to the remaining household members to establish whether the death was associated with an acute febrile illness.

The study was approved by Christian Medical College's Institutional Review Board (Ref: 11726) and the London School of Hygiene and Tropical Medicine's Research Ethics Committee (Ref: 16573). The study is registered at clinicaltrials.gov (NCT04506944), where the protocol (including sample size calculation) and statistical analysis plan are available.

The study coincided with the first three SARS-CoV-2 waves in India. Consequently, the originally intended sample size of 40,000 had to be lowered to about 32,000 due to movement restrictions in February/March 2020. For the same reason, one follow-up round was cancelled in May/June 2021.

*Laboratory testing and case definitions*

All blood samples from febrile cases (acute or convalescent) were tested for scrub typhus IgM and IgG using ELISA. An optical density cut-off of ≥1.0 was used to define IgM and IgG sero-positivity (see Supplement). All samples taken during the fever ("acute samples") were tested for the presence of *O. tsutsugamushi* using PCR. All samples from the sero-cohort were tested for *O. tsutsugamushi* IgG using ELISA. As *Orientia*-specific antibodies can be present at baseline due to past infections, indirect immunofluorescence assays (IFA) were performed to confirm a new infection in paired samples where IgG ELISA was positive at both time points.[18,22] Reversion from IgG ELISA positive to negative was treated as absence of infection without IFA confirmation. In the sero-cohort, IFA was restricted to a random sample of 25% of IgG ELISA-positive sample pairs. The Supplement contains more details on laboratory methods.

The clinical case definition was: self/carer-reported febrile illness plus 1) a positive scrub typhus IgM ELISA, or 2) a positive qPCR for *O. tsutsugamushi*, or 3) a typical eschar if no blood sample could be obtained. A probable case was defined as a case with a positive IgM ELISA or an eschar without ELISA result. A confirmed case was defined as a case with positive PCR, or a case with a positive IgM ELISA and either an eschar or a four-fold or greater increase in IFA or IgG ELISA sero-conversion (from acute to convalescent sample). Severe scrub typhus was defined as the presence of organ involvement, in particular lung, CNS, cardiovascular (shock, myocarditis) and kidneys, and included adverse pregnancy outcomes (miscarriage). For the sero-cohort, serological infection was defined independent of symptoms as: 1) IgG ELISA sero-conversion, or 2) a four-fold or greater increase in IFA titer to at least 1:128.

*Statistical analysis*

The incidence rate of clinical infection was calculated as the number of scrub typhus cases per 1000 person-years. For an individual, time at risk started 2 months prior to the first follow-up visit and ended with the last visit date at which the individual was still in the study. After scrub typhus infection, an individual was considered not at risk for 6 months, reflecting the short-lived protection against re-infection.[23-26] Estimates were adjusted for within-village correlation using jackknife. Logistic regression was used to estimate the probability of IgM-positivity in fever cases where a blood sample was not available (see Supplement). In addition, some people without fever can be IgM positive due to previous asymptomatic infections.[18] The clinical incidence rate was therefore adjusted for the prevalence of IgM positivity in asymptomatic controls using population-attributable fractions (see Supplement).

Risk factors were evaluated using Poisson regression (age- and sex-adjusted). Due to concerns regarding the willingness to report fevers and provide blood samples in the context of SARS-CoV-2 control measures, we conducted an unplanned subgroup analysis where the estimation of the incidence of clinical infection was restricted to participants of the sero-cohort. The incidence of serological infection in the sero-cohort was calculated separately for subgroups who were baseline negative (based on ELISA IgG sero-conversion) or baseline positive (based on ≥four-fold IFA titer increase).

Incidence was calculated using complementary log-log models with the time between blood samples as the denominator and robust standard errors to account for within-village correlation.[27] Individuals without midline samples were excluded. To account for demographic differences between the sero-cohort and the main cohort (Figure S1), sero-incidence was age/sex/year standardised using the main cohort demographics (see Supplement).

**Results**

*Serological study sub-cohort*

5903 participants of the sero-cohort provided at least one sample (Figure 1). ELISA-IgG seroprevalence at baseline was 42.8% (95% CI 35.8%, 50.2%). Seroprevalence increased with age (Figure 2A). 3554 participants provided at least 2 consecutive samples. We observed 265 sero-conversions in 3238 initially sero-negatives and an age/sex/year-standardised incidence of 81.2/1000 person-years (95% CI 70.8, 91.6). The incidence of sero-conversion increased with age (Figure 2B) and was higher in females than in males (age-adjusted RR 1.5, 95% CI 1.2, 1.9). Incidence was 1.6 times higher in the second year than the first (95% CI 1.2, 2.1). The standardised incidence of sero-reversion from positive to negative was 109.5/1000 person-years (95% CI 91.0, 128.0), with lower rates in older age groups and in the second year (Figure S2).

A four-fold or greater IFA titre increase was observed in 50 of 636 initially sero-positive sample pairs tested (incidence rate 81.1/1000 person-years, 95%CI 61.3, 107.5).

*Clinical cohort*

32,279 individuals (Figure S1) from 7619 households were observed over 54,591 person-years (Figure 1) during 13 rounds of follow-up. We observed 6175 fever episodes, with a blood sample taken in 4474 (72%). Of these, 328 episodes (7.3%), occurring in 316 individuals, met the clinical case definition for scrub typhus, and of these, 118 (36.0%, Table S1), the definition of a confirmed case. An eschar was found in 18.6% (61/328) of cases. The proportion of fever cases diagnosed as scrub typhus increased with fever duration and higher care level (Figure 3), with scrub typhus accounting for 29.3% of all fever hospitalizations. The incidence of scrub typhus based on the clinical case definition was 6.0 per 1000 person-years (95% CI 4.8, 7.5). Adjustment for sample unavailability and background IgM sero-prevalence suggested a rate of 6.6/1000 person-years (Supplement). Case numbers showed a marked seasonality (Figure S3), and higher rates in the second year (RR 1.4, 95% CI 1.1, 1.8). Seventy-one cases were hospitalized (incidence rate 1.3/1000 person-years, 95%CI 1.0, 1.7). Twenty-nine cases (8.8% of clinical cases, Table 1) were severe (incidence rate 0.5 per 1000 person-years, 95%CI 0.4, 0.8). Compared to males, females had a higher age-adjusted rate of clinically apparent (RR 1.6, 95%CI 1.3, 2.0), but not of severe infection (RR 1.0, 95%CI 0.5, 2.1). The rate of clinical and severe infection

increased with age (Figure 2). Among clinical cases, diabetes was associated with a 2.4-fold increase in the risk of severe infection (Table S2).

Baseline IgG sero-status was available in 5891 participants of the sero-cohort and 99 clinical infections occurred. In these, baseline IgG positivity did not protect against subsequent clinical illness (RR 1.4, 95%CI 0.9, 2.1). Of the 99 cases, 5 were severe, all of whom were sero-negative at baseline (Fisher's exact p= 0.02).

A verbal autopsy was done in 642 of 645 deaths (Table S3). Five deaths were due to (confirmed) scrub typhus (Table 1), an incidence rate of 0.09/1000 person-years, 95% CI 0.04, 0.2). Thirty-seven fever deaths were due to SARS-CoV-2. Thirty-three fever deaths were unexplained, most of which (but not all) coincided with SARS-CoV-2 waves in India (Figure S4).

Restricting the analysis to participants of the sero-cohort, the standardized incidence of clinical infection was 12.4/1000 person-years (95%CI 8.9, 14.5). The incidence rates of scrub typhus hospitalizations and severe infection were 1.3/1000 person-years (95% CI 0.7, 2.4) and 0.8/1000 person-years (95% CI 0.4, 1.9), respectively.

**Discussion**

This large cohort study conducted in a highly endemic setting yielded estimates of scrub typhus incidence by severity, allowing us, for the first time, to construct a severity pyramid of this important yet neglected vector-borne infection (Figure 4). Most infections caused no or minimal symptoms. Among those with symptomatic illness, however, hospitalisations and severe illness were common. Given that the study was conducted under the unusual circumstances of a pandemic, with SARS-CoV-2 accounting for almost 50% of fever admissions, scrub typhus is likely to be the most common cause of fever admission outside pandemic situations in this setting, confirming earlier hospital-based studies here and in other endemic regions.[4,8,9,12,28]

The study also provides insights into the immunogenicity of the infection. Sero-prevalence and sero-incidence across all levels of severity increased with age (Figure 2), possibly because traditional lifestyles involving high-risk behaviours such as agricultural activities[21,29]

may be more common in those who are older. The increase in IgG sero-prevalence with age is due to the higher rate of sero-conversion and lower rate of re-version in older ages (Figure S2), leading to a gradual build-up of antibodies following repeated infection.[24]

High baseline IgG antibody levels did not protect from clinical infection; in fact, seemed to predispose to it, presumably because high antibody levels reflect frequent, ongoing exposure to the vector. However, despite small numbers, there is a suggestion that baseline IgG sero-positivity confers protection against severe illness. None of the 5 severe cases with available baseline IgG sero-status were IgG sero-positive for scrub typhus prior to the infection. Frequent reversion to sero-negativity after infection means we cannot exclude prior infection in these 5 individuals. On the whole, however, our findings do not support the previous suggestion by our group of antibody-dependent enhancement in the case of scrub typhus.[30] Why older age groups are at the highest risk of severe infection despite high sero-prevalence merits further study. Immuno-senescence and comorbidities, in particular diabetes as demonstrated here and previously,[31,32] may play a role.

In the study area, males and females account for similar numbers of admissions for severe scrub typhus,[30] a finding confirmed by the present study where at population level, males and females were at an equal risk of severe illness. The higher overall risk of infection in females may suggest that if infected, males are at a higher risk of developing severe illness. This pattern is already evident in other infections, most notably SARS-CoV-2.[33] As highlighted by the miscarriages in two women with otherwise non-severe scrub typhus, pregnant women constitute a further vulnerable group.[34-36]

Under-reporting of febrile illness is likely to represent the most important limitation of this study. Given the strict SARS-CoV-2 quarantine measures implemented in the study area, this was to be expected. Due to logistical challenges during the pandemic, we were unable to maintain planned visits every 4 weeks. On the other hand, long visit intervals may have reduced changes in treatment-seeking behaviour among study participants. The subgroup analysis of the sero-cohort participants noted actual rates of clinical infection twice as high as in the main cohort (rates of hospitalisation and severe infection seem to be less affected by under-reporting – Figure 4). Using this estimate in a more selected group suggests that about one in seven infections could be symptomatic, while one in 14 clinical infections may

lead to severe illness (Figure 4). Additional under-reporting of fevers in the sero-cohort sub-population affecting these figures can, however, not be excluded.

Case ascertainment was mainly based on a recombinant antigen ELISA, which has shown high sensitivity and specificity in this setting.[37] Adjustment for IgM sero-positivity in asymptomatic controls using population-attributable fractions lowered the incidence estimates, but this was offset by accounting for fever cases not tested (see Supplement). However, this method does not fully account for the possibility of cross-reactivity with other pathogens. We used IFA-based titration to confirm infection in those IgG ELISA positive at two time points. Among the limitations of this approach is the difficulty of choosing the fold increase to define sero-conversion (for further discussion see Supplement).[38] Sero-conversion based on IgG ELISA is more straightforward to define but is subject to the choice of locally applicable cut-off points.[37,39]

To conclude, this study estimated important epidemiological parameters that may help quantify the burden of scrub typhus infection across endemic regions in Asia where large populations are at risk. Such disease burden estimates will be needed to calculate the cost-effectiveness of vaccines, the development of which has not yet advanced very far.[40] The burden of scrub typhus demonstrated in this study underscores the need to allocate more resources to this disease for research[7,14] and medical education as a common cause of undifferentiated febrile illness.

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**Table 1.** Clinical characteristics of severe cases

| | N (%) | % confirmed case (n/N) | Case details |
|---|---|---|---|
| **Clinical features** | | | |
| ARDS | 19 (66%) | 79% (15/19) | |
| CNS | 4 (14%) | 75% (3/4) | Encephalitis (2x), Meningitis with cranial nerve palsy (2x) |
| Shock | 11 (38%) | 55% (6/11) | |
| Acute kidney injury | 6 (21%) | 50% (3/3) | Dialysis done in 1 case |
| Myocarditis | 2 (7%) | 100% (2/2) | |
| Miscarriage | 2 (7%) | 50% (1/2) | Miscarriage at 8 weeks during fever (1x), Miscarriage at 7 weeks one week after fever (1x) |
| Death | 5 (17%) | 100% (5/5) | Multi-organ failure (4x – 29y female, 42y male, 50y female, 70y male)<br>Sudden death (1x – 80y female) |
| **Respiratory support** | | | |
| High flow O2 via mask/nasal prong | 13 (45%) | 50% (4/4) | |
| Non-invasive ventilation | 3 (10%) | 100% (3/3) | |
| Invasive ventilation | 5 (17%) | 80% (4/5) | |

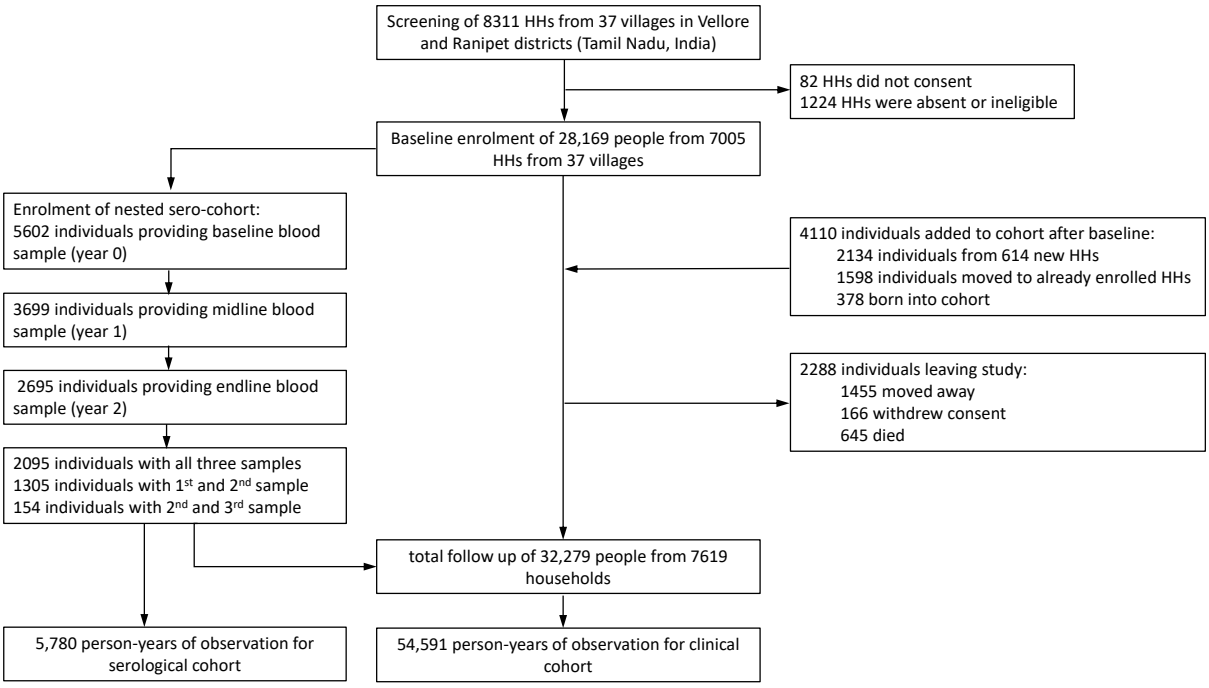

**Figure 1.** Study flow diagram

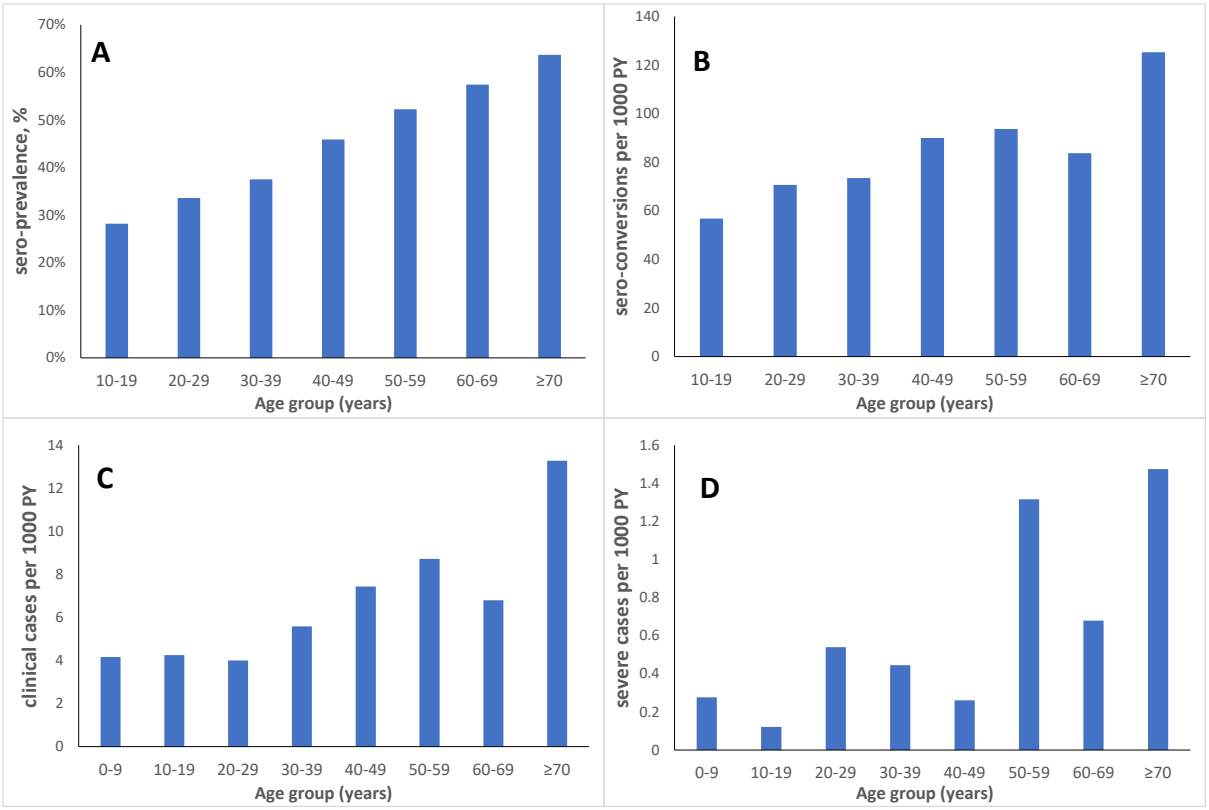


**Figure 2.** Scrub typhus sero-prevalence (A), sero-incidence (B), incidence of clinical infection (C) and incidence of severe infection (D) by age.

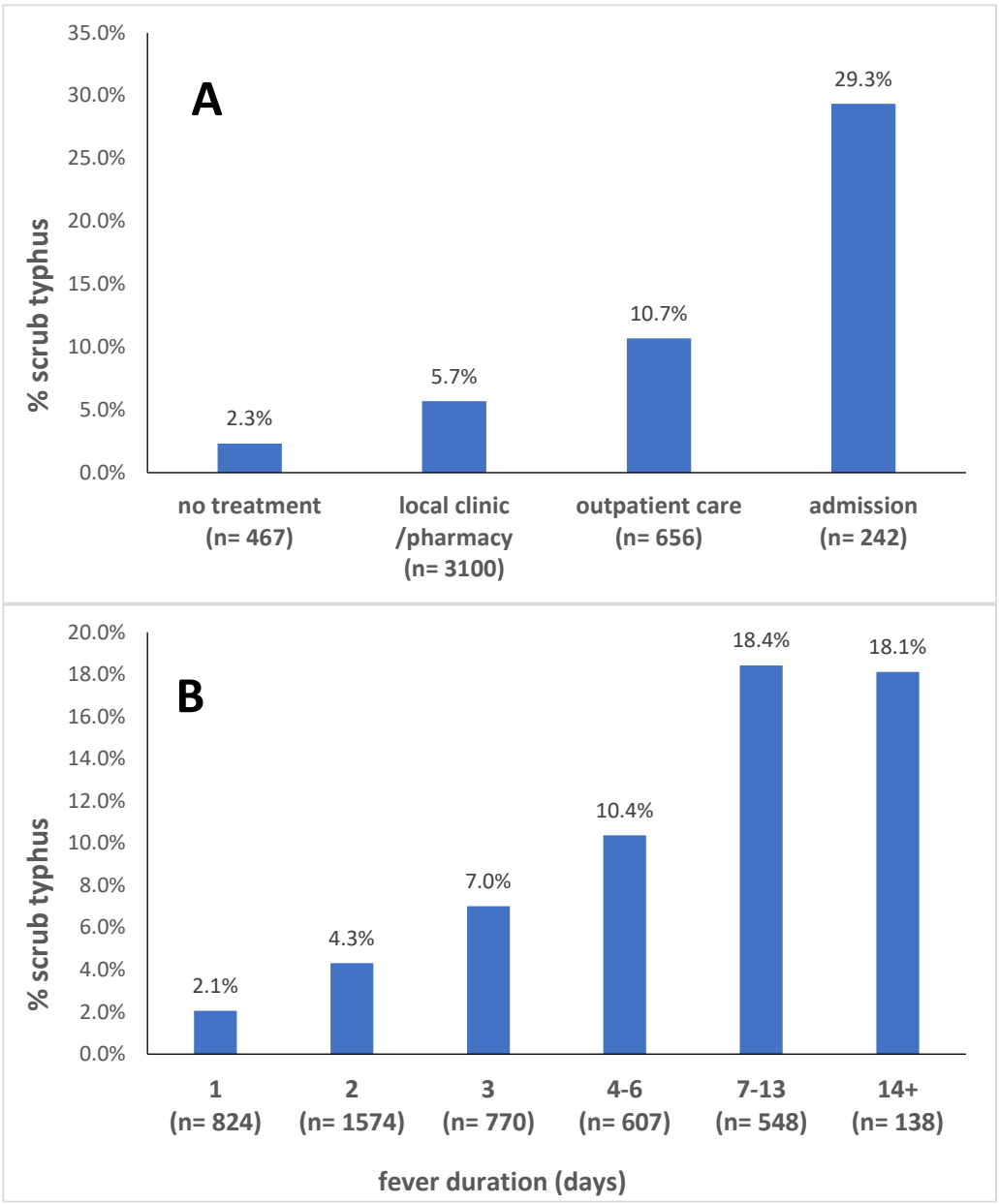


**Figure 3.** Proportion of scrub typhus cases among fever cases by treatment level (A) and total fever duration (B).

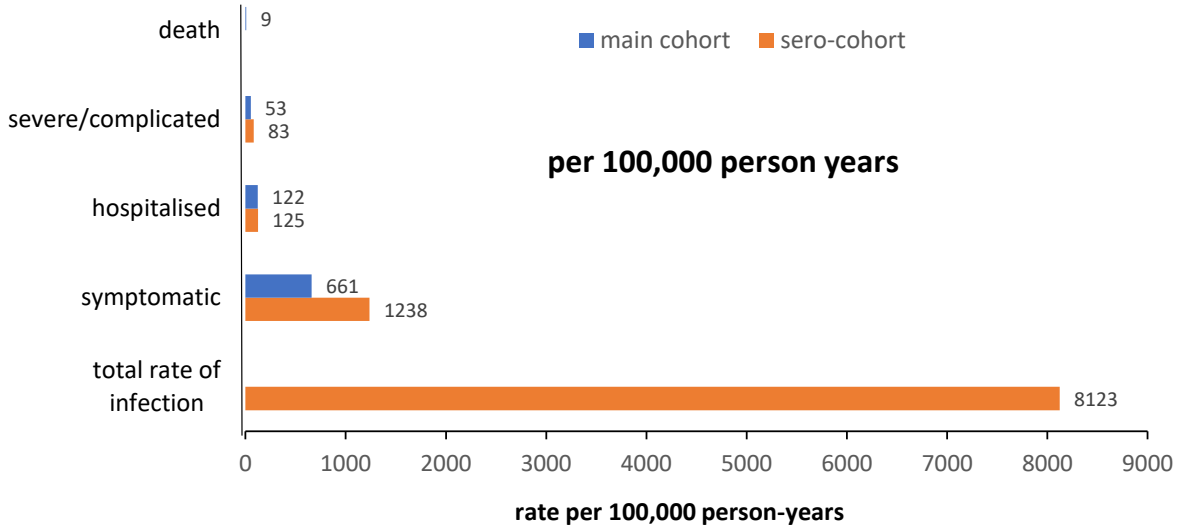


**Figure 4.** Summary of the rates of scrub typhus infection per 100,000 person years by level of severity estimated from the main cohort (blue) and sero-cohort (orange). The estimate for sero-conversion was used to represent total rate of infection.