

Scrub Typhus in Indonesia

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Abstract

Scrub typhus is a neglected and understudied disease. In 1902, Schüffner first described scrub typhus in Indonesia. However, until today, data are lacking and we do not understand its public health significance yet. In this DPhil project, I aimed to (1) characterise scrub typhus epidemiology in Indonesia and its public health importance, and (2) address knowledge gaps in scrub typhus by optimising available data. I mapped and ranked the available evidence on scrub typhus in Indonesia based on the likelihood of sustaining infectious reservoirs. Despite limited data, it appears that scrub typhus does exist and is widespread within the Indonesian archipelago. North Sumatra has the highest number of data points. South Sumatra and Biak have the best evidence in sustaining infective vectors. Serosurvey of archived sera indicated active transmission of scrub typhus in Jambi, Denpasar, and Tabanan. However, the majority of areas in Indonesia did not have any data. To address this, a seroprevalence study to compare exposure to scrub typhus in rural and urban areas of North Sumatra was planned.

Diagnosis seems to be the main challenge hindering scrub typhus research. I reviewed the evidence on the accuracy of scrub typhus point-of-care tests. I also performed a meta-analysis on the limited data. The available immunochromatography tests have good specificity – indicating the potential role for ruling in diagnosis. However, they suffer from relatively low sensitivity and are not widely available. A systematic review on scrub typhus treatment studies demonstrated heterogeneity in methods. The optimal treatment dose and duration for scrub typhus is still uncertain. There is an absence of consensus in treatment and research methodologies. There are still gaps in our knowledge on scrub typhus in Indonesia and globally. One way to improve awareness of scrub typhus is to recognise scrub typhus as a neglected tropical disease. Scrub typhus research is also influenced by economic and political factors. Stakeholder engagement and cooperation are therefore essential to fill these knowledge gaps.

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Last but not least, I would like to thank my family and friends for their relentless support and encouragement. I would not be able to finish this thesis without them.

Author contributions

In this section, I will detail what research activities I performed myself in each results chapter.

I conducted the search, data extraction, analysis, and write up in *Chapter 3. History of scrub typhus in Indonesia*. Dr Marlous Grijsen assisted with extraction of Dutch references.

In *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*, I performed the literature search, data extraction and curation, mapping, analysis, and write up. Dr Marlous Grijsen assisted with extraction of Dutch references.

I coordinated the sample selection and laboratory analysis, prepared and submitted the ethics application, performed data curation, supported the laboratory testing, performed data analysis, and wrote for *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples*. Ampai Tanganuchitcharnchai and Sirada Ongchaikupt performed the enzyme-linked immunosorbent assay (ELISA).

For *Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform*, I performed the search of articles, screened the title and abstract, assessed full texts for inclusion, designed the database, extracted and analysed the data, and wrote the chapter. Dr Sauman Singh and Dr Roland Ngu helped in assessing the full text of articles and extracting data. Brittany Maguire helped in resolving any conflict in article inclusion.

I searched the databases, screened and assessed the eligibility of articles, extracted and analysed the data, and wrote up the results in *Chapter 7. Scrub typhus point-of-care testing: A systematic review and meta-analysis*.

Publications from this thesis

There are two published papers which have arisen from this thesis:

1. Saraswati K, Day NPJ, Mukaka M, Blacksell SD. **Scrub typhus point-of-care testing: A systematic review and meta-analysis**. PLoS Negl Trop Dis. 2018 Mar 26;12(3):e0006330.
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The preliminary results of *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping* were presented in the 2nd Asia-Pacific Rickettsia Conference 2019 and 68th Annual Meeting of the American Society of Tropical Medicine and Hygiene 2019.

Manuscripts based on *Chapter 3. History of scrub typhus in Indonesia*, *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping, Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples*, and *Chapter 8. Serological investigation of risk of exposure to scrub typhus in residence at an oil palm plantation in North Sumatra relative to urban dwellers at Medan, North Sumatra (SEROSCRUB): A study protocol* are in preparation.

Impact of Covid-19 pandemic

The Covid-19 pandemic has affected the research plan and we could not carry out several research activities. The Eijkman Institute was one of the national referral diagnostic centres. Many of the laboratory facilities, spaces, and human resources were redirected towards Covid-19 diagnostic efforts. I volunteered to assist with the diagnostic efforts for a few months. With travel restrictions, we could not transport reagents and samples to perform confirmatory tests on scrub typhus positive samples. We could not gather and test negative control samples from populations that were presumed unexposed to scrub typhus.

We could not undergo the planned study (*Serological investigation of risk of exposure to scrub typhus in residence at an oil palm plantation in North Sumatra relative to urban dwellers at Medan, North Sumatra (SEROSCRUB)*) due to the face-to-face nature of the study. In addition, our collaborating sites were occupied with the Covid-19 response. The ethics clearance process was also delayed as studies on Covid-19 were prioritised. I hope to continue the planned research activities in the future.

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Chapter 1. General introduction

Scrub typhus is a potentially fatal, acute undifferentiated febrile illness caused by obligate intracellular bacteria from the genus *Orientia* [1, 2]. The bacteria are transmitted during feeding by the larva mites from the genus *Leptotrombidium*. The main pathogen in this genus – *Orientia tsutsugamushi*, is mainly prevalent in the Asia-Pacific region in an area called the 'tsutsugamushi triangle'. The 'triangle' is bordered by Afghanistan and Pakistan in the west, north Japan and far east Russia in the north, and northern Australia in the south. In recent years there is increasing evidence of *Orientia* distribution outside of this classical area, including the Middle East, South America, and Africa [1]. More recently, two new candidate species from the genus *Orientia* were discovered: *Candidatus* Orientia chuto and *Candidatus* Orientia chiloensis contracted in Dubai and Chile, respectively [3, 4].

Infection of humans is often described as taking place in a distinct focus of infection called a 'mite island' [2]. Mite islands can be found among scrub (i.e. tall-growing coarse grass), forest, and plantations (paddy, rubber, palm oil) [2]. This gave the name 'scrub typhus' which refers to the 'scrub vegetation' – the secondary growth after mature tropical forest has been cut [5]. Scrub typhus transmission occurs in a variety of ecological settings, from mountains in South Korea to beaches in Australia [6, 7]. Scrub typhus is thought commonly to be a disease of rural areas [8]. However, there is increasing documentation of scrub typhus occurrence in urban areas [8].

Scrub typhus is an understudied and neglected disease [5]. Although fulfilling the criteria of neglected tropical diseases (NTDs) by the WHO, it is not listed as one [9]. This exclusion impacts the research interest and funding available [10]. The lack of scrub typhus data could be attributed to two main problems: first, the absence of accurate and accessible diagnostic methods; second, since it is treatable with widely available antibiotics, stakeholders might believe that there is no need to do further research on treatment despite the varied, or at times absent, treatment recommendations.

Indonesia lies within the tsutsugamushi triangle – the classical depiction of where scrub typhus is prevalent. There was documentation of scrub typhus outbreaks in Indonesia during the Dutch colonisation era in plantations in the east coast of Sumatra [11, 12]. Scrub typhus also affected the Allied and Japanese forces when they landed in Papua and surrounding islands [13]. With close to half the population living in the rural areas where scrub typhus is likely to be prevalent, the amount of available data on scrub typhus in Indonesia is meagre [5, 14]. We know next to nothing about the distribution of scrub typhus in Indonesia or how big of a problem it is.

Through this DPhil project, I utilised different approaches to fill the gaps in scrub typhus research both in Indonesia and globally. I aim to characterise scrub typhus distribution and its public health significance in Indonesia. In addition, to address the two main gaps in scrub typhus research i.e. diagnosis and treatment: I summarised the diagnostic value of the available point-of-care tests; and assessed the feasibility of establishing an

individual-participant level data (IPD) platform, to ultimately investigate the efficacy of available scrub typhus treatment through IPD meta-analyses.

In this chapter I outline important aspects of scrub typhus as a disease, a review on Indonesia, and of scrub typhus in Indonesia.

History

The term typhus originated from the Greek word *tuphos* meaning 'smoke or haze' [15]. According to Chadwick and Mann, as cited in Paris et al 2020, this term was used for the 'confused state' often found in 'high fevers' in 460 BC during Hippocrates' lifetime [16]. As the etymology suggested, the history of scrub typhus identification turned out to be hidden behind 'smoky clouds', complicated by much confusion [15].

In the 1900s, scrub typhus was documented across the Asia Pacific [1, 15]. There were many names used to describe this disease (Table 1) [1]. Because the diagnostic methods were not as developed back then, many of these diseases were considered as separate entities. This has caused confusion about the identity of these many local diseases that were actually caused by the same bacterium. The nature of the disease was similar to other endemic illnesses such as typhoid fever (while not having the same aetiology) and epidemic typhus (while not sharing their epidemic distribution), adding another layer of

confusion to the then mysterious disease [15]. The history of scrub typhus discovery is discussed further in the next chapter (*Chapter 3. History of scrub typhus in Indonesia*).

Table 1. Different names used to describe scrub typhus

Synonyms	**Country**
Shashitsu	China
Tsutsugamushi	Japan
Kedani disease	Japan
Japanese river fever	Japan
Flood fever	Japan
Island fever	Japan
Akamushi disease	Japan
Shimamushi disease	Japan
Pseudotyphoid	Indonesia
Chigger-borne rickettsiosis	Ubiquitous
Mite-borne typhus	Ubiquitous
Mite fever	Ubiquitous
Rural or “K” form of tropical typhus	Malaysia
Fiévre exanthématique avec ulcére primaire	French Indo-China
Indian mite typhus	India
Mijtekoorts	Indonesia
Mossman fever	Australia
Sarina fever	Australia
Tropical typhus	Malaysia
Rural typhus	French Indo-China

Modified from: Richards and Jiang, 2020 [1]

One name for scrub typhus, *shashitsu*, has been used for more than a thousand years in China. According to Kawamura et al (as cited by Richards et al), the earliest reference to scrub typhus is to be found in a Chinese clinical manual written by Hong Ge in 313 AD

[1, 17]. A description of the characteristics of scrub typhus was included in a poem written in 610 AD by Yuan-Fang Cao [1]. In the book 'Ben Cao, Gang Mu', Shi-Zhen Li, a physician, outlined the clinical manifestations of *shashitsu*. In the earliest account of scrub typhus by Hong Ge, it was described as being caused by 'the bite of a mite' that was often found in summer in areas that were 'flooded by the spring rains' [17]. The bite was followed three days later by high fever and the formation of a pustule at the site of 'bite' [17]. Early descriptions already identified the concept of 'mite islands', where infections only happened to people that entered certain areas [17].

The term *tsutsugamushi* has been used 'since the earliest historical times' in Japan [17]. In Japanese, *tsutsuga* means disease or illness and *mushi* means insect or bug [1]. Though recognised for a long time, scrub typhus was first described in the literature by Hakuju Hashimoto in 1810 as a deadly disease in the Niigata prefecture [18]. Much work was done by the Japanese researchers to find the aetiology of scrub typhus [17]. There were several schools of thought on the potential cause: bacterium, protozoa, or toxin [17]. Palm and Bälz, in 1878 and 1879 respectively, wrote the first articles on scrub typhus published in European journals [17].

In Indonesia, scrub typhus was first reported as 'pseudotyphoid' (*pseudotyphus* in Dutch) by Dr Schüffner in plantations of Deli, North Sumatra in 1909 [12, 19]. The Institute of Medical Research (IMR) in Malaysia started research on a febrile illness they called 'tropical typhus' in 1924 [1]. The term tropical typhus was later found to

encompass both scrub typhus and murine typhus [20]. The IMR played important roles in the history of scrub typhus both in the diagnosis, through the development of Weil-Felix test to diagnose scrub typhus, and treatment, through the seminal clinical trial of the antibiotic chloromycetin [15].

During World War II, it was estimated that there were at least 16,000 cases in Allied soldiers when they were deployed to the areas within the tsutsugamushi triangle [13]. There is thought to have been a similar number of cases in Japanese soldiers [13].

There is increasing evidence of scrub typhus presence outside tsutsugamushi triangle [21]. In addition to the findings of novel *Orientia* species in Dubai and Chile, there have also been reports of scrub typhus circulation in Africa. In 1951, Giroud and Jadin reported several acutely ill Africans showing positive reactions to skin and complement fixation tests against *O. tsutsugamushi* antigen in the Belgian Congo (in present day Rwanda and Burundi) [22]. Antibodies against *O. tsutsugamushi* were also found in febrile patients in Peru [23].

In addition, scrub typhus's incidence in countries within tsutsugamushi triangle is also increasing. Passive surveillance in South Korea, Japan, Thailand, and China have reported increase in incidence [8]. This increase is also observed in urban areas [8]. Though the increase could also be influenced by increased awareness and improved

diagnostic capabilities and accessibility, it is likely that more and more cases will be found.

Aetiology

In the beginning, protozoa was suspected to be the aetiological agent of scrub typhus [13]. In 1910, *Gregarina tsutsugamushi* was described as the potential agent [13]. In 1920, Hayashi described a 'protozoan-like' microorganism named *Theileria tsutsugamushi* [24]. As cited by Richards et al 2020, Hayashi later corrected his report in 1924 and clarified that this organism is actually a bacterium (rickettsia), not protozoa – but he did not rename the organism [1]. Another researcher, Nagayo in 1930 also reported the pathogen and called it *Rickettsia orientalis* [1]. Then in 1931, Ogata studied the bacterium and gave it a different name – *Rickettsia tsutsugamushi* [1]. Meanwhile, in the same edition of the journal where Ogata's work was published, Kawamura also reported *Rickettsia akamushi* as the aetiological agent of scrub typhus [1]. Finally, in 1924, Hayashi clarified that the pathogen he found back in 1920 was indeed the same as *R. tsutsugamushi* and *R. orientalis* [1]. After the Bergey's Manual of Determinative Bacteriology, 6th ed, used the name *R. tsutsugamushi*, it gained traction [1, 25]. In 1995, due to 'phenotypic and genotypic differences' with other Rickettsia species, *R. tsutsugamushi* was reclassified into a new genus – *Orientia* [26]. The name *Orientia tsutsugamushi* was proposed to honour Nagayo et al and Ogata. This name is also fitting since the microorganism is prevalent in the 'Orient' [26].

O. tsutsugamushi is Gram-negative, rod-shaped, 1.2 to 3.0 µm in length and 0.5 to 0.8 µm in diameter [26]. The outer envelope of its cell wall is thicker than the inner leaflet; this sets this microorganism apart from species under the genus *Rickettsia* [26]. Its cell wall is also relatively deficient in peptidoglycan, making it very soft and fragile – but also giving it more resistance to penicillin [26]. The 56-kDa protein is the most abundant protein in the cell surface of *O. tsutsugamushi* [27]. Within the host cell, growth mostly takes place in the perinuclear cytoplasm [26]. Then, these newly formed bacteria are released through a 'budding process', where from the inside, the bacteria push out the cytoplasmic membrane of the host cells [26]. This process is similar to that used by enveloped viruses [26], and is one not often observed among *Rickettsia* species. *Orientia* isolates have bigger genomes than most Rickettsiaceae [28]. There is evidence of extensive amplification of repeat elements, as well as immense chromosomal rearrangements between strains [28]. There are at least 24 different strains of *O. tsutsugamushi* identified [29], the prototype strains being Gilliam, Karp, and Kato [29]. Antigenic variation in *O. tsutsugamushi* will be discussed in more detail in the next section.

Antigenic variation of scrub typhus

The Karp strain was isolated from an American soldier named Karp who was deployed in the Buna-Guna areas of present-day Papua New Guinea [30]. He was injured and evacuated from the field to the 42nd US Hospital, in Brisbane, Australia in 1943 [30, 31]. During treatment, he developed acute fever and was diagnosed with scrub typhus [30].

In 1944, the Gilliam strain was isolated from the blood of a United States Public Health Service (USPHS) doctor, Lt. Col. (Dr.) Alexander Gordon Gilliam [30, 32]. He was a senior surgeon and an epidemiologist [30]. He was stationed in Ledo (Assam) region to investigate the CBI (China-Burma-India) fever [30, 33]. In December 1943, he became ill with scrub typhus.

The Kato strain was isolated from a 15-year-old Japanese boy from Kurosawa village, Naka-Kanbara district, Niigata Prefecture [30, 34]. In 1952, he was acutely febrile and deemed 'a classic human scrub typhus case' [30, 34].

Isolates antigenically similar to these strains are present in differing prevalence and proportion in different places, i.e. each geographical location has its own predominant strains [29]. For example, in Thailand and Japan, Karp-like strains predominate. Based on my findings in *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*, there is no study determining the *O. tsutsugamushi* strains present in Indonesia yet [11, 12, 35-83].

In one study, when reviewed serologically, about 50% of *O. tsutsugamushi* isolates analysed were reactive to Karp antisera, while about 25% were reactive to Gilliam antisera [29]. When looked at molecularly, there is more diversity [29]. Using molecular methods, about 40% of all isolates were phylogenetically similar to Karp strain, while

18% are similar to the JG genotype group – a group serologically alike, but genetically different to Gilliam strain [29]. About 10% each of the isolates were Kato-related, Kawasaki-like, and TA763-like [29]. It is important to consider this large range of strain antigenic variation in future vaccine development [29]. The phenotypic and genotypic variation of *O. tsutsugamushi* also complicates the diagnosis of scrub typhus.

Pathogenesis and immunity

During mite feeding, *O. tsutsugamushi* is inoculated in the epidermis and dermis layers of the skin [2, 5]. The bacteria invade dendritic cells, monocytes, and macrophages in the cutaneous layers [2, 5, 84]. Later, the bacteria disseminate presumably through blood and the lymphatic system to endothelial cells and macrophages in other parts of the body [2]. When the bacteria enter and proliferate inside the vascular endothelial cells, they cause a systemic vasculitis [2].

The immune response against scrub typhus involves both the humoral and the cellular immune system [85]. Immunity against scrub typhus gained through natural infection does not last for life, and previously infected individuals can be reinfected. In general, antibodies return to reference value after two years [86]. The IgM response lasts for up to 12 months after infection and the IgG response for up to 36 months [86-88]. Meanwhile, after one year cellular immunity also steeply declines [86].

Vector

Scrub typhus is transmitted by larval trombiculid mites during feeding. Trombiculid mites are ubiquitous ectoparasites on vertebrates. The larval stages of the family Trombiculidae are called chiggers, red bugs (*akamushi* in Japanese), or berry bugs [89]. The mites are red (giving them their nickname), orange, or yellowish [89]. They are small (150-300 µm in size), barely visible to naked eye [89].

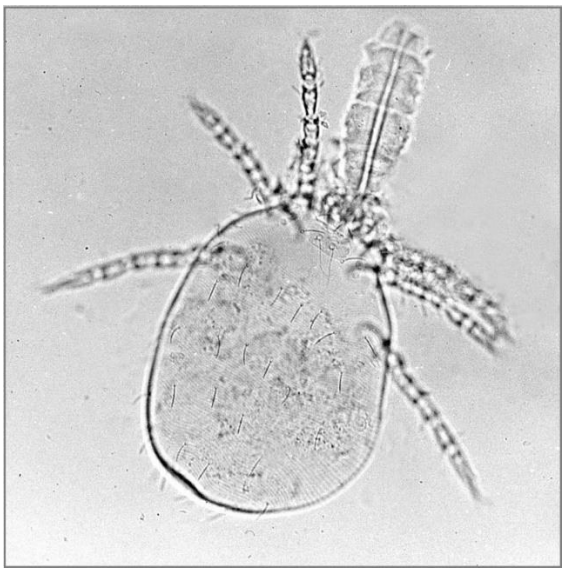


Figure 1. The larva of trombiculid mite with stylostome

Source: Walker, CC BY-SA 3.0, via Wikimedia Commons [90]

Mites from the genus *Leptotrombidium* are the vectors of scrub typhus. Currently, only ten *Leptotrombidium* species are confirmed to be vectors of scrub typhus: *Leptotrombidium akamushi*, *Leptotrombidium arenicola*, *Leptotrombidium chiangraiensis*, *Leptotrombidium deliense*, *Leptotrombidium fletcheri*, *Leptotrombidium gaohuensis*, *Leptotrombidium imphalum*, *Leptotrombidium pallidum*, *Leptotrombidium*

pavlovsky, and *Leptotrombidium scutellare* [91]. *Leptotrombidium deliense* is considered the 'major vector' of scrub typhus since most documented outbreaks are associated with this species [92].

There were several 'criteria of vectorship' that need to be fulfilled before declaring certain species of mites as scrub typhus vectors with clinical importance [92]. First, the candidate vector needs to be proven naturally infected by *O. tsutsugamushi* [92]. Second, there must be evidence that the mite species can transmit the pathogen to a host [92]. Third, the species need to be present in the areas when scrub typhus is prevalent [92]. Fourth, concordantly, naturally infected human hosts should also be present in these areas [92]. Fifth, the candidate mites must also feed on humans [92]. In addition, the species should be able to acquire the pathogen from one host and transmit it to another [92].

There are seven stages in the life cycle of trombiculid mites: egg, deutovum, larva, protonymph, deutonymph, tritonymph, and adult [91, 92]. The complete life cycle of *Leptotrombidium* usually takes 2-3 months [92]. Typically, trombiculid mites lay eggs on the top layer of the soil [92]. After 5-7 days, the trombiculids enter the deutovum, a 'quiescent phase', inside the eggs [92]. The mites continue to grow for 5-7 days and emerge as 6-legged larvae [92]. Usually, after 2 days, they are ready to 'swarm' any mammal or bird in contact [92]. Humans are a dead-end incidental host. During feeding the larval chigger mites attach to the skin of the host with their chelicerae (mouth part)

[91]. They form a stylostome or feeding tube of solidified saliva that helps in reaching deeper tissue layers to gain more nutrients (Figure 1) [91, 93]. Trombiculids mostly feed on the lymph, serum exudates/tissue fluids of its host, not blood [92]. They usually latch onto thin skin [92]. In humans, the preferred areas are ears, armpits, abdomen, and genitalia [91]. They also prefer areas where clothing is tight or the skin is exposed, such as the waist, ankles, and legs [89, 91]. On humans, feeding can last for 2-12 days [92]. When unfed, the larvae are very difficult to see by the naked eye due to their size [92]. However, after feeding, their size may increase several fold [92]. Following feeding larvae fall off the host and within 3 days transform into protonymphs (nymphopanes), a 'pupa-like' stage [92, 94]. In 7-10 days, the mites change into deutonymphs (pilose) – 'velvety, 8-legged nymphs' [92, 94]. Within 14 days the nymphs then transform into tritonymphs (teleiophanes) – a pupa-like imagochrysalis stage [92, 94]. The mites turn into adult form after a further two weeks [92]. Adults are sexually mature and they have similar form as nymphs, only bigger [92]. Adult mites usually live for at least 15 months [92].

O. tsutsugamushi can be transmitted across generations through the ovum (transovarial transmission) and across live stages (transstadial transmission) of the vector mites [94]. The vector mites usually only feed once [92], so in order to infect humans, mites need to have already acquired *O. tsutsugamushi* [92]. Therefore, the bacteria must be transferred across generations and across the various development stages [94].

Leptotrombidium mites live in humid areas where there is moist soil with vegetation coverage, such as in areas with shrubs or grasses. Based on the work of Audy, Shirasaka, and Sasa as cited by Traub, in high humidity 'near the saturation point' and at a temperature of 25–30°C [92].

The distribution of the trombiculid mites can be described on different scales: mite-colony, mite-island, endemic region, and geographic range [95]. Batches of engorged chiggers often form clusters called a 'mite-colony', where they reproduce [95]. Mite-islands are patches of larger localised units of trombiculid mites within an endemic area [95]. This is what gives trombiculids their 'patchy distribution' [91]. The collection of endemic areas forms the geographic range of the vector [95].

Leeches may also have a role in scrub typhus transmission. This has been speculated on since a scrub typhus patient was reported to have a history of terrestrial leech bite [96]. In one case, a 54 year old man developed scrub typhus after being bitten by leeches in Chiloé, Southern Chile [96]. An eschar developed at the said bite site [96]. There is also a case report on *Rickettsia felis* infection after terrestrial leech bite in Northern Laos [97]. However, the role of leeches as vectors of scrub typhus is not yet confirmed.

Epidemiology and burden

Scrub typhus is traditionally described to be present within the so called 'tsutsugamushi triangle' (Figure 2). Based on very limited data, scrub typhus is estimated to affect one million people annually, with one billion people at risk [8, 98]. Scrub typhus has been showing emergence in areas where it has been unknown before and re-emergence in areas where it has been previously 'silent' [5]. It is emerging in Dubai, Chile, Peru, and across Africa; while re-emerging in India, South Korea, and China.

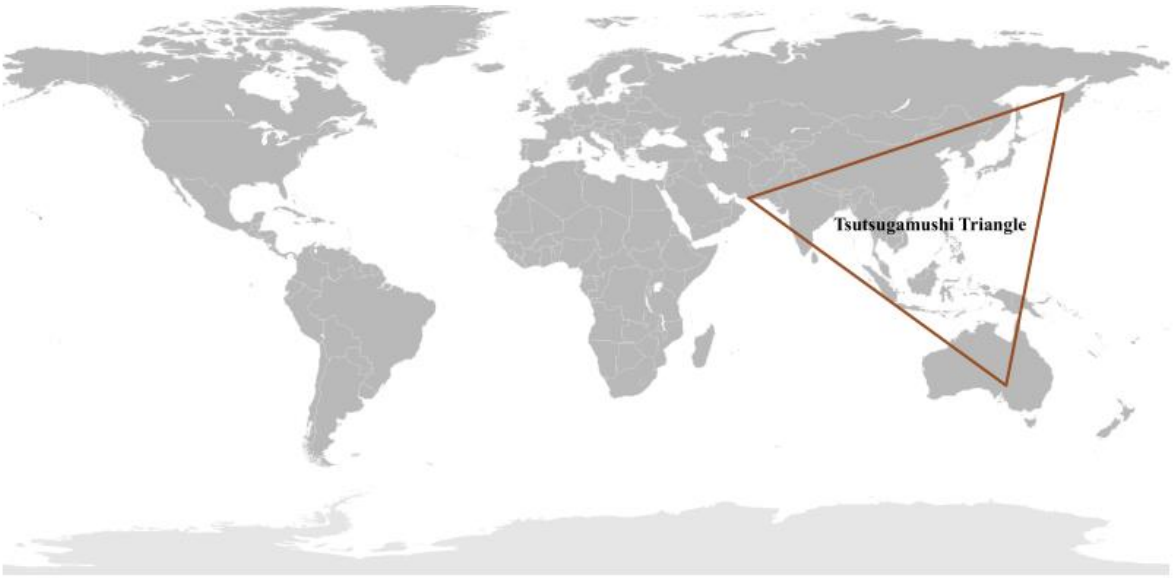


Figure 2. The tsutsugamushi triangle

Source: Richards and Jiang, Tropical Medicine and Infectious Disease 2020, Vol. 5, Issue 2, page 6 of 16, CC BY 4.0 [1]

Scrub typhus is showing an increase in incidence as captured by the passive surveillance systems of South Korea, Japan, Thailand, and China [8]. This could be caused by a genuine increase in the number of cases or due to increased diagnostic/surveillance

efforts and awareness. A report on record from the Korea Center for Disease Control and Prevention (KCDC) described close to a four-fold increase in incidence in 2013 compared with 2001 [99]. In Japan, the National Epidemiological Surveillance of Infectious Diseases (NESID) data showed that there was a six-fold increase in incidence from 2000 to 2008 [100, 101]. In Thailand, the incidence rate increased more than 3-fold from 2005 to 2013 [102]. In China, the number of cases has increased more than 12-fold between 2006 and 2014 [103].

Seroprevalence data from Indonesia, Malaysia, Laos, Bangladesh, Sri Lanka, and Papua New Guinea, has a median seroprevalence of 22.2% (range 9.3%-27.9%) [8, 104-109]. This indicates that there is active transmission of scrub typhus in these countries. However, these studies are not indicative of the seroprevalence of the whole country i.e. cannot be generalised, since the distribution of scrub typhus is patchy. Data from prospective acute fever studies in healthcare centres in Cambodia, Laos, Nepal, and Kenya have shown that scrub typhus is the cause of 1.8%-22.3% of visits [110-114].

There is also evidence indicating scrub typhus transmission outside of the tsutsugamushi triangle [21]. There were several 'presumptive cases' from the Republic of Congo, Cameroon, and Tanzania [115-117]. There were also reports of seropositivity in febrile patients in Kenya and abattoir workers in Djibouti [110, 118, 119]. In addition, a microorganism similar to *O. chuto* was discovered in a chigger pool from Kenya [120]. '*O. tsutsugamushi*-like sequence' has also been found in dogs in South Africa [121]. In

2011, Balcells et al published a report on an acute case of scrub typhus in Chile [96]. Sequencing showed about 97% similarity between the causal agent and O. tsutsugamushi [96]. In Peru, there was serologic evidence of scrub typhus in retrospectively analysed blood sample of febrile patients, with one sample showing evidence of 'recent active infection' [23].

Scrub typhus is known to be mainly prevalent in rural areas, however, there is increasing evidence that scrub typhus is also present in urban areas. The epidemiology of scrub typhus in South Korea has shown some shift. There has been an increase of cases in urban areas, including in the capital, Seoul [7, 99]. This strengthens the view that there is no 'typical' habitat for scrub typhus.

Calculations of DALYs attributable to scrub typhus are mostly not available. Pubmed (https://pubmed.ncbi.nlm.nih.gov/) search results as of 12 January 2022 showed that only one study has calculated DALYs associated with scrub typhus. This study was done in the Laiwu province, China [122]. Here, it was reported that scrub typhus caused 13 DALYs lost [122]. However, generalisation of this data to other settings might not be appropriate since there were no deaths reported in this province [122].

This scarcity of data is also the case with cost calculation/economic analysis. One cost-benefit analysis done in South Korea calculated that a scrub typhus prevention

programme applied in primary health centres could save in total $6.66 million per year [123].

Clinical manifestations

Scrub typhus is typically marked by acute fever that may be accompanied by the pathognomonic sign of eschar – a painless, necrotic skin lesion at the site of vector inoculation. Eschars are often small and in occult sites, requiring a thorough physical examination. Although highly specific for scrub typhus infection [124], eschar presence is highly variable [125, 126]. Other clinical manifestations include headache, myalgia, nausea, vomiting, macular or maculopapular rash, lymphadenopathy, and hearing loss [2, 127]. Due to lack of distinguishing features that can reliably set scrub typhus apart from other acute febrile illness causes, it is challenging to diagnose scrub typhus clinically. Therefore, laboratory testing plays a central role in diagnosing scrub typhus.

The differential diagnosis includes diseases from the rickettsial spotted fever group and typhus group, malaria, typhoid fever, dengue, chikungunya, leptospirosis, relapsing fever, meningococcal disease, and other fevers with macular rash e.g. infectious mononucleosis [2].

Delayed treatment may lead to lethal complications, such as acute respiratory distress syndrome, meningoencephalitis, disseminated intravascular coagulation, and

multiorgan dysfunction [127]. If untreated, scrub typhus mortality may reach 70% (median 6%) [128]. If treated, the median mortality is 1.4% (range 0-33.3%) [128].



Figure 3. Eschar

Modified from Cho et al, Pathogens 2021, Vol. 10, Issue 4, page 4 of 9, CC BY 4.0 [129]

Diagnosis

In the early modern period of the discovery of scrub typhus, the Weil-Felix test played a fundamental role in defining and distinguishing scrub typhus from other endemic acute febrile illness such as murine typhus [15, 16, 20]. In the context of the Asia-Pacific, the Weil-Felix test helped in demonstrating that what was described as a more fatal disease i.e. tsutsugamushi disease, and the often milder scrub typhus, are the same disease [15]. It is a non-specific test based on the cross-reactivity between the bacteria from the genus *Proteus* and rickettsiae first described by Edmund Weil and Arthur Felix. The

serum of patients with scrub typhus showed positive reaction in the form of agglutination to the Weil-Felix test OXK antigen (*Proteus mirabilis*) and negative reaction to the OX19 antigen (*Proteus vulgaris*) [15, 20]. However, this test suffers from low sensitivity and specificity particularly in single acute samples [130, 131]. Despite lacking accuracy, this test is relatively cheap and accessible. Therefore, it is still being used for diagnosis in resource-limited settings [131].

Serology has been the cornerstone of scrub typhus diagnosis and remains so to this day [132]. However, many of the diagnostic tests available perform poorly or may be unavailable [132]. Challenges in diagnosing scrub typhus also contributes to the meagre amount of epidemiology data available.

In addition to the Weil-Felix test, *O. tsutsugamushi* detection by staining was also historically used in the clinical setting [131]. However, the low specificity and sensitivity renders this approach not practical for use in clinical settings and is thus reserved for use in research centres to monitor bacteria growth [133].

The complement fixation test was also used in the past [134]. However, this test is 'strain-specific' [135]. Therefore, its use is limited because every strain present in the area would need to be included in the test. This test is also considered time-consuming compared to newer diagnostic methods [131].

The indirect immunofluorescence assay (IFA) is generally considered as the gold standard for serological diagnosis [132]. However, it is an imperfect standard. More 'unbiased' Bayesian latent class models accounting for an imperfect gold standard show that the 'true' sensitivity and specificity of paired IgM IFA are 70.0% and 83.8%, respectively [124]. The IFA endpoint result interpretation can be subjective since it is evaluated by human eyes and interpretation; hence it suffers from inter-operator variability [136]. The IFA requires relatively sophisticated equipment and considerable training that are often not available in resource-limited settings, and it is not suitable to be used as a bedside point-of care test.

A more accessible alternative to the IFA is the immunoperoxidase test (IIP). Since this test utilises the simpler light microscopy, the need for fluorescent microscopy is eliminated, making it more accessible especially in resource-limited areas [131]. However, the IIP test does not seem to be much used or discussed in scrub typhus research compared to the IFA. As of 8 January 2022, comparing search results of ("scrub typhus"[Title/Abstract]) AND (IFA) and ("scrub typhus"[Title/Abstract]) AND (IIP) in Pubmed (https://pubmed.ncbi.nlm.nih.gov/), there were 98 for IFA and 8 for IIP – suggesting that there are substantially more papers on scrub typhus discussing IFA compared to IIP.

The enzyme-linked immunosorbent assay (ELISA) is simpler to perform than IFA or IIP. Use of ELISAs in scrub typhus diagnosis and research has been increasing over the years

(*Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform*) [137]. ELISA is high throughput, relatively cheaper than IFA, and more reproducible (using an automated plate reader, giving an objective optical density value) [131]. ELISA also has high sensitivity and specificity [138, 139]. Both whole cell and recombinant antigens have been used in *O. tsutsugamushi* ELISAs [131]. The production of whole cell antigen is time consuming and needs to be done in a biosafety level 3 (BSL-3) laboratory. Due to the obligate intracellular nature of *O. tsutsugamushi*, culture needs to be done in 'eukaryotic host cells', requiring more advanced expertise and facilities [140]. Production of reagent could be considered a 'high risk' activity that requires the biocontainment provided by a BSL-3 laboratory. The growth of *O. tsutsugamushi* is relatively slow, with the median 'time to isolation' of 27 days [141]. Recombinant antigens are being studied and used in the available commercial IgM ELISA kits [83, 142, 143]. These kits used the p56-kD type-specific antigens [142, 144].

Serology tests are also available in the form of dipstick, immunochromatographic tests (ICTs), and immunodot assays. These tests have been used as point-of-care tests. They are suitable for use in resource-limited settings because they are easy to perform, provide 'rapid results' (usually in around 15 minutes), and relatively cheap. A meta-analysis on limited data of ICTs (*Chapter 7. Scrub typhus point-of-care testing: A systematic review and meta-analysis*) found sensitivity of 66.0% (95% CI 0.37 – 0.86) and specificity of 92.0% (95% CI 0.83 – 0.97) [145]. The high specificity suggests low false

positive rates, indicating the value of ICT to rule in diagnosis [145]. Bayesian latent class models showed that when used together, IgM ICT and the presence of eschar have good accuracy – sensitivity 88.6% and specificity 95.9% [124]. This therefore, also highlights the importance of performing a thorough physical examination to identify eschars.

Serological approaches have limitations. They can only diagnose scrub typhus when antibodies have been formed and are above detectable levels i.e. starting 5-10 days after presentation [133]. Therefore, serological diagnosis has limited utility for directing the treatment of acute cases. Since IgM and IgG antibodies can be detected for months and years respectively, it is important to also collect convalescent samples in addition to the acute samples when using serology for diagnosis [86-88, 131, 146]. With serology, we also have to define the optimal cut-off. Diagnostic cut-offs should be adjusted based on the background immunity at each location. At the moment, there is also lack of standardisation on how cut-off values for serologic methods are defined [147, 148].

Nucleic acid-based diagnostic methods such as polymerase chain reaction (PCR) can circumvent the limitations of serology-based tests. Molecular assays such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) assays have been developed for scrub typhus diagnosis. PCR techniques used include conventional PCR, nested PCR (nPCR), and quantitative real-time PCR (qPCR) [131].

PCR for *O. tsutsugamushi* is usually based on the detection of the 56-kDa type-specific antigen (TSA) gene, 58-kDa antigen (GroEL heat shock protein) gene, 47-kDa high-temperature requirement A (HtrA) gene, or 16S ribosomal RNA (16S rRNA) gene [124, 149, 150].

As with ELISA, the use of PCR in clinical studies has also shown an increasing trend (Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform) [137]. Although PCR is a sensitive method, the limited duration of rickettsaemia is a limiting factor [131]. nPCR has higher sensitivity than conventional PCR [131]. However, due to the methodologies of nPCR, there is a higher chance of contamination since there is a need to open vials to retrieve the template for the next round of PCR [131]. In terms of sensitivity, qPCR is comparable to nPCR [133]. However, it has less risk of contamination [133]. In addition, it provides a quantitative result in real time [133].

LAMP assays are a cheaper and simpler alternative to PCR. Since LAMP is performed at a single temperature, this eliminates the need for a thermocycler. The results can also be read by human eyes, therefore eliminating the need for a reader machine. The reaction can be performed in a single vial and it can provide relatively rapid results – making LAMP a suitable point-of-care test. Though not as sensitive as PCR, the accuracy is still relatively good. Studies evaluating LAMP show sensitivity ranging from 72.6% to 100% and specificity ranging from 80% to 100% [151-153]. One study evaluated LAMP

in combination with IgM ICT, with sensitivity and specificity between 66.7% to 77.2% and between 63.2% to 90.6%, respectively [154].

Restriction enzyme fragment (RFLP) analysis, multilocus sequence typing/analysis (MLST/MLSA), multispacer typing (MST), and whole-genome sequencing are used for identification or characterisation of orientiae. Culturing orientiae is difficult because of the obligate intracellular nature of the organisms and biosafety requirements of laboratory (BSL-3) [131, 155]. One study showed only about 50% success rate in culturing samples of patients diagnosed with *O. tsutsugamushi* IFA [141]. Immunohistochemical testing of tissue samples is usually only performed in a handful of reference laboratories [131, 156].

Orientia spp., along with *Rickettsia* spp., are classified as a risk group or hazard group 3 pathogen (RG3) in several countries, including the UK [155, 157]. In the areas where it is endemic, there seems to be a lack of clear guidelines on the biocontainment level of orientiae [155]. To be classified as an RG3 pathogen, the disease caused is severe, can be a 'serious hazard' to laboratory personnel, has the potential to spread to the community, but there are effective prophylaxis or treatment [157]. The classification of *Orientia* spp. as an RG3 pathogen has caused work on the bacteria to be highly regulated and limited [155]. Building and sustaining the operation of a BSL-3 laboratory requires large financial commitment that might not be achievable in resource limited settings [155]. This significant restriction also hinders research [158].

Blacksell et al recommended to reclassify *Orientia* spp. and *Rickettsia* spp. as RG2 pathogens and apply a 'risk-based approach' to biocontainment based on the types of activities performed [155]. An RG2 pathogen may cause disease and hazard for laboratory personnel, but 'unlikely to spread to the community', with efficacious prophylaxis and treatment present [157]. This risk-based approach is in line with the WHO Laboratory Biosafety Manual [159]. For example, low risk activities such as 'small-scale' patient sample processing or passage of reference cultures could be performed in a BSL-2 laboratory [155]. However, high risk activities that could generate aerosol or involves high concentration of bacteria would still need to be performed in a BSL-3 facilities [155]. This approach would facilitate sustainable research in resource limited settings where scrub typhus is endemic.

When selecting and interpreting scrub typhus diagnostic tests clinicians need to consider where the patient is in the natural history of the infection (Figure 4) [133]. However, it may be difficult to tell in which stage the patient is at presentation. Therefore, there is value in combining both the nucleic acid- and serology-based tests.

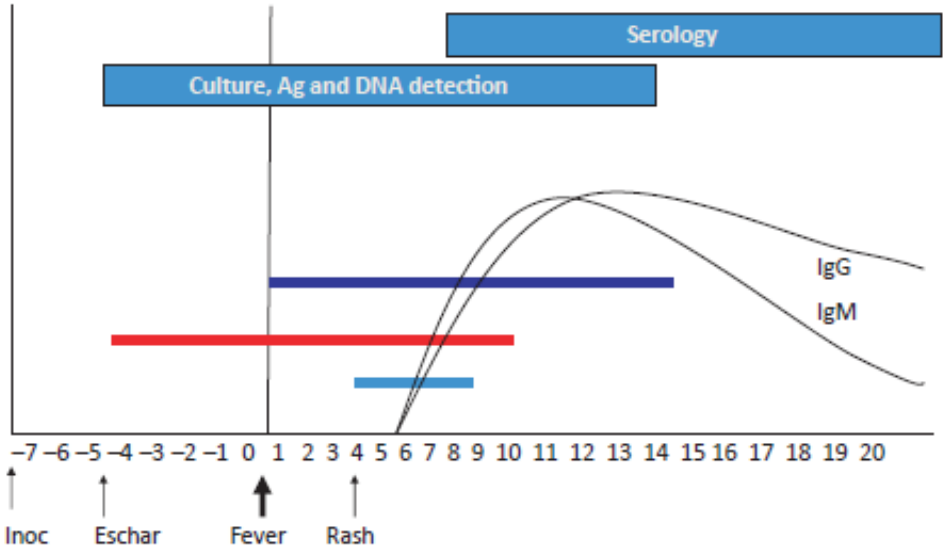


Figure 4. The timeline of rickettsial infection and suitable diagnostic methods

Source: Richards, FEMS Immunology & Medical Microbiology 2021, Volume 64, Issue 1, page 108 [133]. This article is a US government work and is in the public domain in the USA (Public Domain Mark 1.0).

'Inoc' represents inoculation time, in the case of scrub typhus, by the *Leptotrombidium* mites. The red, light blue, and royal blue bars represent eschar, rash, and fever, respectively. Ag = antigen. DNA = deoxyribonucleic acid.

Treatment

In 1947, Dr Paul R. Burkholder was studying about 20,000 types of mould from around 6,000 soil sample in his lab in Yale University [15]. He managed to isolate several that showed antibiotic properties [15]. Further investigations on the moulds and their antibiotic properties was performed by Joslyn, supervised by J. Ehrlich form Parke, Davis & Company [15]. One mould, *Streptomyces venezule,* was isolated from the soil from

Caracas, Venezuela [15, 160]. The culture of this actinomycete in agar streak was found to inhibit the growth of both Gram positive and negative bacteria, including *Staphylococcus aureus* and *Escherichia coli* [160]. Bartz and colleagues managed to isolate the pure crystalline form of the antibiotic from the mould [15]. The extracted antibiotic was named chloromycetin. Early tests also suggested chloromycetin activity against *Rickettsia prowazekii* [160]. Consequently, chloromycetin was also tested for activities against *O. tsutsugamushi* (*Rickettsia orientalis* at the time) using embryonated eggs infected with the Gilliam strain and mice infected with Karp strain; these studies showed that chloromycetin was active against *O. tsutsugamushi* [161].

Due to its efficacy in scrub typhus and no known toxicity in humans a clinical trial on 37 scrub typhus patients was done in 1948 in Kuala Lumpur, Malaysia [162]. Among 25 treated participants there were no complications or fatalities [162]. Meanwhile, within the untreated group (12 participants), one participant each developed parotitis and pneumonia, and there was one fatality [162]. A large reduction in the number of febrile days and days spent in the hospital were observed. With chloromycetin (now more commonly referred to as chloramphenicol – its chemical name), the dreaded mortality of scrub typhus was not perceived to be as concerning as before. Although no longer the drug of choice, chloramphenicol is still used for scrub typhus treatment, particularly in children.

Though resistant to beta-lactam antibiotics, scrub typhus is also treatable with select antibiotics including doxycycline, tetracycline, azithromycin, and rifampicin [2, 163]. Though efficacious, rifampicin should not be used as the first line treatment of scrub typhus in tuberculosis-endemic areas, as it may induce *Mycobacterium tuberculosis* resistance against rifampicin – a part of the first line combination treatment for tuberculosis. Typically, doxycycline is the first line treatment [2]. However, in pregnancy, azithromycin is usually the drug of choice [2].

Several reports have indicated reduced clinical response to doxycycline [164]. Watt et al in 1996 reported cases in northern Thailand with long fever clearance times after being treated with doxycycline [165]. Another strain of *O. tsutsugamushi* (AFSC-4) isolated in western Thailand has also been reported to have lower susceptibility to doxycycline [166]. Recently, Wangrangsimakul et al has raised the possibility that resistance to doxycycline may be a misconception and reduced response to doxycycline is likely to be caused by 'other bacterial, host, and pharmacological factors' [167]. A network meta-analysis showed that among eight antibiotics studied (doxycycline, chloramphenicol, azithromycin, tetracycline, telithromycin, rifampicin, minocycline, and clarithromycin), there is no one antibiotic that was significantly more efficacious or safe [168].

At the moment, the treatment recommendations available are varied or simply not available [163, 169]. There is no WHO guideline on therapy. Likewise, there is no national treatment recommendation in Indonesia. When guidelines are available, the

recommended antibiotic is usually consistent [170-173]. However, there are differences in the recommended dosage and duration of treatment [170-173]. These recommendations were based on a few heterogeneous trials, most with high risk of bias [163]. The strain variation and severity of disease may contribute to the variation. However, the varied treatment regimens and the absence of WHO recommendations reflects the fact that uncertainty remains around the optimal dosage and duration of treatment for scrub typhus patients.

Prevention

Wearing protective or insecticide-treated clothing may prevent the trombiculid mites from latching on the skin. Likewise, application of insecticides on exposed skin may also protect from mite bites.

Vaccines have been developed, but none has been effective [2]. In the past, *O. tsutsugamushi* killed with formalin have been used. In 1945, a classified project to produce scrub typhus vaccine in large scale was started [2, 174]. It was called 'Operation Tyburn'. In 1946, Henderson-Begg and Fulton (as cited by Paris and Day in 2014) reported that 300,000 doses of vaccines made from Burmese strains were produced [2]. However, when used in soldiers in Malaysia, it did not give satisfactory results [2]. Live vaccines were not well-tolerated [85]. Co-administration of antibiotic with live vaccine did not succeed in producing cross-immunity to other strains and lacked protective

longevity [85]. Attenuating the *O. tsutsugamushi* with radiation was also not very effective [85]. Subunit vaccines using recombinant protein are being investigated [85].

Doxycycline has been trialled as pharmacological prophylaxis [175, 176]. In one randomised double-blind study with 1,125 participants, once a week 200 mg doxycycline during exposure showed a positive effect in preventing scrub typhus, but was not statistically significant (p-value = 0.11) [175]. However, when 'per protocol' analysis was done, the effect became statistically significant (p-value = 0.04) [175]. In another randomised double-blind challenge study, 20 participants were exposed to infected *L. fletcheri*. Seven doses of 200 mg doxycycline once a week taken before exposure and continued for six weeks was claimed to be protective [176]. However, no p-value was given for the main outcome and the small number of participants would most likely not have enough statistical power to confirm efficacy and safety [176].

Indonesia

Indonesia is a vast tropical country with varying landscape, from mountainous areas with active volcanoes to tropical rainforest and coastal mangrove forests. There are also rural areas traditionally described as the classical scrub typhus habitat. Close to 120 million Indonesians live in rural areas [14]. They are particularly more vulnerable to health problems due to higher poverty rates and less access to health infrastructure [177].

Previous reports have documented scrub typhus cases and the existence of its vector and reservoir in Indonesia [178-180]. Therefore, we can conclude that scrub typhus is present in Indonesia. In addition, scrub typhus is a public health problem in neighbouring countries including Malaysia and Australia [127]. It is likely that scrub typhus is causing considerable morbidity and mortality. However, we do not have a clear picture of how big the problem is as data remain scarce. Without reliable data, it is difficult to design and apply public health measures to reduce scrub typhus morbidity and mortality.

Indonesia lies between the Indian and Pacific Oceans, connecting mainland Asia and Australia (Figure 5). Indonesia is the world's largest archipelagic country with more than seventeen thousand islands spread over an area of 5,100 km west to east by 1,700 km north to south [181]. The distance between the westernmost to the easternmost point in Indonesia is greater than the distance from London to Cairo. Indonesia's total area (land and water area included) is about 1.9 million km^2 [181]. Including the exclusive economy zone, Indonesia claims about 7.9 million km^2 of total sea area [181].



Figure 5. Indonesia's location in the world map

Source: Rei-artur, CC BY-SA 3.0, via Wikimedia Commons [182]

Indonesia has five main islands: Sumatra, Java, Kalimantan (Borneo), Sulawesi, and Papua (New Guinea). Among these five islands, two are shared with neighbouring countries: Borneo with Malaysia and Brunei; Papua with Papua New Guinea. Two other islands also shared land borders with other countries: Timor with Timor Leste; Sebatik with Malaysia. Other smaller islands are grouped into two main archipelagos: the Nusa Tenggara or Lesser Sunda Islands and the Maluku Islands (Moluccas); and 60 smaller archipelagos [181].

The archipelago spans over and along the equator, giving Indonesia its tropical climate. There are two seasons: rainy (December to March) and dry (June to September) season

[181]. Temperatures stay quite constant over the year. Yearly temperature averaging at 28°C in the lowlands, 26°C in the highlands, and 23°C in the higher mountainous areas [181].

Indonesia sits within the Pacific 'ring of fire' – areas in the Pacific Rim where many tectonic plates meet resulting in a string of active volcanoes and many earthquakes. Indonesia was affected by the 2014 Indian Ocean earthquake and tsunami. This disaster killed over 160,000 Indonesians and displaced more than 200,000 people [181].

Indonesia has some of the most biodiverse areas in the world. Indonesia's unique location, the meeting of two continents, gave Indonesia its rich biodiversity: having both flora and fauna species from Asia and from Australasia, as well as species endemic to Wallacea in between (Figure 6).

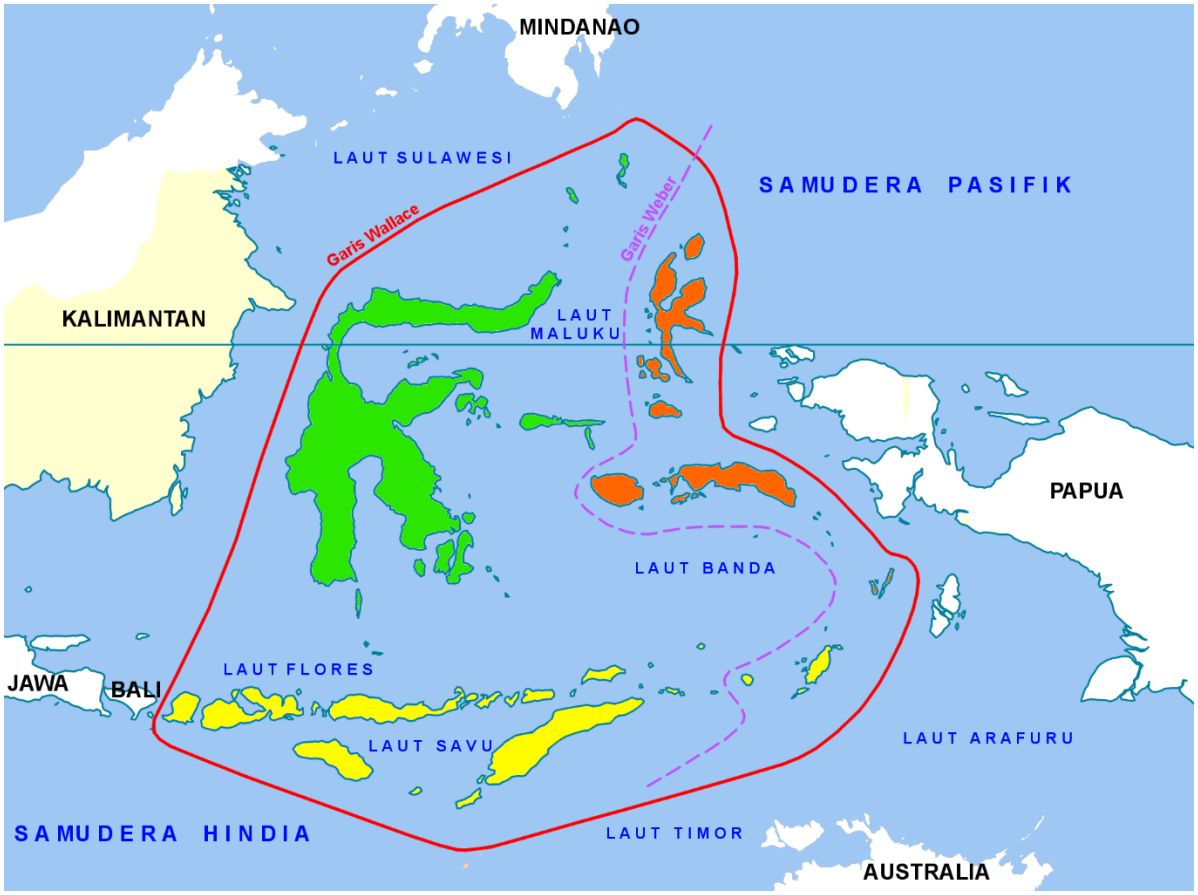


Figure 6. Wallacea

Source: Ariefrahman, CC BY-SA 3.0, via Wikimedia Commons [183]

The red line encircles Wallacea. Wallace's Line runs between the Borneo (Kalimantan) and Sulawesi, and Bali and Lombok. The purple dash represents Weber's Line.

Indonesia is the fourth most populous country in the world. Based on the national census, Indonesia had a population of 270 million in 2020 [184]. About 56% of Indonesians live on the island of Java [184]. There are about 350 ethnolinguistic groups in Indonesia, with one estimate of 550 languages spoken in the country – that is about

‘one-tenth of all the languages in the world’ [181, 185]. Javanese, Sundanese, and Malay are the three largest ethnic groups [185].

Indonesia’s human development index (HDI) in 2019 was 0.718 – ranking 107 out of 189 countries [186]. The literacy rate in adults (age 15 and older) for 2019 was 95.7% [186]. The percentage of adult population 25-year-old or older with at least some secondary education was 50.9%.

Indonesia is the largest economy in Southeast Asia [187]. Globally, Indonesia is the ‘tenth largest economy in terms of purchasing power parity’ [187]. The annual gross national income (GNI) per capita for 2020 was US$3,870 – placing Indonesia as a lower-middle income country [188]. Indonesia was briefly included in the upper-middle income country bracket with a 2019 GNI per capita of US$4,050. However, due to the COVID-19 pandemic, Indonesia observed a 2.1% reduction in GDP growth [188]. In the first semester of 2021, Indonesia’s poverty rate was 10.14% [189]. The provinces with the highest poverty rates were Papua (26.86%), West Papua (21.84%), and East Nusa Tenggara (20.99%) [190].

Indonesia’s age structure in 1990 was pyramid shaped [191]. By 2019, the shape was showing changes and started to shift to an inverted pyramid shape [191]. In 2100, Indonesia is forecast to have an inverted pyramid shape age structure [191]. This is

accompanied by a decreasing fertility trend [191]. Total fertility (representing how many children a woman delivers on average) in 1990 was 3.1 [191]. It decreased to 2.4 in 2017 and is forecast to be 1.7 in 2100 [191]. The estimated maternal mortality ratio per 100,000 live births for 2017 was 177 [192]. In 2019, the neonatal mortality rate per 1,000 live births was 12.4 and the under-5 mortality rate was 23.9 per 1,000 [193, 194]. The under-5 and under-1 mortality rate have shown a consistently decreasing trend [191]. The life expectancy at birth for 2020 was 72 years [189]. This is about an 8 year increase from 1990 [189].

In addition to demographic transition, Indonesia is also experiencing epidemiological transition. The top three causes of deaths in 2019 (in descending order) were stroke, ischaemic heart disease, and diabetes [191]. In 2009, diabetes was ranking sixth [191]. Tuberculosis was the third largest cause of death back in 2009 and in 2019 it ranked fifth [191]. Non-communicable diseases are of increasing importance.

The total disability-adjusted life years (DALYs) attributable to 'communicable, maternal, neonatal, and nutritional' problems had halved from 43.8 to 18.1 million between 1990 and 2016 – a 58.6% decrease [195]. One DALY means that 'one year of full health' is lost; this is calculated by summing the years of life lost (YLLs) and years lived with a disability (YLDs) due to the disease or condition [196]. The total years lost due to injury also decreased [196]. On the other hand, DALYs caused by non-communicable diseases

increased [196]. The foremost risk factor linked to the DALY burden was dietary risk [195].

Though showing declining trends, infectious diseases, undernutrition, and maternal and neonatal diseases remain important public health concerns. Indonesia is still burdened by neglected tropical diseases including soil-transmitted helminths infection, lymphatic filariasis, leprosy, and yaws [197]. Dengue has also burdened health care facilities during outbreaks that typically happen around the rainy season [198]. Dengue is the most common aetiology amongst hospitalised patients with acute febrile illness [199]. Indonesia still struggles with high levels of stunting – with 31% children under the age of 5 years suffering from it in 2018 [200]. On the other hand, obesity is rising – doubling over the past 20 years [201].

One of the Sustainable Development Goal #3 (Good Health and Well-being) indicators is the UHC services coverage index of essential health services [202]. It is a composite index 'computed by averaging service coverage values across 16 tracer indicators' that covers four categories: reproductive, maternal, newborn and child health; infectious diseases; noncommunicable diseases; service capacity, access and health security. It is expressed in percentages, with 100% meaning 'full coverage' encompassing the four categories. Indonesia's for 2019 was 65% [202].

Indonesia is a democratic republic with a president as both the head of state and government. The first level of administrative unit is the province. There are currently 34 provinces in Indonesia with 354 districts with 'decentralised governance' [203]. Indonesia was under the authoritarian rule of a centralised government for decades. In 1998, reforms towards a democratic republic were started. The health system has become decentralised since then. Health service delivery is also organised in a decentralised manner, limiting the capability of the Ministry of Health to align policy in the health system [204]. There are still regional discrepancies in health outcomes and the accessibility of health services [204].

In 2014, the government of Indonesia rolled out a universal health coverage programme – the Jaminan Kesehatan Nasional (National Health Insurance System; JKN) [203, 204]. By 17 September 2021, the JKN covered 226.3 million participants (83.5% of the Indonesian population), making it the 'largest single-payer system in the world' [203, 205]. In a single-payer system, one agency (usually public, or quasi-public) is responsible to collect premiums and finance the health services [206]. Health services may be provided by the public sector together with or without private healthcare agencies. In the case of Indonesia, the government through the Badan Penyelenggara Jaminan Sosial Kesehatan (Social Security Agency for Health; SSAH) administers the financing of healthcare for participants [203]. Healthcare services are provided by both the public and private sectors. The public health service providers were arranged into primary

health care clinics in subdistricts, district hospitals, provincial hospitals, and national referral centres.

The lack of epidemiological data on scrub typhus in Indonesia (and elsewhere), stems from the lack of diagnostic capacity. In addition to the global problem of the absence of widely available, affordable, and accurate point-of-care tests, scrub typhus diagnostic modalities are not routinely available in hospitals. This is actually a more systematic challenge in Indonesia. There is a general lack of infectious diseases diagnostic capacity and when capacity is available, utilisation. In the case of bacterial infection, cultures are not utilised as much as it should be to direct antibiotic treatment. Compared to Thailand, which has comparable per capita health expenditure, Indonesia has a relative lack of infectious disease diagnostic capacity. For example, in one comparative study the proportion of inpatients sampled for blood cultures in a referral hospital in South Sulawesi was significantly lower than in Ubon Ratchathani, Thailand: 9% and 13% respectively [207]. Another study showed that about half of patients receiving antibiotics did not have cultures taken or had negative culture results [208]. Cultures are important to ensure appropriate antibiotic treatment. In addition, underutilisation of cultures may also cause underestimation of the presence of antimicrobial resistant organisms [207].

This lack of diagnostic capacity proved to be a challenge during the Covid-19 pandemic [209]. At the beginning of the pandemic, Indonesia had only one referral laboratory i.e.

the National Institute of Health Research and Development (NIHRD) under the Ministry of Health [209]. However, through 'cross-sectoral collaboration', within 12 months of the first Covid-19 case diagnosed in Indonesia, the country has managed to increase the capacity to 685 laboratories spread across 34 provinces [209]. By mid-January 2021, Indonesia had also fulfilled the WHO standard to test 1,000 people per one million population per week [209]. This demonstrates that diagnostic capacity building is possible. Hopefully the increased awareness of infectious diseases and the improved molecular diagnostic capacity will lead to an improvement in scrub typhus diagnostic capacity as well.

Beta-lactam antibiotics, including amoxicillin and third generation cephalosporins, were the most commonly prescribed antibiotics in Indonesia [210, 211]. Doxycycline and chloramphenicol were not the typical second-line antibiotics prescribed. Therefore, in the face of diagnostic challenges in resource-limited settings, understanding the distribution and risk of acquiring scrub typhus is essential for ensuring early diagnosis and prompt treatment. On the other hand, since commonly prescribed antibiotics i.e. beta-lactams, are not effective against *O. tsutsugamushi*, their prescription in the absence of diagnostic testing may also lead to increased antibiotic resistance [132].

Chapter 2. Aims and objectives

In this chapter, I discuss the approaches used in my DPhil projects and their rationale. At the end of this chapter, I present the aims and objectives of this thesis.

My DPhil revolves around scrub typhus both in the Indonesian and global context. The overarching aims of my DPhil studies are twofold: (1) to characterise scrub typhus epidemiology and its public health importance in Indonesia and (2) to address knowledge gaps in diagnosis and treatment of scrub typhus by optimising available data.

Through this DPhil project, I utilise different approaches to fill the gaps in scrub typhus research. This DPhil project seeks to characterise scrub typhus epidemiology and its public health significance in Indonesia by: (1) summarising the available evidence of scrub typhus transmission in Indonesia, (2) conducting a serosurvey of archived fever study samples, (3) conducting a seroprevalence study in a presumed high-risk population living in close proximity to palm oil plantations and compare it to a presumed low-risk population living in urban area.

In addition to understanding the public health importance of scrub typhus in Indonesia, this DPhil project also aims to address knowledge gaps related to the two main challenges in global scrub typhus research and control: diagnosis and treatment.

Widely available, affordable, and accurate diagnostic methods for scrub typhus are still absent. Point-of-care tests (POCTs) would play an important role in diagnosis and treatment especially in resource-limited settings. Therefore, this DPhil project also aims to address our gap in understanding the diagnostic value of scrub typhus POCTs.

One way to understand the efficacy of currently available treatment regimens is through establishing an individual-level participant data (IPD) platform covering global treatment studies data. This will allow analysis of larger numbers of participants globally while also accounting for the granularity of data [212]. In the context of investigating the efficacy of available scrub typhus treatment, this DPhil project also aims to assess the feasibility of establishing an IPD platform, to allow more in-depth analyses of scrub typhus treatment studies data.

The objectives are:

(1) To describe the history of scrub typhus in Indonesia,

(2) To map and rank (based on the likelihood of sustaining infectious reservoirs) the available evidence on scrub typhus geographical range in Indonesia,

(3) To perform a scrub typhus serosurvey of archived fever study samples as proxy of active transmission,

(4) To assess the characteristics of scrub typhus treatment studies and explore the needs and feasibility of establishing an individual-level data (IPD) platform,

(5) To summarise the existing evidence on the accuracy of scrub typhus POCTs and assess their diagnostic value,

(6) To compare the seroprevalence of, and factors associated with, exposure to scrub typhus in healthy adult residents of a palm oil plantation and of urban Medan, North Sumatra.

These objectives translate to five results chapters and one protocol chapter in the thesis:

Chapter 3. History of scrub typhus in Indonesia

Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping

Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples

Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform

Chapter 7. Scrub typhus point-of-care testing: A systematic review and meta-analysis

Chapter 8. Serological investigation of risk of exposure to scrub typhus in residence at an oil palm plantation in North Sumatra relative to urban dwellers at Medan, North Sumatra (SEROSCRUB): A study protocol

Chapter 3. History of scrub typhus in Indonesia

The history of scrub typhus in Indonesia entangles with colonial and world history. Early reports of cases showed distributions overlapping with the economic and military interests of the Dutch colonial government. These reports came from the Dutch colonial institutions such as plantations, military posts, or health facilities. Outbreaks reported from northern Sumatra occurred in plantation workers. Plantations for palm oil, rubber, tobacco, cinchona, cloves, and others constituted vital economic activities to the colonial authorities. Endemic infections threatening those activities received priority. The same priority applied to infections among the armed security forces. More sporadic cases as occurred in Aceh, Palembang, and Batavia (Jakarta) were associated with military activities of the Dutch colonial government (Figure 7). A large outbreak of scrub typhus in Netherlands Papua occurred among Allied troops during World War II. These foci of attention by the colonial government give us awareness of the possibility of overlooked niches of infection through those earliest years of investigations.

Scrub typhus in Indonesia was first described in the area of *Senembah Maatschappij* (Senembah Company) Deli, Sumatra by Dr Wilhelm Schüffner, the chief medical officer of Senembah Company. Schüffner went on to fame as a malariologist (pioneering longitudinal studies involving microscopic blood film examinations), and for pioneering work in leptospirosis. He first reported encountering scrub typhus in 1902 [12, 19].

The Senembah Company was a tobacco company in northeast Sumatra. This company was established in 1889 (Janssen, 1914, as cited in Darini and Anggraeni, 2021), following the success of *Deli Maatschappij* (Deli Company) [213, 214]. Jacobus Nienhuys pioneered tobacco planting in Sumatra and founded the Deli Company [213, 214]. The Agragrian Law, issued in 1870, prompted much investment in Deli and facilitated economic growth [213].

The mortality of workers in these plantations was high [213, 214]. Between 1890 and 1894 the mortality rate in the Senembah Company was 71 per 1,000 workers [213]. In the early years of the company, the conditions were difficult as there were many lands to be reclaimed, cleared, and cultivated [214]. Their living conditions were also 'inadequate', with overcrowded windowless barracks and a lack of clean water access [213]. In addition, there were many infectious diseases circulating in the area: malaria, dysentery, and cholera [213-215]. The workers also suffered from anaemia and beri-beri [213-215]. Cholera epidemics were the highest cause of deaths among workers [213]. In 1897, Schüffner was employed to improve the plantation workers' health [215]. He 'modernised' the plantation hospital in Tandjong Morawah (Tanjung Morawa), Deli (Figure 8) [215]. The use of laboratory equipment helped the plantation doctors to understand disease epidemiology and design health interventions [215]. Due to the health interventions applied and the developments in modern medicine, the mortality rate of the workers decreased [215].



Figure 7. Map of Indonesia with provinces named and relevant locations annotated

The locations of interest are represented with red triangles. NTB: Nusa Tenggara Barat (West Nusa Tenggara), NTT: Nusa Tenggara Timur (East Nusa Tenggara).



Figure 8. The Senembah Company Hospital in the tobacco plantation of Tanjung Morawa
Source: Tropenmuseum, part of the National Museum of World Cultures, CC BY-SA 3.0, via Wikimedia Commons [216]

In 1902, Schüffner found a disease clinically similar to enteric fever that through laboratory investigation was proven to have different aetiology [12, 19]. Due to its similarities with typhoid fever, Schüffner referred to this disease as pseudotyphoid (*pseudotyphus* in Dutch) [12]. He described that first an 'initial lesion' appeared as a small 'flat vesicle' surrounded by hyperemic skin [12]. Underneath the vesicle there was 'a small area of dermal necrosis' that then ulcerated and accompanied by enlargement of lymph nodes draining the respective areas [12]. The 'initial lesion' or eschar was found in 39% of patients. Schüffner also observed rashes with raised boundaries [12]. The fever

pattern was described as similar to that of enteric fever [12]. Severe cases had progressed to the 'typhoid state' with drowsiness, delirium, and restlessness [12]. Though pseudotyphoid of Deli had lower mortality, he also noted the similarities in clinical presentations and transmission with the Japanese kedani fever [12, 19]. In 1908, Schüffner described an outbreak of 158 cases among 7,700 workers employed by the Senembah Company [11, 12]. Schüffner noted 3% mortality – lower mortality than the tsutsugamushi disease in Japan that can reach 70% [12].

Based on histories taken from interviews of patients, Schüffner suspected that exposures to ticks or mites might be involved [12]. Schüffner specifically suspected the involvement of the 'Trombidium of Deli' (later named taxonomically as *Leptotrombidium deliense*) in transmission [12].

L. deliense was first described by Walch in 1922 [217]. In his paper, Walch reported seven species of Trombiculae he found in Deli, six of which were new species [217]. He singled out the new species *Trombicula (Leptotrombidium) deliensis* as the most probable carrier of pseudotyphoid [217]. This was because of morphological similarities with *T. akamushi* the vector of scrub typhus in Japan and the fact that the geographical distribution of the mites was consistent with the disease distribution [217].

From 1908 onwards, other researchers including Wolff, Kouwenaar, Walch, Keukenschrijver, and Heineman also identified pseudotyphoid, mite fever, and scrub typhus cases in the plantations area of Senembah Company [11, 45, 46].

In 1924 Walch and Keukenschrijver described an outbreak of scrub typhus among coolies at the Good Year Tyre and Rubber Company, also located in northeastern Sumatra. The outbreak started around October 1922 and lasted up to August 1923, during which there were 111 pseudotyphoid cases among 2,500 workers [11]. However, different from what Schüffner had done when noting the cases (he was initially unconvinced that eschar is always present in pseudotyphoid), Keukenschrijver only counted cases with eschar [11]. Walch and Keukenschrijver also noted the similarities of pseudotyphoid and tsutsugamushi disease [11]. The mortality of the outbreak in 1922 was slightly lower than in 1908, 4% and 9%, respectively [11].



Figure 9. Workers clearing the overgrown area within the plantation

Source: Walch and Keukenschrijver, Geneeskundig Tijdschrift voor Nederlandsche Indie 1924, Vol. 64, Issue 2, page: 250-251 [11]

Some areas of rubber plantation were overgrown with secondary vegetation, especially with *lalang* (grass from *Imperata* species) (Figure 9) [11]. All diagnosed cases occurred in workers assigned to clearing these areas [11]. In November 1922, the first *L. deliense* larva was collected from a man [11]. From December 1922 onward, they checked around 200 workers working in the 'dangerous zone' infested with *Leptotrombidium* larvae and recorded whether they had been bitten and if yes, the location of the bite [11]. Pseudotyphoid cases admitted to the hospital were cross-checked for documentation of earlier exposure to mites [11]. Finally, Keukenschrijver described a case where three

weeks earlier, he had recovered a *L. deliense* larva from the scrotum of the patient [11]. An ulcer had developed precisely where he had found the mite [11]. The larvae of *L. deliense* were also found in the ears of 50% of rats captured in the plantation area [11]. They noted correlation between *L. deliense* being found on humans and rats with where pseudotyphoid cases occurred [11]. They also found *L. deliense* feeding on two bird species: *Centropus javanicus* and *Rhinortha chlorophaea* [11].

During this early era of discovery, the Weil-Felix test played a key role in the diagnosis of scrub typhus and other rickettsial diseases. Accordingly, it was pivotal in the early descriptions of scrub typhus in Indonesia. The next few paragraphs describe the history of development of the Weil-Felix test and its interconnections with the history of rickettsial diseases, especially scrub typhus.

In the search of the causative agents of typhus, many organisms were isolated from patients' urine. The organisms were then mixed with the sera of typhus patients to see if the sera agglutinated [15]. In 1915, Edmund Weil and Arthur Felix isolated *Bacillus proteus* from the urine of a patient during a louse-borne typhus outbreak [15]. When mixed with the sera of patients with louse-borne typhus, the sera agglutinated [15, 16]. This cross-reaction was then used as a diagnostic method for louse-borne typhus. Later in 1916, Felix recovered the OX19 strain – a 'very sensitive and stable' strain of *B. proteus*, making test standardisation possible [15]. After further investigation, this strain was reclassified and is now named *Proteus vulgaris* [16].

In 1924, Kingsbury brought to Malaya a strain which was thought to be the OX19 strain [15]. This new strain caused confusion since it agglutinated the sera of scrub typhus patients instead of louse-borne typhus patients [15]. After further investigation, this new strain was characterised as a different strain that could be used to diagnose scrub typhus. It was named the 'Kingsbury' strain (OXK) [15]. With the OXK strain, the Weil-Felix test became the cornerstone of scrub typhus diagnosis [16]. The OXK strain is now classified as a different species from the OX19 strain, *Proteus mirabilis* [16].

With the Weil-Felix test, 'tropical typhus' was further characterised and separated into two diseases: rural and urban typhus [15]. The rural (scrub) typhus had localised outbreaks, was prevalent in rural areas, affected people who worked outdoors e.g. cowherds, and showed positive OXK and negative OX19 reactions [15]. The urban (shop) typhus distribution was not localised. It was prevalent in a more urban setting and showed positive OX19 and negative OXK reactions [15]. The rural and urban form are now referred to as scrub and murine typhus, respectively. We know now that the causative agent of murine typhus is *Rickettsia typhi*. The separation of rural (scrub) typhus and urban (shop) typhus had become clearer at the time, however, there was still confusion about rural (scrub) typhus and tsutsugamushi disease. Even up to 1930, rural (scrub) typhus and tsutsugamushi disease were still considered as separate diseases [15].

In the Dutch East Indies, in addition to pseudotyphoid, 'mite fever' was also used to describe scrub typhus. In his 1927 publication, van Driel used the term *mijtekoorts* (mite fever) [35]. He mentioned that Schüffner had thought about a term to replace *pseudotyphus* (pseudotyphoid) to avoid confusion with paratyphus and other types of fever resembling typhus [35]. Subsequent publications started to use the term mite fever instead of pseudotyphoid.

In 1936, Wolff and Kouwenaar published an article in the Geneeskundig Tijdschrift voor Nederlandsch-Indië (Medical Journal for Dutch East Indies) titled *Onderzoekingen over Sumatraansche Rickettsiosen* (On Rickettsia Diseases in Sumatra), reviewing cases from plantations in northeast Sumatra including the Senembah Company [45]. They described three diseases: Sumatran mite fever, scrub (rural) typhus, and shop (urban) typhus [45]. Sumatran mite fever was considered as 'a variant of tsutsugamushi disease', that is a distinct disease from scrub typhus [45]. They noted that mite fever and scrub typhus have similar epidemiology, clinical manifestations, and pattern of Weil-Felix reactions [45]. However, Wolff separated these diseases based on the presence of 'primary ulcer' or eschar: mite fever (with eschar) and scrub typhus (without eschar) [45]. Wolff and Kouwenaar suggested that the differences between these two diseases might be caused by differing means of transmission. They thought that scrub typhus may have been transmitted by ticks [45]. Here, we can see that the term 'mite fever' now had a different case definition from the pseudotyphoid described by Schüffner, that included both fever with and without eschar.

In the Dutch East Indies there were a number of terms used to describe scrub typhus (Box 1) [218].

1. With primary lesion (eschar)
    a. Mijtekoorts (mite fever)
    b. Sumatran mite fever
    c. Tsutsugamushi (of Malaya/Sumatra/Java)*
    d. Kedani fever (of Malaya/Sumatra/Java)*
    e. Pseudotyphus/pseudotyphoid of Sumatra**
    f. Pseudotyphus/pseudotyphoid of Deli**
2. Without primary lesion (eschar)
    a. Scrub typhus
    b. Rural typhus
    c. Tropical typhus
    d. Pseudotyphus/pseudotyphoid of Sumatra**
    e. Pseudotyphus/pseudotyphoid of Deli**

Box 1. The terms used in the Dutch East Indies to describe scrub typhus [218]

*Differentiated from tsutsugamushi and kedani fever of Japan because it was previously thought that the disease in Malaya/Sumatra/Java was different from the one in Japan because it had lower case fatality.

**Pseudotyphus/pseudotyphoid was used to describe both cases with eschar and without eschar

In addition to Deli and the surrounding areas, scrub typhus was also documented in Aceh. It was first reported by Emanuels in 1932 [40]. He reported cases diagnosed in the

military hospital of Koeta Radja (Kuta Raja), Banda Aceh, Aceh [40]. He encountered fever cases that were negative for malaria and suspected tropical typhus. Wolff and the pathology laboratory in Medan assisted Emanuels to obtain the *Proteus* species needed to perform the Weil-Felix test [40]. He then diagnosed this series of cases as 'tropical typhus' (defined as having positive Weil-Felix OXK reaction) and 'mite fever' (defined as having eschar) [40]. These patients were soldiers and forced laborers who had returned from patrol in Seulimeum, Kroëng Raja (Krueng Raya), and Lho-nga (Lhoknga) area in Aceh [40].

In 1940, Kotter also reported how scrub typhus affected the military operation in Aceh [49]. He observed that from 1932 to 1937 there were more patients who died from mite fever compared to malaria (four and none, respectively) [49]. In January 1938, mite fever paralysed a brigade for nearly two months [49].

Likewise, Dr van der Schroeff, who was based in the military hospital in Lhokseumawe (the second largest city in Aceh province, located about 270 km east of the capital Banda Aceh) also reported cases of mite fever and rural (scrub) typhus in palm oil plantation workers [50]. These workers were tasked to clear an abandoned coffee plantation overgrown with lalang [50]. Van der Schroeff also suspected mite fever and rural (scrub) typhus were the same disease. He thought that eschar might always be present, but were not noticed all the time [50].

Scrub typhus was also documented in south Sumatra [44]. In 1935, van Waardenburg reported four cases of rural (scrub) typhus in Bengkulu [44]. Other main islands also had reports of scrub typhus. In 1935, Bessem and Kuypers reported cases in Singkawang and Sintang, West Borneo [41, 43].

The earliest account of scrub typhus in Java came from Dinger and Lentjes from the military hospital in Weltevreden (part of present day Jakarta) [42]. They documented mite fever in Tangerang, Banten in 1935 [42]. In 1939, Wolff and de Graaf also reported mite fever cases in central Batavia [47]. They also trapped rodents and performed Weil-Felix tests on them and found positive OXK reactions [48]. In the article, Wolff and de Graaf mentioned that mite fever and rural (scrub typhus) were not often found in Java [47].

Meanwhile, in Malaya, Lewthwaite and Savoor conducted animal experiments on the 'typhus-like fevers of Malaya' and concluded in a 1936 article that the differentiating eschar was not only present in tsutsugamushi disease, but also present in rural (scrub) typhus, it just that the lesion had 'varying degrees of development' and may already disappeared when patients were examined [15, 219]. Further investigations also showed that microscopically the pathogen looked identical to the *Rickettsia orientalis* (the aetiology of tsutsugamushi disease) slides sent by Nagayo, and they concluded that rural (scrub) typhus and tsutsugamushi disease are 'identical' [15, 219]. They also urged to stop using the term 'rural typhus' and 'scrub typhus' in favour of the term

'tsutsugamushi disease' [15, 219]. However, the term 'scrub typhus' gained greater traction throughout World War II due to its easy pronunciation (and probably non-Japanese identity), and being meaningful in English [15].

In 1942, the Japanese Empire forces successfully invaded the Dutch East Indies. The power in Indonesia shifted from the Dutch to the Japanese by that cataclysm. The Empire of Japan also invaded the island of New Guinea that had been under the administration of the Netherlands and Australia (Dutch New Guinea and Australian New Guinea, respectively). The Allied forces halted the Japanese encirclement of Australia at Guadalcanal in the Solomon Islands, and then moved offensively toward New Guinea. That large island was an essential stepping stone for the assault on occupied Philippines and then southern Japan itself [220]. New Guinea island was thus of great strategic importance in World War II.

'Military necessity' during World War II forced the scientific community to improve their understanding of scrub typhus [13]. Scrub typhus caused 'medical casualties second only to malaria and was more dreaded by the men' and therefore impeded or threatened military operations [13]. This disease was causing the most deaths among the communicable diseases in the American forces in the Southeast Asia Theatre [221]. Among the American, British, and Australian troops, scrub typhus had caused over 16,000 cases and over 600 fatalities [13]. For perspective, malaria had caused 113,256 cases and 90 deaths in the US Navy and Marine forces [222]. Though the information

available was ‘fragmented’, based on recovered medical records and prisoners’ interviews, it was reasonable to believe that scrub typhus also affected the Japanese forces in ‘epidemic proportions’ [13]. The Japanese did not realise until late 1944 that the disease they encountered was the same disease as tsutsugamushi disease back at home [13].

In 1945, Griffiths reported a scrub typhus outbreak among Allied forces in Dutch New Guinea (now Papua and West Papua province) [51]. American soldiers had landed on the northern coastline between Cape Sansapor and Cape Opmarai on 30 July 1944 [51]. The first case was diagnosed on 6 August 1944 [51]. By 30 September 1944, there were 931 cases and 34 fatalities [51]. The majority of cases – more than 500, caught scrub typhus in ‘semi-cleared areas’ that might have been abandoned villages or plantations [51]. The other patients contracted scrub typhus in ‘overgrown coconut groves’ and the meeting of beach and forest. Griffiths also suspected that rain forest might have a role in the transmission, but failed to connect any case with this kind of environment [51]. He concluded that areas with grass and ‘low dense ground cover’ were linked with scrub typhus [51]. The soldiers also deployed several disease control efforts including clearing the areas by removing grass and scrub, ‘rat control’, and treating clothing with insecticides [51]. In 1947, Griffiths published a follow up paper. He also reported finding *L. deliense* in Sansapor [52].

In 1948, Dr Smadel, chief of the Division of Virus and Rickettsial Diseases of the Walter Reed Army Medical Department Research and Graduate School at Washington DC, led a team to conduct a clinical trial to test the effect of chloromycetin for the treatment of scrub typhus [162]. Chloromycetin was found to be efficacious [162]. The effect was dramatic enough that Mr Dorall, the administrative assistant to the director of the Institute for Medical Research in Malaya at the time – Dr Lewthwaite, drew a cartoon depicting the end of scrub typhus (Figure 10) [223]. The caption of the cartoon read: 'Laying the bogey of scrub typhus to rest in Malaya in 1949 at the successful conclusion of trials of chloromycetin (chloramphenicol), the first drug ever to cure typhus infections.' [223].

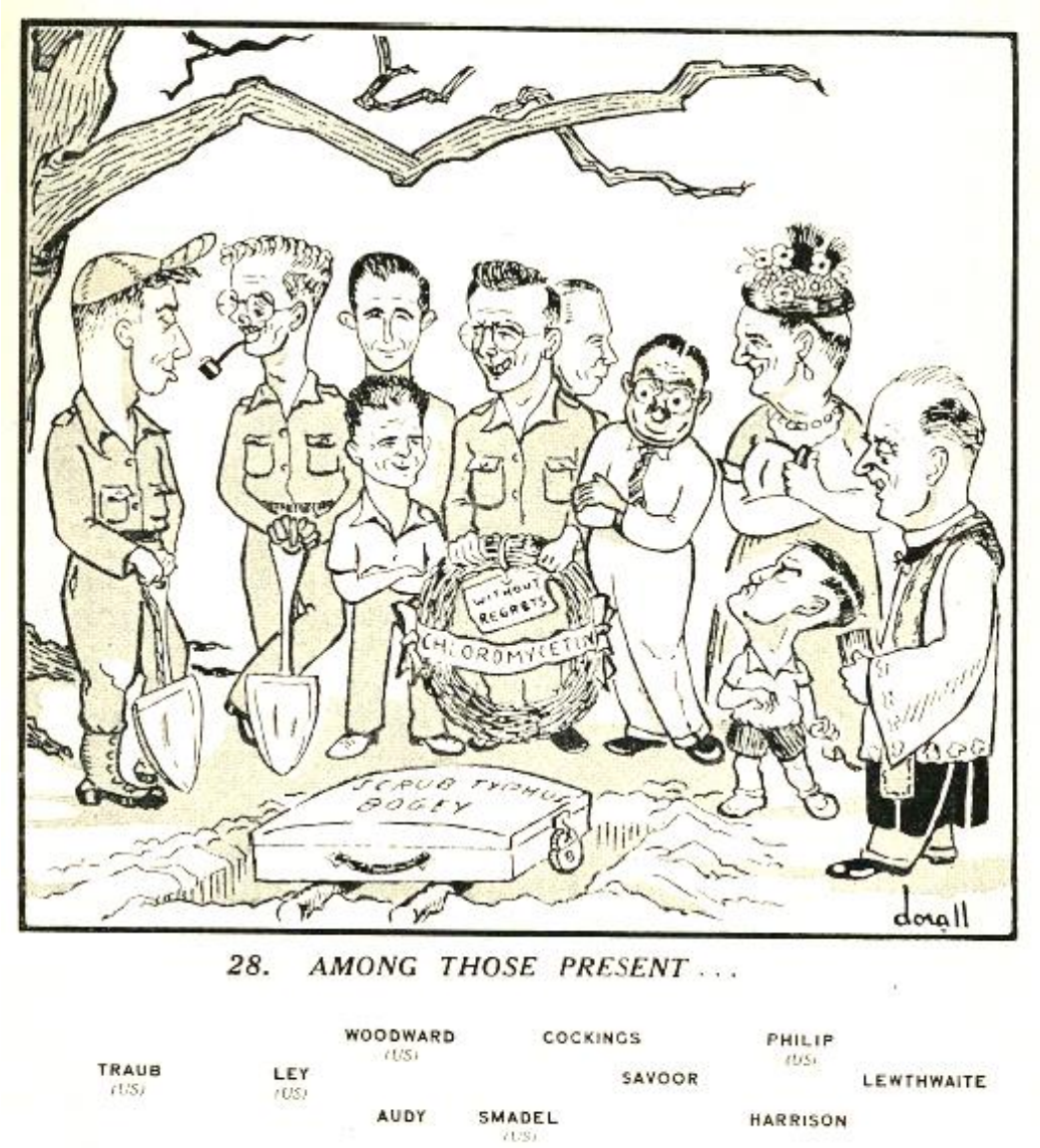


Figure 10. The cartoon drawn by Dorall, depicting the end of scrub typhus after chloromycetin was proven efficacious

Source: The Institute for Medical Research, 1900-1950: Studies from the Institute for Medical Research, Federation of Malaya no. 25, page 83 [223]

After World War II, scientific reports more consistently used the term 'scrub typhus'. In 1950, Dr Plooij from the military hospital in Palembang reported 40 scrub typhus cases

after exposure to *alang-alang* (*lalang*) fields, with only four patients having eschar [55]. Plooij tried to treat the patients with para-amino benzoic acid (PABA) that was said to have some effect, but he found that it was not as significant when compared to previous documentations in the literature [55]. In the early 1950s, Hoogerheide and Ensink documented two clusters of cases in Biak and Noessi (Nusi) islands off the north coast of Papua [56]. Patients were treated with chloromycetin and all recovered [56].

At the end of World War II and the fall of Imperial Japan, Indonesia declared independence on 17 August 1945. However, the Dutch still considered the Dutch East Indies as a colonial possession and launched a military campaign to regain control, initially aided by the British forces sent to disarm and repatriate Japanese soldiers (they later disengaged and supported Indonesian independence). After a bloody guerrilla warfare struggle, the Dutch government finally conceded sovereignty on 27 December 1949. However, that concession excluded Dutch New Guinea which was retained as a colonial possession.

Aside from several studies in Dutch New Guinea, there was a gap in the data on scrub typhus in the then Indonesian area from 1951 to 1970. This period was an unstable time in Indonesia history. In the early period of independence, Indonesia was faced with many political and economic challenges. In 1965, a coup d'état took place. This movement was followed by an anti-communist mass killing that lasted from late 1965 to early 1966. This massacre might have killed from 300,000 to 3,000,000 people.

Eventually, in 1967, Sukarno – the first president was overthrown and replaced by General Suharto. All of this turmoil had deeply impacted medical research within Indonesia.

In 1962, Indonesia started to govern western New Guinea under the supervision of the United Nations. From 14 July until 2 August 1969, a plebiscite was done and 1,025 people chosen by the Indonesian authorities voted for integration of western New Guinea into Indonesia. Eventually, on 19 December 1969, western New Guinea was formally united into the republic and named Irian Jaya province. Since then, Irian Jaya province was divided into West Papua and Papua provinces.

In the 1970s, Indonesia had become more stable and medical research was starting to be conducted. One of the institutions that works on scrub typhus was the Balai Besar Penelitian dan Pengembangan Vektor dan Reservoir Penyakit (B2P2VRP) or the Centre for Disease Vector and Reservoir Research and Development. I went to their headquarters in Salatiga, Central Java and catalogued their collection of microscopic slides of mite vector samples. B2P2VRP was first established in 1976 in collaboration with the WHO Vector Biology and Control Research Unit (VBCRU) [224]. It is a part of the Badan Penelitian dan Pengembangan Kesehatan or the Indonesia National Institute of Health Research and Development (NIHRD), Ministry of Health of Republic of Indonesia [224]. This institution conducts studies on zoonotic disease vectors and reservoirs to inform disease control efforts [224]. Unfortunately, evaluation of scrub

typhus pathogen and vector were not included yet in the study design of their latest and biggest survey: Riset Khusus Vector dan Reservoir Penyakit – Disease Vector and Reservoir Special Research (Rikhus Vektora) [225].

The oldest slide in the collection was from 1971, documenting an *L. deliense* specimen collected in South Borneo. In 1972, they documented *L. deliense* from West Java, Central Java, and North Sumatra. In a series of slides dated 1978 to 1981, B2P2VRP documented *L. deliense* in Jambi, Lampung, Bengkulu, South Borneo, and Papua. In 2004, Ristiyanto et al from B2P2VRP reported the presence of *L. deliense* and *L. fletcheri* in Central Java [78]. Ristiyanto et al also published a paper in 2016, summarising the results of small mammal ectoparasite surveys conducted from 2004 to 2011 across five provinces in Java: Banten, West Java, Jakarta, Central Java, and East Java [81]. The investigators found 16 genus and 28 species of trombiculid mites [81]. In the *Leptotrombidium* genus, the vector of scrub typhus were documented: *L. deliense*, *L. fletcheri*, *L. akamushi*, *L. arenicola*, *L. imphalum*, and *L. scutellare* [81].

In 1981, Pusat Penelitian Ekologi Kesehatan (Centre for Ecology Research) of the NIHRD performed a study in Central Sulawesi, where Hadi and Nalim documented *L. deliense* presence and positive *O. tsutsugamushi* IFA in rodents (no species or genus identified) [68].

In the 1970s, the Indonesian government extended its transmigration programme (a practice dating to colonial rule), involving 'transettlement' of people from the more populated islands of Java, Bali, and Lombok to other main islands with recently opened agricultural sites where the population was not as dense [62]. Gandahusada et al surveyed the transmigrant communities in Lampung using *O. tsutsugamushi* IFA and found seropositivity in the communities [62]. In one of the sites, the seropositivity reached 20% [62]. Another survey in transmigrant communities in South Sumatra was conducted in 1977 [57]. This was a collaboration survey between the Zoonoses Sub Division of the Indonesia NIHRD and WHO Vector and Rodent Control Research Unit, No. 2 (VRCRU-2) [57]. They recovered *L. deliense* and *L. fletcheri* in the trapped rodents [57]. In a survey performed over 1982-1983, Hadi et al also studied scrub typhus in a transettlement area in Lampung [67]. Here, *L. deliense* and *L. fletcheri* presence was documented [67]. Caught animals including *Maxomys surifer*, *Niviventer bukit*, *Rattus argentiventer*, *R exulans*, *R. rattus diardii*, and *R. tiomanicus* showed positive reactions in *O. tsutsugamushi* IFA [67]. Furthermore, the people in the communities also had positive *O. tsutsugamushi* IFA with 11.9% seropositivity [67]. In addition to the southern part of Sumatra, this transmigration programme also targeted Sulawesi and Borneo Island as resettlement areas. In a survey done by the Indonesia NIHRD in 1983, Hadi et al documented *L. deliense* in East Borneo and Central Sulawesi [65]. The rodents trapped in Central Sulawesi also had positive reactions in *O. tsutsugamushi* IFA [65]. In 1985, Nurisa et al studied the bionomics of scrub typhus in a transmigration community in Jambi [72]. *L. deliense* was discovered in the trapped rodents (mainly *Rattus tiomanicus*) [72].

In 1975, a survey was conducted in and around the Mount Bromo area, East Java. Hadi et al reported the discovery of *L. fletcheri* and *L. scutellare* [66]. In the same year, Dennis et al reported *O. tsutsugamushi* isolation from vectors (*L. arenicola*) and non-human hosts (*R. argentiventer* and *R. rattus diardi*) found in Ancol, Jakarta in a survey started in 1975 [61]. They also performed IFA on the community living in the area; the seroprevalence of scrub typhus was 0.36% [61]. Hadi et al discovered *L. deliense* and *L. fletcheri* in Biak and Owi Island off the north coast of Papua in 1976 [58]. *O. tsutsugamushi* was also isolated from the spleens of *R. exulans* and *R. rattus septicus* [58]. In West Sumatra, *L. deliense* has also been documented as ectoparasites of small mammals in 1976 [63]. In 1980, Liat et al reported finding *L. deliense* in rodents caught in Tanjung Priuk, a then suburban area in north Jakarta [59]. In 1977-1978, the WHO Vector Biology and Control Research Unit conducted a study in Ciloto area, West Java [60]. They found *L. deliense* in the area [60].

In 1981, a survey on vector and reservoir was conducted in Batam Island (about 10 km south of Singapore) [64]. There were 54 animals trapped, including rodents: *Rattus exulans*, *R. rattus*, *R. rattus diardii*, and *R. surifer* [64]. In this survey, Hadi et al also found *L. arenicola* in the island [64]. In 1985, Hadi et al surveyed the Sunter area in north Jakarta. Here, they documented *L. deliense* [69].

In a 1992-1993 survey, Corwin et al investigated rickettsial infections in Indonesian military personnel [74]. They were tested for exposure to *R. typhi*, *O. tsutsugamushi*,

and *R. conorii*. Using ELISA, the seropositivity for scrub typhus was 7.88% [74]. In 1993, Richards et al documented antibody against scrub typhus in rodents in Papua [76]. Among 72 trapped rodents, 12.5% were positive in *O. tsutsugamushi* ELISA [76]. Richards et al also surveyed military communities in Malang, East Java in 1994 and found antibodies against scrub typhus in both trapped rodents and human participants [75]. 5.1% of the trapped rodents and 1.3% of participants were showing positive reaction in *O. tsutsugamushi* ELISA. In 1997, Richards et al documented 9.4% seropositivity in participants in Gag Island – a small nickel-producing island about 150 km to the west of the coast of the island of Papua [77].

In addition to studies specific to scrub typhus or rickettsial diseases, data on scrub typhus were also generated through acute febrile illness studies which included scrub typhus in their diagnostic panel. In 1997, an acute febrile illness study was done in north eastern Papua by Punjabi et al [75]. Using ELISA, they reported positive antibodies against scrub typhus in 4.42% participants [75].

Many of these projects were conducted or written by investigators from the US Naval Medical Research Unit No. 2 Detachment (NAMRU-2), Jakarta. NAMRU-2 Jakarta was established in 1970 through a bilateral agreement between the US and Indonesian government that was supposed to last 30 years [226]. In the end, NAMRU-2 operated in Indonesia for about 40 years and made 'substantial contributions to local, global, and military medicine' [226]. NAMRU-2 had contributed to capacity building in terms of both

training human resources and donating laboratory facilities. NAMRU-2 also participated in public health interventions such as malaria control effort in Central Java, the digital Early Warning Outbreak Recognition System (EWORS), and avian influenza (H5N1) diagnosis [226].

Between July 2005 and December 2007, 116 human cases of H5N1 were diagnosed in Indonesia – the highest number of any country in the world at the time [227]. It also adversely impacted the poultry industry with deaths reaching 16 million [227]. In the beginning of the outbreak, Indonesia referred the clinical samples to laboratories within the WHO global influenza surveillance network (GISN) – now the Global Influenza Surveillance and Response System (GISRS) [227, 228]. The samples were sent to the US Centers for Disease Control and Prevention (CDC) in Atlanta, which was a WHO International Influenza Reference Laboratory, through NAMRU-2, which was the WHO Collaborating Center for the Southeast Asia region [227]. The samples were also sent to the WHO H5 Reference Laboratory at the Hong Kong University [227]. However, starting January 2007, the Indonesian government stopped sending their clinical samples out of the country [227]. A series of incidents led to this decision [227]. The analysis results of the samples sent to the laboratories abroad were presented and written up without the knowledge of the Indonesian government [227]. The Indonesia Ministry of Health was informed by a journalist that an Australian company was planning to develop a vaccine using a virus strain shared by the Indonesian government with the WHO. This had prompted ‘drastic action’ [227].

On the one hand, the Indonesian government felt that their sovereignty was violated and advocated for 'transparency of the global system' and 'equity between developed and developing nations' – a demand that was also voiced by other less resourced countries [227]. On the other hand, this dispute took place during a public health crisis, where data and sample sharing is important to inform control effort [226]. Indonesia began withholding their samples [226, 227]. This set a precedent for future crises [226]. NAMRU-2 with its role as the WHO Collaborating Center for the Southeast Asia region, eventually was dragged into this debate [227]. The health minister at the time, Siti Fadilah Supari also accused NAMRU-2 of 'espionage, biological weapons research, and development of "new diseases" to enhance pharmaceutical companies' profit margins' [227]. Within Indonesian society there was also 'larger political pressure' that contributed to making the relationship fall apart [227]. One of them was that some Indonesians viewed NAMRU-2 as a remnant of former dictator president Suharto [227]. In 2010, NAMRU-2 finally moved its headquarters to Hawaii, briefly, and then to Singapore where it remains today [227].

NAMRU-2 had contributed considerably to scrub typhus research in Indonesia. Among 51 articles with primary data on scrub typhus in Indonesia included in *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*, 14 (26.9%) articles listed authors from NAMRU-2.

In addition to NAMRU-2, WHO, and the NIHRD, the Smithsonian Institution also performed a study on ectoparasites of rodents in North Sulawesi [73]. Durden et al extracted a *L. deliense* from a person [73]. Authors from Indiana State University and Vanderbilt University also did a study under 'Project Wallace' in 1985 and reported *L. deliense* presence in North Sulawesi [71]. In the same year, Goff from the University of Hawaii and Durden from Vanderbilt University also documented the presence of *L. deliensis* in North Sulawesi [70].

From 2007 to 2008, Widjaja et al studied the distribution of rickettsiosis in Java, Sumatra, Sulawesi, and Borneo [82]. In North Sulawesi and East Borneo, they detected *O. tsutsugamushi* in *Leptotrombidium* mites using PCR [82]. They also found positive antibodies against *O. tsutsugamushi* in trapped small mammals (rodents) across North Sulawesi, West Sumatra, and East Borneo [82]. In 2008, Ristiyanto et al documented the presence of *L. deliense* and *L. fletcheri* in the trapped *R. tanezumi* and *R. exulans* in the slopes of Mount Merapi, Central Java [79].

From 2013 until 2016, a multicentre acute febrile illness cohort study was conducted [83]. Investigators collected data from eight tertiary hospitals across seven cities in Java, Bali, and Sulawesi [83]. IgG and IgM against *O. tsutsugamushi* were tested for using commercial ELISA [83]. There were no acute cases of scrub typhus found, but they found positive IgG in Jakarta, West Java, Yogyakarta, Central Java, East Java, and South

Sulawesi [83]. Acute febrile illness studies including scrub typhus in their diagnosis panel are still lacking in Indonesia.

Looking at the history of scrub typhus in Indonesia, we can see how wider factors can influence research and knowledge generation. In the early period of discovery, colonial interests dictated research and data gained were concentrated in areas important to the colonial regime. Likewise, during World War II, military necessities pushed the research communities to gather more knowledge faster. Subsequent political turmoil and economic challenges were reflected in a void of scrub typhus research. Fortunately, the political and economic atmosphere in Indonesia is now relatively stable. Therefore, it is time to invest in research funding and mechanisms to ensure continuous medical research, not only on scrub typhus, but also on other important health problems in Indonesia.

The data on scrub typhus laid out in this chapter will be elaborated and analysed further in *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*.

Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping

Introduction

Scrub typhus is understudied. The great difficulty in differentiating scrub typhus from other endemic febrile illnesses with similar clinical presentation, along with poor diagnostics, largely explains why we do not know much about its burden. This uncertainty occurs even within its classical distribution region, the tsutsugamushi triangle in Asia and western Pacific.

Indonesia is located within the tsutsugamushi triangle. Documentation of scrub typhus outbreaks in Indonesia exists, most notably among plantation workers in east Sumatra in 1900s and Allied Forces in western Pacific during World War II [1]. Prevalent scrub typhus also occurs in the surrounding countries with which Indonesia shares land borders, namely Malaysia and Papua New Guinea [8]. This sparse, dated, and proximal evidence suggests that scrub typhus occurs in Indonesia and, as it invariably does elsewhere, causes morbidity and mortality. In the absence of reliable measurements, the extent of harm done by scrub typhus in Indonesia will remain almost wholly unknown.

This paucity of information drives a vicious cycle of under recognition and underfunding; there is no funding because there is no evidence, and there is no evidence because there is no funding. The work presented here aims broadly at this dilemma by gathering rare evidence in order to define the geographical distribution of scrub typhus at subnational levels in Indonesia. This represents a crucial first step in identifying knowledge gaps, guiding future studies, and, ultimately, mitigating the morbidity and mortality caused by this dangerous infection. Defining the distribution of scrub typhus infection risk without case data from reliable and accessible point-of-care tests nonetheless essentially informs empirical treatment guidelines for prompt and rational antibiotic treatment. Credible knowledge of the distribution of scrub typhus transmission would certainly aid the rational use of empiric antibiotic treatment and survival odds.

Human cases or seroprevalence may not be the sole means of demonstrating scrub typhus transmission risk. The presence of confirmed vectors of *O. tsutsugamushi* and infection of non-human hosts may be assessed to indicate areas of risk to humans. A similar approach has been applied to define the geographical distribution of *Plasmodium knowlesi,* a mosquito-borne zoonotic malaria that occurs in humans [229]. Published data and grey literature on the location of *O. tsutsugamushi* infection in human and non-human hosts, as well as presence of vectors (infected or not), can be collected through a systematic search and engagement with stakeholders to compile a map of that evidence. Then, by giving different weights based on the diagnostic quality and how contemporary the evidence may be, we can categorically rank areas based on their

apparent potential to maintain an infectious reservoir. The resulting map would not chart the prevalence or incidence of human infections, but it would summarise existing evidence of risk transmission and it would identify gaps where data is lacking or not available.

As of August 2021, I could not find any review summarising the available evidence of scrub typhus in Indonesia. This evidence mapping exercise would be the first. This project aims to map and categorically rank geographic areas for likelihood of sustaining infectious vectors and reservoirs, thereby generating an evidence-based map illustrating the likely geographic distribution of scrub typhus in Indonesia. Furthermore, I want to identify areas where evidence on *O. tsutsugamushi* infection and presence of vectors is lacking.

Methods

Search strategy

Thirteen international electronic databases including Embase, Global Health, Medline, Scopus, and Index Medicus for South-East Asia Region (IMSEAR) were searched, along with three Indonesian electronic databases, those of the Indonesian National Institute of Health Research and Development (NIHRD), Ministry of Health, and of the Ministry of Research, Technology and Higher Education. Four local university and research centre websites were also reviewed and a manual search of literature from Dutch colonial era

held at the Eijkman Institute library was conducted. In addition, five Dutch journal and museum websites were also searched or contacted.

Searches were undertaken in English and Dutch (for colonial era articles). No Indonesian language search term was used because there is no Indonesian word for scrub typhus and pilot searches showed that all Indonesian articles can be captured by the English and Dutch search strategy. No language or publication date restrictions were applied. The search terms were combinations of terms related to the bacterium (*Orientia tsutsugamushi*); scrub typhus disease e.g. mite typhus, kedani fever, chigger borne rickettsia; the mite vector e.g. trombiculid, *Leptotrombidium*, chigger; and terms related to Indonesia e.g. Netherlands East Indies, Dutch East Indies. The detailed search strategy is provided in Appendix 1. No specific searches for the other candidate species i.e. *candidatus* O. chuto and O. chiloensis were done since there are no reports of their circulation in Southeast Asia so far and the current strategy should have also captured these articles because of the inclusion of the term '*Orientia*'. If available, I searched the reference list of the relevant articles. If there were any relevant review articles, I also screened the reference lists of these, even though they were not eligible.

In addition, I visited the Balai Besar Litbang Vektor dan Reservoir Penyakit (B2P2VRP) – the Institute of Vector and Reservoir Control Research and Development operated by Ministry of Health. During the visit, I made an inventory of the mite vector samples available in the form of microscopic slides. I catalogued information written on the slides

including slide number, genus and species name, slide date, genus and species of hosts where the mites were found, locality, province, and sampling date. These data were also included in this evidence mapping.

I also included the results from *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples* as data points. This was a cross-sectional serosurvey of human archived sera using indirect ELISA to detect IgM and IgG against *O. tsutsugamushi*. The samples were collected during acute febrile studies conducted at hospitals in Jambi, Denpasar, and Tabanan. The Jambi site is located in Jambi province in southern Sumatra, while the Denpasar and Tabanan sites are located in Bali province. The samples from Jambi, Denpasar, and Tabanan were collected in December 2014–December 2015, January 2017–July 2018, and January 2017–July 2018, respectively. The Jambi and Denpasar sample collection hospitals were located in urban settings, and the Tabanan hospital was in a semi-urban setting. The seropositivity rates for each site were as follows: Jambi 9.71%, Denpasar: 7.95%, Tabanan: 8.96%.

Eligibility criteria

Evidence on *O. tsutsugamushi* detection and antibodies against *O. tsutsugamushi* in either humans or animals, and the survey-based presence or absence of vectors were eligible for inclusion. Data of interest included the location of infection and methods used to detect *O. tsutsugamushi* or antibodies against it. There was no publication date

restriction. There was no limit on the minimum sample size of collection for inclusion. However, in ascertaining evidence of absence or very low risk of scrub typhus, the sample size had to be at least 400, to achieve the upper limit of 95% confidence interval on prevalence of less than 1% when all samples are negative. That is, if a survey found no positive samples and its sample size is 400, the upper limit of 95% of confidence interval would be 0.009 and thus we can be 95% certain that the true proportion of positives in the population is less than 1%.

Data extraction

Data were extracted into a Microsoft Excel spreadsheet (Supplement 1). Each data point contained unique information on evidence for *O. tsutsugamushi* circulation and presence of its vector at a specific location during a specified year. If one location was sampled three times, three observations or data points were created. If there was more than one location mentioned in the extracted article, the location used as the data point is the more likely infection location i.e. where the patient was located for two weeks (14 days) before clinical signs and symptoms started. If more than one paper refers to the same location and time period but with different case numbers, observation, and description, these papers were deemed as separate sources. Data points were extracted from both papers and counted as different data points.

In case reports or case series, the admission or presenting date was counted as the study start date, and where this is not clear, the date of first observation mentioned was used.

Where the last day of observation or date of discharge was not available, study end dates were entered as the last day of observation or the date of discharge. Where there was aggregated data e.g. by location or time and it could not be disaggregated, the aggregate data was entered. If the data could be disaggregated, it was separated to the smallest location or time unit possible. In the case of reinfection, each infection episode was inputted as an event data point. Where the study month and year were unknown, the publication month and year were inputted instead.

The geographic coordinates of each data point were also extracted. The latitudes and longitudes were recorded in decimal format. If the relevant paper provided coordinates for the survey, this was entered. In case the coordinates were given in the degree format i.e. in 'degrees, minutes and seconds' (e.g. 8° 39' 0.0000'' S, 115° 13' 0.0012'' E), conversion to decimal format was done using online converters http://www.calculatorcat.com/latitude_longitude.phtml. If sketch maps were available in the papers, these were analysed for contextual and landmark information provided and then searched on online gazetteer (https://www.google.com/maps primarily and http://www.geonames.org/) and coordinates were extracted. If the paper provided only the name of the collection site, that was searched in online gazetteers to obtain the coordinates. Historical papers in English and Dutch exhibited site names with different spellings which were entered to the online gazetteer as appropriate. The site information available in these papers was recorded and used as the basis for cross-checking.

Each data point was classed as a point or polygon. A point is defined as a site that can be geopositioned to an accuracy of ≤25km^2 (i.e. 5km x 5km) or has ≤25km^2 area size [229]. A polygon is a site that cannot be geopositioned to an accuracy of ≤25km^2 or has >25km^2 area size [229]. The coordinates of the approximate centre of the polygon were used. If the survey site coordinate information was not given or could not be located with the online gazetteer, but information on higher level administrative division (e.g. subdistrict or district) was available, this was entered as the site location.

Data analysis

The extracted data was mapped by geographic coordinates to each subnational unit. These data were synthesised using descriptive statistics, graphs, maps, and narrative synthesis. Proportions and frequencies were used to summarise and describe categorical variables. The analysis was done on Microsoft Excel 2013, StataBE 17, R version 3.6.2 and R Studio Version 1.2.5033 [230, 231].

Defining the area of study

In the mapping process, Indonesia was divided into subnational units. Though division based on administrative boundaries would be useful for governments since public health data are usually structured based on these, the distribution of hosts and vectors does not follow administrative boundaries. Here, we used a mixed approach where the size of the islands and their distance from each other was also taken into account [229]. The largest islands in Indonesia i.e. Sumatra, Java, Borneo, Sulawesi, and Papua were

divided based on the level one administrative (provincial) boundaries [229]. Indonesia is the world's largest archipelagic country, sometimes provincial boundaries span across islands even though they were far from each other [229]. Islands larger than 200 km^2 and further than 25 km from the largest islands were considered as separate units [229]. If the islands were within 10 km of each other, they were categorised into the same unit [229]. When smaller islands (less than 100 km^2) were 'more than 10 km from any other islands', they were 'disregarded' [229]. The subnational unit division can be seen in Figure 11. As of July 2021, there were 77 subnational units for Indonesia. This included the whole Timor Island which partly belongs to Timor Leste. Each subnational unit was then assigned a score based on the likelihood of sustaining vectors which are infectious to humans.



Figure 11. The division of Indonesia into subnational units

Assigning an evidence score to each subnational unit

A score was assigned to each subnational unit. The scores reflected the subjective weight of evidence based on the level of certainty for the presence of human infection risk by *O. tsutsugamushi*.

The evidence was ranked in descending score order as follows: confirmed human case, probable human case, possible human case, seroprevalence in humans, infection in vector (e.g. *Leptotrombidium* spp), infection in other hosts (e.g. rodents) and presence of vector, infection in other hosts but presence of vector unknown, presence of vector but presence of the pathogen unknown.

The ranking of evidence and scores in detail are below (Figure 12):

1. Confirmed case of human scrub typhus (score +24)
2. Probable case of human scrub typhus (score +22)
3. Possible case of human scrub typhus, diagnosed by Weil-Felix test (score +19)
4. Possible case of human scrub typhus, diagnosed clinically (score +18)
5. Seroprevalence of >20% in community (score +16)
6. Seroprevalence of >10% and ≤20% in the community (score +15)
7. Seroprevalence of >5% and ≤10% in the community (score +14)
8. Seroprevalence of 5% or less (but >0) in the community (score +13)

9. Infection confirmed in vector (score +10)
10. Infection confirmed in other hosts and presence of vector (score +8)
11. Infection confirmed in other hosts but presence of vector is unknown (score +6)
12. Presence of vector but presence of *O. tsutsugamushi* is unknown (score +3)

In addition, all evidence of presence gathered were given a score based on the year when sample collection or observation ended i.e. study end year:

1. 1945 or older: 0
2. 1946 to 1965: score +1
3. 1966 to 1985: score +2
4. 1986 to 2005: score +3
5. 2005 to present: score+4

If the exact sampling date was available, the year of sampling was used as the start and end study year. I used the study end year because it was more representative than the publication date. Higher scores were given to newer studies to account for possible ecological changes in the sites over time.

Confirmed cases must be diagnosed with at least one laboratory examination, including: bacterial culture and isolation, sequencing, indirect immunofluorescence test (IFA),

indirect immunoperoxidase test (IIP), polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), or immunochromatography test (ICT). Probable cases were diagnosed when eschar was found during physical examination. Eschar is a small painless necrotic skin lesion at the site of *O. tsutsugamushi* inoculation by a mite and it is a highly specific sign of scrub typhus [2, 124]. I categorised possible cases into two: first, cases with positive OX-K antigen Weil-Felix reaction; second, cases diagnosed clinically based on clinicians' suspicion (with no eschar found). Weil-Felix test for scrub typhus is a test based on the cross-reaction of the *Proteus mirabilis* Kingsbury OX-K antigen with antibodies against *O. tsutsugamushi* [131]. This test suffers from low sensitivity and specificity, but was still being utilised in several resource-limited settings [131].

In 'vectors', I only included confirmed vectors in the analysis. Confirmed vectors fulfilled the 'criteria of vectorship' (*Chapter 1. General introduction*) [92]: (1) the candidate vector needs to be proven naturally infected by *O. tsutsugamushi*; (2) there must be evidence that the mite species can transmit the pathogen to a host; (3) the species need to be present in the areas when scrub typhus is prevalent; (4) naturally infected human hosts should also be present where scrub typhus is prevalent; (5) the candidate mites also feed on humans; (6) the species should be able to acquire pathogen from one host and transmit it to another. The confirmed vectors are: *Leptotrombidium akamushi*, *Leptotrombidium arenicola*, *Leptotrombidium chiangraiensis*, *Leptotrombidium deliense*, *Leptotrombidium fletcheri*, *Leptotrombidium gaohuensis*, *Leptotrombidium*

imphalum, *Leptotrombidium pallidum*, *Leptotrombidium pavlovsky*, and *Leptotrombidium scutellare* [91].

Using the longitude and latitude information, first I mapped the data points in QGIS. Then I created a new variable in the dataset – 'subnational unit'. This variable contained the information as to which subnational unit each data point belongs. After sorting the data points based on subnational unit, I assessed what kind of evidence is available in each subnational unit.

Where there is more than one data point in one subnational unit, the evidence ranked with a higher score was used and the lower score was set aside. An algorithm was developed as a means of scoring each subnational unit for certainty based on weight of evidence as ranked and scored above (Figure 12). I used a Microsoft Excel spreadsheet to calculate the score of each subnational unit (Supplement 2). The spreadsheet incorporated the algorithm for evidence ranking (Figure 12). The scores for the level of certainty and sample collection year were added to calculate the final scores. A map was generated using QGIS Desktop 3.8.3. Each subnational unit was coloured with gradation corresponding to its score, i.e., the higher the score, the darker the colour.

Reporting

Although this study is an evidence mapping exercise and not a systematic review per se, there are similarities in the methodologies and reporting standards. Therefore, there is value in abiding to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines as this ensures transparent and clear reporting [232]. The reporting of this evidence mapping thus followed PRISMA guidelines when applicable.

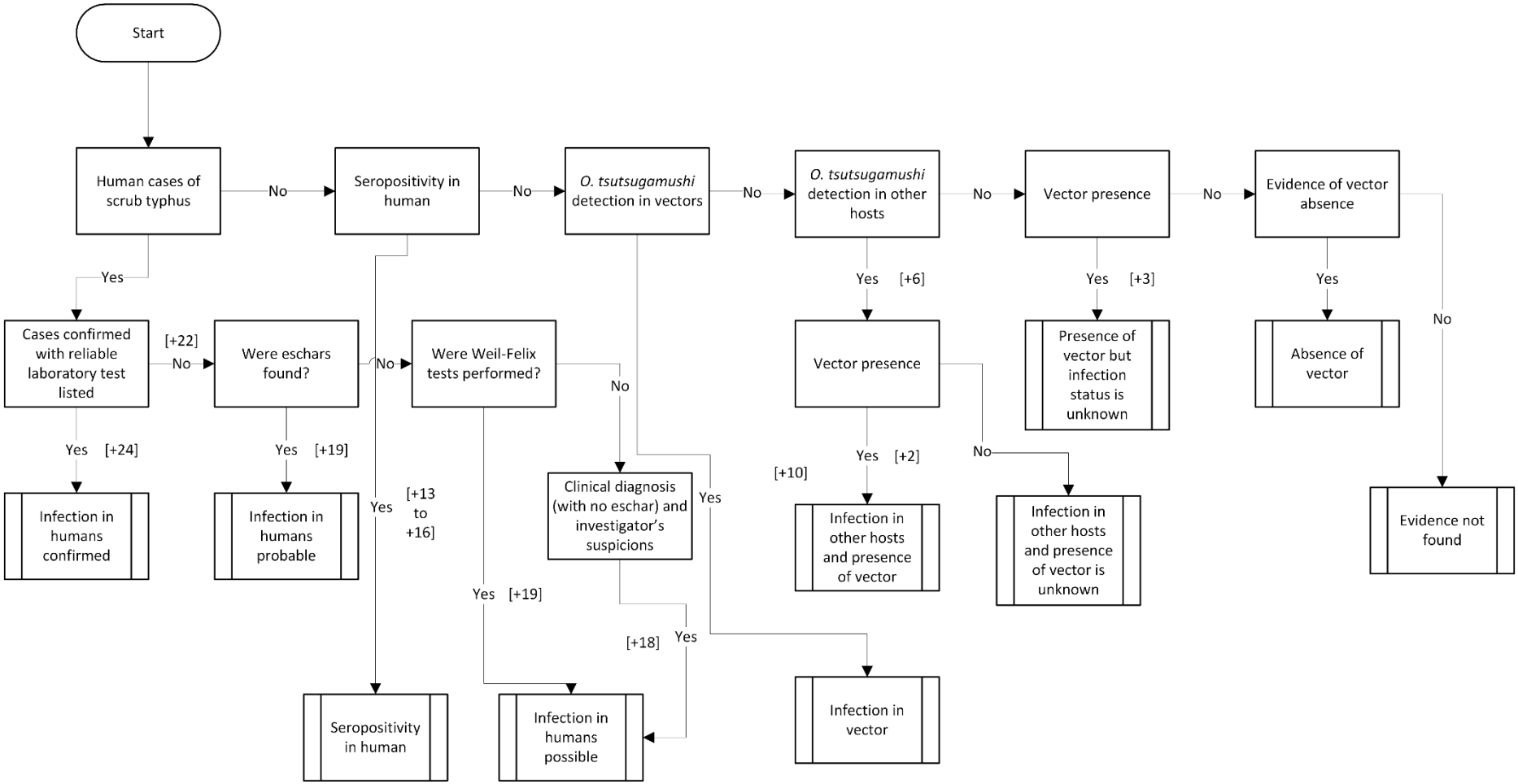


Figure 12. The scoring algorithm applied to score each subnational unit

Results

Search results

The initial database and website searches were performed on 7 November 2018, with an update search conducted on 15 June 2021. I identified a total of 426 citations, which resulted in 263 citations to be screened after duplicates were removed (Figure 13). I excluded 192 non-relevant studies and proceeded with eligibility assessment of 71 full text articles. Twenty studies were further excluded because there were no extractable data points (n = 14, for example a relevant paper did not have any location or study time mentioned, and the full texts could not be accessed after extensive attempts i.e. requesting the help of the Bodleian Libraries (n = 6). Finally, I included 51 studies for the evidence mapping [11, 12, 35-83]. The list of included studies can be viewed in Appendix 2. In addition to published studies, I also included the results from *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples* and unpublished data from B2P2VRP. There were 354 data points in total extracted from these sources.

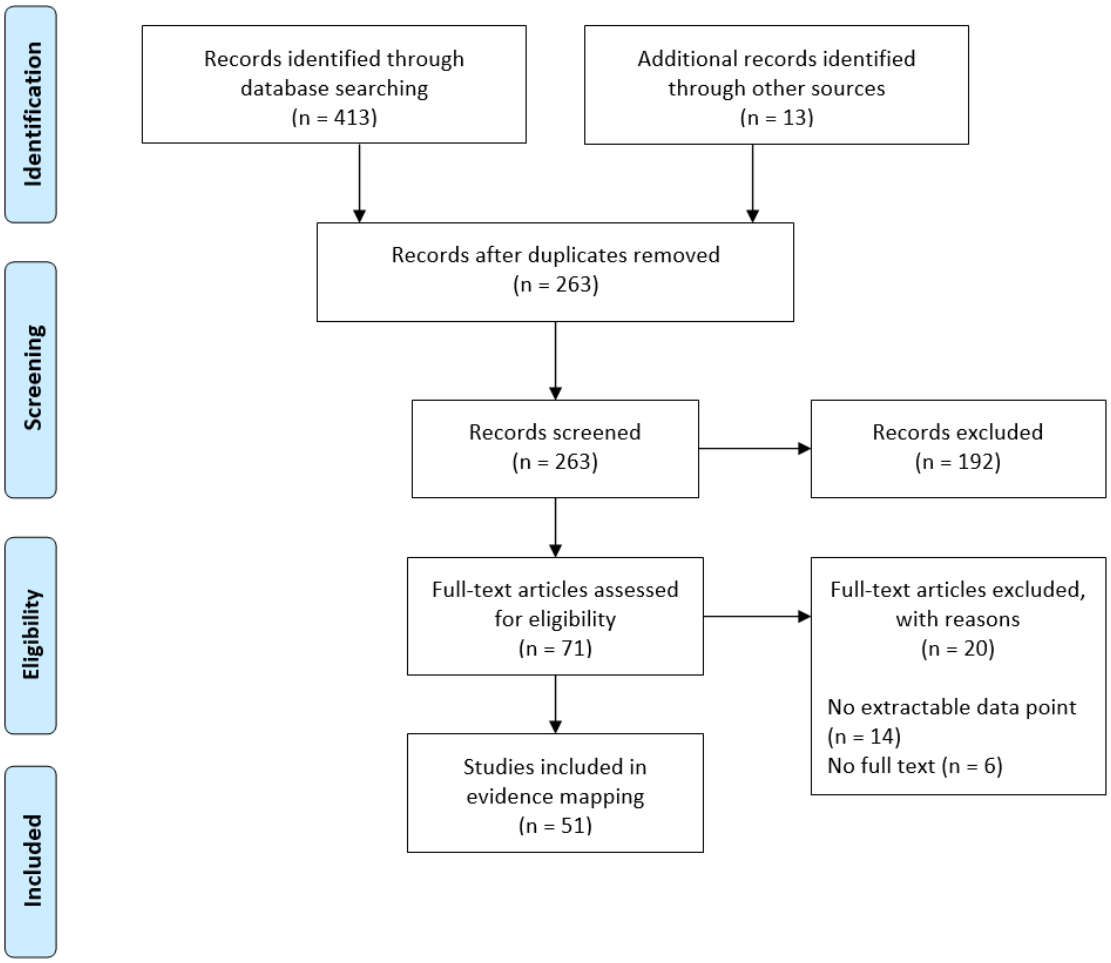


Figure 13. The PRISMA flowchart describing the numbers of articles identified in the searches, screened, assessed for eligibility, and included in the evidence mapping

Study end year

The included study end dates range from 1908 to 2018 (Figure 14). Most of the sample collection periods ended between 1931 and 1945 (n = 128/354, 36.2%) and between 1916 and 1930 (n = 75/354, 21.2%). Only 11 (3.1%) data points collection ended between 1946 and 1960, as well as the 1961 to 1975 period. The majority of data points (n = 223, 63.0%) were collected before 1946. Only 61 (17.2%) are from the last thirty years.

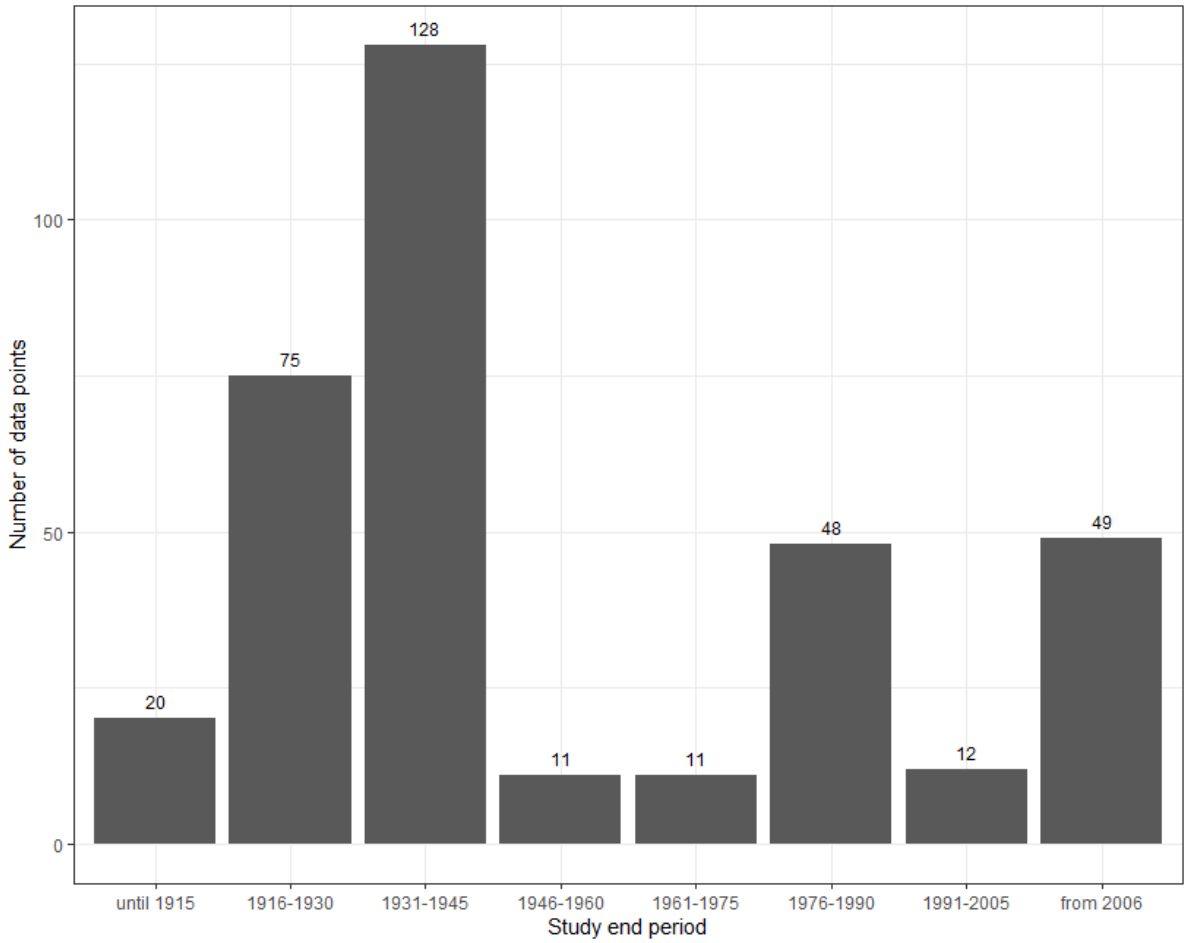


Figure 14. The number of data points collection ending in each period

Figure 15 shows the number of data points in each category over time. All probable and possible cases data points were collected by studies ending in 1954 or older. Conversely the data for human seropositivity and infection in vectors were only collected from 1975 and 1976 onwards, respectively. The period of data points collection on infection in other hosts and presence of vectors were the two widest.

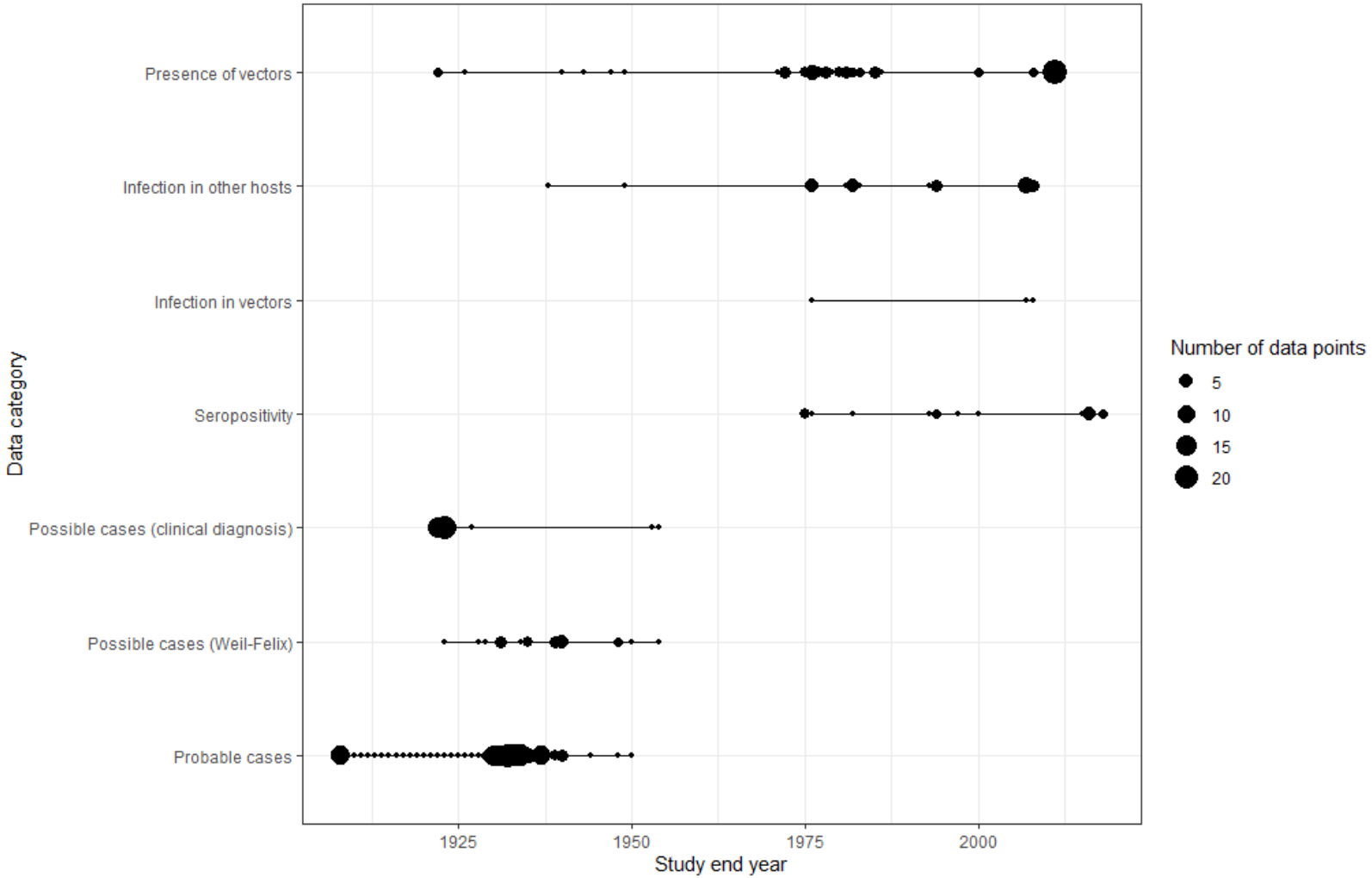


Figure 15. The number of data points in each category over 110 years

Distribution of data points

There were 354 data points extracted from included studies (Table 2). These data points were distributed across 25 out of 77 subnational units (32%), with most data points in North Sumatra (n = 149, 42.1%), followed by Aceh (n = 64, 18.1%) and Jakarta with 23 data points (6.5%). The remaining subnational units (n = 22, 88%) had less than 20 data points. The median of total data points in each subnational unit was 4 (range 1–169).

Figure 16 shows the distribution of extracted data points plotted on the map. The three largest point clusters were of 148 (41.8%), 50 (14.1%), 25 (7.1%) data points, which were located in North Sumatra, Aceh, and Jakarta, respectively. The North Sumatra cluster was located close to the east coast of northern Sumatra, where plenty of colonial Dutch plantations were located. Likewise, the Aceh cluster was located in an area where Dutch plantations were frequent. Jakarta, known as Batavia under Dutch colonial rule, was the capital of Dutch East Indies and is now the capital of Indonesia.

Many data points were extracted in the same location; therefore, I chose point cluster symbology so that the overlapping data points can be represented. I used natural breaks (Jenks) classification for this map. This method was chosen since it would minimise variance within classes by grouping similar values, while emphasising the differences between classes [233, 234]. The range of the number of data points from each subnational unit was 1 – 169 and the median was 4, which could be considered as a wide range with most of the subnational units having way less than 169 data points (22 out

of 25 (88.0%) of the subnational units had less than 20 data points). Natural breaks can present the wide range clearly while still providing contrasts between the classes of subnational units with smaller number of data points.

Table 2. The number of data points in each subnational unit

Subnational unit	Probable case	Possible case (Weil-Felix)	Possible case (clinical diagnosis)	Sero-prevalence	Infection in vector	Infection in other hosts	Presence of vector	Number of data points
North Sumatra	103	3	39	0	0	0	4	149
Aceh	50	13	0	0	0	0	1	64
Jakarta	2	3	0	2	1	5	10	23
East Java	0	0	0	4	0	4	9	17
Lampung	0	0	0	4	0	6	6	16
Biak	0	1	1	0	0	3	5	10
West Java	0	0	0	1	0	0	9	10
Central Java	0	0	0	1	0	0	9	10
North Sulawesi	0	0	0	0	1	4	3	8
East Borneo	0	0	0	0	1	4	1	6
Papua	0	0	0	1	0	1	3	5
West Borneo	2	2	0	0	0	0	0	4
Bengkulu	0	1	0	0	0	0	3	4
Jambi	0	0	0	1	0	0	3	4
Central Sulawesi	0	0	0	0	0	2	2	4
West Sumatra	0	0	0	0	0	4	0	4
West Papua	1	0	1	0	0	0	1	3
South Sumatra	1	1	0	0	0	0	1	3
Banten	0	1	0	0	0	0	1	2
Bali	0	0	0	2	0	0	0	2

Subnational unit	Probable case	Possible case (Weil-Felix)	Possible case (clinical diagnosis)	Sero-prevalence	Infection in vector	Infection in other hosts	Presence of vector	Number of data points
South Borneo	0	0	0	0	0	0	2	2
Yogyakarta	0	0	0	1	0	0	0	1
South Sulawesi	0	0	0	1	0	0	0	1
Waigeo+	0	0	0	1	0	0	0	1
Bintan+	0	0	0	0	0	0	1	1
Total data points	159	25	41	19	3	33	74	354

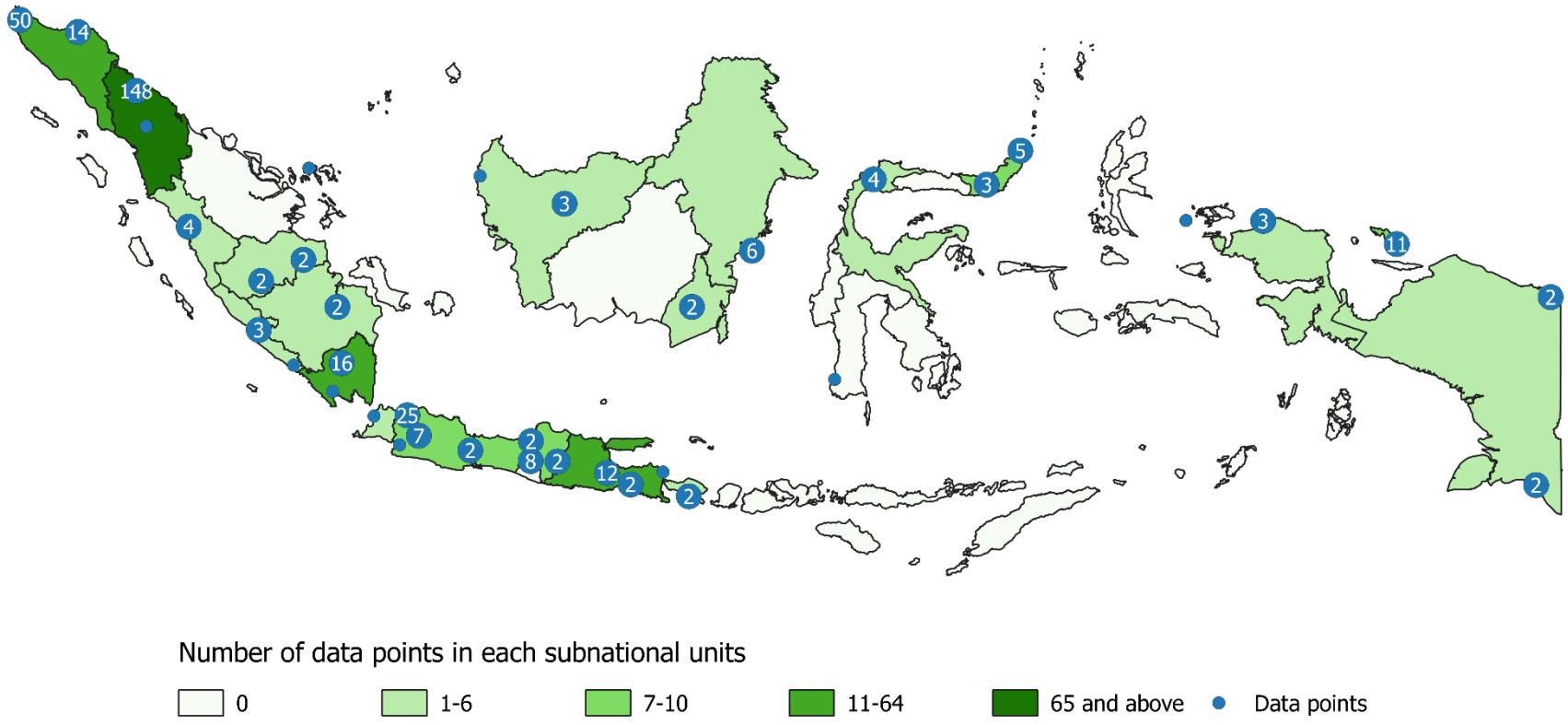


Figure 16. The distribution of data points across the subnational units in Indonesia

The colour of the subnational units represented the total number of data points within the boundaries of each subnational unit. The blue dots represented each data point, and if there are any overlapping data points, these were displayed as a cluster (a blue dot with the number of overlapping data points).

Confirmed case

One article reported an acute scrub typhus case confirmed by more than four times increase in ELISA IgG titre between acute and convalescent samples [235]. This patient also developed an eschar-like skin lesion. The patient lived in Jakarta, however, he travelled to the Banda Islands, Maluku within the disease incubation window [235]. Therefore, the infection site could not be pinpointed and this data were not mapped.

Probable case

North Sumatra had the highest number of probable case data points (n = 103, 29.1%), followed by Aceh with 50 data points (14.1%) (Table 2). Out of the total 354 data points, Jakarta and West Borneo had two data points each. West Papua and South Sumatra had one data point each.

There were 2,780 probable cases counted. North Sumatra had the highest number of probable case counts (n = 1,651, 59.4%). Followed by West Papua with 931 probable cases (33.5%) – although it had only one data point [51]. Aceh had 189 probable cases (6.8%) with 50 data points. South Sumatra, West Borneo, and Jakarta had four, three, and two probable cases respectively.

The studies included as the probable case data points also had utilised other methods of diagnosis in addition to the presence of eschar, including Weil-Felix test, animal

passage (with negative results), clinical diagnosis with physician's suspicion (Supplement 1).

All probable case data points were extracted from surveys done in 1950 or before (Figure 15, Supplement 1), with the earliest survey ended in 1908. One data point in West Papua noted that 34 out of 931 scrub typhus patients died during the survey period. All probable case data points were extracted from 12 articles [12, 38-40, 43, 45, 46, 48-51, 55].

Possible case (Weil-Felix)

In this section I will elaborate the data points and number of possible cases diagnosed with Weil-Felix test. There were 25 data points in total representing this category with Aceh having the highest number of data points (n = 13/25, 52.0%), followed by Jakarta and North Sumatra with three data points each (12.0%), and West Borneo (n = 2/25, 8.0%). Banten, Bengkulu, Biak, and South Sumatra had one data points each (4.0%).

There were 158 cases counted. Aceh had the largest number of possible cases diagnosed with Weil-Felix test (n = 77/158, 48.7%). South Sumatra (n = 36/158, 22.8%) and Jakarta (n = 29/158, 18.4 had the second and third highest number of possible cases, respectively. Biak had eight (5.1%), North Sumatra had four (2.5%), and West Borneo had two (1.3%) possible cases. Banten and Bengkulu had one (0.6%) possible case each.

Most of the data points (n = 6/25, 24.0%) collection ended in 1940 (Figure 15, Supplement 1). Sixteen (64.0%) data points collection ended within the 1935-1950 period. The most recent study end year was 1954.

Possible case (clinical diagnosis)

North Sumatra had the highest number of data points (n = 39/41, 95.1%) and possible cases diagnosed clinically (n = 119/147, 80.9%). West Papua and Biak had one data point each (2.4%), with 21 (14.3%) and 7 (4.8%) cases respectively.

Most of the data points were collected in the 1920s (n = 39/41, 95.1%), with 1954 being the most recent study end year (Figure 15, Supplement 1).

Seropositivity

There were 11 subnational units with seroprevalence data (Table 3). Lampung and East Java had four data points (n = 4/19, 21.1%) each. Followed by Bali and Jakarta with two data points (n= 2/19, 10.5%) each. Waigeo, West Java, Jambi, Yogyakarta, Papua, South Sulawesi, and Central Java had one data point each (n = 1/19, 5.3%).

The range of seroprevalence found across the subnational units was 0.36% to 20%, with a median of 6%. The highest seroprevalence (20%) was found in Lampung, while the lowest (0.36%) was found in Jakarta. There were 3,192 people tested, with 205 counted

as seropositive. The most commonly used diagnostic methods were ELISA (n = 14/19, 73.7%), followed by IFA (n = 5/19, 26.3%).

Table 3. The distribution of data points with seropositive participants across subnational units

Subnational unit	Number of data point(s)	Percentage	Seroprevalence (%)
East Java	4	21.1	0.63, 1.3, 3.65, 7.88
Lampung	4	21.1	5.7, 6.6, 11.9, 20
Bali	2	10.5	7.95, 8.96
Jakarta	2	10.5	0.36, 3.2
Waigeo+	1	5.3	9.4
West Java	1	5.3	9.3
Jambi	1	5.3	9.71
Yogyakarta	1	5.3	6
Papua	1	5.3	4.42
South Sulawesi	1	5.3	2.9
Central Java	1	5.3	2

There were three data points documenting absence, however, these data points did not fulfil the minimum sample size to rule out presence (i.e. 400). There was one data point each in East Java, Bali, and Central Sulawesi [68, 75, 83]. Studies in East Java and Bali both used ELISA to test 168 and 48 participants respectively, while the study in Central Sulawesi used IFA to test 91 participants.

The earliest data collection ended in 1975, while the latest ended in 2018 (Figure 15, Supplement 1). Most of the data points collection ended in the 2010s (n = 9/19, 47.4%).

Infection in vector

There were three data points for infection in vector – one data point (n = 1/3, 33.3%) each in Jakarta, North Sulawesi, and East Borneo. One data point recorded infection in *L. arenicola* found in Jakarta diagnosed with animal passage and isolation. Two other data points from North Sulawesi and East Borneo described infection in *Leptotrombidium spp*; these were tested using rtPCR. The studies in Jakarta, North Sulawesi, and East Borneo ended in 1976, 2007, and 2008, respectively (Figure 15, Supplement 1).

Infection in other hosts

There were 33 data points in total for infection in other hosts. Lampung had the highest number of data points (n = 6/33, 18.2%), followed by Jakarta with five data points (15.2%). East Java, East Borneo, North Sulawesi, and West Sumatra had four data points each (12.1%). Biak, Central Sulawesi, and Papua had three (9.1%), two (6.1%), and one (3.0%) data point(s) respectively.

All documented infected hosts were rodents (Table 4). There were seven (21.2%) and four (12.1%) data points documenting *Rattus tanezumi* and *Rattus tiomanicus* infection, respectively. In addition to *Rattus*, other recorded documented hosts included the following genus: *Bandicota*, *Chiropodomys*, *Maxomys*, *Niviventer*, and *Leopoldamys*. The *Rattus* and *Maxomys* species were not identified in one data point (3.0%) each. In two data points (6.1%) only the order was documented i.e. hosts documented as 'rodents'.

The earliest and latest conducted studies ended in 1938 and 2008, respectively (Figure 15, Supplement 1).

Table 4. The number of data points for each non-human host species

Host	Number of data point(s)	Percentage
Rattus tanezumi	7	21.2
Rattus tiomanicus	4	12.1
Rattus rattus	4	12.1
Rattus exulans	3	9.1
Rattus argentiventer	3	9.1
Rattus norvegicus	2	6.1
Rodents	2	6.1
Bandicota indica	1	3.0
Chiropodomys gloroides	1	3.0
Maxomys sp	1	3.0
Maxomys surifer	1	3.0
Niviventer fulvescens	1	3.0
Leopoldamys sabanus	1	3.0
Maxomys whiteheadi	1	3.0
Rattus sp	1	3.0
Total	33	100.0

Most of the data points used ELISA as the diagnostic method (n = 17/33, 52%), followed by IFA (n = 11, 33%), isolation (n = 7, 21%), and Weil-Felix test (n = 1, 3%). The data point using Weil-Felix test was taken in 1938. Three data points used more than one diagnostic method (isolation and IFA), therefore, the sum of all the previously mentioned percentage is more than 100%.

Presence of vector

There were 74 data points describing presence of vectors (Table 5). The subnational units with the highest number of data points were Jakarta (n = 10/74, 13.5%), followed by West, Central, and East Java with nine data points each (12.2%). Lampung had six data points (8.1%). The other subnational units had five or less data points. The data points collection spanned from 1922 to 2011 (Figure 15, Supplement 1).

Table 5. The distribution of data points on vector presence across subnational units

Subnational unit	**Number of data point (s)**	**Percentage**
Jakarta	10	13.5
West Java	9	12.2
Central Java	9	12.2
East Java	9	12.2
Lampung	6	8.1
Biak	5	6.8
North Sumatra	4	5.4
Bengkulu	3	4.1
Jambi	3	4.1
Papua	3	4.1
North Sulawesi	3	4.1
South Borneo	2	2.7
Central Sulawesi	2	2.7
Banten	1	1.4
Bintan+	1	1.4
East Borneo	1	1.4
Aceh	1	1.4
West Papua	1	1.4
South Sumatra	1	1.4
Total	74	100.0

The most commonly present vector species was *L. deliense* – found in 55 out of 74 (53%) data points. *L. fletcheri* had ten (13.5%) data points (Table 6). The other four species: *L. scutellare*, *L. arenicola*, *L. akamushi*, and *L. imphalum*, had less than five data points.

I also found 11 data points documenting the presence of *Trombicula* (*Leptotrombidium*) *pseudoakamushi*, eight on *Trombicula* (*Leptotrombidium*) *schuffneri*, one on *L. miculum*, and one on *L. keukenschrijveri*. In three data points, only the genus was identified (recorded as *Trombiculae*). These data points were not included in the main analysis since these were not on confirmed vectors of scrub typhus.

Table 6. The number of data points for each vector species

Host	**Number of data point(s)**	**Percentage**
Leptotrombidium deliense	55	74.3
Leptotrombidium fletcheri	10	13.5
Leptotrombidium scutellare	4	5.4
Leptotrombidium arenicola	2	2.7
Leptotrombidium akamushi	2	2.7
Leptotrombidium imphalum	1	1.4

Scores

The distribution of scores across subnational units can be viewed in Figure 17. We documented data points across 25 out of 77 (33%) subnational units. The lowest and highest scores were 23 and 5 respectively, with median of 18. South Sumatra and Biak

had the highest score with 23 points, while South Borneo and Bintan had the lowest score with 5 points. These scores were reflected in the map (Figure 18).

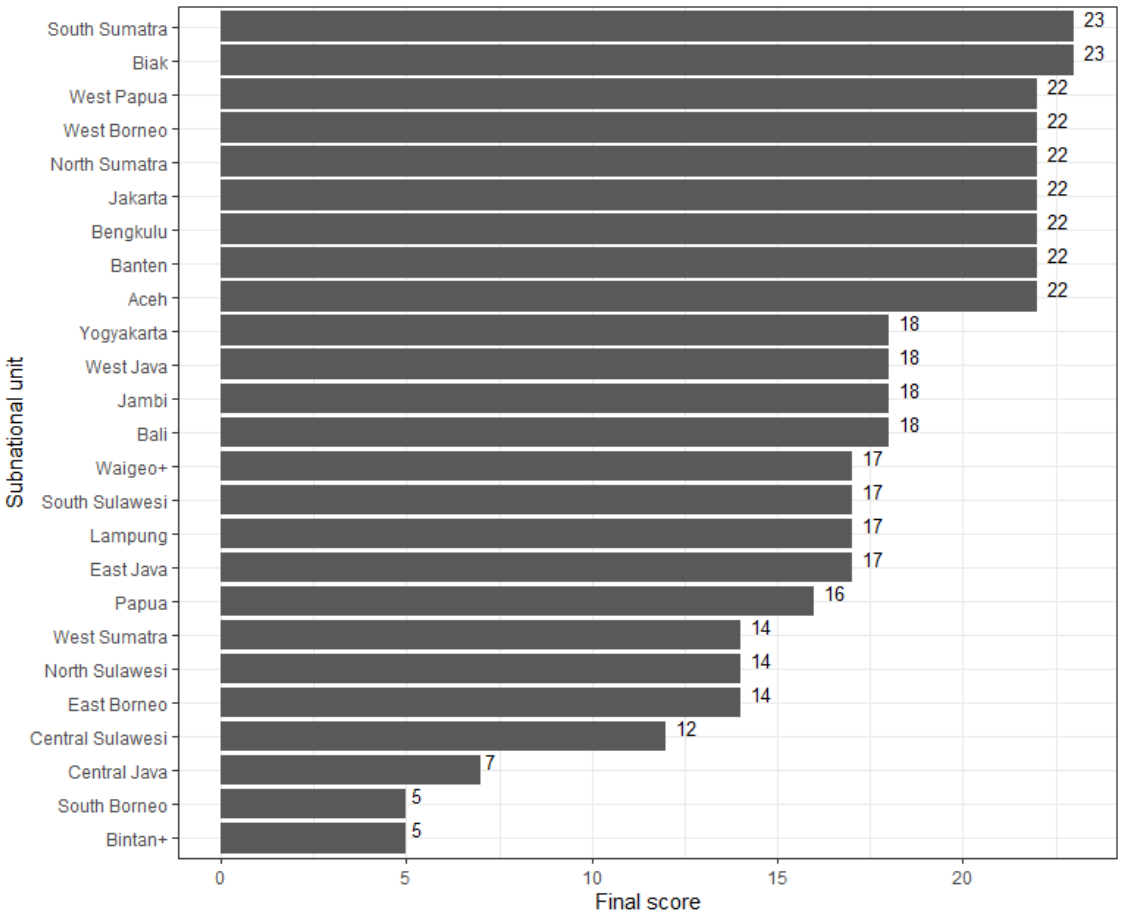


Figure 17. The distribution of scores across subnational units

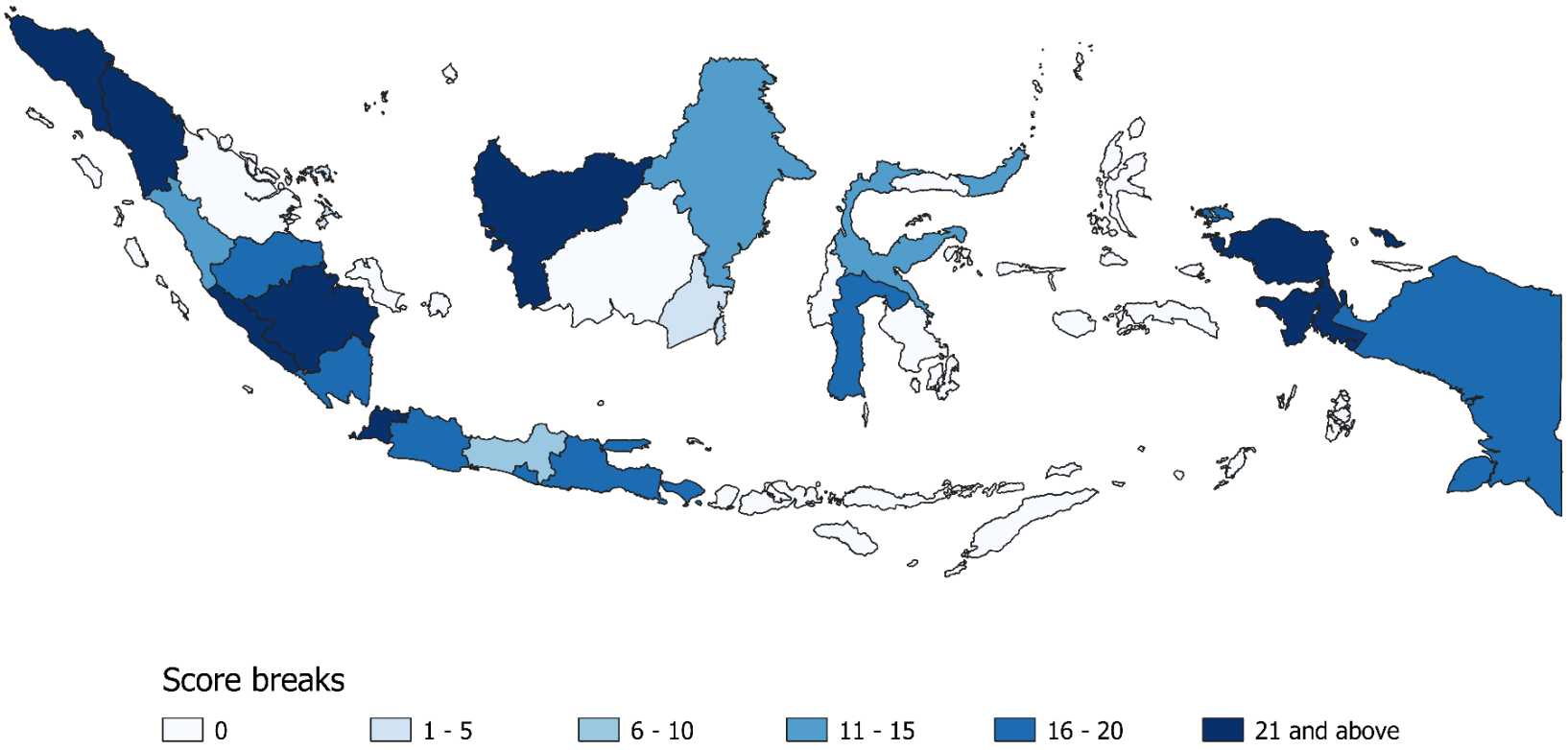


Figure 18. The map of subnational units' evidence scores

The darker the colour the higher the scores. Considering the range and distribution of the scores, 'pretty breaks' i.e. classification using round numbers as class limits, with interval of five (defined interval) were selected for visualisation [233, 234].

Table 7 showed the comparison between number of data points and scores in each subnational unit.

Table 7. Scores and number of data points in each subnational unit

Subnational unit	**Score**	**Number of data points**
Biak	23	10
South Sumatra	23	3
North Sumatra	22	149
Aceh	22	64
Jakarta	22	23
West Borneo	22	4
Bengkulu	22	4
West Papua	22	3
Banten	22	2
Jambi	19	4
West Java	18	10
Bali	18	2
Yogyakarta	18	1
East Java	17	17
Lampung	17	16
South Sulawesi	17	1
Waigeo+	17	1
Papua	16	5
North Sulawesi	14	8
East Borneo	14	6
West Sumatra	14	4
Central Sulawesi	12	4
Central Java	7	10
South Borneo	5	2
Bintan+	5	1

Discussion

Through this exercise, I have reviewed the available evidence and summarised the literature on the geographical distribution of *O. tsutsugamushi* and its trombiculid mite vectors in Indonesia. Evidence from various sources on scrub typhus in Indonesia were gathered and evaluated; these data were used to inform a summary evidence map. Finally, I extracted 354 data points from 51 studies, the Centre for Disease Vector and Reservoir Research and Development (B2P2VRP) collection, and from the study conducted in *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples*. These data points were spread across about a third of subnational units and five major islands in Indonesia i.e. Sumatra (Sumatera), Java (Jawa), Borneo (Kalimantan), Celebes (Sulawesi), and Papua. This study also highlighted the lack of evidence in the other two thirds of subnational units. The assessed evidence showed that South Sumatra and Biak had the best evidence of sustaining infective vector. There was one confirmed acute scrub typhus case, however, I could not include this as a data point since the site of infection was unknown. I documented 2,780 probable human scrub typhus cases. *L. deliense* was the most commonly found vector species. All non-human infected hosts were rodents. Almost two thirds of data points were collected before 1946.

The exhaustive search of the literature over a period of about 110 years encountered no published review of scrub typhus in Indonesia. This study represents the first known attempt to systematically map the likely risk of scrub typhus transmission in an

evidence-based manner. The search conducted was extensive – including international and Indonesian electronic databases, local university and research centre websites, Dutch journal and museum websites, and manual search in the Eijkman Institute library were searched (Appendix 1). I also searched in English and Dutch languages, allowing inclusion of historical Dutch articles from Indonesia's pre-independence era (1945 and older).

Because the search was performed in English and Dutch only, there could be data in other languages not captured. Although this is less likely, as publications usually mention the main etymology of scrub typhus: the main aetiology – *Orientia tsutsugamushi* or the term 'tsutsugamushi' in the text, and therefore can be captured by the broad search terms (Appendix 1). Even in article written in Japanese script, these terms would still usually be typed in Latin alphabet. For the Dutch articles, I was able to work with a medical translator who was also involved with article screening, this also served as quality control of the article screening stage.

Another limitation of this work was that the scoring of each subnational unit was an aggregation of the scores of several points or polygons within each subnational unit. For example, if there is one data point in a subnational unit, the whole subnational unit – be it a province, island, or islands, would be given the same score. Therefore, the scores of each subnational unit are not reflective of the evidence available in each pixel or specific area within. This is important to keep in mind when interpreting the results of this study,

particularly given that the distribution scrub typhus is often described as patchy in the form of 'mite islands' that may be both ephemeral and interspersed [94]. The sites of data collection were described in Figure 16 – this could provide more context in interpreting the evidence map (Figure 18).

So, what do the scores mean? The scores assigned to each subnational area reflected the strength of evidence. The scores, however, did not reflect the volume of data collected in the respective subnational area. For areas with no evidence of presence or absence, the likelihood of sustaining infectious reservoirs is unknown. We do not know whether this is because of lack of evidence and there actually are scrub typhus cases in these areas or it is simply not circulating there. The lack of evidence supporting absence of cases and infectious reservoirs (keeping in mind that to be able to declare absence much bigger sample size is needed) supports the thinking that zero evidence score is more of a problem of lack of evidence instead of real absence of scrub typhus risk.

The weighting of the scores were designed to reflect the risk of *O. tsutsugamushi* infection in human. I gave infection in human the highest score to give weight to areas where scrub typhus is of public health importance. This, however, could introduce bias as there are many factors that can influence patient presentation to health care facilities. For example, health seeking-behaviour, accessibility of health care facilities, and timing of diagnosis in relation to the national history of the disease.

This evidence map highlights the evidence gap in about two thirds of subnational units in Indonesia. For these areas, we should pilot research in locations traditionally described as the classical scrub typhus vector habitats. The area of priority should include areas with most people living in rural areas. For example, serosurveys or including scrub typhus diagnostics in acute febrile illness studies would help lay groundwork to better our understanding. Regions surrounded by areas with evidence of *O. tsutsugamushi* presence e.g. Riau in east Sumatra and Central Borneo could also potentially bear scrub typhus burden. The evidence was also lacking in the groups of smaller islands such as the Nusa Tenggara islands, including the Nusa Tenggara Barat (West) and Nusa Tenggara Timur (East) province, and Maluku (Moluccas) islands. This may reflect the relatively few research/healthcare facilities available or lack of research interest in the area.

Almost two thirds of the data points were collected before 1946, and may no longer be representative of the current situation. There might have been changes over time in the ecology and land use in the study sites. In addition, some of the diagnostic methods used then were not the standard diagnostic methods currently used. This was, to a degree, accounted for in the scoring process of each subnational unit by weighting; that is by giving higher scores for cases diagnosed by methods with higher accuracy. In addition, more recent data were also awarded higher scores.

One confirmed acute case of scrub typhus was documented [235]. However, the location of infection is inconclusive, therefore, this could not be mapped. All data points on probable and possible cases were collected in 1954 or before – showing that current data on human infection is lacking. Seropositive data points provided more recent data – the data collection started in 1975 until 2018. Despite this, there were still gaps as these data points were only available in 11 out of 76 (14%) subnational units. Therefore, more serosurveys to determine the spread and understand factors influencing *O. tsutsugamushi* infection are still needed. Furthermore, studies to determine prevalence of acute infection also need to be prioritised.

The lack of data points on confirmed human infection could be due to the low awareness of medical practitioners and the lack of accurate and accessible diagnostic modalities. Scrub typhus and other rickettsial diseases were not mentioned in the Indonesian standard list of medical graduate competencies [236]. We need to raise awareness for clinicians and include rickettsial diseases in the empiric treatment guideline of acute fever as one of the causes of intractable fever after given beta-lactam antibiotics. The lack of scrub typhus diagnostics in health facilities is not an isolated problem, but rather a systemic problem. Globally, there is a lack of widely available and affordable scrub typhus point-of-care diagnostics. In addition, the lack of infectious diseases diagnostic capacity and utilisation in general is a systemic challenge in Indonesia. Bacterial cultures tend to be underutilised in Indonesia [207, 208]. In the beginning of the Covid-19 pandemic, Indonesia also struggled to scale up SARS-CoV-2 diagnosis [209].

It is possible that the incidence of acute scrub typhus in Indonesia is low or is absent in most areas. However, given the presence of infective vectors and seropositive participants, as well as the current lack of survey activities and data, this may be less probable. Furthermore, scrub typhus is certainly present in nearby nations, even those sharing land borders with Indonesia such as Malaysia and Papua New Guinea.

Establishing scrub typhus and other rickettsial diseases as neglected tropical diseases recognised by WHO can also increase awareness to these diseases and possibly open more research funding opportunities [10]. This could further improve control efforts and clinical treatment. The WHO recounted four criteria to classify a disease as a neglected tropical disease (NTD): (1) primarily causing significant morbidity and mortality in populations living with poverty; (2) mainly affecting 'tropical and sub-tropical' populations; (3) feasible strategies for control available; and (4) 'neglected by research' [9]. At the moment, scrub typhus satisfies three out of four criteria (1, 2, and 4). Then when feasible control methods are available, scrub typhus would be able to fulfil criterion 3. Not recognising scrub typhus as an NTD has deprived researchers of funding opportunities, in turn causing further under representation and widen knowledge gaps. This also hinders feasible control methods development, presenting us with a vicious cycle. Recognising scrub typhus as an NTD could potentially break this endless loop and then reduce morbidity and mortality, as well as economic losses associated with this disease [10].

Presence of vectors appeared to be the most consistent category studied over time. Identifying the presence of vectors can be done morphologically and thus does not necessarily require laboratory tests. However, identifying the *Leptotrombidium* mites morphologically to the species level is challenging and there are not many experts in Indonesia. The unclear disease burden and unfamiliarity with scrub typhus are likely to have dimmed the interest in pursuing this expertise. The use of distance learning methods such as video modules can be helpful to train entomologists [237]. Recent developments in medical entomology such as the use of molecular methods and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) for species identification, might help tackle this challenge [238].

L. deliense is thought to be the major vector of scrub typhus [92]. This is consistent with the findings in this review as well: *L. deliense* was the dominant vector species noted. Its presence has also been documented in outbreak locations: North Sumatran plantations; and Sansapor, West Papua, where the Allied soldiers landed and camped [11, 52].

I found two data points documenting *L. akamushi* presence. Gispen in 1950 reported the presence of *Trombicula* (now *Leptotrombidium*) *akamushi* in Jakarta [54]. According to Vercammen-Grandjean and Langston (1976), *L. akamushi* distribution 'does not overlap' with the distribution of *L. deliense* [239, 240]. They also challenged the idea of *L. akamushi* prevalence in Southeast Asia [239, 240]. There is a chance that these specimens might have been misclassified. It had been documented previously that

specimen classified as *T. akamushi* was actually a subspecies of *L. fletcheri* [240]. Subsequent to Gispen's work, *L. fletcheri* was considered a separate species from *L. akamushi* [240].

With the current state of data, it is difficult to identify hotspots of infection accurately, as there are still many areas with no evidence. However, based on the available data, North Sumatra has the highest number of data points as well as a high likelihood of sustaining infected vectors. The data points from North Sumatra units were from rural areas fitting the classical scrub typhus habitat. However, since the data points on human cases were more than 50 years old, the current situation may be different from when these data points were collected because of changes in ecology e.g. land use (although most of the survey locations in North Sumatra are still used as plantations), climate, or human behaviour e.g. workers wear better protective clothing. Therefore, there is a need to target these areas too in future studies using current standardised diagnostic methods to confirm the prevalence of scrub typhus infection. To address this, I planned a seroprevalence study in palm oil plantations of North Sumatra (*Chapter 8. Serological investigation of risk of exposure to scrub typhus in residence at an oil palm plantation in North Sumatra relative to urban dwellers at Medan, North Sumatra (SEROSCRUB): A study protocol*). The presence of infected vectors has also been confirmed with rtPCR in East Borneo and North Sulawesi. In both areas, no human scrub typhus infection has been documented. These areas should also be targeted in future studies to confirm if scrub typhus is an important public health concern.

It is difficult to distinguish areas of absence. The currently available studies had sample sizes that were too small, making the results not enough to declare absence. In addition, for scrub typhus, there is no definitive list of vectors yet. It is important to keep this in mind when designing studies especially in areas with no data points; studies need to have enough sample size and use sensitive diagnostic methods, so the results can help define with higher certainty areas where scrub typhus is not circulating.

There are inherent limitations to an evidence map. To better understand the infection risk in an area, niche modelling incorporating ecological parameters to predict presence and absence in areas without data points would help in filling the knowledge gap [241]. This could also give a higher resolution map and thus could be helpful in selecting areas to do further research and direct what kind of research would be most useful.

The available data supports a reasonable conclusion that *O. tsutsugamushi* occurs in Indonesia. The presence of vectors that can sustain infection is well documented. However, this evidence mapping has also identified areas where data are lacking. Therefore, this evidence mapping highlighted the need to improve our understanding of scrub typhus and confirmed that scrub typhus is indeed a neglected tropical disease.

Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples

Introduction

In Indonesia, given the confirmed presence of infectious scrub typhus vector and acute cases summarised in *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*, we may surmise that endemic scrub typhus occurs in Indonesia. It is thus likely that scrub typhus imposes burdens of morbidity and mortality in Indonesia, but of almost wholly unknown geographic distribution, frequency, or magnitude. Unrecognised and unmeasured harm cannot be acknowledged and mitigated, thus imposing potentially dangerous underestimation of harm.

Scrub typhus diagnosis is not performed routinely for acutely febrile patients. Aside from a very few referral laboratories or in a research setting, laboratory tests to diagnose scrub typhus are simply not available. Scrub typhus treatment guidelines are not available and it is not usually included in the differential diagnosis of acute febrile cases. Many Indonesian clinicians are not familiar with clinical scrub typhus diagnosis and treatment. This lack of awareness and the scarcity of confirmatory diagnosis contributes largely to the meagre data available on scrub typhus distribution in Indonesia.

Through this study, we aimed to perform a scrub typhus serosurvey of archived fever study samples as a proxy indicator of active transmission. The purpose is to begin to understand more about where endemic scrub typhus might be occurring in Indonesia. Assessing seroprevalence as a proxy of exposure and active transmission is a starting step in understanding the epidemiology of scrub typhus in Indonesia. The use of archived sera samples of participants with acute fever instead of random samples of healthy people added complication in that we did not simply measure the background seroprevalence alone, but also the possibility of scrub typhus as a cause of acute febrile illness. Given the limitations of our resources and the unavailability of convalescent samples, we could not perform other comparative diagnostic tests or compare the acute and convalescent samples, therefore whether seropositivity just meant exposure or acute infection, the two were not easily separated.

In this study we analysed archived sera samples from acute febrile illness cases. Analysing archived sera by-passed expensive and time-consuming clearance application and sample collection, therefore, optimising the samples already collected. The data generated through this project can help gauge the scrub typhus level of exposure in proximity to the study sites, and potentially generalisable to areas with similar ecology and demography. This study can also serve as the stepping stone that provides preliminary data for future more advanced studies that can help inform empiric antibiotic treatment guidelines and public health measures of risk mitigation.

In this project, ELISA was used to analyse the serum samples. ELISA is a more standardised, objective, and reproducible scrub typhus diagnostic method compared to the gold standard IFA [131, 242]. In the context of scrub typhus diagnosis, ELISA is often used semi-quantitatively – a cut-off value is used to determine negative and positive samples. On the other hand, IFA is often restricted to the use of quantitative serology. Though IFA requires human image interpretation that might influence reproducibility, human pattern recognition is helpful in differentiating specific and non-specific fluorescence. ELISA allows high throughput testing and does not require the advanced training needed for IFA. It also does not require individual test interpretation as the resulting optical density is output directly from the reader machine (IFA invokes operator interpretation of image), making ELISA results more easily reproducible [131, 242]. With lower cost, the sensitivity and specificity of ELISA are also comparable to those of the IFA [131, 242]. This could be an ideal diagnostic method in a referral laboratory in resource limited settings. More researchers have used ELISA for diagnosis in scrub typhus treatment studies over time as shown in Figure 49 *of Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform*.

Due to limitations imposed by the Covid-19 pandemic in Indonesia, we could not (as planned) test additional samples from rural and urban areas of North Sulawesi, South Sulawesi, and North Sumatra. In addition to reducing the number of sites analysed, I also could not get access to samples from urban areas which would have individuals with

presumed no prior exposure to scrub typhus. The mean plus two or three standard deviations of the optical density values of the 'presumed unexposed' or 'negative control' samples was usually used as the cut-off to determine seropositivity [243]. There were no established scrub typhus ELISA cut-offs in Indonesia or the sites analysed. Due to the unavailability of these samples, I used other methods to determine cut-offs, including visual inflection point, change point analysis, and finite mixture models.

Table 8. The characteristics of each AFI study

Sites	Jambi [244]	Denpasar [245]	Tabanan
Sample collection time	December 2014–December 2015	January 2017–July 2018	January 2017–July 2018
Hospital	Private hospital in the provincial capital	Public district hospital in the provincial capital	Public district hospital
Inclusion criteria	Patients presenting within 5 days of onset with fever >38°C and at least one of dengue-like clinical manifestations including retro-orbital pain, joint pain, malaise, and rash.	Patients 14 years and older presenting with fever >38°C and at least one of dengue-like clinical manifestations including retro-orbital pain, joint pain, malaise, and rash.	Patients ≥ 6 months presenting with fever (oral temperature ≥38°C and/or history of fever) for <7 days and leukocyte count ≤10,000
Exclusion criteria	None	History of chronic diseases including diabetes mellitus, human immunodeficiency syndrome, chronic kidney, liver, lung, and heart disease.	Illness is considered as respiratory or gastrointestinal infection.
Number of samples	352	415	268

In this project, we analysed archived fever study samples from three regions, namely Jambi (Sumatra), Denpasar (Bali), and Tabanan (Bali). We analysed archived serum samples with an in-house ELISA method used previously [138]. Through this project we have also conducted training on in-house ELISA in a referral laboratory in Jakarta i.e. the Eijkman Institute for Molecular Microbiology, further building scrub typhus diagnostic capabilities in Indonesia.

Methods

This was a cross-sectional study on seropositivity of archived sera. This study was approved by the Eijkman Institute Research Ethics Committee (approval No. 128). This study received a waiver from the Oxford Tropical Research Ethics Committee (OxTREC) as the project analysed samples obtained in a clinical study previously cleared and the samples analysed were anonymised.

Study size calculation

To ensure sample sufficiency and validity, we only analysed samples with volume of 100 µl or more. To make sure that we would not miss seropositive samples when there is low seropositivity, the minimum number of sera to be analysed per site was set at 200 – to achieve upper limit of 95% confidence interval of seropositivity of less than 2%. If in a site there were no seropositive samples and the sample size of the site is 200, the upper limit of 95% confidence interval – based on binomial distribution, calculated with *cii* command in STATA – would be 0.018 and thus we can be 95% certain that the true seropositivity in the population is less than 2%.

Serum samples collection

Serum samples collected from 2014 to 2018 in three sites in Indonesia were retrospectively analysed. The sites were Jambi (urban, Sumatra), Denpasar (urban, Bali), and Tabanan (semi-urban, Bali). The sera were collected as a part of acute febrile illness (AFI) studies across three hospitals. We tested 1,035 sera: 352 from Jambi, 415 from Denpasar, and 268 from Tabanan (Table 8). The results of the primary study from Jambi and Denpasar sites have been published [244, 245]. The datasets collected by the previous AFI studies included demographic variables e.g. age and sex as well as clinical history, signs and symptoms, and laboratory values. These samples were previously tested for dengue infection with flavivirus PCR and dengue NS1 [244, 245].

Laboratory procedures

Indirect ELISA to detect IgG and IgM against *O. tsutsugamushi* was performed in the Biosafety Level (BSL) 2 laboratory in the Eijkman Institute for Molecular Biology in Jakarta [138]. The assays were performed with on-site expert technical guidance from Ampai Tanganuchitcharnchai and Sirada Ongchaikupt.

The 96-well microtitre plates were divided into two; half of the wells were coated with *O. tsutsugamushi* antigen while the other half were not coated with antigen (Figure 19). Forty-eight wells in each 96-well microtitre plate were coated with *O. tsutsugamushi* whole-cell antigen from four strains (Karp, Kato, Gilliam, TA 716) diluted at 1:8,000 with 1X phosphate buffered saline (PBS) with pH 7.4. The whole-cell antigens used were pre-titrated and standardised against reference serum. Therefore, each antigen batch is unique because the

final concentration of the antigenic composition would vary from batch to batch. 100 µl of antigen per well was added. One hundred microlitres of PBS only were added to each well in the other half of the plate. After tapping gently, the plates were stored in moist chamber at 4°C for 36 – 48 hours.

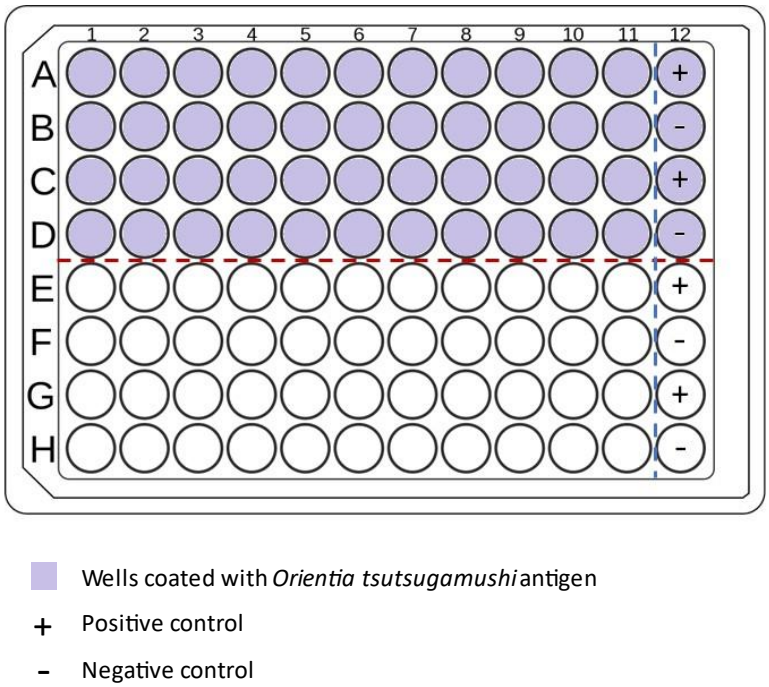


Figure 19. The 96-well plate diagram

Modified from: Nessa Carson, CC0 1.0, via Wikimedia Commons [246]

We first prepared the wash buffer 0.1% Tween 20 in 10X PBS (PBST). Then, we prepared the blocking solution with 5% skim milk in PBST. After the liquid in the plates was aspirated, we pipetted 200 µL of blocking solution into each well and incubated the plates in moist chamber at room temperature for one hour. Then, the plates were washed three times.

We diluted 10 µl of participant sera in blocking buffer (5% skim milk in PBST) in deep-well plate to make 1:100 dilution. We put 100 µl of diluted participant sera in one well with and one well without, antigen coating. We used 100 µl of 5% skim milk as negative control. Previously characterised participant sera were used as positive controls. The negative control was added first, then positive control in 1:100 dilution was added as shown in Figure 19. After tapping gently to mix, the plates were then incubated for one hour in moist chamber at room temperature and washed four times.

Next, we prepared the horseradish peroxidase (HRP) conjugated anti-human IgM and IgG. Goat anti-human IgG (gamma chain) cross-adsorbed secondary antibody, HRP (catalogue number 62-8420) and goat anti-human IgM secondary antibody, HRP (catalogue number 31415) from ThermoFisher Scientific were used. For IgM and IgG conjugated antibody, we used 1:8,000 and 1:1,000 dilution, respectively. We added 100 µl conjugated antibody in each well, then incubated the plates for one hour in a moist chamber at room temperature and washed four times.

Room temperature tetramethylbenzidine (TMB) substrate was added (100 µl per well). TMB chromogen solution (for ELISA) (catalogue number 002023) produced by ThermoFisher Scientific was used. The plates were incubated for 15 minutes in the dark at room temperature and then 100 µl 1 M HCl stop solution was added into each well.

The plates were read at 450 nm and 620 nm. The resulting optical density readings at 450 nm were subtracted from the readings at the reference value of 620 nm. Then, we subtracted the optical density of wells coated with antigen with wells without antigen to subtract background/non-specific absorbance.

Data analysis

The data were presented in graphs, tables, and text. Continuous variables were summarised in medians (Q1 – Q3). Categorical variables were summarised in proportion and frequencies. The association between categorical and continuous variables with the scrub typhus seropositivity was analysed with Chi square and logistic regression, respectively. In this thesis, p-value of less than 0.05 was considered statistically significant. Analysis was done with Microsoft Excel 2019, R version 3.6.2, R Studio Version 1.2.5033, and StataBE 17. The .do file and R script containing the codes and commands used in this study can be viewed in Supplement 4 and Supplement 5. The reporting of this study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement.

Cut-off selection

We initially planned to collect 'presumed unexposed' and 'negative control' samples from an urban population. However, due to the Covid-19 pandemic, we could not gather these samples. Therefore, we could not use the resulting optical density values of this population as the reference for determining cut-offs. We could not construct the receiver operating characteristic (ROC) curve to determine cut-offs as the samples were not tested with the gold standard i.e. IFA.

Various scrub typhus ELISA cut-offs have been used in previous studies [147]. These geographical variations were caused by the differences in prevalence and pathogen characteristics of each particular setting. There was no consensus on scrub typhus cut-offs for Indonesia. One study including samples from Denpasar used commercial ELISA kit (InBios); however, this article did not mention the cut-offs used [83].

When there are no negative control sera, there are several approaches that can be used to determine the cut-off [243], including visual inflection point, change point analysis, and finite mixture modelling. In this section, I explain the concepts of each method as well as their limitations. In this study, I used finite mixture modelling to determine the IgM and IgG cut-off since this is deemed as the more objective method compared to visual inflection point and change point analysis. I also performed the two other methods (visual inflection point and change point analysis) as comparisons to the finite mixture models, to see if there is agreement on the resulting cut-offs.

Visual inflection point

First, the optical density is sorted from the lowest to the highest. Then the point where the curvature of the graph is changing is identified visually by eyeballing [243, 247]. This point divided the observations into samples with lower and higher optical density. The optical density should not increase regularly among the seronegative and seropositive samples. There should be a change point where there is a 'step' that differentiates these two groups.

Although quite 'intuitive' and relatively easy to apply, this method suffers from lack of reproducibility since it is subjective and individuals asked may be biased due to their background or prior knowledge [243]. In addition, if the differences between the negative and positive samples are not pronounced or clear, identifying the point by eyeballing can be difficult.

To determine the inflection point visually, I purposively selected ten people with a range of backgrounds – including clinical medicine, biomedical sciences, public health, statistics; and range of career levels – including PhD student, post-doctoral researcher, clinical resident. The respondents were asked to eyeball line graphs of sorted IgM and IgG optical density values (Figure 24 and Figure 25) and identify the inflection point and the corresponding optical density value of that point. The inflection point was defined as the point where the curve changed from 'predominantly horizontal to predominantly vertical' [247]. Then I gathered all values identified and calculated the mean. The mean of these values would be the cut-off.

Change point analysis

A change point is a point where there is a sudden change in the statistical characteristics in the observed sequence [248, 249]. This analysis therefore can be used as a proxy to identify the cut-off [248]. The concept is similar to identifying the visual inflection point. When the optical density values were sorted from lowest to highest, the change point should be able to differentiate between negative and positive samples. The change point would represent the 'step' in the visual inflection point method, when there is an abrupt change in statistical characteristics of the sequence.

We used a Bayesian Poisson model using random-walk Metropolis-Hastings sampling to identify the change point in Stata using the *bayesmh* command [250].

We fitted the following model for IgM:

$$igm \sim Poisson(\mu 1), if\ number < cp$$

$$igm \sim Poisson(\mu 2), if\ number \geq cp$$

The parameter 'igm' being the IgM optical density values and 'number' being the order number corresponding to each value in the sorted sequence of IgM optical density values. 'µ1' and 'µ2' are the mean of the estimated negative and positive subpopulation. The parameter of interest is 'cp' i.e. the change point.

For IgG, we fitted the following model:

$$igg \sim Poisson(\mu 1), if\ numberigg < cp$$

$$igg \sim Poisson(\mu 2), if\ numberigg \geq cp$$

The parameter 'igg' being the IgG optical density values and 'numberigg' being the order number corresponding to each value in the sorted sequence of IgG optical density values. 'µ1' and 'µ2' are the mean of the estimated negative and positive subpopulation. The parameter of interest is 'cp' i.e. the change point.

This method is more objective and reproducible than identifying the inflection point visually. However, there is still a degree of subjectivity since the prior is something we defined by

ourselves. There are ways to ensure prior selection is appropriate, such as using information from previous studies, integrating expert opinions, or using the results of visual inflection point method. In this chapter, I inputted the results of visual inflection point method as the prior. I used flat priors for the means. For the change point, I used a uniform prior on the 'number' and 'numberigg' value corresponding to the mean minus one standard deviation and mean plus one standard deviation (mean - 1 SD and mean + 1 SD) of the resulting visual inflection point cut-offs. For the initial 'cp' parameter value I used the mean from visual inflection point results. We modelled the mean of the distribution as a mix of μ1 and μ2 [250].

Finite mixture model

Populations often consist of two or more subpopulations [243, 251]. These subpopulations or groups are defined by certain characteristics such as age brackets, sex, economic status, or education levels [251]. However, there are cases when the identifying variable is missing, difficult to collect, or unobservable [251, 252]. In this case, we can use finite mixture modelling to differentiate groups within a population. Finite mixture models can, in a population with known or suspected two or more subpopulations, model the probability of each observation fitting in each group [251, 252]. It can classify samples based on disease status when clinical measurements or laboratory parameter values are available, but 'individual class memberships are not available' or too difficult or expensive to gather [252]. This method may not be suitable to be used in populations with the same level of exposure e.g. when all individuals within the population is exposed [243].

In the context of our study, this approach would be able to categorise the data into two groups: the seronegative and seropositive group. The seronegative group would have lower optical density values [243]. Thus, if there are two groups with normal distribution, the lower distribution would belong to the seronegative population. The finite mixture models estimate the means and variances of subgroups (negative or positive in this case). I assumed that the mean of the lower class is essentially representing the mean for the negative controls (with some uncertainty of course). Therefore, I got the mean and variance from the FMMs for the lower class and calculated the standard deviation from that variance. I used the mean plus three standard deviations (mean + 3SD) of the lower distribution as the cut-off [243].

The models were done in Stata using the *fmm* command [251]. We fitted a mixture of two linear regression models for the optical density (continuous variable) for the two serologic statuses i.e. seronegative and seropositive groups.

In order to demonstrate where the cut-off is based on the FMMs, I superimposed the line of cut-off on the distributions by using simulations to create the two distributions. The parameters for the simulations were obtained from the lower class of the resulting FMMs. These are the same parameters that are used to determine the cut-off.

Simulations were performed to generate the distributions of the lower class (seronegative subpopulation) and upper class (seropositive subpopulation) values for IgM and IgG based on the FMMs. A normal distribution was used to simulate the data in Stata. The means and

standard deviations that were used for simulations were taken from the FMMs outcomes. The overlapping tails of the seronegative and seropositive distributions were considered to belong to both distributions. The overlapping histograms were plotted and a line of cut-off from the FMMs was superimposed to visually show the negative and positive distribution and where the cut-off divide the data. The mean plus 3 SD formula was used to determine the cut-off, where the mean is the sample mean for the negative group from the FMMs. This sample mean is assumed to represent the mean for the negative controls.

Results

There were two samples with missing age data; these samples were not included in the data analysis. Therefore, 1,033 samples were included in the data analysis: 350 from Jambi, 415 from Denpasar, and 268 from Tabanan (Table 9). Accounting for all variables, there were 40 participants with incomplete observations. The complete dataset can be viewed in Supplement 3.

Participants' characteristics

More than half (n = 538/1,033, 52.1%) of the included samples were from male participants (Table 9). Most of the participants were in the 16–30 and 31–45 age bracket, both with 325 (31.5%) participants. Over 9 in 10 participants (n = 990, 95.8%) had history of fever. Over half of participants also reported malaise (n = 706, 68.3%), nausea and/or vomiting (n = 695, 67.3%), and headache (n = 635, 61.5%). The median of haemoglobin, haematocrit, and leukocyte value were within normal range. The median platelet level was low.

Table 9. Participants' characteristics

Characteristics	Jambi	Denpasar	Tabanan	Overall
Sex				
Male – n (%)	177 (50.57%)	207 (49.88%)	154 (57.46%)	538 (52.08%)
Female – n (%)	173 (49.43%)	208 (50.12%)	114 (42.54%)	495 (47.92%)
Age				
0–5 – n (%)	53 (15.14%)	66 (15.90%)	43 (16.04%)	162 (15.68%)
6–15 – n (%)	72 (20.57%)	81 (19.52%)	48 (17.91%)	201 (19.46%)
16–30 – n (%)	115 (32.86%)	135 (32.53%)	75 (27.99%)	325 (31.46%)
31–45 – n (%)	76 (21.71%)	76 (18.31%)	44 (16.42%)	325 (31.46%)
46–60 – n (%)	27 (7.71%)	39 (9.40%)	43 (16.04%)	109 (10.55%)
>60 – n (%)	7 (2.00%)	18 (4.34%)	15 (5.60%)	40 (3.87%)
Clinical manifestations				
Fever – n (%)	332 (94.86%)	402 (96.87%)	256 (95.52%)	990 (95.84%)
Malaise – n (%)	300 (85.71%)	304 (73.25%)	102 (38.06%)	706 (68.34%)
Nausea and/or vomiting – n (%)	239 (68.29%)	309 (74.46%)	147 (54.85%)	695 (67.28%)
Headache – n (%)	248 (7086%)	227 (54.70%)	160 (59.70%)	635 (61.47%)
Loss of appetite – n (%)	138 (39.43%)	250 (60.24%)	74 (27.61%)	462 (44.72%)
Myalgia – n (%)	125 (35.17%)	127 (30.60%)	82 (30.60%)	334 (32.33%)
Abdominal pain – n (%)	187 (53.41%)	40 (9.64%)	21 (7.84%)	248 (24.01%)
Arthralgia – n (%)	41 (11.71%)	132 (31.81%)	58 (21.64%)	231 (22.36%)
Retroorbital pain – n (%)	15 (4.29%)	49 (11.81%)	31 (11.57%)	95 (9.20%)
Rash – n (%)	47 (13.43%)	27 (6.51%)	9 (3.36%)	83 (8.03%)
Bleeding – n (%)	2 (0.57%)	42 (10.12%)	6 (2.24%)	50 (4.84%)
Dyspnoea – n (%)	6 (1.71%)	17 (4.10%)	2 (0.75%)	25 (2.42%)
Altered consciousness – n (%)	1 (0.29%)	0 (0.00%)	1 (0.37%)	2 (0.19%)
Laboratory parameters				
Haemoglobin – median (Q1 – Q3)	13 (11.90 – 14.10)	13.1 (11.80 – 14.10)	14.3 (13.11 – 15.70)	13.3 (12.10 – 14.68)
Haematocrit – median (Q1 – Q3)	38 (34.90 – 40.70)	37.9 (34.30 – 41.60)	42.8 (39.35 – 47.45)	43.8 (37.8 – 184.00)
Platelet – median (Q1 – Q3)	228 (181.75 – 281.00)	163 (118.50 – 239.00)	189 (154.00 – 230.50)	127 (40.08 – 199.00)
Leukocyte – median (Q1 – Q3)	7.67 (5.28 – 10.47)	5.32 (3.60 – 7.08)	6.16 (4.68 – 7.82)	6.25 (4.39 – 8.41)
Total	350	415	268	1033

Optical density values distribution

IgM

The IgM value ranged from -1.07 to 0.97, with mean (SD) of 0.18 (0.17) and median (Q1 – Q3) of 0.16 (0.07 – 0.27). The histogram of the distribution of IgM values (Figure 20) looked close to normal distribution. I sorted the IgM values in ascending order from the lowest to the highest in Figure 21.

IgG

The IgG value ranged from -0.89 to 1.09, with mean (SD) of 0.05 (0.08) and median (Q1 – Q3) of 0.03 (0.02 – 0.06). The histogram of the distribution of IgG values (Figure 22) showed close to normal distribution. I sorted the IgM values in ascending order from the lowest to the highest in Figure 23.

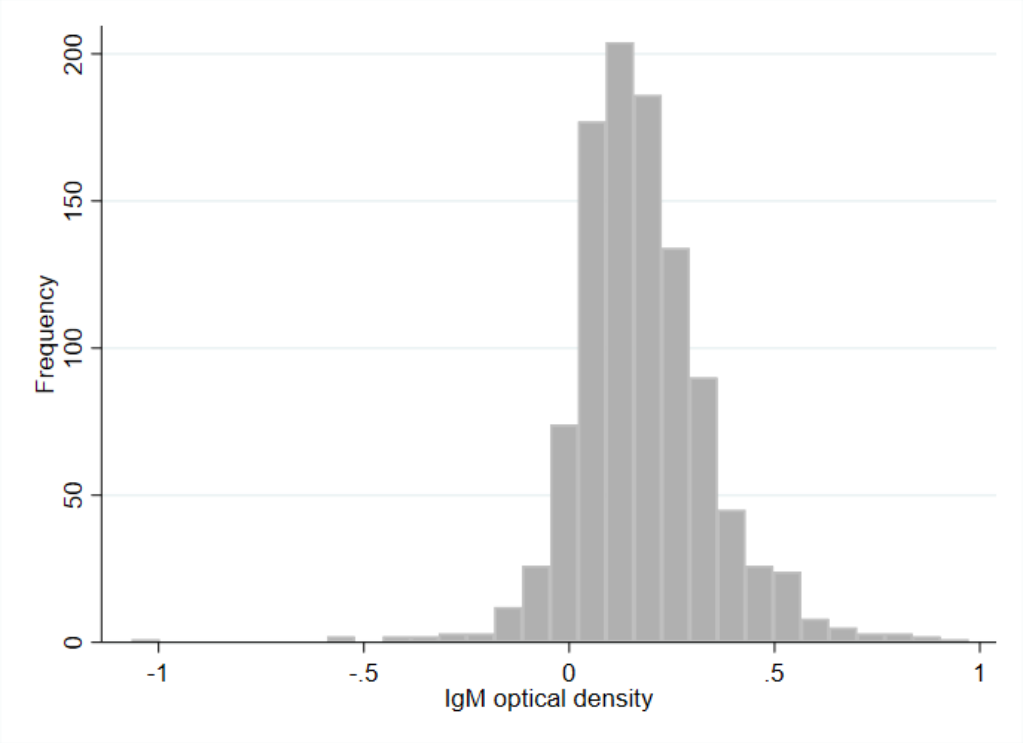


Figure 20. The distribution of IgM optical density values

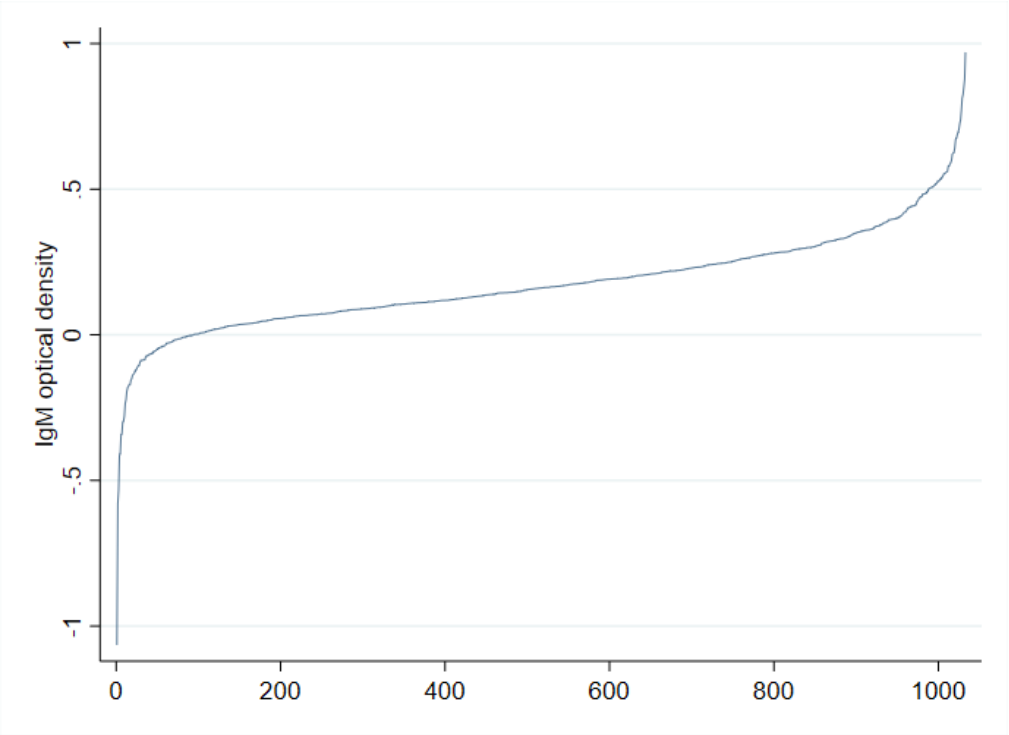


Figure 21. Sorted IgM optical density values

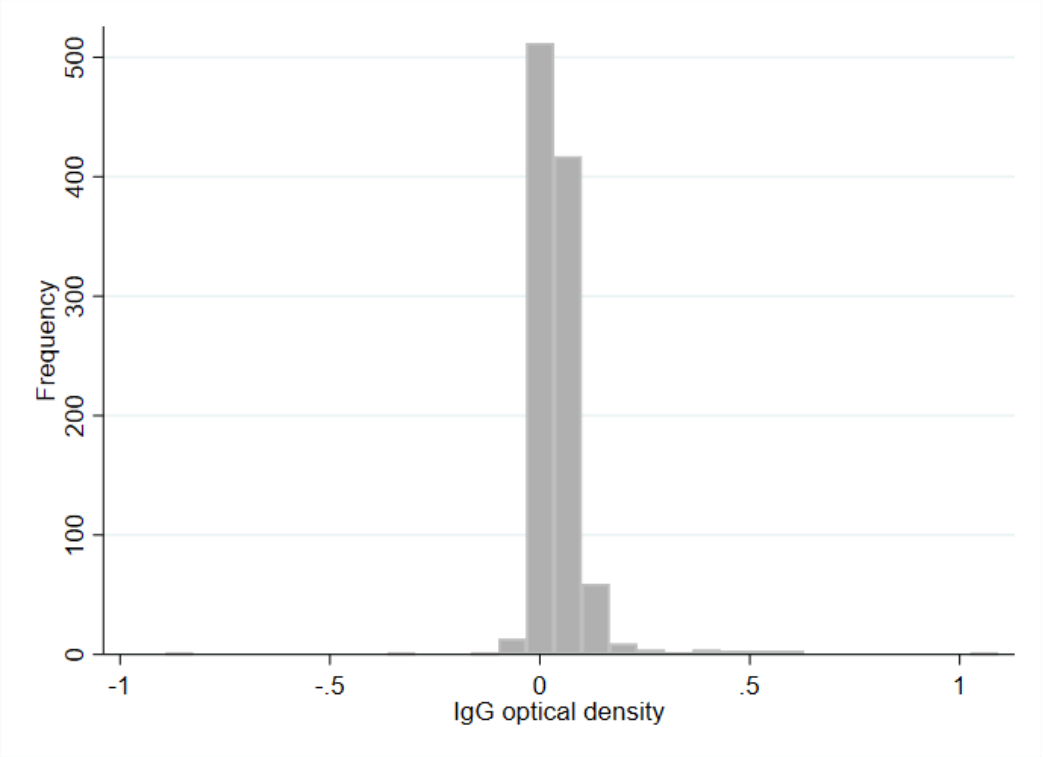


Figure 22. The distribution of IgG optical density values

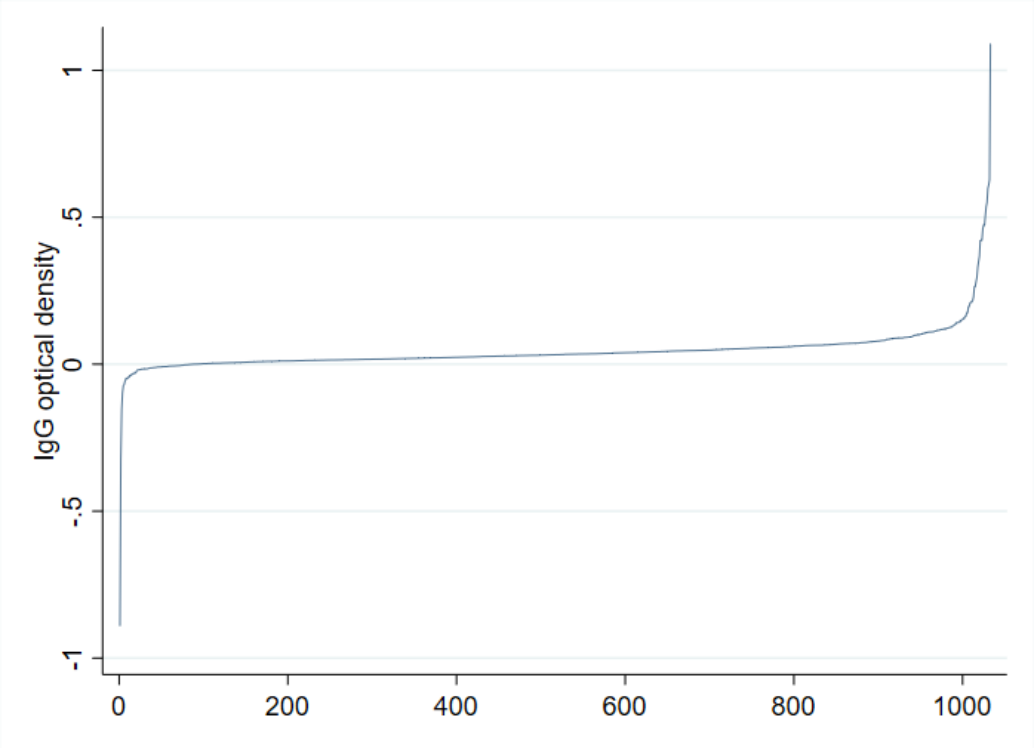


Figure 23. Sorted IgG optical density values

Cut-off selection

Visual inflection point

I asked ten people from varying background and career level to identify the inflection point visually in the graphs of sorted IgM and IgG values (Figure 24 and Figure 25). I explained that the inflection point was the point where the curve changed from 'predominantly horizontal to predominantly vertical' [247].

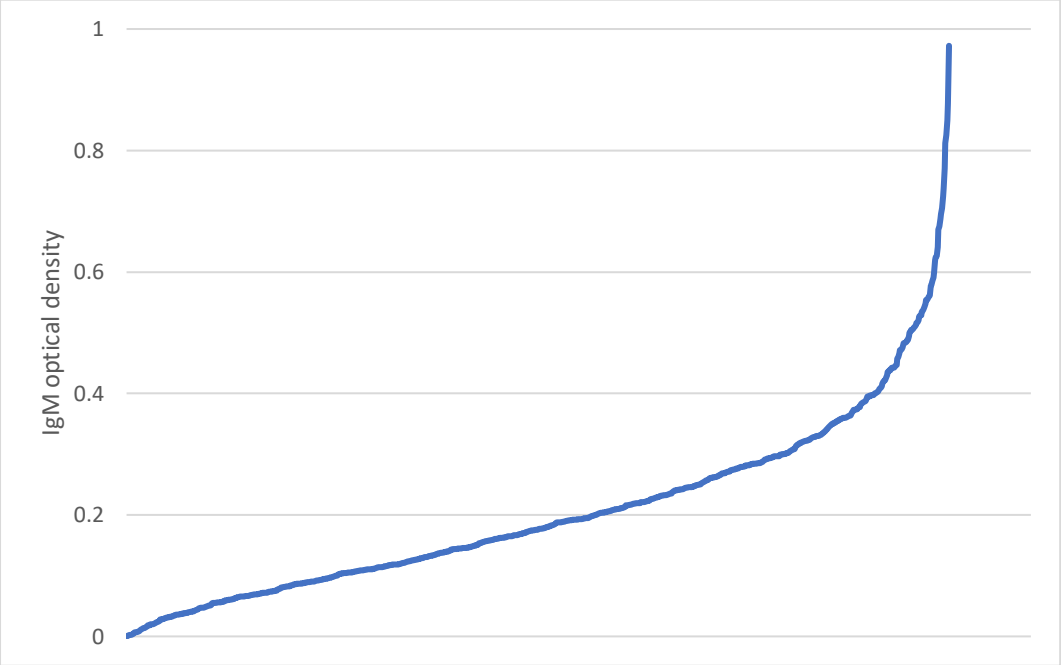


Figure 24. Sorted positive IgM optical density

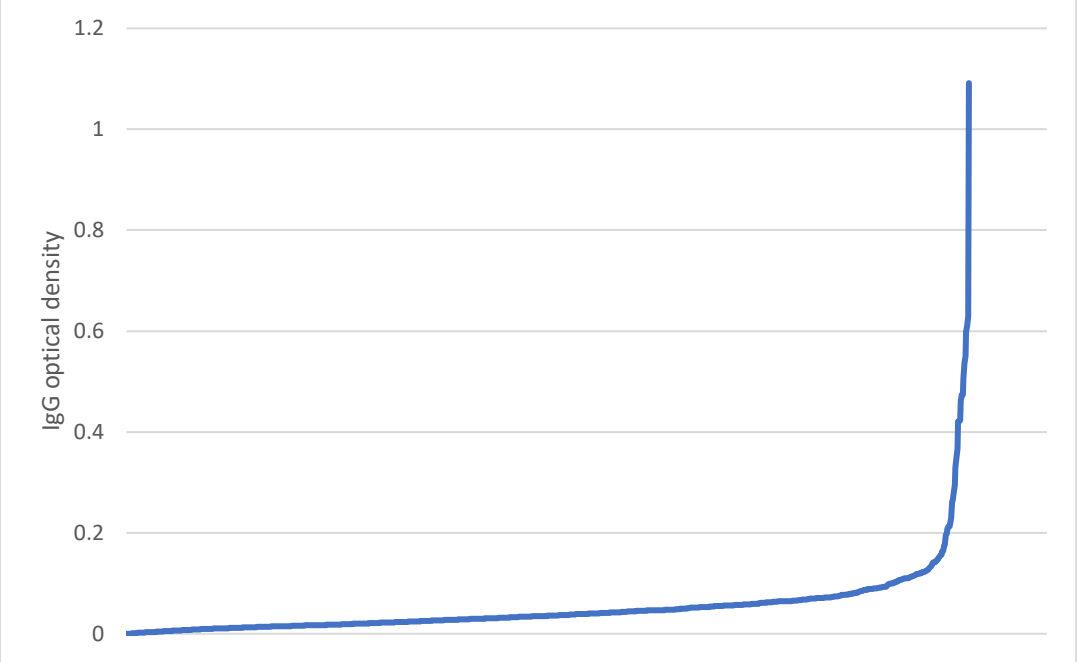


Figure 25. Sorted positive IgG optical density

Table 10 showed the visual inflection points identified by the respondents. The mean of IgM and IgG values selected was 0.47 and 0.18, respectively.

Table 10. The visual inflection points identified by the respondents

Respondent	IgM OD cut-off	IgG OD cut-off
1	0.45	0.18
2	0.40	0.20
3	0.57	0.17
4	0.30	0.10
5	0.47	0.18
6	0.50	0.15
7	0.55	0.18
8	0.50	0.30
9	0.60	0.20
10	0.40	0.18
Mean	0.47	0.18
SD	0.09	0.05

Change point analysis

I incorporated the cut-off values (mean (SD)) calculated through the visual inflection point method as priors in the change point analysis i.e. 0.47 (0.09) for IgM and 0.18 (0.05) for IgG (Table 10). The 0.47 optical density value corresponded with the 976th observation in the sorted IgM values i.e. 0.4658 ≈ 0.47 (Figure 26). The 0.18 optical density value corresponded with the 1006th observation in the sorted IgG values i.e. 0.176 ≈ 0.18 (Figure 27). 976 for IgM and 1006 for IgG was inputted as the initial 'cp' parameter. For the change point, a uniform prior on [929, 1011] and [986, 1013] for IgM and IgG, respectively, was used. The (mean - 1 SD, mean + 1 SD) for IgM cut-off value were (0.38 and 0.56) – this corresponded with 'number' parameter (929, 1011). The (mean - 1 SD, mean + 1 SD) for IgG cut-off value were (0.13 and 0.23) – this corresponded with 'numberigg' parameter (986, 1013). The commands used can be viewed in Supplement 4.

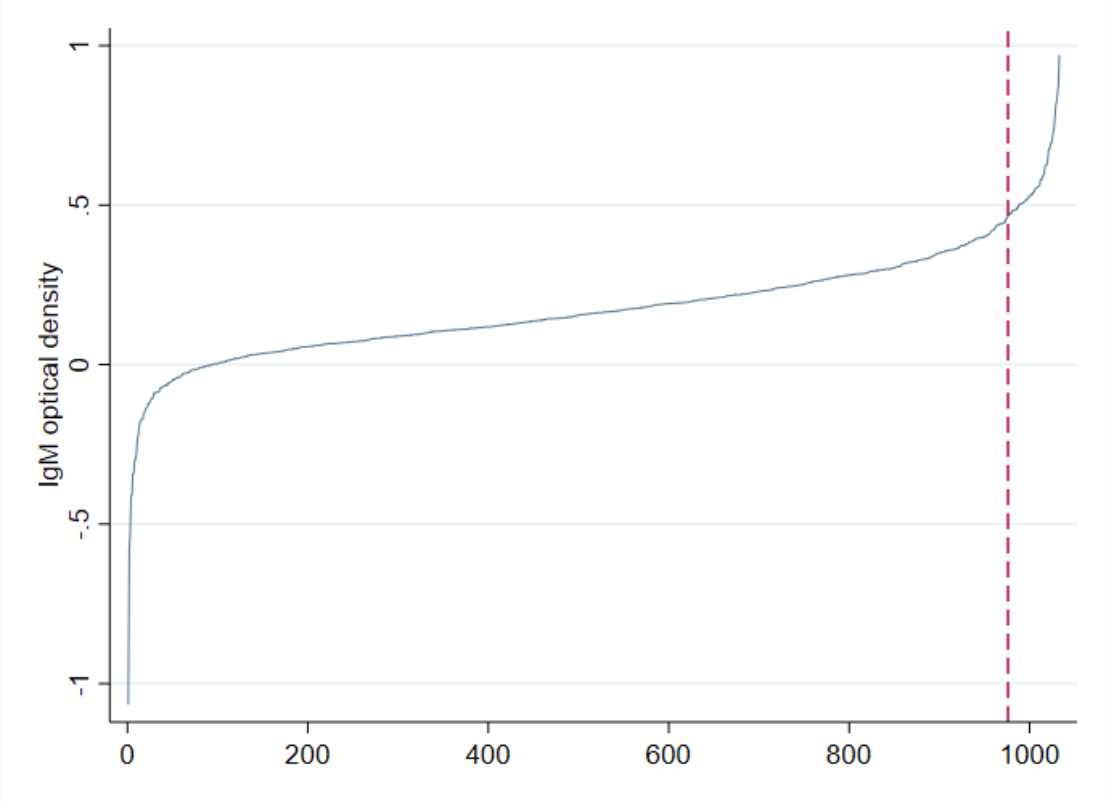


Figure 26. Line graph of sorted IgM optical density values

The red dash line represents the 976th observation.

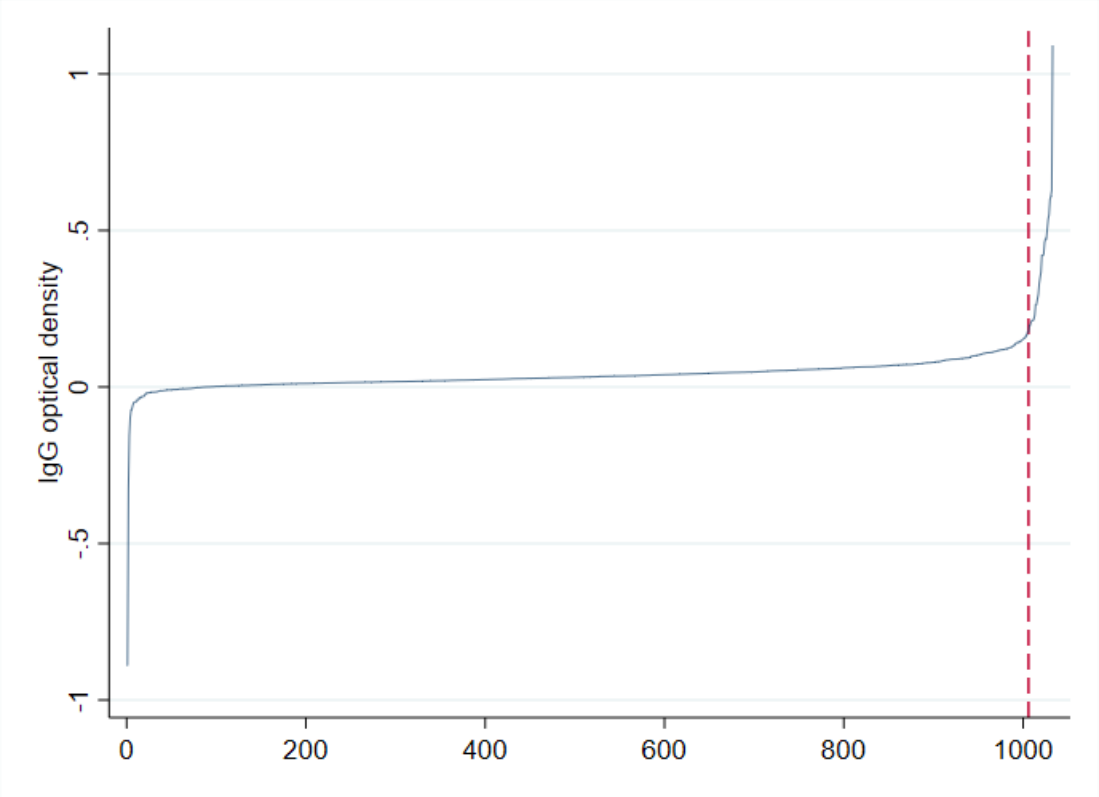


Figure 27. Line graph of sorted IgM optical density values

The red dash line represents the 1006th observation.

The mean change point for IgM cut-off was 978.89 with 95% credible interval of 932.35 – 1010.03. The optical density value corresponding to the 978th observation is 0.4721 ≈ 0.47 (Figure 28).

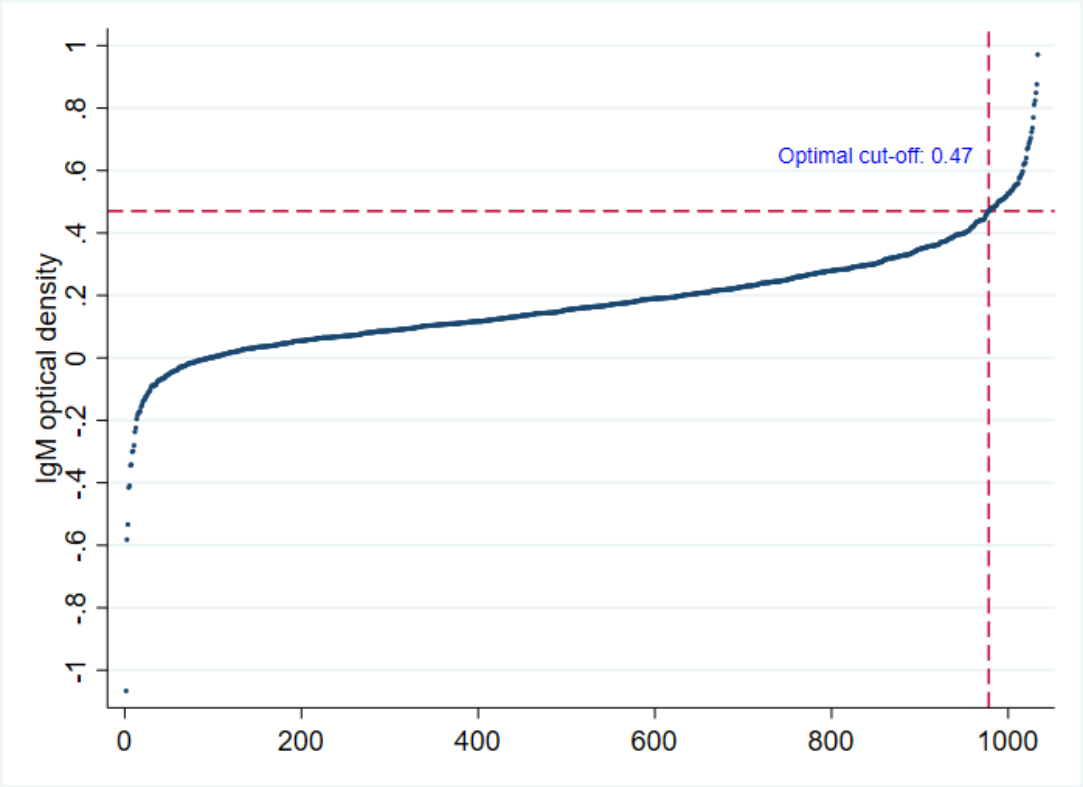


Figure 28. The sorted IgM optical density values with the resulting change point cut-off

The vertical red dash line represented the 978th observation and the horizontal red dash line represents the 0.47 optical density value (optimal cut-off).

Figure 29 showed how the change point cut-off divided the data into seronegative and seropositive samples.

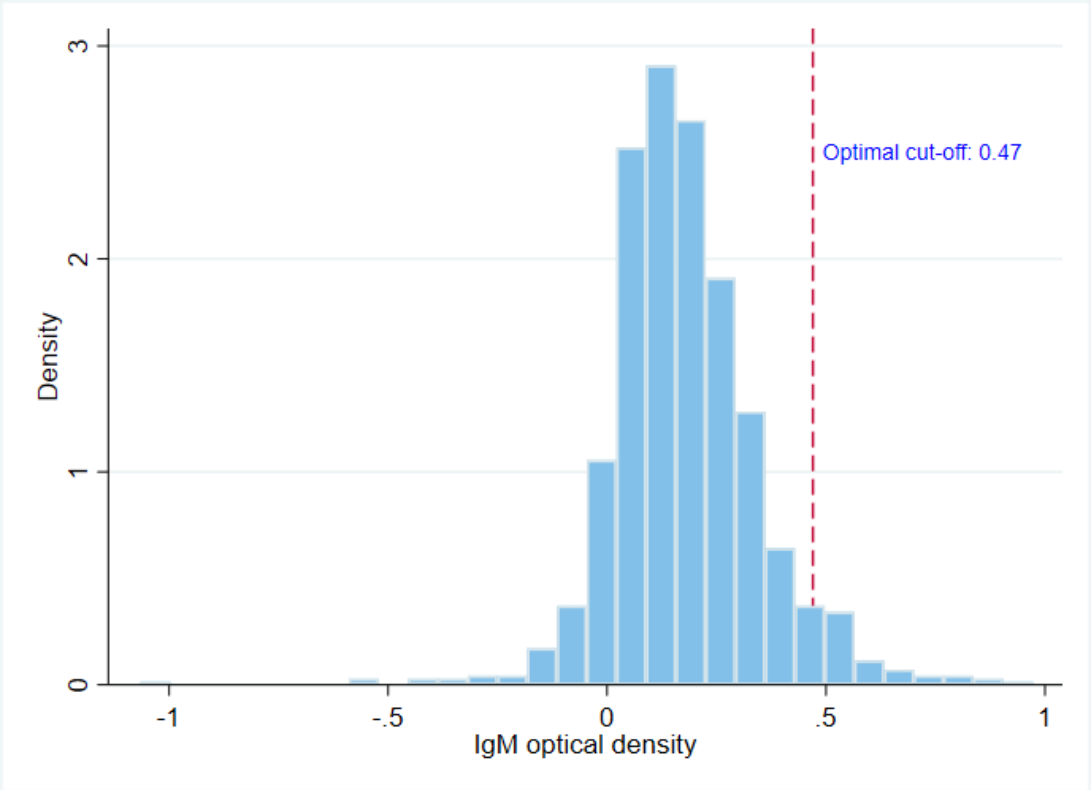


Figure 29. The histogram of IgM optical density distribution and resulting change point cut-off

The mean change point for IgG cut-off was 1012.99 with 95% credible interval of 934.34 – 1032.81. The optical density value corresponding to the 1012th observation is 0.2167 ≈ 0.22 (Figure 30).

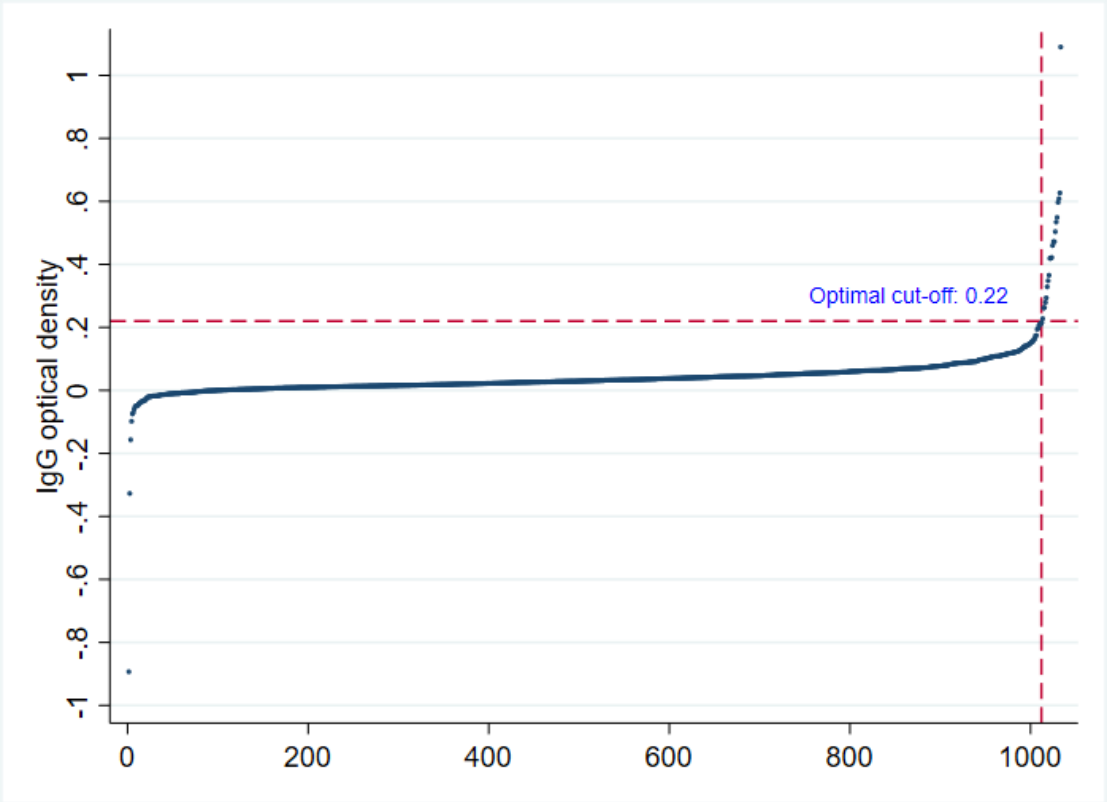


Figure 30. The sorted IgG optical density values with the resulting change point cut-off

Figure 31 showed how the change point cut-off divided the data into seronegative and seropositive samples.

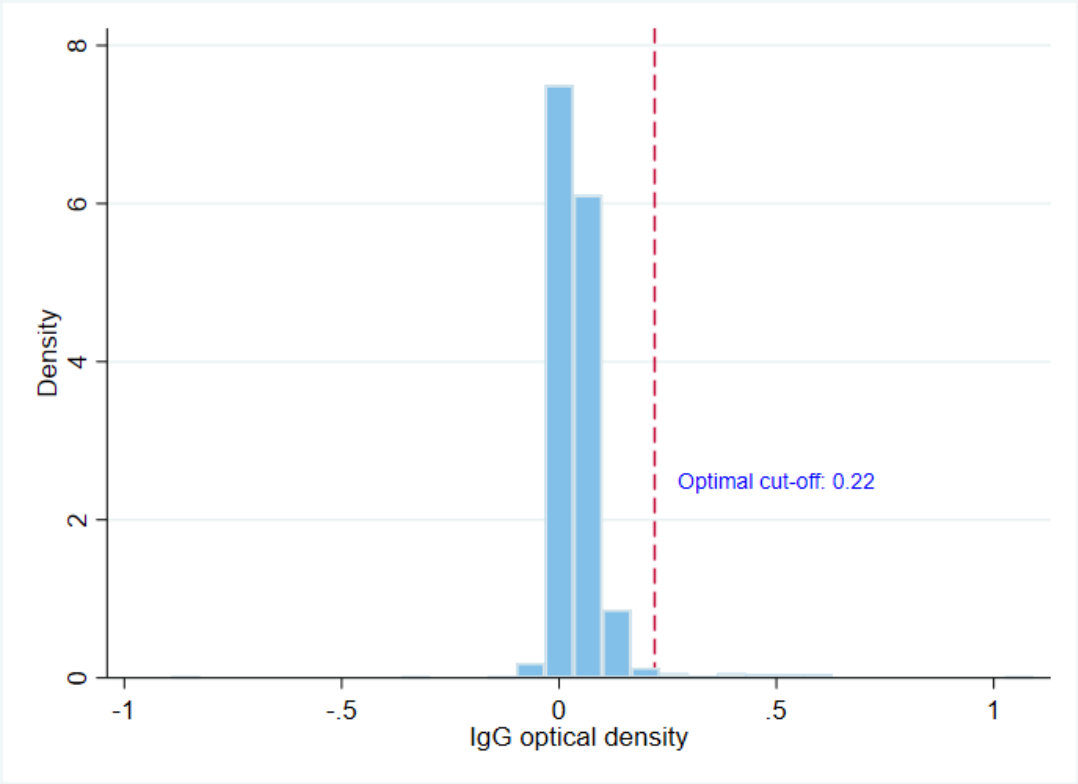


Figure 31. The histogram of IgG optical density distribution and resulting change point cut-off

I also ran an analysis using a more 'non-informative' priors, that is inputting a wider possible range for the cut-offs. I used [200, 1033] and [400, 1025] for the IgM and IgG, respectively. The mean change point for IgM cut-off was 996.46 with 95% credible interval of 873.55 – 1032.71. The optical density value corresponding to the 996th observation is 0.5163 ≈ 0.52. The mean change point for IgG cut-off was 1008.60 with 95% credible interval of 947.57 – 1024.77. The optical density value corresponding to the 1,008th observation is 0.1985 ≈ 0.20.

Finite mixture models

Table 11 showed the outcome of the finite mixture models of IgM optical density values. The mean of the lower class i.e. modelled seronegative subpopulation was 0.15 (95% CI 0.14 to 0.17). The cut-off for IgM was 0.4891 ≈ 0.49. I plotted a histogram of the collected original IgM data and where the resulting cut-off divide the distribution (Figure 32). I also simulated the IgM data based on the outcome of FMMs parameters to visualise the seronegative and seropositive distribution (Figure 33). The side-by-side comparison of the original and simulated data can be viewed in Figure 34.

Table 11. Finite mixture models outcome for IgM data

IgM	**Mean**	**95% confidence interval**		**Variance**
		Lower limit	**Upper limit**	
Lower class	0.15	0.14	0.17	0.01
Upper class	0.25	0.21	0.29	0.08

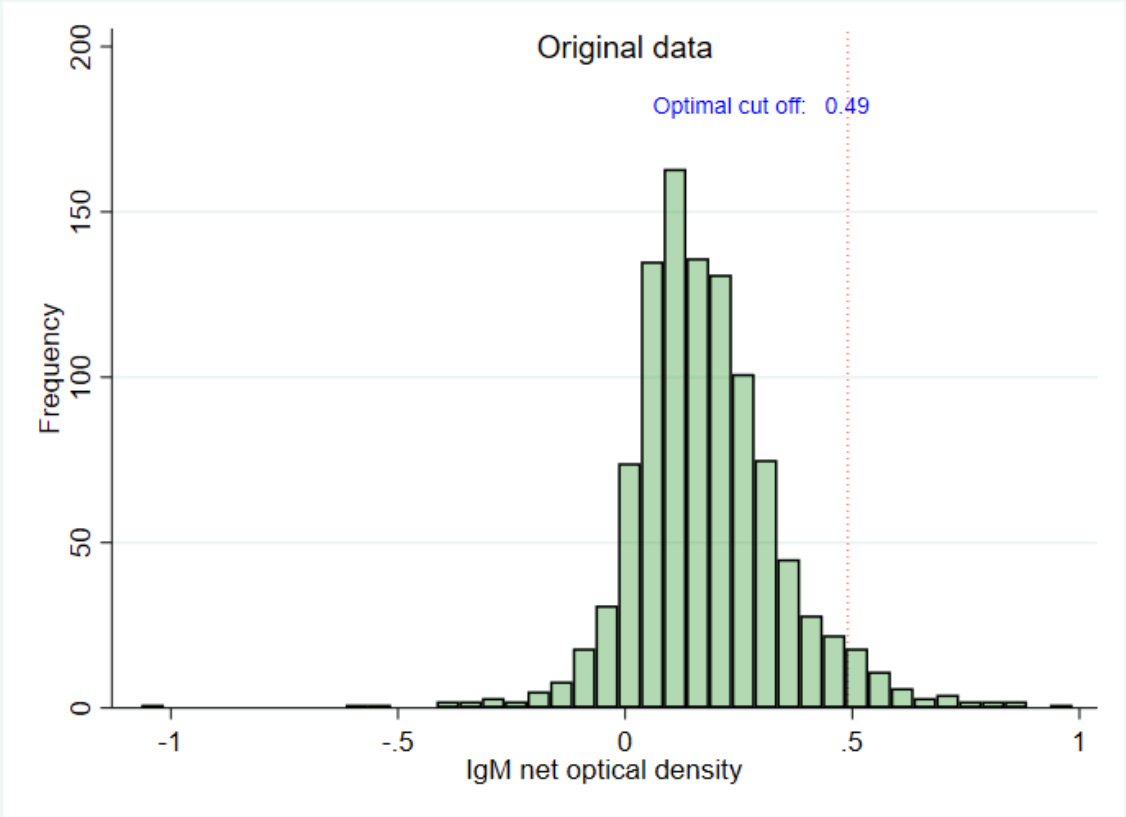


Figure 32. The histogram of original IgM data

The red dotted line represented the 0.49 cut-off.

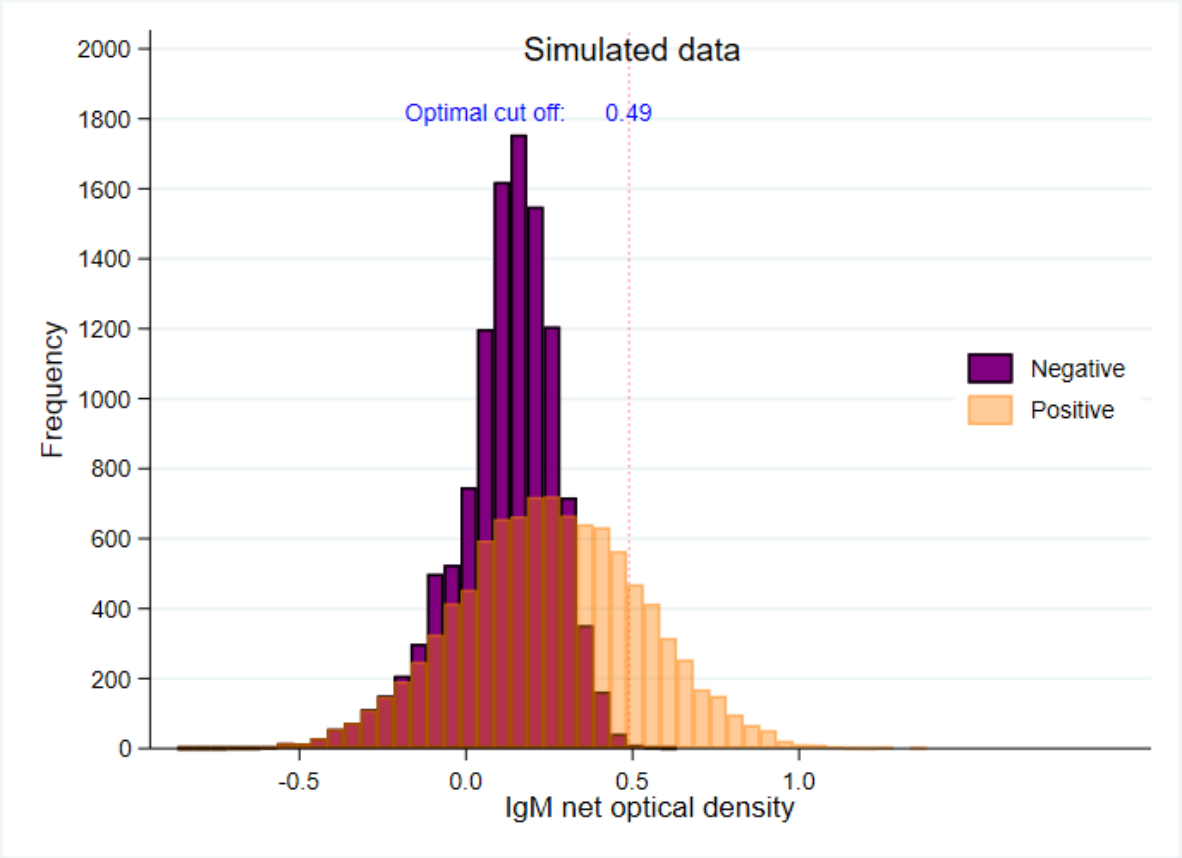


Figure 33. The histogram of simulated IgM values

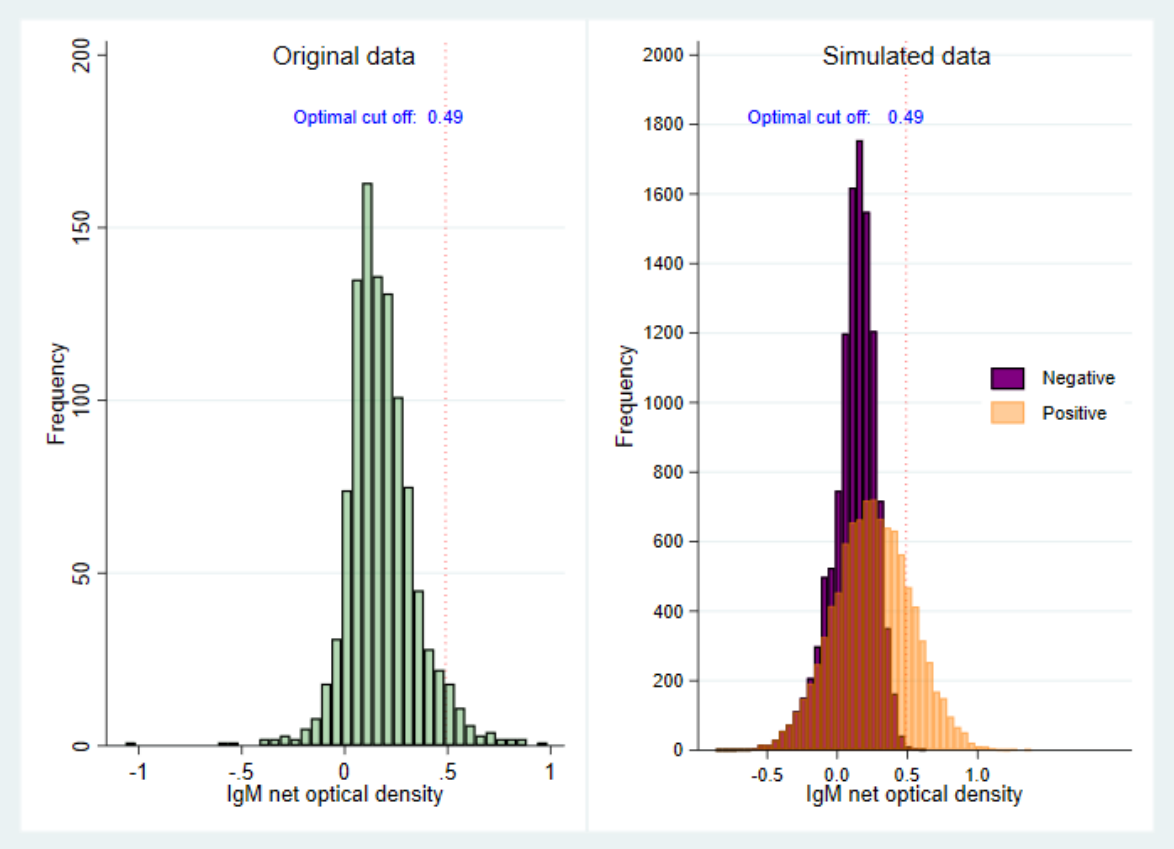


Figure 34. Comparison of original and simulated IgM data

Table 12 showed the outcome of the finite mixture models of IgG optical density values. The mean of the lower class i.e. modelled seronegative subpopulation was 0.03 (95% CI 0.03 to 0.04). The cut-off for IgG was 0.1257 ≈ 0.13. I plotted a histogram of the collected original IgG data and where the resulting cut-off divide the distribution (Figure 35). I also simulated the IgG data based on the outcome of FMMs parameters to visualise the seronegative and seropositive distribution (Figure 36). The simulated data distribution looked similar with the original data, suggesting that the resulting outcome parameters of the FMMs are appropriate. The side-by-side comparison of the original and simulated data can be viewed in Figure 37.

Table 12. Finite mixture models outcome for IgG data

IgG	Mean	95% confidence interval		Variance
		Lower limit	Upper limit	
Lower class	0.035	0.03	0.04	0.001
Upper class	0.19	0.12	0.25	0.06

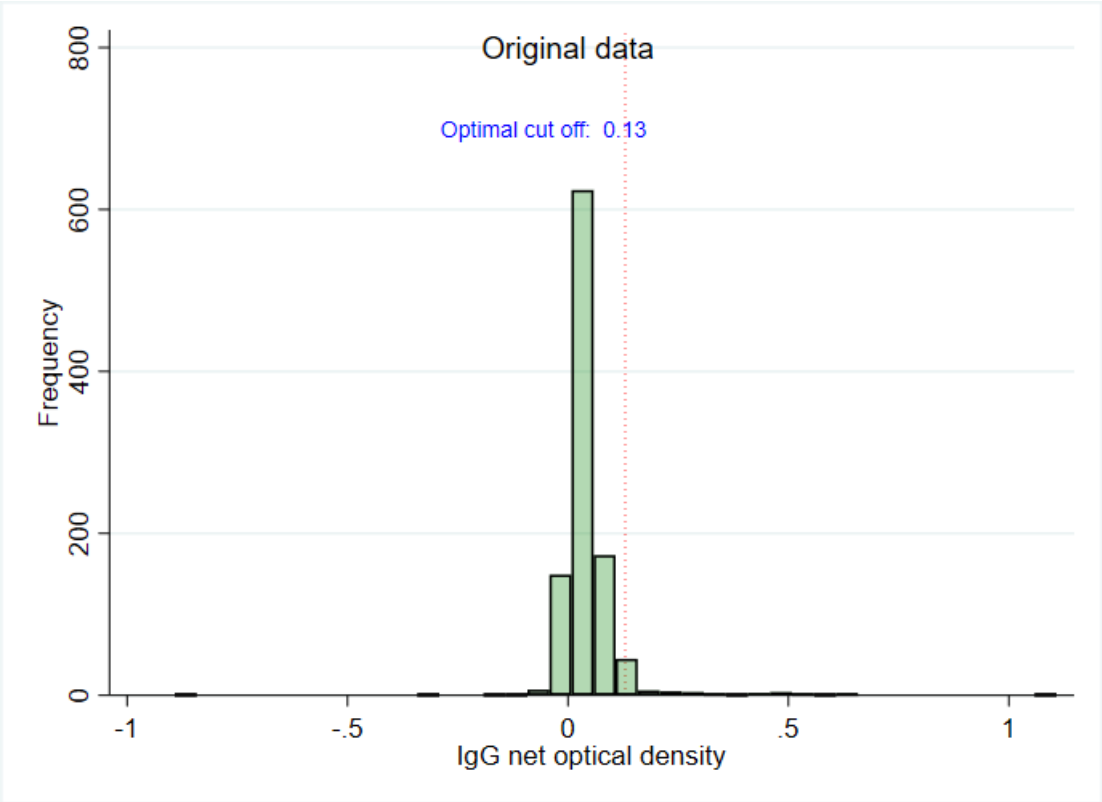


Figure 35. The histogram of original IgG data

The red dotted line represented the 0.13 cut-off.

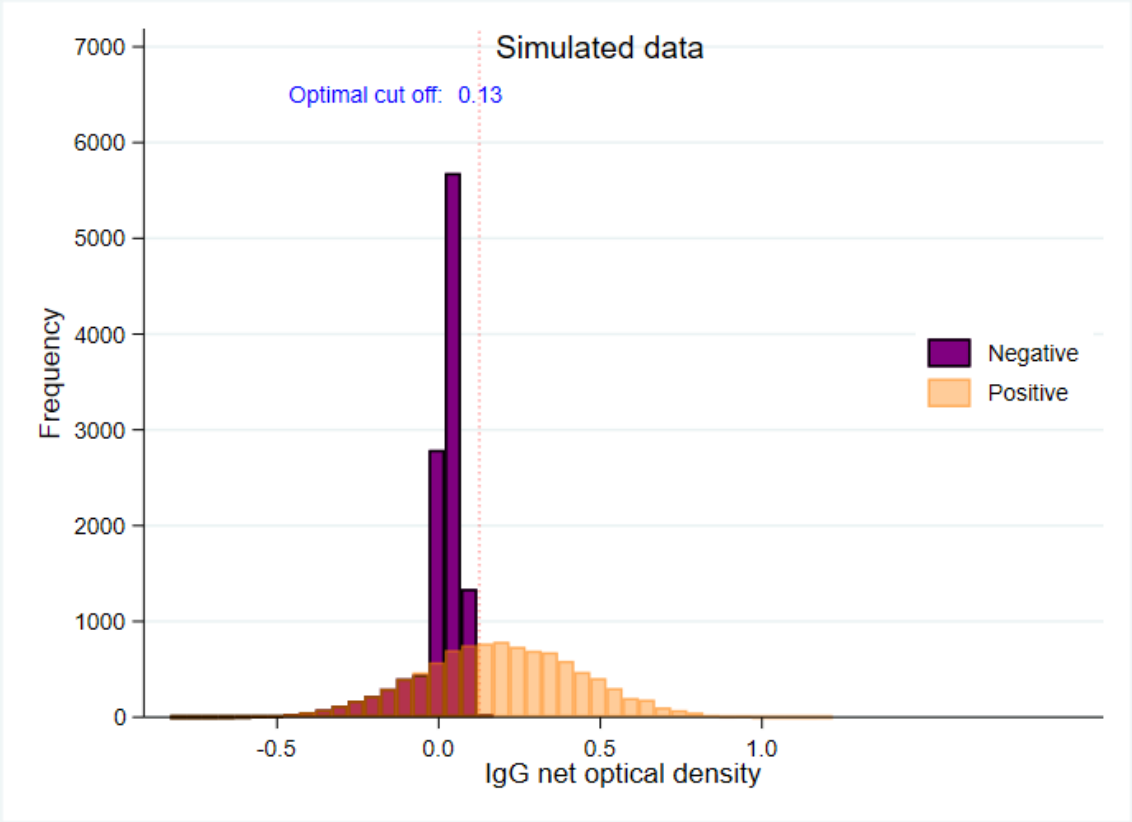


Figure 36. The histogram of simulated IgG values

The red dotted line represented the 0.13 cut-off.

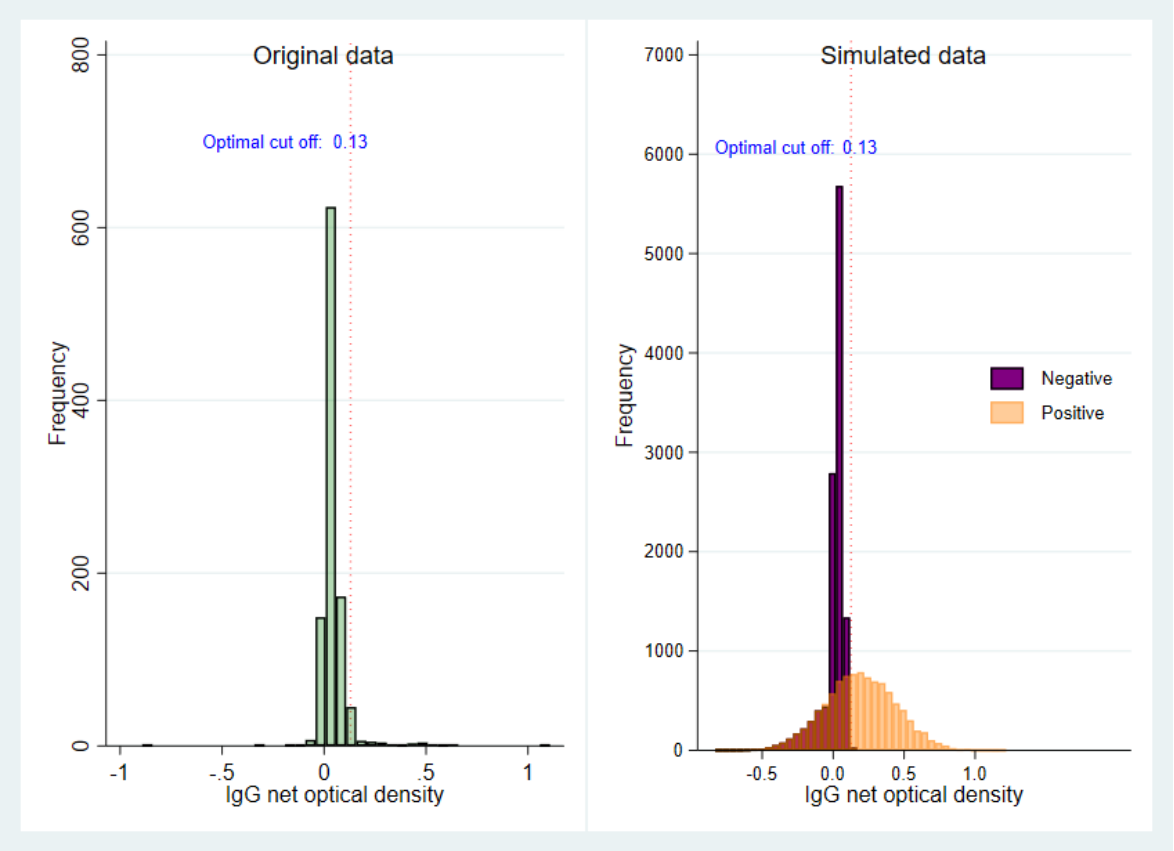


Figure 37. Comparison of original and simulated IgG data

For IgM, the cut-off from FMMs was the highest. The difference with the other methods was 0.02 (Table 13). The proportion of IgM seropositive samples was therefore lowest when this cut-off was used. For IgG, the cut-off from FMMs was the lowest. The differences between IgG cut-off calculated using FMMs and other methods were less than 0.1. Consequently, the proportion of seropositive samples for IgG was the highest when using the FMMs cut-off as well. Overall seropositivity was also highest when the FMMs cut-offs were applied.

Table 13. The comparison of cut-offs from three methods

	Visual inflection point	**Change point analysis**	**Finite mixture models**
IgM cut-off	0.47	0.47	0.49
IgG cut-off	0.18	0.22	0.13
IgM seropositivity	57 (5.52%)	57 (5.52%)	47 (4.55%)
IgG seropositivity	27 (2.61%)	21 (2.03%)	45 (4.36%)
Overall seropositivity*	83 (8.03%)	78 (7.55%)	91 (8.81%)

*The proportion of samples with positive IgM and/or IgG against scrub typhus

Main results

The overall seropositivity, that is when IgM and/or IgG against scrub typhus was positive, was 8.81% (n = 91/1,033). The proportion of IgM positive samples was higher than the IgG positive samples i.e. 47 (4.55%) and 45 (4.36%) for IgM and IgG, respectively.

When split by sample collection sites (Table 14), the number and proportion of overall seropositivity was highest in Jambi (n = 34/350, 9.71%). The IgM seropositivity was highest in Jambi as well (n = 20/350, 5.71%). For IgG seropositivity, all of the sites had 15 positive samples each, but the proportion was highest in Tabanan (n = 15/268, 5.60%). When the proportions of overall positive samples from each site were compared using Chi-square test, the difference was not statistically significant (p-value = 0.69). Therefore, there was no association between the overall positivity status and sample collection sites.

Table 14. Seropositivity proportion of each site

	Jambi	**Denpasar**	**Tabanan**	**Overall**
IgM positive	20 (5.71%, 95% CI 3.73% to 8.66%)	18 (4.34%, 95% CI 2.76% to 6.75%)	9 (3.36%, 95% CI 1.78% to 6.26%)	47 (4.55%, 95% CI 3.44% to 6.00%)
IgG positive	15 (4.29%, 95% CI 2.61% to 6.95%)	15 (3.61%, 95% CI 2.20% to 5.88%)	15 (5.60%, 95% CI 3.42% to 9.03%	45 (4.36%, 95% CI 3.27% to 5.78%)
Overall positive*	34 (9.71%, 95% CI 7.04% to 13.27%)	33 (7.95%, 95% CI 5.72% to10.96%)	24 (8.96%, 95% CI 6.09% to 12.98%)	91 (8.81%, 95% CI 7.23% to 10.69%)

*The proportion of samples with positive IgM and/or IgG against scrub typhus

95% confidence interval calculated using Wilson score interval

There was 40 out of 538 (7.43%) males and 51 out of 495 (10.30%) females with overall positive status. When the two proportions were compared using Chi-square test there were no statistically significant association between overall positivity status and sex (p-value = 0.10, 95% CI -0.06 to 0.01).

I plotted a boxplot of IgM optical density values and sex in Figure 38. I used two sample t-test to compare the means in the male and female group. There was statistically significant difference between the two groups (p-value = 4.65×10^{-5}, 95% CI -0.06 to -0.02), suggesting lower IgM values in males.

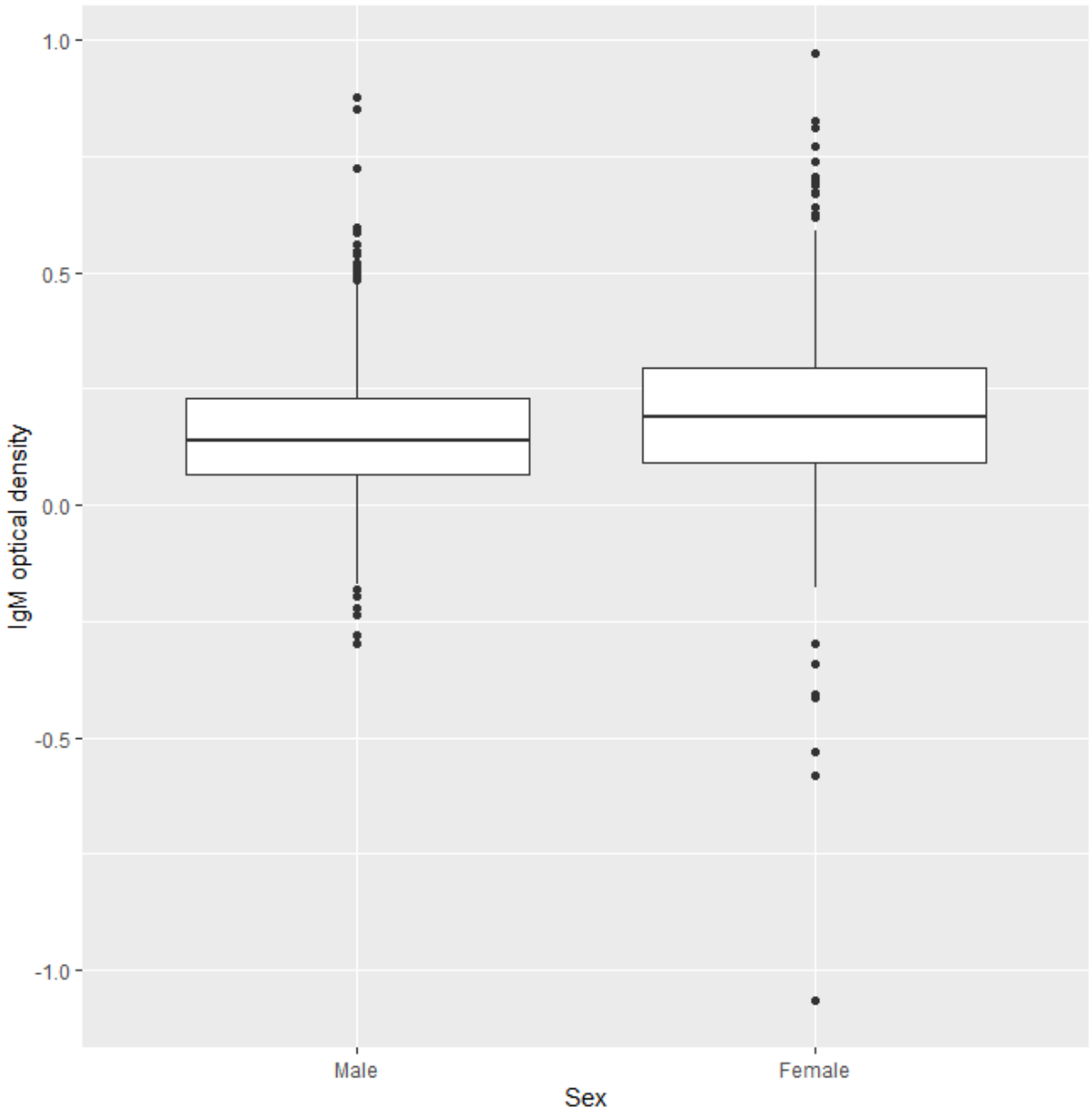


Figure 38. The boxplot of IgM optical density values and sex

I plotted a boxplot for IgG optical density values and sex in Figure 39. I also used two sample t-test to compare the means in the male and female group. The difference between the two groups was not statistically significant (p-value = 0.54, 95% CI -0.01 to 0.01).

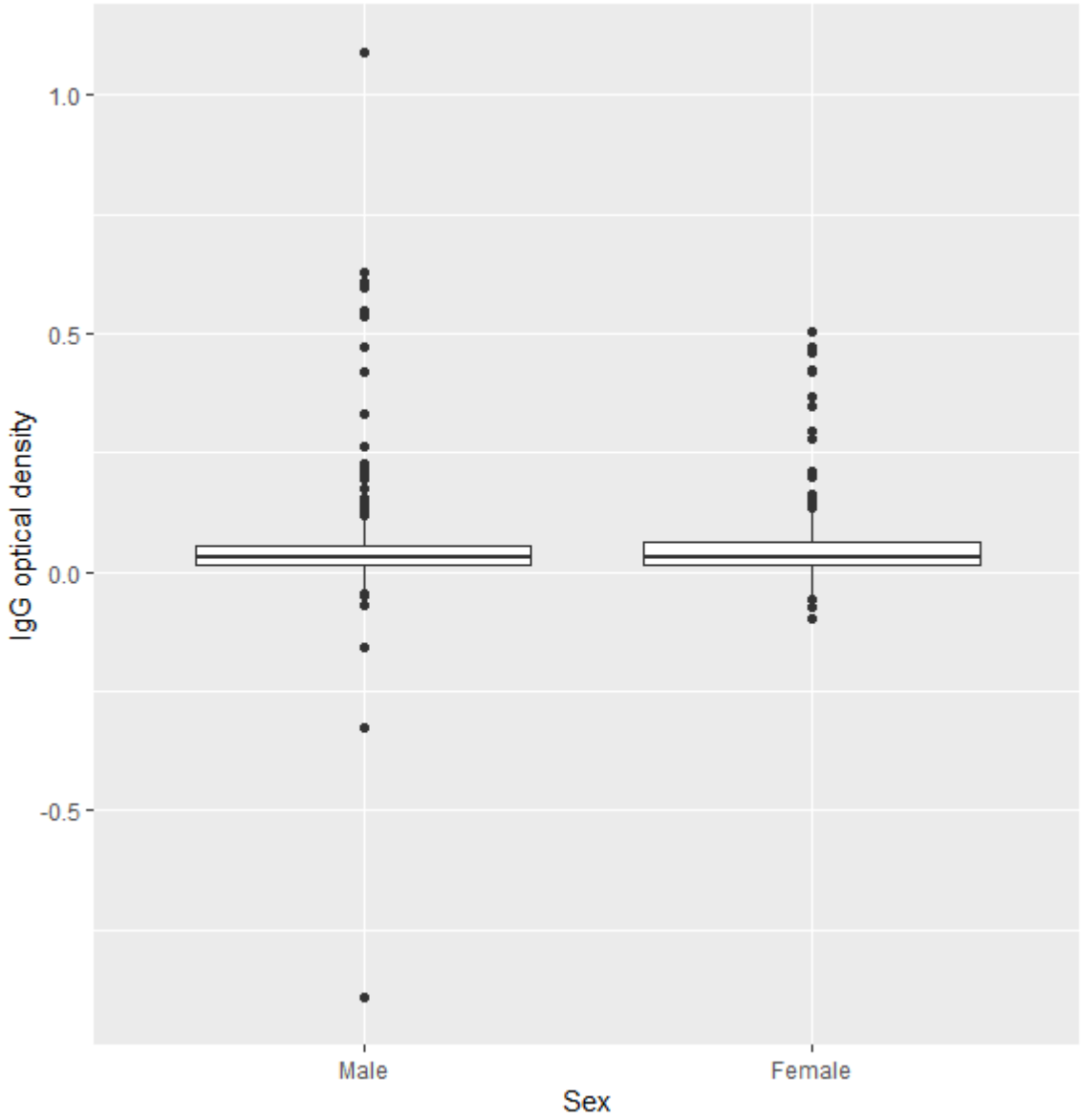


Figure 39. The boxplot of IgG optical density values and sex

I plotted the IgM optical density values and age in a scatter plot (Figure 40). The scatter plot did not show a strong linear relationship between both variables. Before assessing the correlation between age and IgM values, I plotted the histogram of age to see whether age was normally distributed or not (Figure 41). The histogram did not show normal distribution. This is in concordance with the result of Shapiro-Wilk test for normality (p-value < 2.20×10^{-16}). Therefore, to assess the correlation between IgM values and age, I calculated the

Spearman's rank correlation rho in R. There was a negative correlation between the IgM values and age (rho = -0.07, p-value = 0.02). I also computed a simple linear regression to investigate the correlation between IgM values (optical density) and age (year) (Table 15). The simple linear regression showed a significant relationship between age and IgM values (p-value = 0.003). The slope coefficient for age was -0.0009, so the IgM optical density, on average, decreases by 0.0009 for every one year increase in age. The R-squared value was 0.0083, so only 0.83% of the variation in IgM values can be explained by the model containing only age.

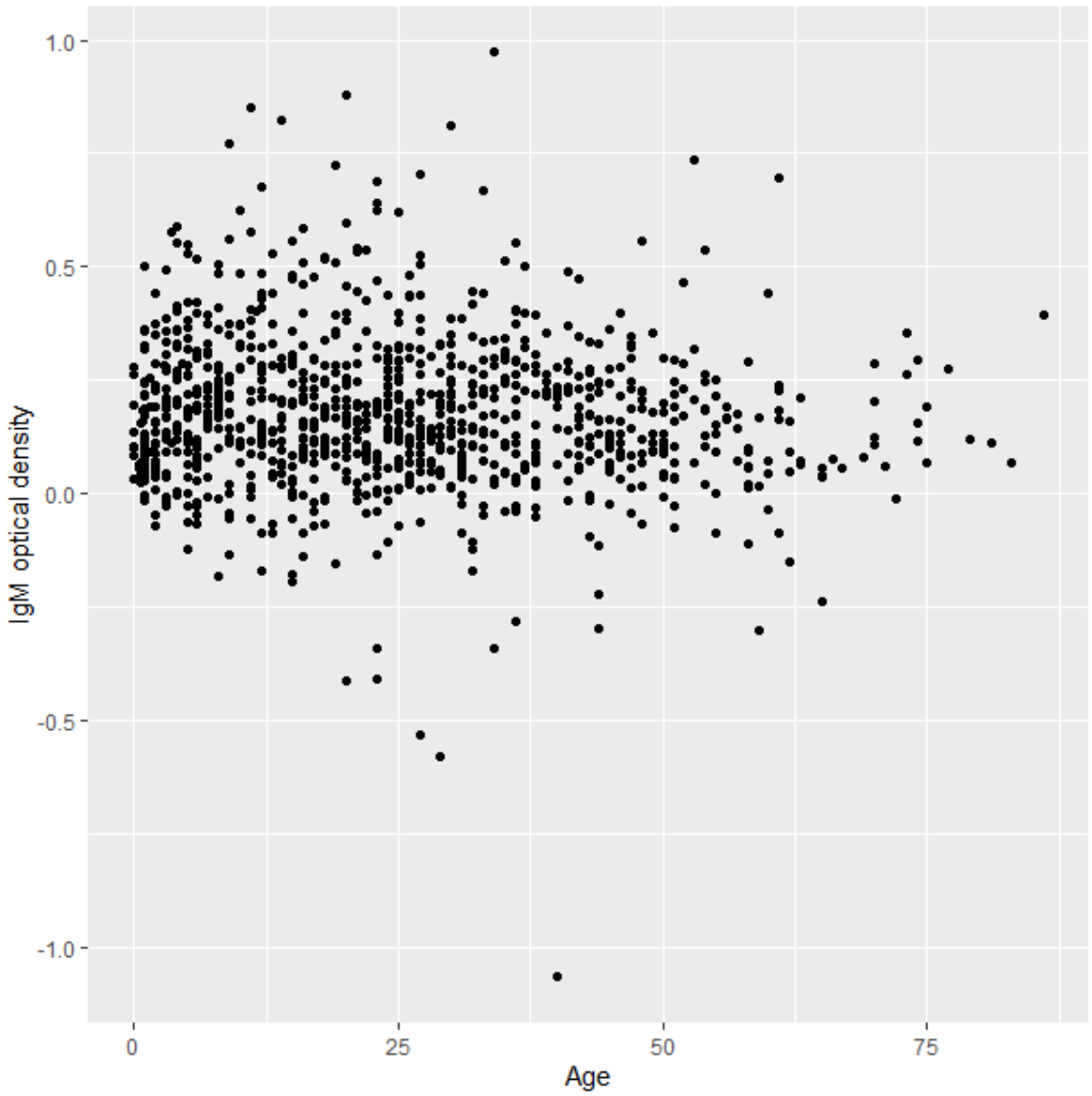


Figure 40. The scatter plot of IgM values and age

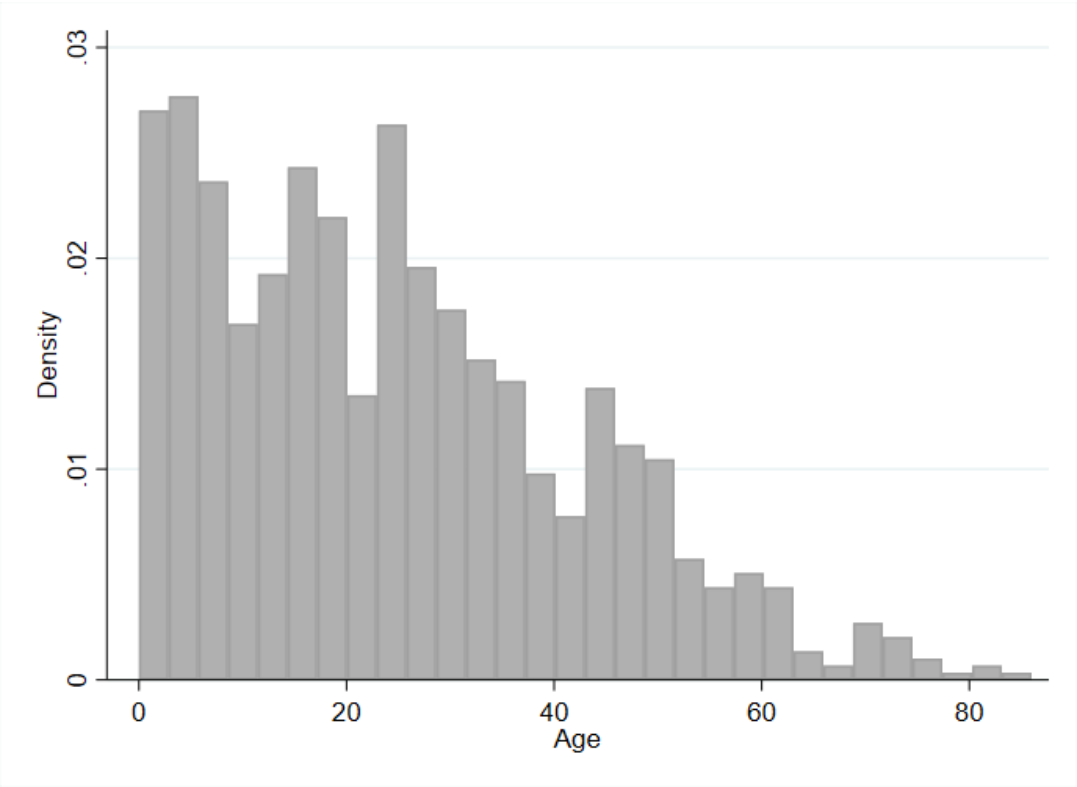


Figure 41. The histogram of age

Table 15. Linear regression summary for age predicting IgM value

	Estimate	**95% CI**	**p-value**
Intercept	0.20	0.18 to 0.22	$<2 \times 10^{-16}$
Age	-0.0009	-0.002 to -0.0003	0.003

R-squared: 0.0083, adjusted R-squared: 0.0074

I also plotted the IgG optical density values and age in a scatter plot (Figure 42). The scatter plot did not show a strong linear relationship between both variables. I calculated the Spearman's rank correlation rho in R to assess the correlation between IgG values and age. There was a positive correlation between the IgG values and age (rho = 0.23, p-value = 2.92×10^{-14}). I also computed a simple linear regression to investigate the correlation between IgG

values (optical density) and age (year) (Table 16). The simple linear regression showed a significant relationship between age and IgG values (p-value = 1.61 x 10^{-10}). The slope coefficient for age was 0.0009, so the IgG optical density, on average, increases by 0.0009 for every one year increase in age. The R-squared value was 0.0389, so only 3.89% of the variation in IgG values can be explained by the model containing only age.

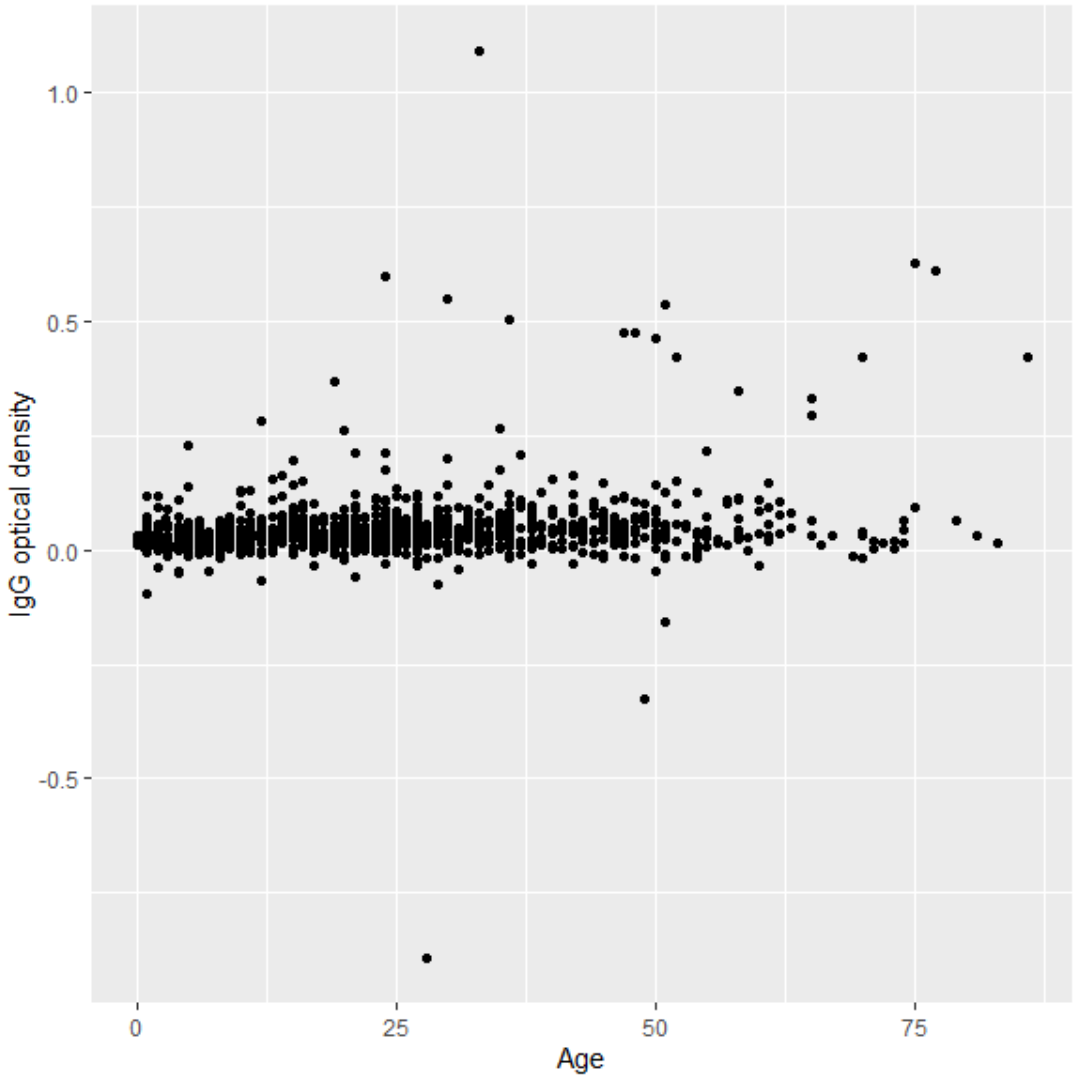


Figure 42. The scatter plot of IgG values and age

Table 16. Linear regression summary for age predicting IgG value

	Estimate	**95% CI**	**p-value**
Intercept	0.02	0.01 to 0.03	8.70×10^{-8}
Age	0.0009	0.0006 to 0.001	1.61×10^{-10}

R-squared: 0.0389, adjusted R-squared: 0.0380

I also explored the relationship between age and overall positivity status using logistic regression (Table 17). There is statistically significant relationship between age and having overall positive status. With one year increase in age, the odds of having overall positive status are increased by 1%.

Table 17. Logistic regression for the probability of having overall positive status based on age

Characteristic	**Odds ratio**	**95% CI**	**p-value**
Age	1.01	1.00 to 1.02	0.03

The samples were also previously tested for dengue infection [244, 245]. There were 166 dengue positive samples among the total 1,033 (16.07%, 95% CI 13.88% – 18.45%). Table 18 showed the number of samples and their overall scrub typhus serology and dengue infection status. There were 19 samples with both overall scrub typhus seropositivity and dengue infection (n = 19/1,033, 1.84% (95% CI 1.18% – 2.85%)). The proportion of dengue positive samples within the overall seropositive scrub typhus sample was 20.88% (95% CI 13.79% to 30.32%). There was no statistically significant association between overall scrub typhus seropositivity and dengue infection (p-value = 0.19, 95% CI -0.02 to 0.08).

Table 18. The scrub typhus overall seropositivity status and dengue infection

	Dengue positive	**Dengue negative**	**Total**
Scrub typhus overall seropositive	19	72	91
- Scrub typhus IgM positive*	8	39	47
- Scrub typhus IgG positive*	11	34	45
Scrub typhus overall seronegative	147	795	932
Total	166	867	1,033

*Numbers not counted again in totals

Discussion

We used in-house optimised and validated ELISA to test for IgM and IgG against scrub typhus to analyse 1,033 serum samples: 350 from Jambi, 415 from Denpasar, and 268 from Tabanan. We used the finite mixture modelling to determine the cut-off. The resulting cut-offs for IgM and IgG were 0.49 and 0.13, respectively. The IgM seropositivity across all sites was 4.55% (95% CI 3.44% to 6.00%), while the IgG seropositivity was 4.36% (95% CI 3.27% to 5.78%). The overall seropositivity, that is the proportion of samples with positive IgM and/or IgG, was 8.81% (95% CI 8.11% to 11.74%). The overall seropositivity for Jambi, Denpasar, and Tabanan were 9.71% (95% CI 7.04% to 13.27%), 7.95% (95% CI 5.72% to10.96%), and 8.96% (95% CI 6.09% to 12.98%) respectively. Just under one sixth (16.07%, 95% CI 17.53% to 22.39%) of the samples were positive for dengue infection. Among the scrub typhus seropositive participants, just over one fifth (20.88%, 95% CI 13.79% to 30.32%) had dengue infection.

There was no association between the proportion of overall positive samples and sites or sex. There was a weak relationship between overall positivity status and age. The odds of

becoming overall seropositive increase by 1% with one year increase in age. The IgM values were lower in males than females. There was a weak correlation between age and IgM values, where IgM values decrease by 0.0009 with every year gained in age. There was also weak correlation between age and IgG values, where with one year increase in age, the IgG values increase by 0.0009. The R-squared values for the correlation between age and both the IgM and IgG values were less than 4%, suggesting that age may not be a main factor determining IgM and IgG optical density values. There was no association between scrub typhus overall seropositivity status and dengue infection.

Seropositive status in this study would indicate that there was exposure to and active transmission of *O. tsutsugamushi*. Positivity might also indicate acute infection, particularly in the dengue negative and scrub typhus IgM positive group (n = 39/1033, 3.78%). However, that remains uncertain because we did not have convalescent samples and were thus unable to observe any increase in IgM or IgG level. We also did not have other diagnostic methods e.g. IFA or PCR for active scrub typhus to confirm what is inferred by the ELISA results on the acute samples. If we want to ensure less false positive and be more conservative, we could increase the cut-off to diagnose an acute case. However, I think the cut-off used here is already specific, as it was calculated by adding mean and three times standard deviation of the assumed negative distribution, instead of one- or two-times standard deviation. However, to distinguish the acute infection for sure, we would need to test the samples with gold standard diagnostics. Contingent on funding and permit approval, we planned to re-check the positive samples with IFA. However, due to the Covid-19 pandemic, we could not do this yet.

Positive IgG would be indicative of past exposure or active infection if there was prior exposure. The level of IgG seropositivity in the sample collection sites in acute febrile participants was slightly higher than in one recently published study from Indonesia (4.36% vs 3.8%) [83]. This could be caused by the wider eligibility criteria applied in the previous study i.e. no exclusion on people presenting with focal signs/symptoms e.g. gastrointestinal or respiratory symptoms. The number of samples with positive IgM in the previous study was not mentioned in the published results [83]. However, it was concluded that there was no participant with confirmed scrub typhus diagnosis – defined as 'the detection of 47-kD omp gene of O. tsutsugamushi and/or sero-conversion or four-fold increase *O. tsutsugamushi* IgM and/or IgG by ELISA' [83]. The previous study collected samples from tertiary referral hospitals [83], while this study population also included district hospital in a semi-urban area (Table 8).

We found samples with very low negative values e.g. -1.07 was the lowest for IgM. These negative net OD values resulted from substantially higher OD in the wells without compared to the OD in the wells with antigen coating. Water has been shown to cause problems during assay standardisation across laboratories, even though the same reagents are used [253]. Here, we used identical reagents and plates as has been used in the reference laboratory. However, since this did not affect all of the plates, this is most likely not a systemic problem. This could be caused by random contamination, pipet or pipetting errors, or washer problems [253]. It may also be caused by nonspecific binding of the serum sample or antihuman conjugate [253]. Unfortunately, we could not transport the samples to the reference laboratory for cross examination with reference test i.e. IFA due to difficulties in acquiring material transfer agreement. We decided not to exclude such values from the analysis, since

these samples would likely still be seronegative. Excluding these samples would compromise the sample size and may overestimate the seropositivity because that would decrease the denominator.

Many previous studies used 0.5 as the optical density cut-off [147]. Here, we used 0.49 for IgM and 0.13 for IgG. In addition to using the results of finite mixture models (FMMs), we also performed two other methods as comparison. The results of the comparative methods, namely visual inflection point and change-point analysis, largely agree with the results of FMMs. Therefore, I think using the above cut-offs was reasonable for this study. However, I am not sure if this cut-off can be directly used for other studies in different sites; because different locations may have different background immunity.

We can conclude that there has been exposure to scrub typhus at all sites, but was improbably the main cause of acute febrile illness in these patients. Jambi and Denpasar are provincial capitals (urban areas); this might be one of the reasons. Although not a main cause of acute fever, keeping in mind that there was active transmission, it is still important to be familiar with the disease – especially when there are cases of acute fever that did not respond to beta-lactam antibiotic treatment. Clinicians also need to do a thorough physical examination to look for eschars in these cases.

I performed visual inflection point, change-point analysis, and finite mixture modelling to determine the cut-off. These three approaches can be used to determine the cut-off when

there is no sample from an unexposed population or when the gold standard diagnostic method is unavailable.

The visual inflection point method is simple and does not need any calculations, models, or software. In this project, the mean of the respondents' answer is quite close to the results from other methods. However, if we look at individual responses, some are quite far from the mean. Ensuring there are enough respondents asked could be important when using this technique. Setting a minimum number of respondents would be useful. Here, with ten respondents, the resulting mean for IgM and IgG was similar with the other methods. It is also important to define and explain what an inflection point is as the respondents might not be familiar with it – demonstrating this with graphs might be helpful.

We also performed a change-point analysis using Bayesian approach. The resulting cut-offs are also in agreement with the cut-offs gained from FMMs. I think it is important to ensure that we inputted appropriate and specific priors, since the results of this method can change according to what priors were used. Here, we used the results from the visual inflection method as the prior. I also ran a model with different priors and the resulting cut-offs were different by -0.05 for IgM and 0.02 for IgG. This demonstrated that though changed, the resulting cut-offs between these two sets of priors were not drastically different.

I used FMMs to determine the cut-offs. FMMs require use of statistical software, therefore, this method is not as straightforward as identifying the inflection points visually. I need to

define one number in the command, that is the number of subpopulations. Knowledge of the disease is very important to be able to define this number appropriately. Since we do not need to define as many parameters as with the change-point analysis, I found this method to be less complicated and more objective. The results are also more intuitive to interpret.

The presumed unexposed population samples continue to be collected. We hope to also test and analyse these in the future. We also hope to be able to test the positive samples with IFA.

This is the first study to use in-house ELISA methods to investigate scrub typhus in Indonesia. This method is cheaper than using the available commercial kits. This project also incorporated capacity building with the set-up of the in-house ELISA method. We have used archived samples here, optimising the collected sera. This study also provided scrub typhus data for Tabanan. I could not find any previous data on Tabanan (*Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*). This project contributed to the understanding of scrub typhus in Indonesia.

The use of archived sera may have optimised the value of the samples. However, this also means that we did not have control over the primary study design, including the sampling, and recruitment process. The primary study was also not specifically designed for this study. However, due to the limited resources and the COVID-19 pandemic making primary clinical research difficult, we chose to use these archived samples. The data sets from each primary studies were heterogeneous, although there were commonly collected variables.

In this study, we have managed to differentiate seropositive patients and seronegative patients. However, there was still uncertainty if the seropositive samples were from acute scrub typhus participants. We did not have convalescent samples and gold standard for comparison. For acute samples, other diagnostic methods could be more ideal given the timing i.e. PCR or other nucleic acid detection based methods. However, the overarching aim of this study was to understand the distribution of scrub typhus in Indonesia. Therefore, the use of serology method was justified. Serologic methods can detect IgG and therefore previous exposure.

The seropositive proportion might not reflect the seroprevalence of scrub typhus in the community. Since the samples were from acute febrile participants consecutively recruited in hospitals, it might select people who are febrile due to scrub typhus, and therefore increasing the proportion.

The use of archived febrile study samples might explain the findings in this survey. In the case of dengue, seroprevalence studies in non-febrile participants e.g. blood donors in endemic locations usually showed higher IgG compared to IgM seropositivity [254-256]. In this study, the IgM was higher than the IgG seropositivity. Since the samples were collected in participants with acute fever, this could possibly reflect that scrub typhus was the cause of fever in some of these febrile cases.

In the future, seroprevalence surveys in the community and including scrub typhus in the diagnostic panel of acute febrile illness studies in more areas (particularly rural areas) would be crucial in improving our understanding of scrub typhus distribution in Indonesia. We have planned to conduct a community seroprevalence study in both rural and urban areas (*Chapter 8. Serological investigation of risk of exposure to scrub typhus in residence at an oil palm plantation in North Sumatra relative to urban dwellers at Medan, North Sumatra (SEROSCRUB): A study protocol*). However, due to the Covid-19 pandemic this study has been delayed. Those studies are funded and planned for late 2022 (contingent on pandemic development). In addition to seroprevalence surveys and acute febrile illness studies, exploring factors associated with exposure to scrub typhus is also important. Accurate and accessible point-of-care-tests would be invaluable in supporting future scrub typhus studies.

We can conclude that *O. tsutsugamushi* exposure in humans occurs at all sites analysed. Though scrub typhus might not be the most common cause of acute fever in Jambi, Denpasar, and Tabanan, it is still important to consider it as one of the aetiologies, particularly in cases not responding to beta-lactam antibiotics. We still need further studies in other regions of Indonesia, particularly in rural areas.

Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform

Introduction

Scrub typhus largely affects rural populations for whom access to health care facilities and laboratories with diagnostic capacity are limited [2]. It is difficult to diagnose clinically, therefore, laboratory tests play an essential role in diagnosis. The gold standard for diagnosis is the indirect immunofluorescence assay (IFA), which requires sophisticated laboratory facilities and skilled personnel [132]. There are no reliable and widely available point-of-care tests yet. The difficulty in diagnosis is one of the main reasons for the limited epidemiological knowledge on scrub typhus. The IFA has been used to diagnose scrub typhus since at least the 1960s [148], but although considered the gold standard, its accuracy is limited [124]. With scant and scattered data, it is difficult to identify the most at-risk population, the regional distribution, and the optimal treatments to optimise disease control efforts, which to date rely on limited evidence. Data are even rarer for specific populations including pregnant women and children. Despite its mortality and morbidity impact, scrub typhus is not considered in the current WHO list of Neglected Tropical Diseases (NTDs).

Antibiotics, including doxycycline, tetracycline, chloramphenicol, and azithromycin, are the current mainstays of treatment [2, 163]. Delayed response to antibiotic treatment has been reported [257], although the contribution of antimicrobial resistance is uncertain [167].

Although the antibiotic choice used to treat scrub typhus is virtually consistent, the recommended dosing and duration of treatment varies. For example, the recommended azithromycin treatment duration for pregnant women is three and five days in the Thailand and India guidelines, respectively [172, 173]. Another example, the United States CDC guidelines and the Indian Academy of Paediatrics Guidelines use different weight cut-offs in their dosage guidelines for doxycycline therapy in children [170, 171]. It is unlikely that these different treatment regimens are all optimal. The treatment regimens used at the moment are based on limited evidence from few randomised clinical trials [163]. The evidence is also of low certainty [163]. Even in the countries within the classical tsutsugamushi triangle, such as Indonesia and Malaysia, I could not find any treatment guidelines for scrub typhus up to September 2021. Likewise, WHO treatment recommendation is absent. This reflects the lack of high-quality supportive evidence for optimal treatments [163, 169].

Existing data collected from clinical trials and longitudinal patient observational studies are the sources of our current evidence-based knowledge. However, there have been no recent reviews of the existing literature that assessed the landscape and focused on the methodology of scrub typhus treatment studies, and we hypothesise that in the context of scarce data, they are an underutilised source of information to address research priorities and bridge knowledge gaps. This systematic review aims to assess the characteristics of scrub typhus treatment studies and explore the need for and the feasibility of addressing knowledge gaps through pooling scrub typhus individual patient data.

This work was done in collaboration with the Infectious Diseases Data Observatory (IDDO). This systematic review was published in the Public Library of Science (PLOS) Neglected Tropical Diseases in October 2021 (DOI: 10.1371/journal.pntd.0006330). All associated data, including the full dataset, variable and data dictionary were published as supporting information with the article and hosted in the IDDO website (https://www.iddo.org/scrub-typhus).

Methods

Search strategy

We conducted a systematic literature review and wrote a manuscript following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Appendix 3) [232]. The review is registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the reference CRD42018089405. Six electronic databases (Ovid Embase, Ovid Medline, Ovid Global Health, Cochrane Library, Scopus, Global Index Medicus), ClinicalTrials.gov, and WHO ICTRP were searched using a search strategy detailed in the supporting information (Appendix 4). The search terms used were the combinations of: (1) scrub typhus and its synonyms with different spelling variations such as “mite typhus”, “kedani fever”, “chigger borne rickettsia”; and (2) the aetiology *Orientia tsutsugamushi*. The search was limited to publication dates from January 1998 up to February 2020 due to the significantly reduced likelihood of gathering individual participant-level data with time since publication [258]. No language restriction was applied at the search stage or initial screening of literature for eligibility. The reference lists of systematic reviews and meta-analyses (including Cochrane reviews) identified during the systematic search were screened for any

additional studies that met the inclusion criteria to ensure comprehensive inclusion of articles.

Eligibility criteria and study selection

Prospective clinical studies in humans with scrub typhus where treatment was administered and participants were followed up for at least 24 hours (i.e. clinical trials and longitudinal observational studies) were eligible for inclusion.

The exclusion criteria were as follows: studies on scrub typhus published earlier than 1 January 1998, conference abstracts published earlier than 1 January 2016 (as older conference abstract would most likely be published as a full paper), secondary research, non-longitudinal studies e.g. cross-sectional studies, case report/series, studies including five patients or less, studies on co-infection cases only, studies using non-definitive diagnosis i.e. not using confirmatory laboratory test, studies not yet completed, and studies without an accessible full-text. Although the search strategy and title/abstract screening were not restricted by language, due to the review team's proficiencies, potentially relevant studies identified in languages other than English, French, and Spanish were not included for data extraction and further analysis.

Titles and abstracts were screened by one researcher (Kartika Saraswati). Full texts were independently reviewed by two authors (Kartika Saraswati and Sauman Singh) with Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia). When conflicts

arose, a third researcher (Brittany Maguire) was consulted. Inclusion was not limited to any minimum quality assessment to ensure a complete assessment of the research landscape.

Data extraction and analysis

Relevant information from included studies was extracted using a piloted REDCap (Research Electronic Data Capture) database [259]. Extracted data included details on study design, treatment tested, patient characteristics, diagnostic methods, geographical location, outcome measures, and statistical methodology. The full list of variables extracted can be viewed in Appendix 5 and Supplement 6. Data extraction was performed by one of three reviewers (Kartika Saraswati or Sauman Singh or Roland Ngu). For each publication, a second alternative reviewer (Kartika Saraswati or Sauman Singh or Roland Ngu) cross-checked every data variable entered by the first reviewer for quality control to identify missed or discrepant information. The third reviewer (Brittany Maguire) resolved any data interpretation differences that arose between the two reviewers.

For the purposes of this review, we use the term “study arm” to refer both to a study arm from a clinical trial and patient cohorts from observational studies where treatment is administered.

Analysis was done with R version 3.6.2 and R Studio Version 1.2.5033 [230, 231]. The extracted data were synthesised using descriptive statistics, graphs and narrative synthesis.

Categorical variables were summarised with proportions and frequencies. Continuous variables were summarised as quartiles, minimums and maximums.

Results

Search results

Formal database searches were first conducted on 23 March 2018, with a subsequent update search completed up to 17 February 2020, which identified a total of 6,134 citations. After removing duplicates across the primary and update searches with EndNote, we screened the title and abstract of 3,100 articles (Figure 43) (Supplement 7). There were 660 full texts assessed for eligibility. From these, 533 articles were excluded, among those 357 were case reports or case series. We found 20 papers not in English: four in Korean and 16 in Chinese. We could not access the full text of six papers despite extensive attempts. We included 127 relevant studies in the analysis [114, 260-385]. There were seven interventional studies and 120 observational studies included (Appendix 6). The full dataset can be viewed in (Supplement 8).

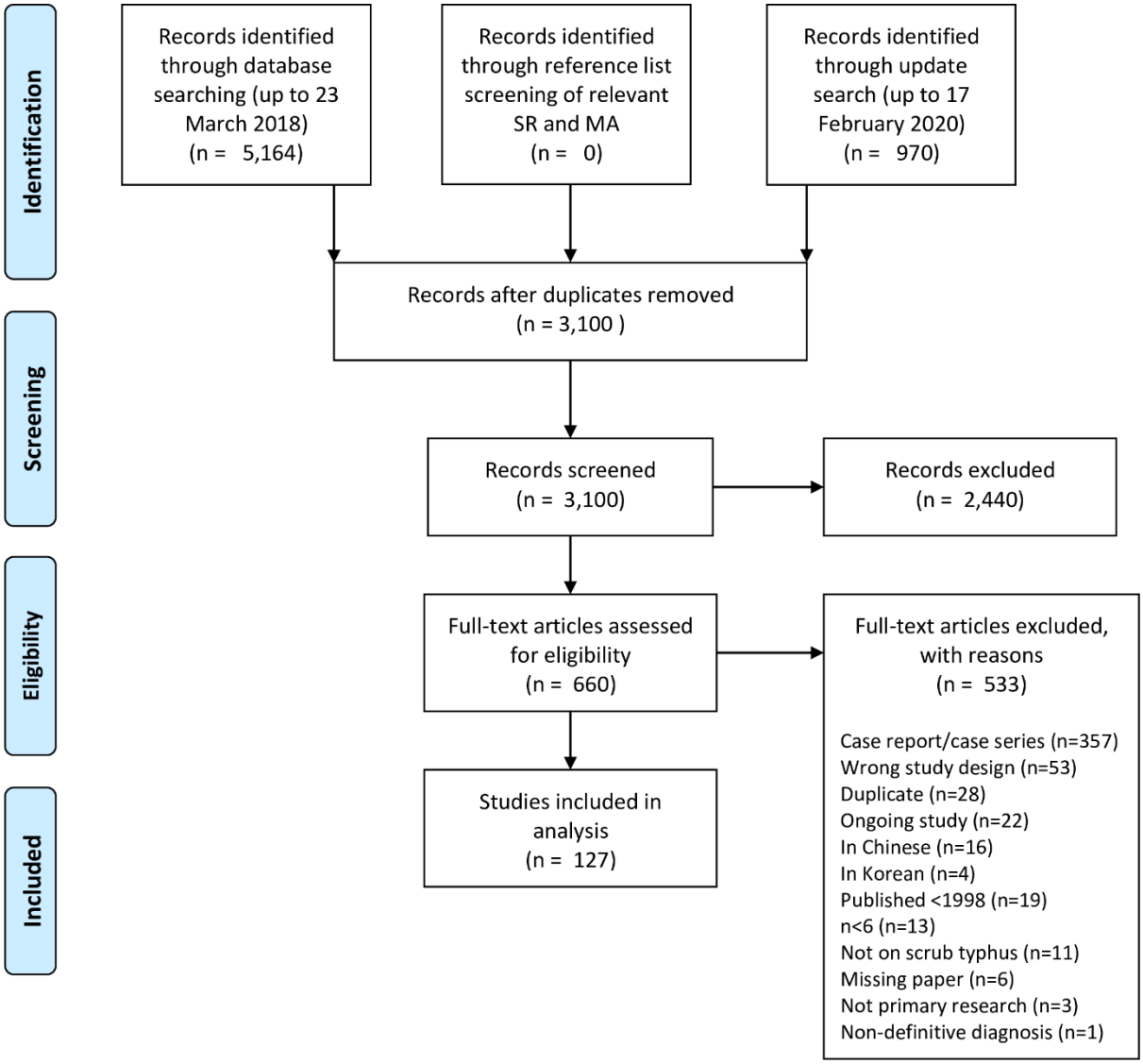


Figure 43. PRISMA flowchart

MA = meta-analyses, SR = systematic review

Study settings and population

The distribution of included studies is depicted in Figure 44. All studies were conducted in countries within the classic tsutsugamushi triangle. Of the 12 countries represented, India had the highest number (n= 84/127, 66%) of studies, followed by the Republic of Korea (South Korea) (n=14/127, 11%) and Thailand (n= 11/127, 9%) (Figure 45). This review identified no

published antibiotic treatment data for *Candidatus* Orientia chuto and *Candidatus* Orientia chiloensis.

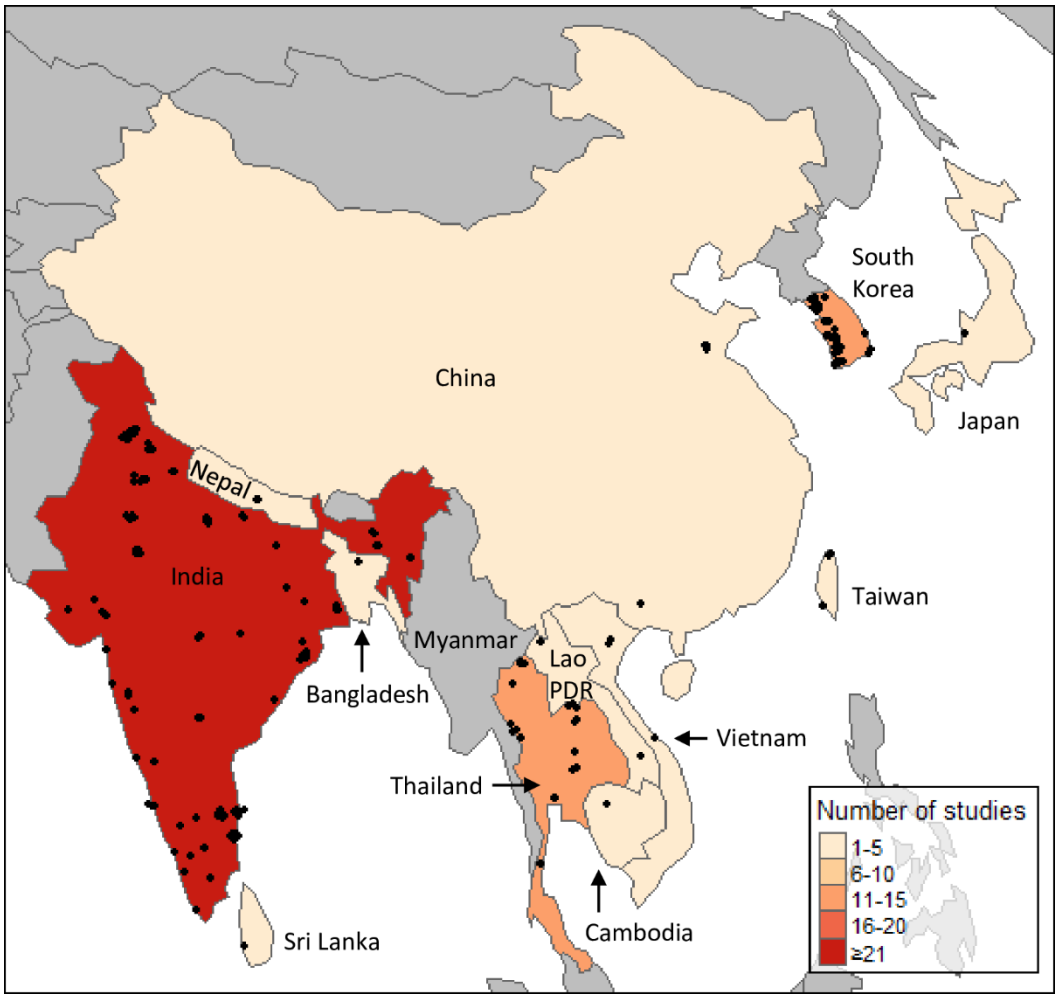


Figure 44. Number of eligible studies from each country

Jittered points represent each study location. Vector and raster map data from naturalearthdata.com.

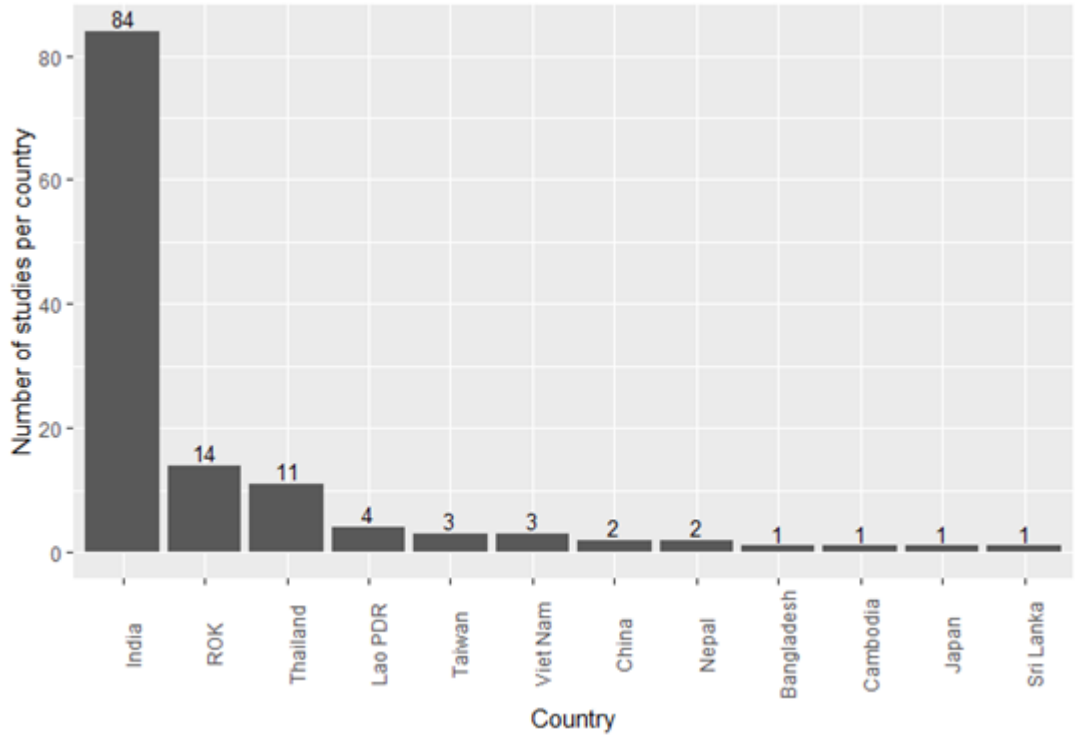


Figure 45. Number of studies per country

Lao PDR = Lao People Democratic Republic, ROK = Republic of Korea (South Korea).

There were 141 study arms included in the 127 studies. In total, there were 12,079 participants enrolled in the studies, with most studies (n = 84) and over two-thirds of participants recruited in India (n = 8,377, 69%). The 14 and 11 studies conducted in South Korea and Thailand recruited 1,592 (13%) and 707 (6%) participants, respectively (Figure 46). The median number of scrub typhus participants recruited in the studies was 57 (range 6–502) (Figure 47). Most of the studies (n=109/127, 86%) recruited less than 200 participants.

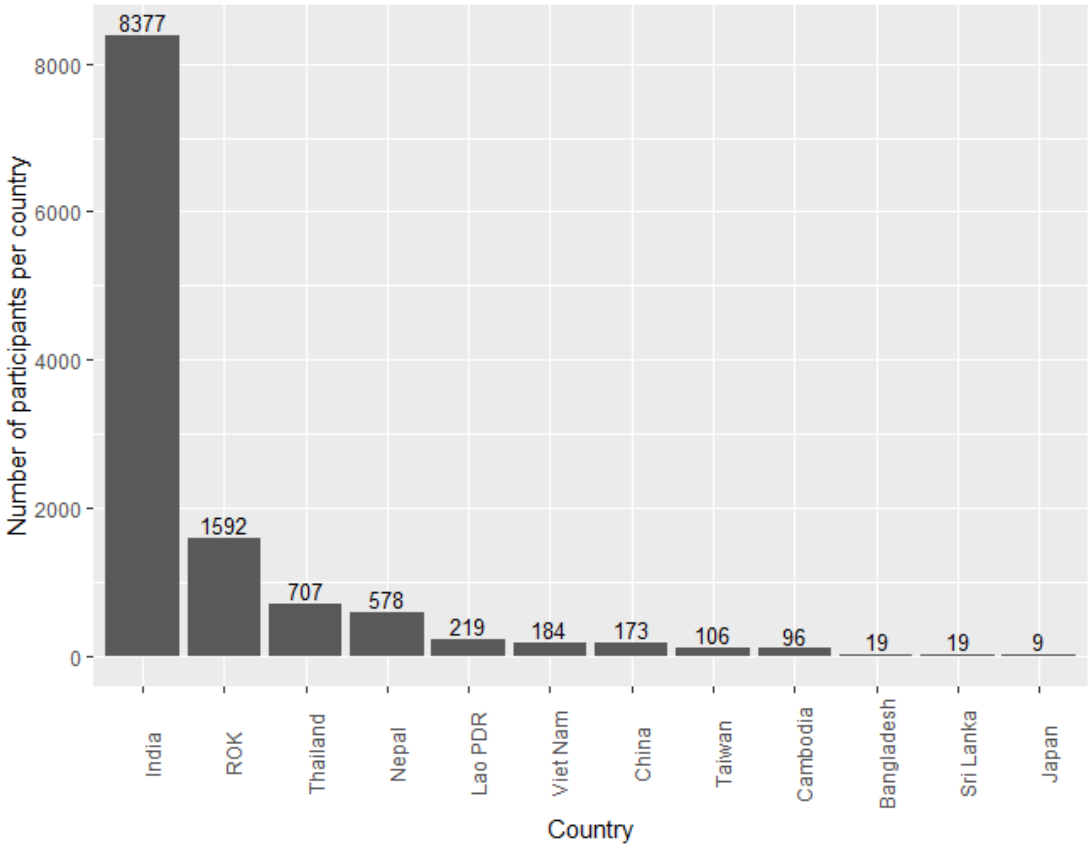


Figure 46. Number of participants per country

Lao PDR = Lao People Democratic Republic, ROK = Republic of Korea (South Korea).

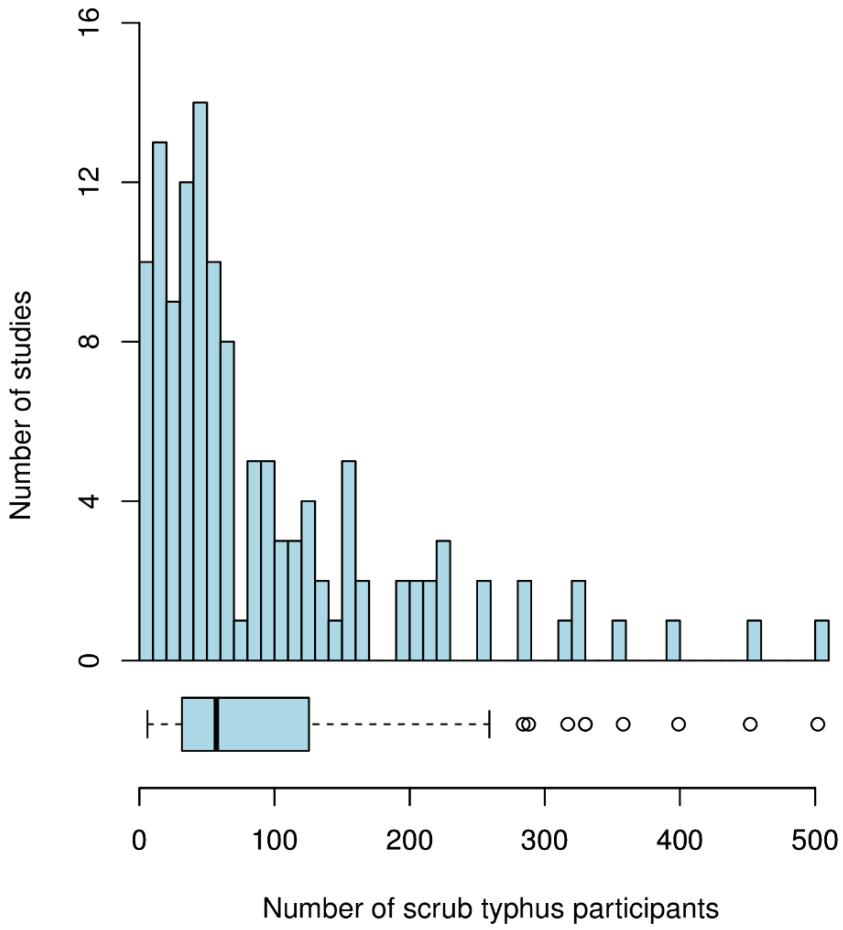


Figure 47. Number of scrub typhus participants enrolled per study

Bin width is 10. The box denotes the lower and upper quartiles with the median indicated by the thick vertical line. Capped bars represent the minimum and maximum values within 1.5*interquartile range of the first and third quartiles, respectively. Circles indicate values outside this interval.

The majority of the included studies were conducted in lower middle-income countries as defined by the World Bank (n = 98/127, 77%) (Figure 48) [386]. Most studies were conducted in urban areas (n = 98/127, 77%), and almost all studies recruited participants from a hospital/health-centre (n = 125/127, 98%) (Figure 48). One study recruited participants from a school (n = 1/127, 1%) and one from antenatal clinics (n = 1/127, 1%). Most of the studies

did not mention in which settings the participants included lived (n = 97/127, 76%). Among the studies that provided this information, there were 29 (97%), 18 (60%), 5 (174%) studies with participants from rural, semi-urban, and urban areas, respectively. There were no interventional studies that indicated inclusion of any pregnant women and only three interventional studies included children (age <18 years). Pregnant women and children were included in 14 and 75 observational studies, respectively. There were at least 5,995 female and 5,392 male participants included. Twenty-nine studies (23%) did not specify clearly the number of female and male participants with scrub typhus.

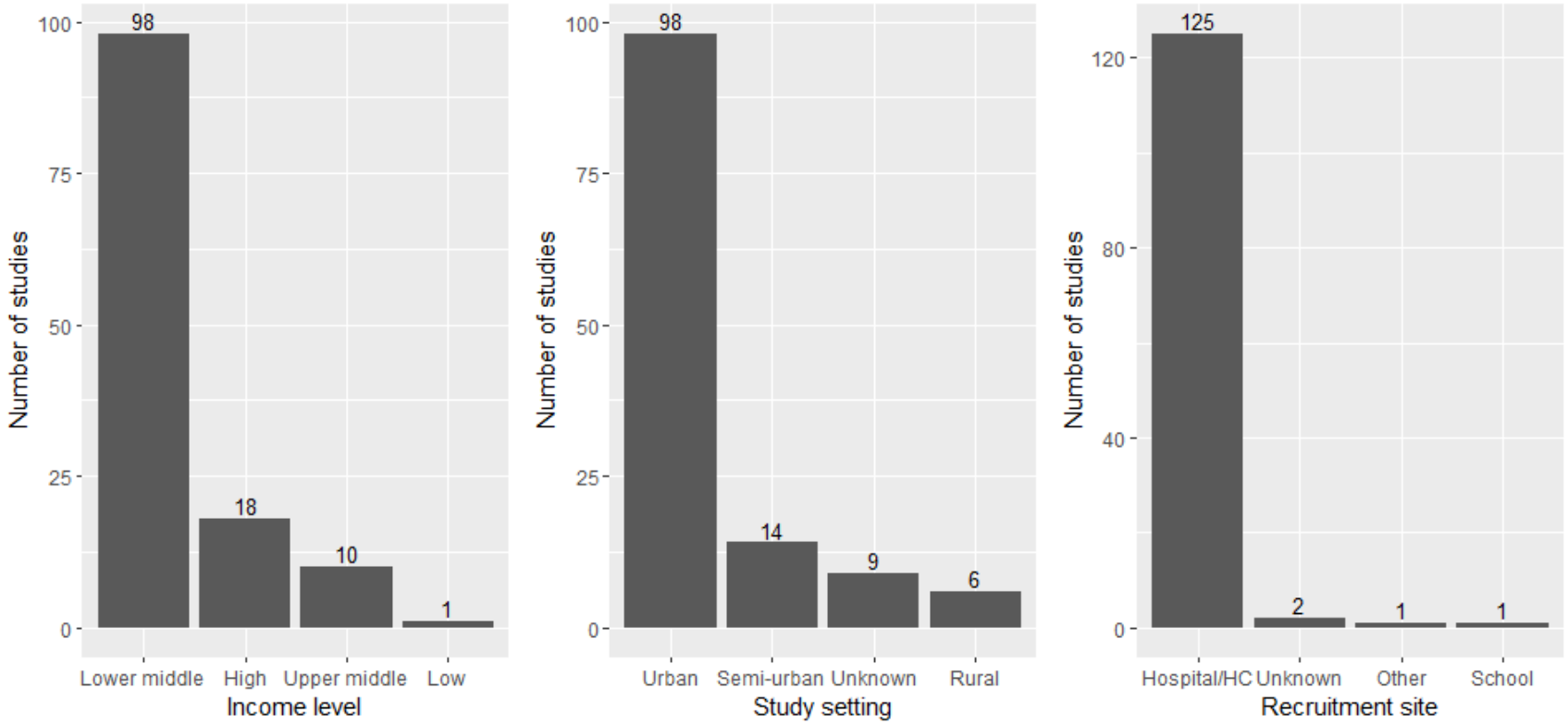


Figure 48. Number of studies for each income level, study setting, and recruitment site

Diagnostic methods

The methods used to diagnose scrub typhus varied substantially across the included studies (Table 19). The most commonly used diagnostic method was enzyme-linked immunosorbent assay (ELISA) (n = 65/127, 51%). Indirect immunofluorescence assay (IFA), which is typically considered as the gold standard, was used by 33 studies (n = 33/127, 26%). Polymerase chain reaction (PCR) was used in 30 (n = 30/127, 24%) studies; Weil-Felix in 25 (n = 25/127, 20%); immunochromatography test in 16 (n = 16/127, 13%); eschar presence in 13 (n = 13/127, 10%); other clinical presentation in 6 (n = 6/127, 5%); indirect immunoperoxidase test in 4 (n = 4/127, 3%); cell culture isolates in 3 (n = 3/127, 2%). Six (n = 6/127, 5%) studies used other diagnostic methods, including hemagglutination assay (PHA), dotblot immunoassay, microimmunofluorescence, and favourable response to antibiotics. No studies used the scrub typhus infection criteria (STIC) [151]. Nine observational studies, including 186 participants, gave no details on the diagnostic methods used. For interventional studies, IFA was the most commonly used diagnostic method (n = 4/7, 57%). While for observational studies, the most commonly used diagnostic method was ELISA (n = 65/120, 54%).

The proportion of studies using culture and isolation were consistently under 12.5% over the years (Figure 49). Similarly, ICT and other clinical presentation aside from eschar were used in less than 25% of studies across the years. The use of IFA, IIP, eschar, and Weil-Felix showed decreasing trends, while the proportion of studies using PCR and ELISA showed an increasing trend over time.

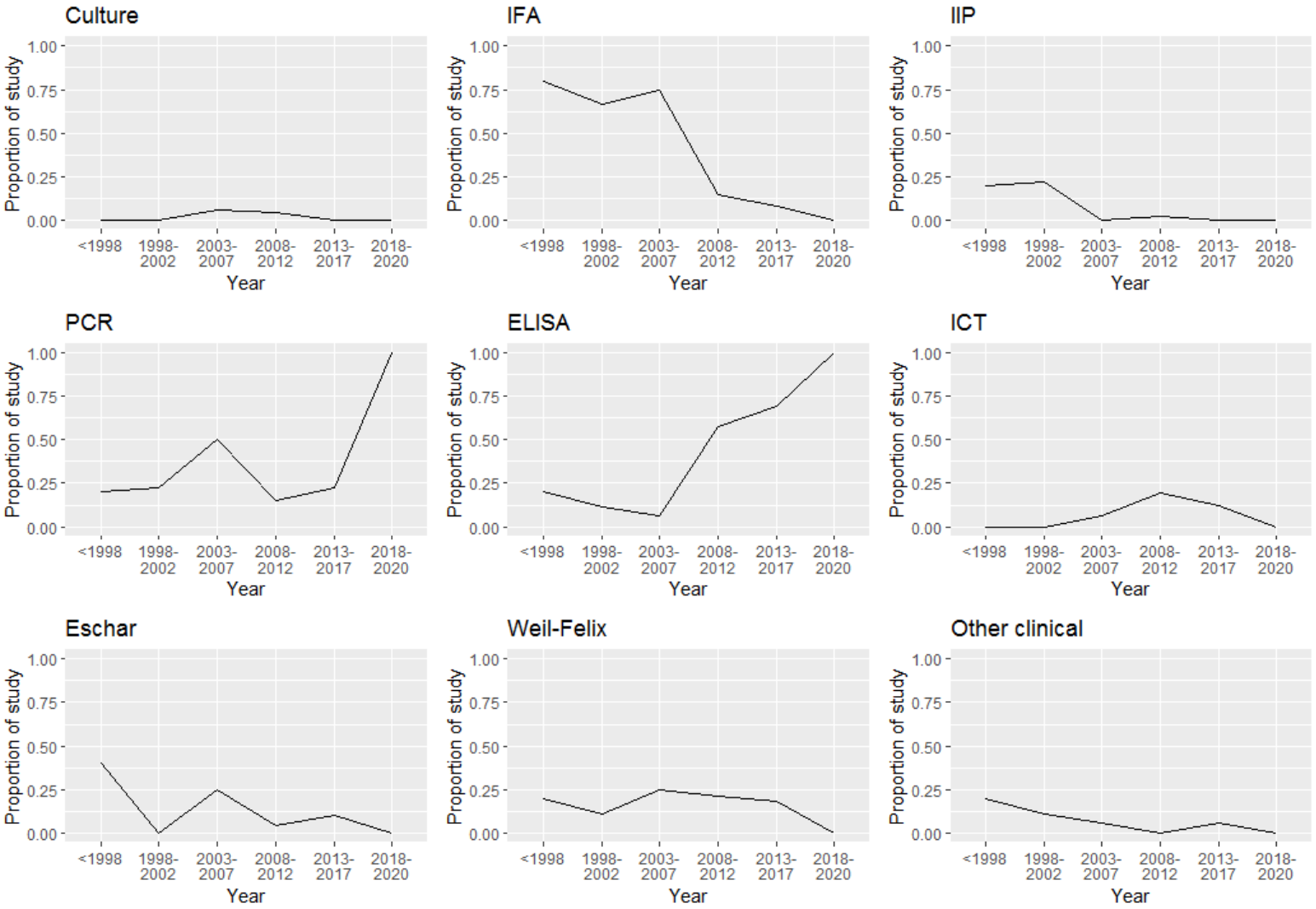


Figure 49. The proportion of studies using each diagnostic methods divided by the number of studies in each year group

Table 19. Diagnostic methods distribution across studies and participants

Diagnostic method	Interventional studies		Observational studies		All studies	
	Number of studies	Number of participants	Number of studies	Number of participants	Number of studies	Number of participants
ELISA	1	126	64	9,527	65	9,653
PCR	1	158	29	3,683	30	3,841
Eschar presence	2	153	11	2,543	13	2,696
Weil-Felix	0	0	25	1,555	25	1,555
ICT	1	57	15	760	16	817
Other*	1	118	5	270	6	388
Other clinical presentation**	1	61	5	270	6	331
IIP	1	126	3	52	4	178
Cell culture isolate	0	0	3	137	3	137

ELISA = enzyme-linked immunosorbent assay, ICT = immunochromatography test, IFA = indirect immunofluorescence assay, IIP = indirect immunoperoxidase test, PCR = polymerase chain reaction.

*The other diagnostic methods used were hemagglutination assay (PHA), dotblot immunoassay, microimmunofluorescence, and favourable response to antibiotics

** Clinical presentation with no eschar presence component

Treatment

There was substantial heterogeneity in treatment, with 14 types of antibiotics administered in the included studies. Doxycycline was the most commonly administered antibiotic (94/141 study arms with 8,062 participants), followed by azithromycin and chloramphenicol, which

were used in 55 (5,855 participants) and 20 (1,801 participants) study arms, respectively (Figure 50). All other antibiotics were administered to less than ten study arms and less than 1,000 participants.

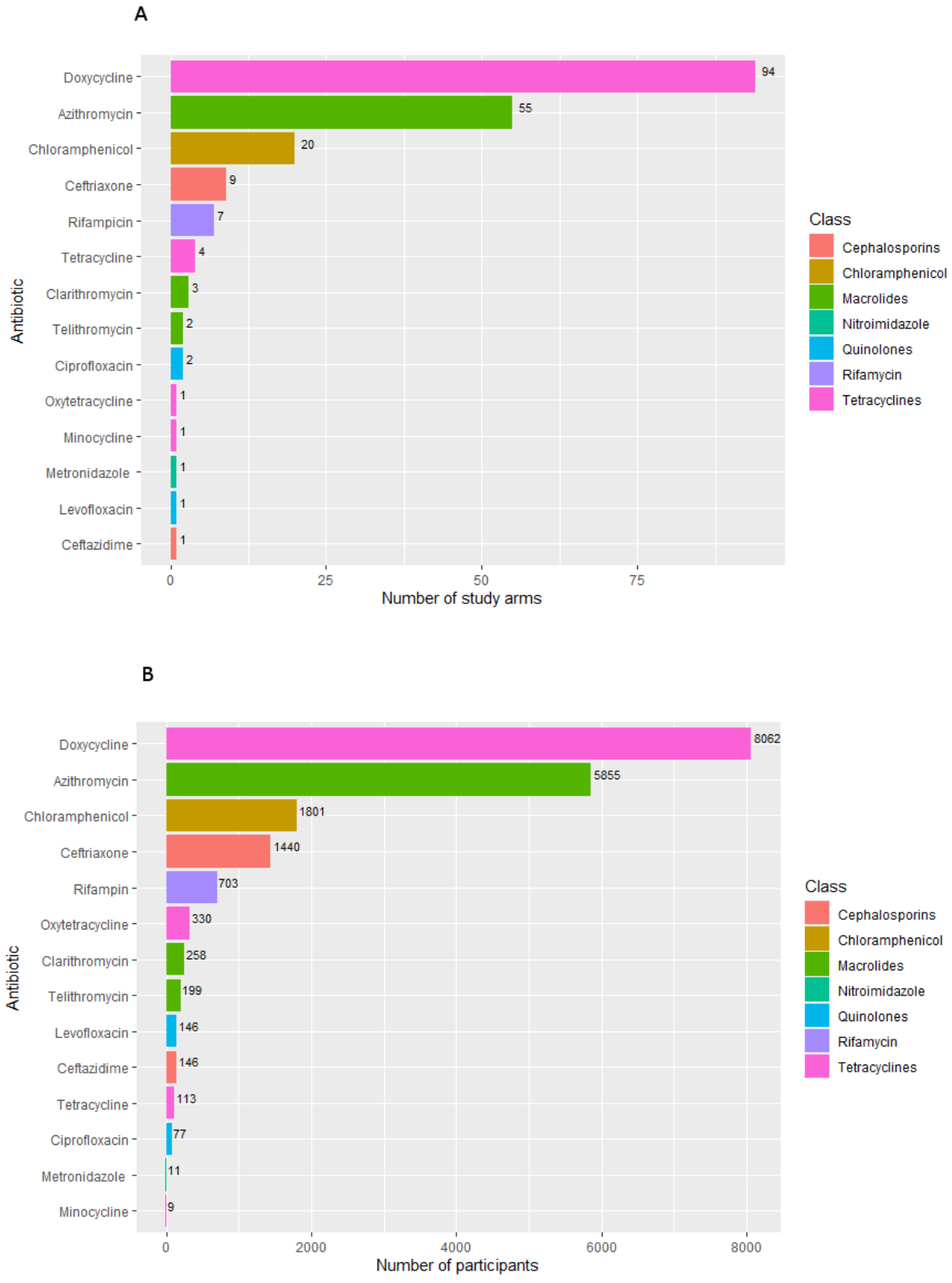


Figure 50. Number of study arms (A) and participants (B) within studies administering each antibiotic

The dosage regimen administered in the studies varied substantially. I identified 52 described combinations of drug, dose, and frequency. When the duration of antibiotic treatment was also considered, there were 66 unique combinations of drug, dose, frequency and duration. There were ten dosage regimens reported for doxycycline: one with loading dose and nine with no loading dose. Two study arms administered 200 mg doxycycline followed by a 7-day course of 100 mg twice daily or every 12 hours. Among the reported dosage regimens with no loading dose, the most often used doxycycline regimen was 100 mg twice daily (n = 23/35, 64%) (Figure 51). There were six dosage regimens reported for azithromycin: two with loading dose and four with no loading dose. One study arm used 20 mg/kg/dose loading dose, followed by 10 mg/kg/dose for two days. One study arm administered 1 g followed by 500 mg once daily for two days. Within the dosage regimens without loading dose, the most commonly administered azithromycin regimen was 500 mg once daily (n = 7/13, 54%) (Figure 51). For chloramphenicol, there were three identified dosage regimens (administered in one study arm each): 500 mg four times daily, 500–1,000 mg every six hours, and 25–50 mg/kg/day four times daily.

For pregnant participants, azithromycin 500 mg once daily was used in four studies. Two studies administered azithromycin for five days, one study administered it as a single dose, while one study did not mention the duration of treatment. In paediatric participants, antibiotics administered included doxycycline (4, 4.5, and 5 mg/kg/day), azithromycin (10 mg/kg/day), and chloramphenicol (100 mg/kg/day). The duration of treatment for paediatric participants was frequently unreported, but one study administered azithromycin 10 mg/kg/day for 7 days.

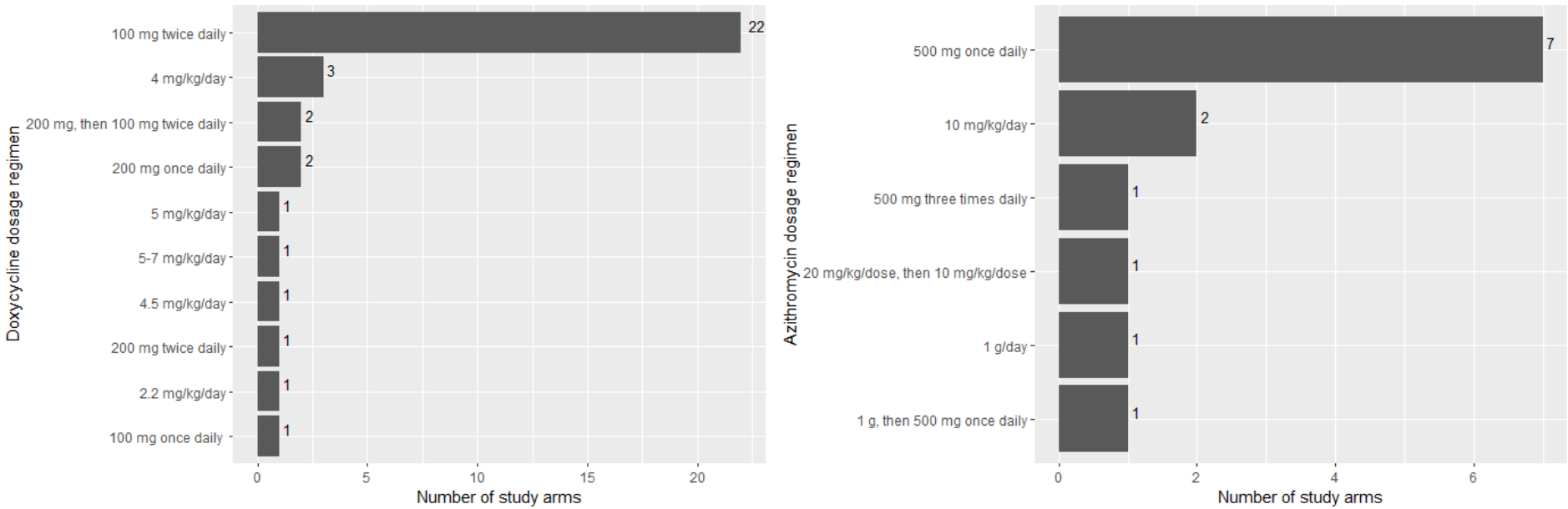


Figure 51. Number of study arms administering each doxycycline and azithromycin dosage regimen

Patient outcomes

Mortality, complications, adverse events and clinical responses were assessed in the majority of studies with 106 (10,794 participants), 99 (10,103 participants), 93 (9,688 participants), and 87 studies (7,895 participants) reporting these outcomes, respectively (Figure 52, Table 20). They were also the most commonly assessed outcomes among the observational studies with 100 (10,211 participants), 95 (9,770 participants), 87 (9,105 participants) and 80 (7,251 participants) studies, respectively. Conversely, clinical response, fever clearance, and fever clearance time were the most commonly assessed outcomes among interventional studies (7 studies each, 644 participants).

Table 20. Number of studies and participants within studies reporting each outcome

Outcome	Interventional studies		Observational studies		All studies	
	Number of studies	Number of participants	Number of studies	Number of participants	Number of studies	Number of participants
Mortality	6	583	100	10,211	106	1,0794
Complication	4	333	95	9,770	99	1,0103
Adverse events	6	583	87	9,105	93	9,688
Clinical response	7	644	80	7,251	87	7,895
Hospital stay	1	93	45	4,689	46	4,782
Fever clearance	7	644	41	3,554	48	4,198
Fever clearance time	7	644	40	3,543	47	4,187
Improvement of lab parameters	2	185	23	1,488	25	1,673
Clinical response besides fever clearance	0	0	9	767	9	767
Relapse	6	486	2	58	8	544
In vitro outcome	1	126	1	10	2	136
Imaging improvement	0	0	3	77	3	77
Pharmacokinetics	0	0	0	0	0	0

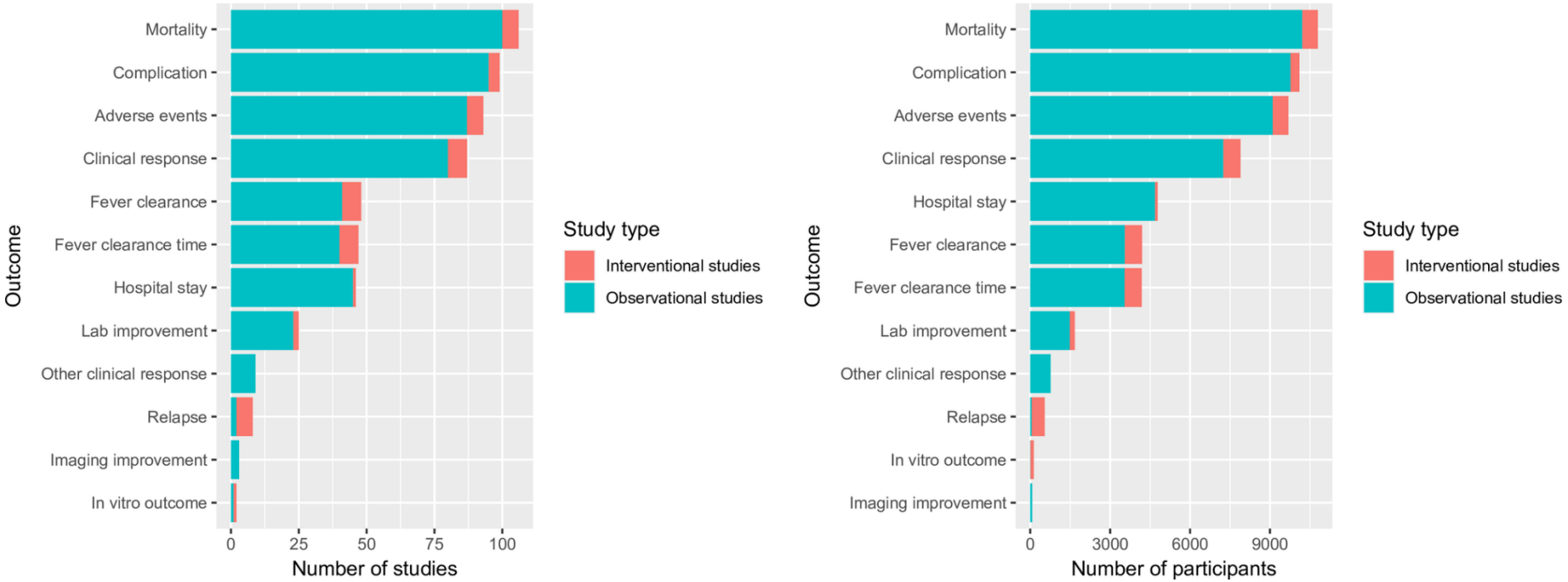


Figure 52. Number of studies and participants within studies assessing each outcome

The interventional studies used varying definitions of fever clearance time and relapse. Thirty-nine out of 47 studies recording fever clearance time (FCT) as an outcome did not specify the definition of FCT. There was substantial variability in the definition of FCT in the other eight studies concerning temperature cut-off, treatment, antibiotic, antipyretic, time (within 24-hour and 48-hour) and two times temperature measurement to confirm fever clearance (Appendix 7). Relapse was defined in two out of seven studies noting this outcome; both studies defined relapse as the reappearance of fever and other scrub typhus signs and symptoms within one month (30 days) in the absence of other causes.

Discussion

After screening 3,100 records, we identified and reviewed 127 scrub typhus treatment studies comprised of 141 study arms (referring both to a study arm from a clinical trial or a patient cohort from an observational study where treatment is administered) and 12,079 participants. All of these studies were conducted within the classical tsutsugamushi triangle. However, in concordance with the findings of another review on non-malarial febrile illness in South and South East Asia conducted by the Infectious Diseases Data Observatory (IDDO) [387], it is worth noting that even within the tsutsugamushi triangle, information is lacking – many countries did not have any data points, e.g. Myanmar and Pakistan. Substantial heterogeneity was observed in diagnosis methods, antibiotic treatment regimens, and observed outcomes.

Despite being one of the most commonly reported pathogens in South and South-East Asia [387], the review illustrates the limited number of interventional studies and very sparse data collected on specific groups such as children and pregnant women, reflecting that scrub typhus is indeed a neglected disease in terms of research to inform policy. Although scrub typhus predominantly affects rural regions, most of the studies were conducted in urban settings. This could be a reflection of the imbalanced location of research sites and/or healthcare facilities in rural and remote areas relative to urban areas – further highlighting the relatively limited access to healthcare of rural patients [388]. Participants from rural areas were included in about a fifth of the studies, though the majority of the studies did not mention explicitly where the participants lived. Many of the studies were identified to be from heath facilities in urban areas that could include patients seeking care there from rural areas, hence the location of studies does not necessarily reflect the locations of homes of patients nor where patients contracted scrub typhus. Other studies have also shown that scrub typhus trials have a high risk of bias as assessed by the Cochrane risk of bias tool [163, 169]. Most of the studies included were deemed to have high risk of performance and detection bias in association with blinding process [163, 169]. These findings illustrate specific knowledge gaps which prospective studies could cover.

However, if assembled, the existing data could answer important research questions through IPD meta-analyses. Mortality, adverse events, complications, and clinical response were captured by the majority of studies, raising the possibility that these studies could be combined and analysed. The creation of an IPD platform would allow some level of

standardisation between studies which would be critical to facilitate pooling of available data and account for the heterogeneity of these studies [389, 390].

To our knowledge, this is the first systematic review exploring the scrub typhus treatment study landscape that is focused on the methodology and characteristics of the studies. I conducted an extensive search in electronic databases and clinical trial registries. We also included observational studies to ensure a more comprehensive identification of IPD. We flagged ongoing studies to allow evaluation of what kind of data would be available in the future. Although no language restrictions were applied during the search, we could not extract data from articles in Korean and Chinese due to limited translation resources. This review only included peer-reviewed published studies. We also specified the search to start in 1998 due to the small probability of obtaining individual patient data from earlier studies [258]. This process might introduce selection bias, and there may be more IPD available in ongoing investigations and grey literature such as government surveillance data that were not identified in this search. Due to resources restrictions, only one reviewer screened the title and abstract. This might introduce classification error in the selection stage. However, we were able to deploy two reviewers in the full-text screening and data extraction stage.

This review highlights the heterogeneity in the methods and reporting of scrub typhus treatment studies. Bearing in mind that this could be influenced by the severity of cases and the local antibiotic sensitivity, there are various treatment regimens administered and outcomes observed. Although IFA (the gold standard) was the most common diagnostic method used among interventional studies, PCR and ELISA (diagnostic methods suitable for

high throughput with a more objective interpretation) showed an increase in their use over time. This might reflect a shift in the preferred diagnostic methods. Due to differences in definitions used, it would be challenging to conduct a useful aggregated data meta-analysis without strong assumptions and access to additional data and information. In this situation, curation and standardisation of IPD would help in pooling available data [389, 390].

In the studies we examined there were 7,835 participants treated with doxycycline, 5,970 with azithromycin, and 2,056 with chloramphenicol – which should be sufficient to estimate their efficacy and potential patient-level factors affecting the outcomes. Therefore, the development of an IPD platform to facilitate the closing of this gap in knowledge is valuable and feasible with the amount of data documented so far.

To optimise the value and utility of data gathered through clinical research, it would be valuable to define a set of data and design standards for RCTs and observational studies to facilitate comparisons across studies. Standards of data collected should include definition of key variables, study design, diagnostic methods, and endpoints. More importantly, these standards need to be implemented consistently. Furthermore, it is essential to adhere to reporting guidelines such as the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) and CONSORT (Consolidated Standards of Reporting Trials) checklists during design and publication [391, 392].

The Clinical Data Interchange Standards Consortium (CDISC) develops data standards to ensure clarity and maximise the interoperability and reusability of data, consequently improving data value and impact. It is required by many regulatory authorities and leading research organisations [393]. This would provide a framework for collecting, organising, analysing, and sharing data. This framework, complemented by consensus on outcomes, can ensure comparability and interoperability of data. The COMET (Core Outcome Measures in Effectiveness Trials) Initiative has compiled resources to develop and apply core outcome sets (COS) for clinical trials [394]. COS is 'an agreed standardised set of outcomes that should be measured and reported, as a minimum, in all clinical trials in specific areas of health or health care' [394]. Such an approach is needed in scrub typhus clinical trials and observational studies to facilitate meta-analysis and comparisons between studies. Concerted efforts from stakeholders will be needed to manifest this.

In the future, further quality assessments of clinical trials may be useful to identify the source of heterogeneity and identify any barriers to develop more standardised methods and reporting. Formulation of standardised definition, procedures to assess treatment efficacy, and reporting through a consensus-based approach within the research community is an important next step. For data sharing to become normalised, mechanisms need to be put in place to ensure a mutually beneficial relationship between people who re-use the data and those who collect them [390]. Engagement and cooperation with the scrub typhus research community and investment in data infrastructure to implement standards and facilitate data sharing and meta-analysis are of utmost importance to establish an IPD platform.

A bibliographic analysis on the relevant papers could also be helpful to identify the researchers and groups/centres with substantial IPDs, which would be useful during stakeholder engagement.

One way to increase awareness of scrub typhus is its recognition as an NTD by the WHO. Scrub typhus fulfils three out of the four WHO suggested criteria for NTD [9]: (1) it causes 'important morbidity and mortality' particularly in rural population with a higher rate of poverty and less access to health care facilities, (2) affecting people in tropical and subtropical areas, and (3) 'neglected by research'. The inclusion of scrub typhus would prompt support for advocacy and development of improved diagnostics tools, treatment, and control strategies [9]. When 'effective and feasible' control strategies are available, scrub typhus can fulfil the last criteria – (4) a disease that can be immediately controlled with public health interventions [9, 395].

There is still considerable uncertainty about the optimal treatment for scrub typhus, reflected by the highly heterogeneous set of treatment regimens used in the studies reviewed over the last 22 years. Despite the low number of scrub typhus treatment trials published, there is likely a reasonable amount of data available with key information to address this uncertainty. This review further supports the case for the development of a scrub typhus IPD platform and we deemed that such endeavour would be feasible with stakeholders' cooperation and funding. This will facilitate pooled analysis of treatment data that will in turn, inform clinical practice and disease control measures. Stakeholder engagement and cooperation will be crucial to the establishment of a scrub typhus IPD platform.

Chapter 7. Scrub typhus point-of-care testing: A systematic review and meta-analysis

Introduction

Scrub typhus is difficult to diagnose clinically due the lack of specific clinical features that can differentiate it from other acute febrile illness common in the endemic region such as dengue and typhoid fever. Laboratory tests play a major role in diagnosing scrub typhus. Scrub typhus is prevalent in rural areas of low- and middle-income countries. Therefore, rapid, accurate, and affordable point-of-care tests would be invaluable to provide early diagnosis and prompt treatment in these resource-limited settings. Failure in diagnosing scrub typhus may result in prolonged illness, complications including pneumonitis, acute respiratory distress syndrome, renal failure, meningoencephalitis, and unnecessary treatment with inappropriate antibiotics [2, 132, 396].

Serology remains the mainstay of diagnosis. The immunofluorescent assay (IFA) and immunoperoxidase test (IIP) are considered imperfect gold standards, in view of their limitations which include high expense, requirement for substantial training to perform, inter-operator variability in result interpretation, and the often-retrospective nature of diagnosis that does not help in directing treatment [2, 124, 132, 148, 397]. Another antibody detection method, the enzyme-linked immunosorbent assay (ELISA) has been developed and shown to have both sensitivity and specificity of greater than 90.0%; however, this is highly dependent on endemicity and the application of a previously investigated and geographically-based cut-

off [2, 142]. Besides antibody-based diagnostics, molecular detection methods, including using the polymerase chain reaction (PCR) to detect various genes targets (e.g. 47 kDa, 56 kDa, *groEL*, 16S rRNA genes) have also been developed, however they have limitations in terms of diagnostic sensitivity due to the limited period of rickettsaemia [2, 124, 132, 397]. PCR is still deemed impractical in resource-limited endemic areas because it requires considerable training and expense [2, 124, 132, 397]. The bacteria can be isolated through *in vitro* and *in vivo* cultivation methods, such as cell culture and mouse inoculation, respectively [2, 132]. These methods need considerable training, biosafety level 3 (BSL 3) laboratory containment facilities for large-scale propagation, and usually take several weeks which contributed to the retrospective nature of the diagnosis [2, 132].

Therefore, there is clearly a need for affordable point-of-care testing (POCT) for scrub typhus diagnosis in endemic settings with resource constraints. There are varied definitions of POCT, but fundamentally POCT should provide quick results to inform patient management and be convenient enough to be performed close to the patient (i.e. not in a central laboratory) [398, 399]. POCT can potentially play an essential role in providing early diagnosis and prompt treatment. However, there was no study exploring the diagnostic value of it yet.

Immunochromatographic tests (ICTs), dot-blot, and loop-mediated isothermal amplification (LAMP) assays all have the principal qualities of POCT. ICTs and dot-blot tests have the same inherent problems of IFA as serology-based tests (e.g. the retrospective nature of diagnosis in cases where diagnosis relies on convalescent samples and delicate cut-off setting), while offering more simplicity and speed [2, 132]. LAMP is an alternative technique which involves

amplification and detection of bacterial DNA. Similar in principle to conventional PCR assays, LAMP assay does not require intricate DNA extraction, a thermocycler, or special equipment to read the result [132, 151, 154].

This study aims to summarise the existing evidence on the accuracy of scrub typhus POCTs to inform clinical practitioners of their diagnostic value when providing care in resource-limited settings where scrub typhus is endemic.

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Methods

Eligibility criteria

I included articles on POCTs that would be feasible in primary health care provider or outpatient settings. Only articles published in English were included. To ensure feasibility in resource-limited settings, I excluded studies evaluating methods which were inherently more complicated, requiring relatively high levels of expertise and/or specialised equipment. I only included articles on POCTs performed on human samples. Studies on the Weil-Felix test were excluded due to its established poor diagnostic accuracy and the lengthy time required to perform [132, 400]. For meta-analysis and meta-regression, the studies must have an extractable diagnostic accuracy 2 by 2 table containing true positive, true negative, false positive, and false negative values.

Search strategy

After a preliminary search, I searched PubMed for articles published up to 2 February 2017, combining the search terms ‘scrub typhus’, ‘immunochromatography’, ‘dot blot immunoassay’, and ‘loop mediated isothermal amplification’ without any other restrictions i.e., "scrub typhus" AND (rapid diagnosis OR immunochromatograph* OR dot blot immunoassay OR loop mediated isothermal amplification). I screened titles and abstracts of the articles and reviewed the full text of relevant articles. In addition, manual screening of the reference list of relevant articles was also performed.

Quality assessment

To assess the quality of the studies, I used the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) [401]. QUADAS-2 can be used as a quality scoring system to determine the risk of bias and the applicability of the article [401]. It evaluates four main areas: ‘patient selection’, ‘index test’, ‘reference standard’, and ‘flow and timing’ [401]. These are assessed by using seven ‘signalling questions’ (e.g., ‘was a case-control design avoided?’) with ‘yes’, ‘no’, and ‘unclear’ answer [401]. The answers to these ‘signalling questions’ were then used to judge whether the risk of bias is low and if there is low concern for the applicability of the research [401]. If the response to the risk of bias and applicability questions were ‘low risk’ or ‘low concern’, the articles were given one point each. The articles were grouped based on their score into high (6–7 points), moderate (4–5 points), and low (0–3 points) quality categories.

Data extraction

I extracted the data from the articles, and where the results were unclear, Prof Stuart Blacksell was consulted. The data was recorded on a form developed through an iterative process to ensure that all the required data could be collected for future reference. The parameters extracted include: citation information, methodology (i.e., study design, participant characteristics, index and reference test details), and the diagnostic accuracy results (including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and if available, numbers required to construct a 2 by 2 contingency table.

Statistical data analysis and reporting

The extracted data were compiled into summary tables and analysed through narrative synthesis. I performed meta-analysis and meta-regression on the commercially available POCT diagnostic accuracy data, excluding studies with low quality (i.e. QUADAS-2 score of 3 or less). Diagnostic tests still in development stage or in the form of prototype were not included in the meta-analysis and meta-regression, but included in the narrative synthesis. If one study derived more than one 2 by 2 table, each table was extracted as separate data entries. However, if one study used more than one reference test cut-off titre, only data using one cut-off value above 1:3,200 were used to ensure accuracy [124]. In performing the meta-regression, relevant signalling questions with 'unclear' as the answers were entered as 'no' to turn these into dichotomous variables. Statistical analysis was done with STATA/IC 14.0 (College Station, TX) using MIDAS and METANDI commands. In the meta-analyses, heterogeneity was assessed using the Chi-square statistic, higher values of the Chi-square (and hence low p-value) being consistent with heterogeneity. In the summary statistics of the

resulting forest plot, overall sensitivity and specificity were estimated and reported alongside the 95% confidence interval. In addition, study specific estimates were provided in the same plot to visualize how the estimates from each of the studies deviate from the overall estimate. Most of the results were presented graphically. The data were analysed, summarised, and presented following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement as much as possible (Appendix 8) [232]. This review was registered in the International Prospective Register for Systematic Review (PROSPERO) with registration number CRD42017056727.

Results

Search results

There were 133 articles in total identified through database searching and reference list screening (Figure 53). After title and abstract screening and full text review, I included 31 relevant articles. There were six articles excluded after full text review. Since this study only focused on human diagnostics, I excluded one article using rabbit sera in its negative sera panel. One study involved DNA extraction, which is not applicable as a POCT in resource-limited settings. Four other papers were excluded due to language (not in English, n = 2) and study design (not experimental diagnostic accuracy studies, n = 2). There were 20 articles on ICTs, eight articles on dot-blot assays, four articles on LAMP assays, and one each on a passive hemagglutination assay, an IgM dot immunobinding assay, and a latex agglutination test. Four articles evaluated more than one type of diagnostic test. There were 11 studies that evaluated diagnostic tests still in development and 21 studies on prototype/commercial tests.

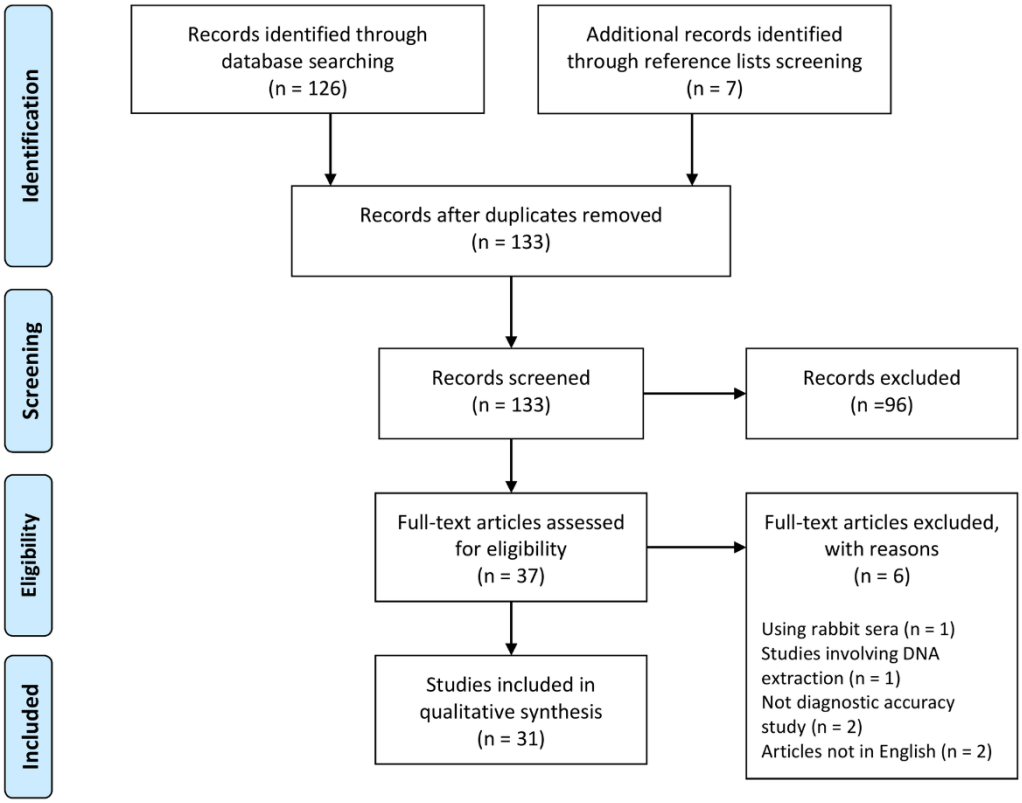


Figure 53. PRISMA flow diagram

Characteristics of the included studies

In total, there were 6,772 samples analysed. The samples were taken from 12 countries, with most studies recruiting in Thailand (n = 15, 48.4% of included studies), India (n = 5, 16.1%), Laos (n = 3, 9.7%), and Korea (n = 3, 9.7%). There was one study each (3.2%) conducted on samples from Sri Lanka, Nepal, Malaysia, Peru, Indonesia, United States of America, and Australia. Five studies (16.1%) have unclear sample collection location. Ten (32.2%) studies collected paired samples (acute and convalescent phase), while 15 studies (48.4%) did not provide sufficient details on sample collection timing (Table 21).

Table 21. Summary of included studies

First author	Year	Sample collection location	Index test assay type	Reference test assay type	QUADAS-2 score
Anitharaj et al [402]	2016	India	ICT IgM	ELISA	5
Blacksell et al [403]	2010	Laos	ICT IgM	IFA	6
Blacksell et al [404]	2010	Laos and Thailand	ICT IgM; ICT IgG, IgM	IFA and/or PCR and/or culture	5
Blacksell et al [154]	2012	Thailand	ICT IgM; ICT IgG, IgM; LAMP	STIC (cell culture isolation, PCR, IFA)	6
Cao et al [405]	2007	China	ICT IgG, IgM; ICT IgG; ICT IgM	Unclear	2
Ching et al [406]	2001	Unclear	ICT IgG, IgM; ICT IgG; ICT IgM	IFA	2
Chinprasatsak et al [407]	2001	Thailand	Dot-blot IgG, IgM	IIP	6
Coleman et al [408]	2002	Thailand	Dot-blot IgG, dot-blot IgM, ICT IgG, ICT IgM	IIP	7
Huber et al [152]	2012	Unclear	LAMP	PCR	2
Kim et al [409]	1993	Unclear	Passive hemagglutination assay	IFA	3
Kim et al [410]	2013	Korea	ICT IgG, IgM; dot-blot IgG	Unclear	2
Kim et al [411]	2016	Korea, Sri Lanka, India	ICT IgG, IgM	IFA	3
Kingston et al [412]	2015	Thailand, Nepal	ICT IgM PAb, MAb	IFA	6
Koay et al [413]	1995	Unclear	IgM dot-immunobinding assay	IIP	3
Lee et al [414]	2014	Korea	ICT IgG, IgM, IgA	IFA	4
Paris et al [153]	2008	Thailand, Laos	LAMP	In vitro isolates, IFA, PCR, ICT IgG & IgM	3
Paris et al [151]	2011	Thailand	ICT IgM, LAMP	STIC (Cell culture isolation, PCR, IFA)	6

First author	Year	Sample collection location	Index test assay type	Reference test assay type	QUADAS-2 score
Pradutkanchana et al [415]	1997	Thailand	Dot-blot IgG, IgM	IFA	4
Prakash et al [416]	2006	India	Dot-blot IgG, IgM	Dot EIA, ELISA, clinical response to doxycycline or chloramphenicol	3
Ramyasree et al [417]	2015	Unclear	ICT IgM	ELISA	5
Rodkvamtook et al [418]	2015	Thailand	Dot-blot IgG, IgM	IFA	5
Silpasakorn et al [419]	2012	Thailand	ICT IgM, ICT IgG	PCR & IFA	4
Silpasakorn et al [420]	2012	Thailand	ICT IgG, IgM, IgA	PCR & IFA	3
Stephen et al [421]	2015	India	ICT IgG, IgM, IgA	ELISA IgM, IgG	5
Stephen et al [422]	2016	India	ICT IgG, ICT IgM	IFA	5
Watt et al [423]	1998	Thailand	Dot-blot IgG, IgM	IFA	5
Watthanaworawit et al [424]	2015	Thailand	ICT IgM; IgG, IgM, IgA	PCR & IFA	5
Weddle et al [425]	1995	Malaysia, Peru, Indonesia, USA	Dot-blot immunoassay IgG, IgM	IFA	4
Wilkinson et al [426]	2003	Thailand, Australia	ICT IgG, ICT IgM	Unclear	2
Wongchotigul et al [427]	2005	Thailand	Latex agglutination test	IFA	4
Zhang et al [428]	2011	China	InBios ICT IgG, ICT IgM	IgM and IgG antibodies detection (tests unclear) and PCR	4

Quality of articles

There were 7, 14, and 10 articles with good, moderate, and low quality, respectively (Table 21). Two articles fulfilled all of the QUADAS-2 main criteria (Appendix 9). Most of the articles (n = 8, 25.8%) scored 5 points. Two articles (6.5%) scored full points for the risk of bias component (i.e. 4 points), although most of the articles scored only 1 point (n = 12, 38.7%). The majority of the articles scored 3 points in the applicability component (n = 22, 71.0%). There were 24.4% (n = 53/217) sub-questions with 'unclear' response. Among the 'unclear' responses, most (n = 45, 84.9%) were observed in the index test and reference standard domain of the risk of bias assessment (Figure 54). The main reason for this is that most of the articles did not mention explicitly whether they performed blinding during the conduct and interpretation of both the index test (n = 23, 74.2%) and reference test (n = 25, 80.6%) (Supplement 9). There were 20 studies (64.5%) with a case-control study design.

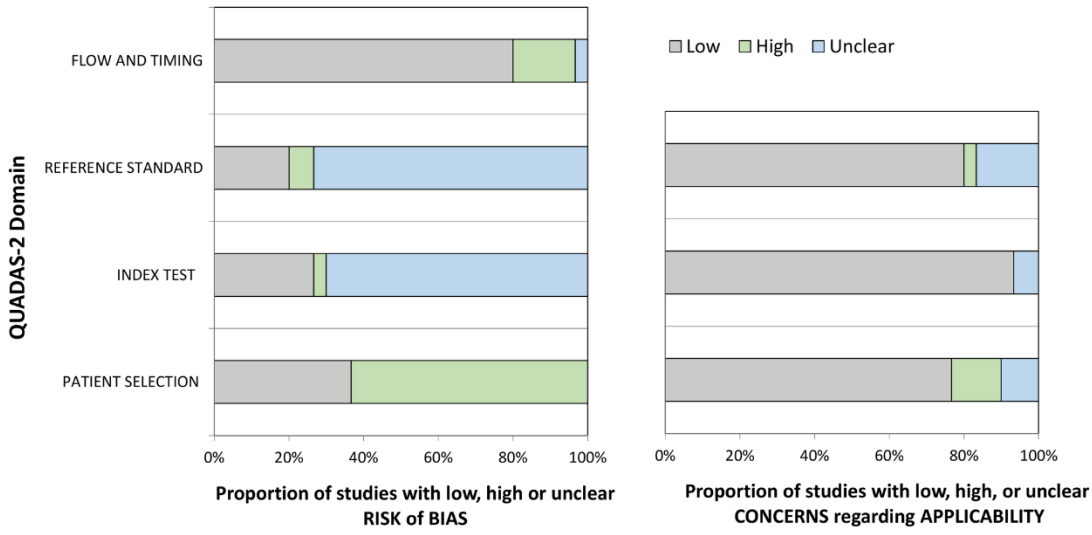


Figure 54. QUADAS-2 finding per domain

Performance of POCT

IgM ICT

There were five manufacturers of IgM ICT identified (Table 22), namely: AccessBio, InBios, PanBio, Standard Diagnostic (SD), and ImmuneMed. There were two studies (6.6%) that assessed in-house tests [405, 406]. The sensitivity ranged from 23.3% to 100.0%, and the specificity ranged from 73.0% to 100.0% (Figure 55), results from Zhang et al, 2011 and Lee et al, 2014 were not plotted since only sensitivity values were presented [414, 428]). The accuracy across data points of the same manufacturers varied across the studies. The InBios IgM ICT tests reported >80.0% sensitivity and >90.0% specificity on average. The ImmuneMed IgM ICT demonstrated sensitivity of 99.0% and specificity of 98.0%, however, there was only one data point for this manufacturer. One of the studies reported sensitivity and specificity for the AccessBio ICT IgM of 97.0% and 93.0%, respectively. However, the other AccessBio studies did not demonstrate such a high degree of accuracy (Figure 55).

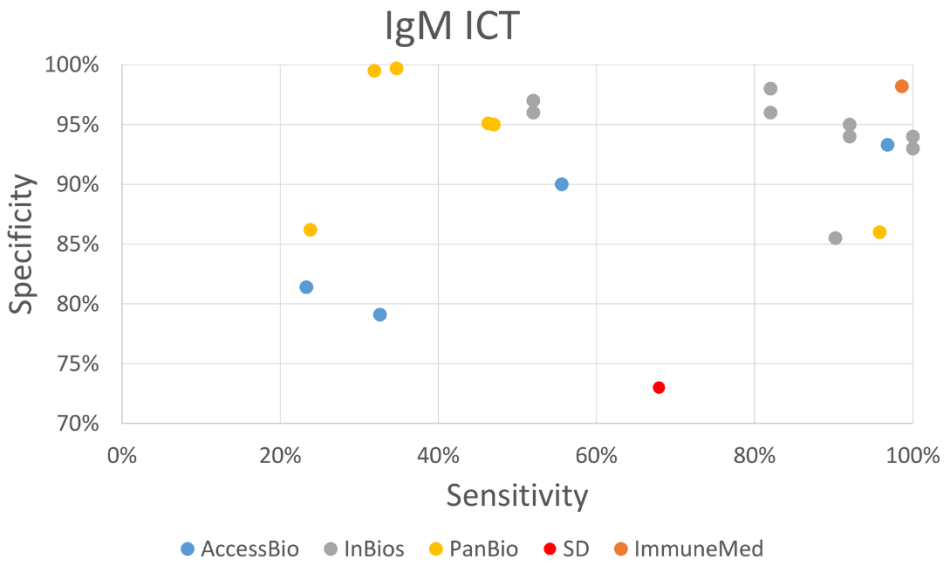


Figure 55. IgM ICT sensitivity and specificity range

Results from Zhang et al, 2011 and Lee et al, 2014 were not plotted since only sensitivity values were presented [414, 428]

Table 22. Summary of ICT IgM

Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
CareStart, AccessBio	Blacksell et al [404]	2010	Laos and Thailand	Acute phase	IFA and/or PCR and/or culture	IFA: 4-fold rise in paired serum samples	96.8/93.3
	Blacksell et al [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC	IFA: an admission IgM titre of 1:12,800, and/or a 4-fold rising IgM titre in paired serum samples	55.6/90.0
	Watthanaworawit et al [424]	2015	Thailand	Acute (median 2 days of fever, IQR: 2–3 days) and convalescent phase (median interval to convalescent sample collection was 14 days, range: 11–30 days).	PCR & IFA	≥ 4-fold increase in IFA IgM titer, 1:25,600	Acute samples: 23.3/81.4 Paired samples: 32.6/79.1
ImmuneMed	Stephen et al [422]	2016	India	Acute phase with partly paired samples	IFA	1:40	87.0/94.6
InBios	Zhang et al [428]	2011	China	Acute phase: 2 to 10 days after onset	IgM and IgG antibodies detection (unclear) and PCR	Unclear	Sensitivity 93.9%

Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
	Silpasakorn et al [419]	2012	Thailand	Acute and convalescent phase	PCR & IFA	IFA: IgM 1:400 or a 4-fold increase	90.2/85.5
InBios prototype	Kingston et al [412]	2015	Thailand, Nepal	Unclear	IFA	1:400	PAb: 52.0/97.0
						1:400	MAb: 52.0/96.0
						1:1,600	PAb: 82.0/98.0
						1:1,600	MAb: 82.0/96.0
						1:6,400	PAb: 92.0/95.0
						1:6,400	MAb: 92.0/94.0
						1:25,600	PAb: 100.0/94.0
						1:25,600	MAb: 100.0/93.0
PanBio	Blacksell et al [403]	2010	Laos	Admission phase: median 6 days of fever (IQR = 5-10 days); median interval to convalescent sampling: 7 days (IQR = 5-10 days)	IFA	≥ 1:400	23.8/86.2
						4-fold increase in paired samples	31.9/99.5
						Combination of the above	34.7/99.7
	Paris et al [151]	2011	Thailand	Mostly paired samples. Median fever before admission = 5 days (IQR: 3-7)	STIC	IFA: an admission IgM titer ≥1:12,800 and/or a 4-fold increase	47.0/95.0

Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
	Blacksell et al [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC	IFA: an admission IgM titer ≥1:12,800 and/or a 4-fold increase	46.3/95.1
PanBio prototype	Wilkinson et al [426]	2003	Thailand, Australia	Unclear	Unclear	Unclear	95.8/86.0
SD Bioline Tsutsugamushi	Blacksell et al [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC	IFA: an admission IgM titer ≥1:12,800 and/or a 4-fold increase	67.9/73.0
	Ramyasree et al [417]	2015	India?	Unclear	ELISA	Unclear	Agreement 97%
In-house tests	Ching et al [406]	2001	Unclear	Unclear	IFA	>1:40	74.0/99.0
	Cao et al [405]	2007	China	Unclear	Unclear	Unclear	81.2%/100.0

Total antibody ICT

The ICTs with IgG, combination of IgG and IgM, and combination of IgG, IgM, and IgA as the detection target were grouped together under 'total antibody ICT' (Table 23). There were five manufacturers identified, namely: AccessBio, ImmuneMed, InBios, PanBio, and SD. The remaining studies (n = 3, 6.6%) assessed the diagnostic performance of in-house tests in development. The sensitivity and specificity ranged from 20.9% to 99.1% and 67.9% to 100.0%, respectively (Figure 56), results from and Zhang et al, 2011 and Lee et al, 2014 were not plotted since only sensitivity values were presented [414, 428]). As in the case of IgM ICT, the accuracy across data points of total antibody ICT of the same manufacturer varies. The ImmuneMed total antibody ICT demonstrated >95.0% specificity and >80.0% sensitivity.

Table 23. Summary table of total antibody ICT

Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
CareStart, AccessBio	Blacksell et al[a] [404]	2010	Laos and Thailand	Acute phase	IFA and/or PCR and/or culture	IFA: 4-fold rise in paired serum samples	97.6/71.4
	Blacksell et al[a] [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC	IFA: an admission IgM titre of 1:12,800, and/or a 4-fold rising IgM titre in paired serum samples	60.5/67.9
ImmuneMed	Kim et al[a] [410]	2013	Korea	Unclear	Unclear	Unclear	99.1/98.9
	Kim et al[b] [411]	2016	Korea[d]	Unclear	IFA IgM	1:10	98.6/98.2
					IFA IgG	1:40	97.1/97.7
			Korea[e]	Unclear	IFA IgM	1:10	98.6/97.6
					IFA IgG	1:40	97.1/97.0
			Sri Lanka	Unclear	IFA	Unclear	92.1/96.1
			India	Unclear	IFA IgM	1:10	Sensitivity 86.0%
					IFA IgG	1:40	Sensitivity 92.0%
	Stephen et al[b] [422]	2016	India	Acute and convalescent phase	IFA	1:80	77.3/86.4

Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
InBios	Zhang et al[b] [428]	2011	China	Acute phase: 2 to 10 days after onset of illness	IgM and IgG antibodies detection (unclear) and PCR	Unclear	Sensitivity 90.9%
	Silpasakorn et al[a] [419]	2012	Thailand	Acute and convalescent phase	PCR & IFA	IFA: IgG 1:800 or a 4-fold increase	66.7%/98.4
PanBio	Coleman et al[b] [408]	2002	Thailand	Acute phase	IIP	1:400	90.4/95.8
Panbio prototype	Wilkinson et al[b] [426]	2003	Thailand, Australia	Unclear	Unclear	Unclear	96.1/93.0
SD Bioline Tsutsugamushi***	Silpasakorn et al[c] [420]	2012	Thailand	Acute phase: median 6 days of fever (range 1-47 days); median interval between obtaining admission and convalescence phase: 13 days (range 3-32 days)	PCR & IFA	IFA: IgM or IgG IFA assay titre > 1:400 or a 4-fold increase	66.7/98.4
	Lee et al[c] [414]	2014	Korea	Unclear	IFA	4-fold rise or single titre ≥ 1:160	Sensitivity 72.6%
	Stephen et al[c] [421]	2015	India	Acute and convalescence phase	ELISA IgM	Unclear	91.7/90.5
					ELISA IgG	Unclear	85.7/100.0
	Watthanaworawit et al[c] [424]	2015	Thailand	Median fever = 2 days (IQR: 2–3 days), and	PCR & IFA		Acute samples: 20.9/74.4

Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
				the median interval between obtaining initial acute-phase specimens and convalescent specimens was 14 days (range: 11–30 days).		≥ 4-fold increase in IFA IgM titre, 1:25,600	Paired samples: 76.7/76.7
In-house tests	Ching et al[a] [406]	2001	Unclear	Unclear	IFA	>1:40	IgG: 86.0/97.0
							IgG, IgM: 89.0/97.0
	Cao et al[a] [405]	2007	China	Unclear	Unclear	Unclear	IgG: 94.6/98.9
							IgG, IgM: 98.2/98.1

[a]Total antibody: IgG & IgM

[b]IgG

[c]IgG, IgM, IgA

[d]Sera from healthy participants were used as control

[e]Sera from participants with diseases other than scrub typhus were used as control

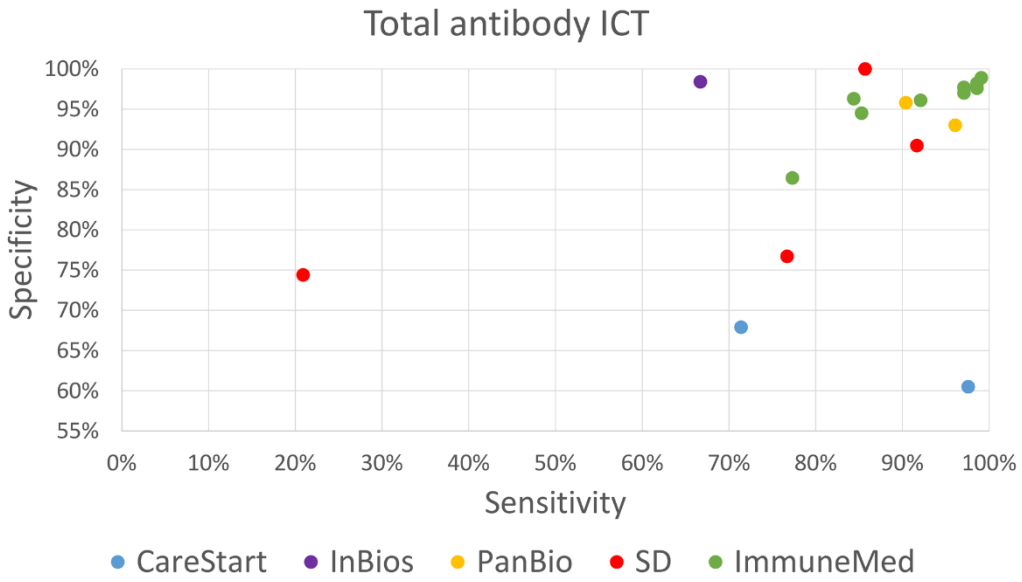


Figure 56. Total antibody ICT sensitivity and specificity range

Results from and Zhang et al, 2011 and Lee et al, 2014 were not plotted since only sensitivity values were presented [414, 428]

Dot-blot

Aside from the in-house tests assessed by four studies, there were two dot-blot assay manufacturers, namely Integrated Diagnostics and PanBio. The range was 59.6% to 100.0% and 83.0% to 98.7% for sensitivity and specificity, respectively (Table 24).

LAMP

Two studies assessed the Loopamp LAMP kit (Eiken Chemical, Japan) (Table 24). The other two studies evaluated in-house tests. The sensitivity ranged from 66.7% to 100.0% and specificity ranged from 63.2% to 100.0%.

Other methods

There was one study each on a passive hemagglutination assay, an IgM dot-immunobinding assay, and a latex agglutination assay (Table 24). These tests were all in the development phase.

Table 24. Summary of articles on dot-blot, LAMP and other assays

Assay type	Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
Dot-blot	Dip-S-Ticks, Integrated Diagnostics	Pradutkanchana et al [415]	1997	Thailand	Unclear	IFA	≥1:400 or a 4-fold or greater rise in IFA titre to at least 1:200	94.0/98.7
		Chinprasatsak et al [407]	2001	Thailand	Acute and convalescent phase	IIP	'Four-fold or greater rise in IIP titre to at least 1:200, or a single IgM antibody titre of ≥ 1:400 and/or IgG antibody titre of ≥ 1:1,600.'	86.7/94.3
		Coleman et al [408]	2002	Thailand	Acute phase	IIP	1:400	IgM: 60.3/97.4 IgG: 59.6/95.3
	Rickettsia Screen, PanBio	Prakash et al [416]	2006	India	Acute phase	Dot EIA, ELISA, clinical response to doxycycline or chloramphenicol	ELISA: ≥16 PanBio units	100.0/93.5
	In-house	Weddle et al [425]	1995	Malaysia, Peru,	Acute phase, unclear for negative panel	IFA	<1:64, >1:128, if in between	90.0/83.0

Assay type	Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
				Indonesia, USA			depends on clinical condition	
		Watt et al [423]	1998	Thailand	High-antigen dipstick: median = 4 days of fever (range = 2-14)	IFA	IgG 1:1,600 and/or IgM 1:400	67.0/98.0
					Low-antigen dipstick: median = 4 days of fever (range = 2-30)	IFA	IgG 1:1,600 and/or IgM 1:400	100.0/98.0
		Kim et al [410]	2013	Korea	Unclear	Unclear	Unclear, only IgG tested	97.7/98.6
		Rodkvamtook et al [418]	2015	Thailand	Acute phase: fever no more than 2 weeks;	IFA	1:400 or 4-fold increase for IgG & IgM	98.5/96.3
					Paired sample. Convalescent phase: collected after 3-14 days after the acute sample.	IFA	1:400 or 4-fold increase for IgG & IgM	98.3/97.0
LAMP	Loopamp kit, Eiken Chemical LAMP	Paris et al [153]	2008	Thailand, Laos	Acute phase for positive panel	IFA	4-fold rise or single titre ≥ 1:160	Sensitivity 72.6%
		Paris et al [151]	2011	Thailand	Mostly paired samples. Median fever before admission = 5 days (IQR: 3-7)	In vitro isolates, IFA, PCR, ICT IgG & IgM	1:400 and four-fold rise of paired serum for IgM IFA	100.0/100.0

Assay type	Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
	In-house LAMP	Huber et al [152]	2012	Not stated	Not stated in detail	PCR	N/A	90.0/80.0
	Combined Panbio ICT IgM and in-house LAMP	Blacksell et al [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC (cell culture isolation, PCR, IFA)	IFA: an admission IgM titre of 1:12,800, and/or a 4-fold rising IgM titre in paired samples	66.7/90.6
	Combined SD ICT IgM and in-house LAMP	Blacksell et al [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC (cell culture isolation, PCR, IFA)	IFA: an admission IgM titre of 1:12,800, and/or a 4-fold rising IgM titre in paired samples	77.2/68.2
	Combined AccessBio ICT IgG, IgM and in-house LAMP	Blacksell et al [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC (cell culture isolation, PCR, IFA)	IFA: an admission IgM titre of 1:12,800, and/or a 4-fold rising IgM titre in paired samples	68.5/84.9
	Combined AccessBio ICT IgM and in-house LAMP	Blacksell et al [154]	2012	Thailand	Acute (median 5 days of fever, IQR 3 to 7 days) and convalescent phase	STIC (cell culture isolation, PCR, IFA)	IFA: an admission IgM titre of 1:12,800, and/or a 4-fold rising IgM titre in paired samples	71.6/63.2

Assay type	Assay	Study	Year	Location of sample collection	Sample collection timing	Reference test assay type	Reference assay positivity cut-off	Sensitivity (%)/ specificity (%)
Others	Passive hemagglutination assay	Kim et al [409]	1993	Unclear	Unclear	IFA	Seroconversion or 4-fold rise	99.0/98.9
	IgM dot-immunobinding assay	Koay et al [413]	1995	Unclear	Unclear	IIP	1:50	90.4/81.4
	Latex agglutination assay	Wongchotigul et al [427]	2005	Thailand	Unclear	IFA	1:400	89.1/98.2

Meta-analysis results

There were 11 data points extracted from four studies included in the meta-analysis (Figure 57). In the resulting forest plot (Figure 57), the top three data were extracted from studies assessing total antibody ICT (Blacksell et al, 2010 and Watthanaworawit et al, 2015) [404, 424]. The rest of the 2 by 2 table data were extracted from studies assessing IgM ICT diagnostic performance. The pooled sensitivity and specificity were 66.0% (95% CI 0.37 – 0.86) and 92.0% (95% CI 0.83 – 0.97), respectively. The overall Chi-square heterogeneity statistics showed significant heterogeneity ($p < 0.001$). There was a high degree of heterogeneity present (I^2 value = 97.48%, 95% CI 96.71 – 98.24 for sensitivity and I^2 value = 98.17%, 95% CI 97.67 – 98.67 for specificity). Meta-regression on several covariates was performed in an attempt to explain this heterogeneity. Significant heterogeneity was observed for total number of samples ($p = 0.01$), study design (whether using case-control design or not, $p = 0.01$), blinding during index test interpretation ($p = 0.02$), and QUADAS-2 score ($p = 0.01$). No significant heterogeneity was observed for the blinding during reference test interpretation ($p = 0.21$) and antibody target detection of the tests ($p = 0.22$). All of these studies used IFA as their reference standard, except Blacksell et al, 2010 which used IFA with the addition of PCR and culture [404]. None of the meta-analysed studies used an IFA cut off lower than 1:400 as the reference comparator.

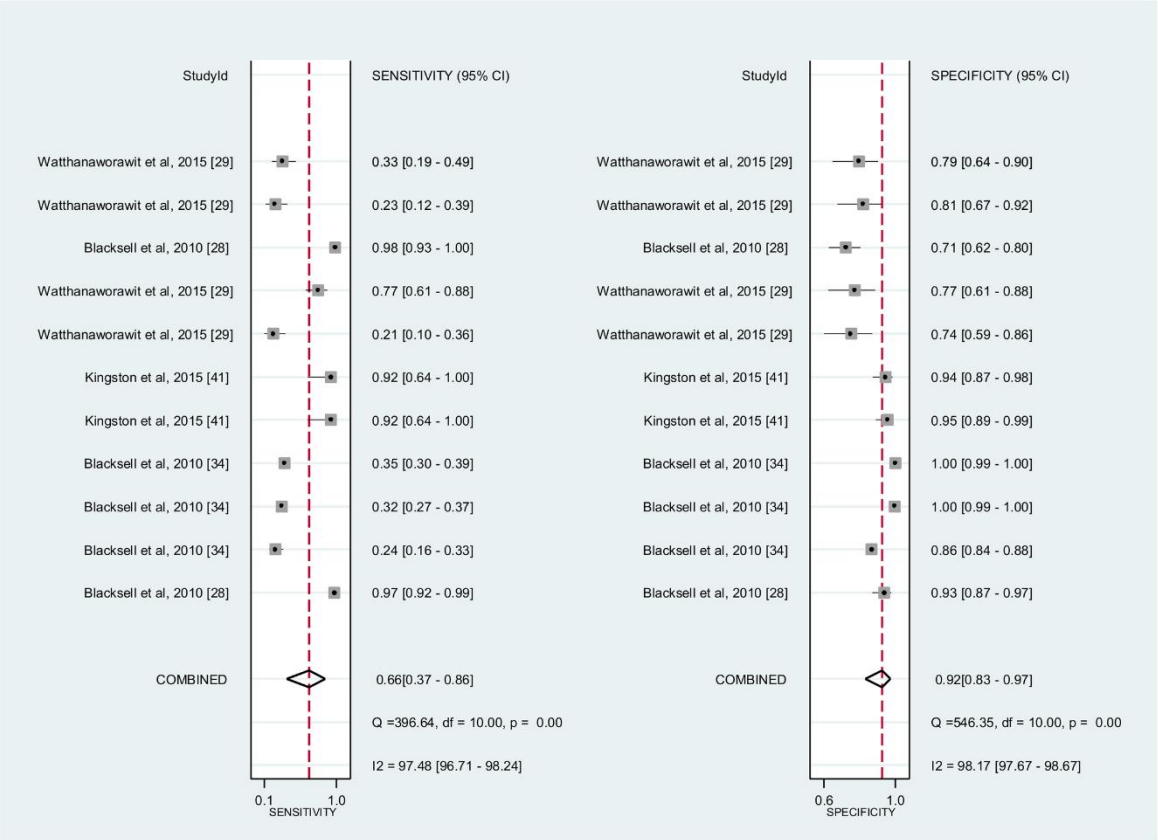


Figure 57. Forest plot of sensitivity and specificity of current commercially available POCTs

Discussion

There were 31 relevant articles included in this review. Almost half of the included articles were of moderate quality. The meta-analysis showed moderately low pooled sensitivity and good specificity of the current commercially available scrub typhus POCTs. However, the studies were heterogeneous with the I^2 value indicating a high degree of heterogeneity. Hence, the pooled sensitivity and specificity value needs to be interpreted with caution.

The systematic review and meta-analysis highlighted the methodological and clinical heterogeneity across scrub typhus POCT diagnostic accuracy studies. It is difficult to pool results and compare studies due to these differences. Meta-regression for other covariates of interest (e.g. sample collection timing) could not be performed because of limited information presented in the original articles.

Almost a quarter of the responses gathered in the main seven questions on QUADAS-2 quality assessment were 'unclear'. Although I did not assess the quality of reporting in this review, this finding indicates that the quality of reporting in the included studies is still arguably poor. Poorly conducted and controlled diagnostic accuracy studies are a waste of time, resources, and effort; moreover, if research is not accurately reported, it can hinder critical appraisal, replication, and meta-analysis of studies [429]. The launch of reporting guidelines such as Standards for Reporting Diagnostic Accuracy (STARD) and PRISMA is a starting point in improving the quality of reporting, although they are not applied as much as they should be [429, 430]. Besides adhering to reporting guidelines, regulations should be adapted to incentivise better and more complete reporting [430]. Creating a reporting infrastructure and building the capability of both authors and reviewers are also necessary to encourage better reporting [430].

Approximately two thirds of the included studies used case-control design. Compared with studies that recruit patients consecutively, case-control study design (evaluating index test in separate diseased population and control group) overestimates diagnostic

performance [431]. Therefore, the results presented need to be interpreted with caution.

Aside from LAMP assay, all of the other POCTs reviewed here relied on antibody detection. Serological diagnosis is problematic in several ways. First, the primary serum collection may not contain sufficient antibodies since it takes time for antibodies to increase to a detectable level creating a 'false negative' result. Second, in endemic populations with significant background immunity, an appropriate cut-off needs to be established to ensure accurate diagnosis otherwise there is the possibility of 'false positives'. Furthermore, the issues regarding the selection of IgM as opposed to whole antibody is very much dependent on the situation. Conventional thought is that whole antibodies may give higher number of 'false positive' results in endemic situations due to the presence of residual IgG from previous scrub typhus infections as was the case with the AccessBio ICTs tested in Thailand and Laos [404]. These shortcomings of serology highlight the need to develop alternative diagnostic strategies.

Although being pooled from heterogeneous studies, the lower limit of the cumulative specificity confidence interval was above 80.0%. This indicates that commercially available ICTs have value in 'ruling in' for the diagnosis of scrub typhus. However, it was difficult to draw conclusions with confidence based on the currently available evidence given the high degree of heterogeneity amongst the studies.

The aim of POCT usage would determine whether high sensitivity or high specificity is the priority. For directing acute treatment, it would be more damaging if we missed treating patient and ending up with complication or mortality. Therefore, we want to minimise the false negatives with POCT with high sensitivity. Likewise, if the POCT is used as a screening tool with other more accurate diagnostic modalities available e.g. combination of PCR and IFA, higher sensitivity would be desirable. POCT with higher specificity would be desired if it is being used to confirm diagnosis after a sensitive screening test. However, based on Bayesian latent class models (that should reflect the 'true sensitivity' when the gold standard is imperfect), the sensitivity of IgM ICT is the highest when compared with other tests when done individually i.e. not in combination [124]. Therefore, we can conclude that given the accuracy of the currently available POCT, improvement in sensitivity is essential.

Another important consideration when performing a diagnostic accuracy study is the choice of reference comparator. Often the IFA, the serological 'gold standard', is selected. However, this test is far from perfect. IFA result interpretation is subjective and there remains a lack of consensus on the optimum positivity cut-off. A cut-off is often adopted without sufficient local evidence, potentially resulting in incorrect diagnostic accuracy measures for the diagnostic under test [148, 154]. To evaluate the true accuracy of IFA, Bayesian latent class models (LCM) have been used. The models showed that the IFA IgM has sensitivity and specificity of 70.0% and 83.8%, respectively; therefore, suggesting it to be unfit as a reference standard [124]. An alternative

reference comparator is the composite Scrub Typhus Infection Criteria (STIC), which have been proposed as a more robust method to diagnose scrub typhus with more confidence, by including a panel of parameters with high specificity [151]. The panel includes: (i) isolation of *O*. *tsutsugamushi*, (ii) at least two positives out of the three PCR assays targeting the 56kDa, 47kDa, and groEL genes, (iii) admission IFA IgM titre of ≥1:12,800, (iv) 4-fold rise in IgM titre from paired samples [124, 151, 154]. At least one of these criteria needs to be fulfilled for a positive scrub typhus diagnosis. However, the Bayesian LCM also showed that STIC's sensitivity and specificity are less than optimal for a reference test (90.5% and 82.5%, respectively).

This study has several limitations. First, the search was performed in one database, and in English only which might have resulted in non-inclusion of relevant articles. Second, the article inclusion and quality assessment were completed primarily by one person, though I attempted to decrease the risk of bias by routine discussion of contentious studies amongst the authors. Third, several studies did not present all of the parameters that I wished to extract, hence limiting the meta-analysis, as I could not include all relevant papers due to the limited information presented in the papers.

In reality, there is only a small number of scrub typhus POCTs that are commercially available. The Panbio POCTs are no longer available and therefore the market is dominated by a few companies including InBios, Standard Diagnostics, and AccessBio. Selecting the most appropriate POCT is very much dependant on a few key factors such

as availability of product locally, the price and the shelf life. In this study I have not examined these local practical aspects that should be considered when selecting POCTs.

In the absence of robust POCTs, the presence of an eschar can be a valuable clinical sign strongly suggesting a diagnosis of scrub typhus. Although its reported presence is very variable, it is still regarded as pathognomonic. An eschar may go unnoticed by the patient since it is painless, often does not itch, can be small, and looks similar to post trauma scab. In addition, it may be located in a concealed area, such as the perineum or under the breasts. This emphasises the importance of performing a thorough physical examination. Developing an algorithm based on the accuracy of eschar and other diagnostic methods to decide which laboratory test to perform and when to initiate treatment would be helpful particularly when resources are limited.

Treating patients empirically based on the pathogen pattern in an area is common practice, but bears the risk of unnecessary or inappropriate treatment (with the attendant risks of side effects) and promotion of antimicrobial resistance. POCTs can play an important role in reducing the number of patients treated empirically and increasing the proportion of patients treated appropriately.

There is an urgent need to develop an affordable and accurate POCT. However, if POCTs still rely on serological measures only, they might not be able to provide diagnosis in

time to inform treatment. Future research should also be directed towards developing new antigen-based tests to improve diagnostic accuracy in the early period of disease.

Chapter 8. Serological investigation of risk of exposure to scrub typhus in residence at an oil palm plantation in North Sumatra relative to urban dwellers at Medan, North Sumatra (SEROSCRUB): A study protocol

Introduction

Scrub typhus is mainly marked by acute fever. Eschar, a necrotic skin lesion in the site of vector inoculation, is a specific sign. However, it is not always present. In some populations, eschar occurred only in 7% of patients [126]. The lack of differentiating features imposes difficulties in setting scrub typhus apart from other infectious causes of acute febrile illnesses. Laboratory tests thus play a vital role in diagnosing scrub typhus. The indirect immunofluorescence assay (IFA) is commonly considered as the gold standard for serological diagnosis, but this test lacks good sensitivity [131, 132]. In addition, such sophisticated techniques are often not feasible to be applied routinely in resource-limited settings [132]. The challenges in diagnosing scrub typhus in turn contributes to the meagre availability of robust epidemiologic evidence of its transmission. That lack of evidence does not infer a lack of transmission, but instead reflects the relative technical difficulty of gathering such evidence.

In the face of diagnostic challenges in resource-limited settings, understanding the distribution and risk of acquiring scrub typhus is essential in ensuring early diagnosis and prompt treatment. Delayed treatment may lead to lethal complications, such as acute respiratory distress syndrome, meningoencephalitis, disseminated intravascular coagulation, and multiorgan disfunction [127]. If untreated, scrub typhus mortality may be as high as 70% [128]. On the other hand, overtreatment without establishing diagnosis may lead to increased antibiotic resistance and poor clinical outcomes due to inappropriate therapies [132]. Although there is limited evidence, the presence of vectors, infected vectors, and scrub typhus transmission in humans have been documented in Indonesia (*Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*). In addition, scrub typhus is a demonstrated public health problem in neighbouring Malaysia and Australia [127]. Scrub typhus is certainly present in Indonesia and it is likely to be causing morbidity and mortality, but direct evidence informing the scale and geographic reach of the infection is almost wholly lacking.

Scrub transmission is reportedly highly focal in nature, occurring within narrowly specific habitats and optimal climatic conditions [2]. Scrub typhus had been recognised in association with cleared lands partially regrown [2]. Changes of forest cover is associated with increased zoonotic and vector-borne diseases outbreaks [432]. Commodity production, including palm oil plantation, have been driving 27% of forest lost globally [433]. Indonesia is the biggest palm oil producer in the world with just over 14 million hectares under commercial cultivation palm oil trees [434]. Palm oil

plantations in Southeast Asia have been identified as also likely foci of scrub typhus transmission [2, 435, 436]. Young palm oil plantations can provide an 'ideal habitat' for scrub typhus vectors and rodents [437]. Grass often grows in between the palm oil trees, providing canopy for ideal breeding ground for the vector mites [437]. Primary forest clearing would also eliminate wild predators of rodents [437]. Grasses and fallen palm leaves provide nesting places for rodents [437]. Rickettsial transmission peaked within two to three years of tree planting [437]. As the trees mature, the canopy closes and grasses do not grow anymore [437]. Therefore, the vector population and disease transmission is reduced [437]. Over 8.2 million palm oil plantation workers in Indonesia could be at substantially higher risk of being exposed to scrub typhus at work compared to other Indonesians [438]. A meta-analysis showed that agricultural workers in Southeast Asia are 1.74 (95% CI 1.47 – 2.07) times more likely to be affected by an infectious disease [439].

On the other hand, there are also more and more data on the emergence of endemic scrub typhus in urban area [7, 99, 108, 440]. In the past, *Orientia tsutsugamushi* infection in human and the presence of its vector has also been documented in the urban areas of Indonesia [48, 61, 441]. Over 150 million Indonesians live in urban areas. Therefore, it is important to understand the risk of scrub typhus in urban areas in Indonesia.

In this study, we aim to measure seropositivity for scrub typhus among healthy adults who live and work in a palm oil plantation in northern Sumatra and compare that rate with urban residents of Medan, a major metropolitan city in the same region. We consider seropositivity as a proxy measure of exposure to scrub typhus transmission and hypothesise relatively high risk of such with permanent residence within a palm oil plantation. We consider this study a logical starting point for beginning to gather the evidence required to gain a more thorough and practical understanding of the epidemiology and ecology of scrub typhus transmission all across the Indonesian archipelago.

The objectives of this study are twofold: to compare the seroprevalence of scrub typhus in healthy adult residents of a palm oil plantation and at urban Medan, North Sumatra and examine factors associated with exposure to scrub typhus.

Design

This will be a cross-sectional seroprevalence survey carried out in healthy adults over a period of 2-3 months. Participants will be recruited from two sites: (1) palm oil plantation and (2) an urban setting, as defined by Biro Pusat Statistik (Statistics Indonesia) [442]. During research team visit, venous blood samples will be collected and a questionnaire (Appendix 10) will be administered. There will be no longitudinal element to this study, i.e. subjects are sampled only once during the venous blood sampling period.

Participant identification and recruitment

Study participants

1,000 healthy adult volunteers at least 17 years of age (500 volunteers from palm oil plantation and 500 volunteers from urban areas) will be recruited for this serosurvey.

Inclusion criteria

The participant will be considered eligible if ALL the following apply:

- At least 17 years of age
- Free of signs of acute febrile illness
- Resident at site for at least 3 years without an absence of more than 6 months in the past 3 years
- Willing and capable to provide informed consent to participate in the study.

Exclusion criteria

The participant will not be enrolled in the study if ANY of the following apply:

- Less than 17 years of age
- Have signs of acute febrile illness
- Resident at site for less than 3 years or having a prolonged absence of more than 6 months during the past 3 years
- Venous blood cannot be withdrawn after three attempts.

Study procedures

Recruitment

Palm oil plantation: The research team will approach designated officials of government-owned palm oil plantation companies called PT Perkebunan Negara 3 (PTPN 3) and PT Perkebunan Negara 4 (PTPN 4) located at Siantar and Aek Nabara, respectively, in order to secure the permission and cooperation of the company in obtaining reasonable and practical access to the residents. There are approximately 600 workers and 1,200 of their families residing permanently around the plantation areas. The company will present a map of their plantation area that includes designated sites of residence of its employees. The research team will request access to no fewer than 5 distinct areas of residence within the plantation. These locations (hamlet, village, etc.) will not be located at peripheries void of palm oil forest but will be surrounded by young or nearly mature palm oil forest where the centre of the settlement is no more than 300 meters from palm oil forest. The residences with lower Covid-19 prevalence will be prioritised for recruitment.

The team will then visit each site and ask to meet with village leaders (senior company or government officers, teachers, religious figures, etc.) in order to explain the survey intent, methods, and risks and benefits. The research team will ask the advice of the leaders in organising a health screening event for adults and their children. The team will accept for screening all who report to the screening centre (local clinic with staff assisting) and offer those services without regard to willingness to provide informed

consent for the serological survey for scrub typhus. The communities will be invited for the health screening with those considered eligible then being offered the opportunity to provide informed consent to participate.

Urban Medan: The research team will approach a company with employees residing in metropolitan Medan, preferably within housing compounds. The research team will liaise with the company to communicate the research aims and procedures and secure permission and cooperation. The research team will ask the advice of the leaders in organising a health survey event in the company facilities for adults and their children. The team will accept for screening all who report to the screening centre (company clinic with staff assisting) and offer those services without regard to willingness to provide informed consent for the serological survey for scrub typhus. Adults will be invited for screening for that survey with those considered eligible then being offered the opportunity to provide informed consent to participate.

Informed consent

The participant must personally sign and date the latest approved version of the Informed Consent Form (Appendix 11) before any study specific procedures are performed.

Written and verbal versions of the Participant Information Sheet and Informed Consent Form (Appendix 11) will be presented to the participants in their native language detailing no less than: the exact nature of the study; what it will involve for the participant; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished (while the team remains on site, i.e. approximately 12 hours) to consider the information, and the opportunity to question the Investigator and research team or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained by the investigator team.

Study visit

The recruitment process will be linked with a health screening offered to all residents of the site. Health screening for adults is aimed to screen for diabetes mellitus (by random blood sugar measurement), hypertension (by blood pressure measurement), and nutritional status (by body mass index (BMI) measurement). Adults who provided

informed consent will first be administered a questionnaire (Appendix 10) and then asked to submit to a physical examination by a qualified and licensed physician (clinical assessment and vital signs collection) and then to allow collection of a 3 mL whole blood by venepuncture conducted by a qualified and licensed phlebotomist. A small volume of blood will also be used for blood sugar point-of-care testing as a part of health screening.

Sample handling

3 mL of venous blood will be withdrawn once. The blood sample will be placed in an EDTA tube. The blood sample will be centrifuged to obtain the serum. Serum samples will be stored temporarily in freezers at the site. Later, batches of serum samples will be sent to Eijkman Institute for Molecular Biology for analysis. Specimen will be stored in freezer under the custodian of EOCRU. All procedures regarding the use of these samples go through ethical clearance from the relevant ethics committee. The specimen will be assayed using in-house enzyme-linked immunosorbent assay (ELISA) against O. tsutsugamushi IgG and IgM [138].

Discontinuation/withdrawal of participants from study

Each participant will have the right to withdraw from the study at any time. Withdrawal from the study will result in exclusion of the data for that participant from analysis.

Definition of end of study

The end of study will be when all the sampling procedures have been finished.

Covid-19 risk mitigation

The standard operating procedure (SOP) to minimise the risk of Covid-19 transmission and mitigate potential disruption during the conduct of study (Appendix 12) must be applied throughout the study period.

Statistics and analysis

Description of statistical methods

Statistical analysis will be done using STATA 17, R, and Microsoft Excel. Seroprevalence data will be cross tabulated with variables collected. Association between categorical variables and scrub typhus seropositivity will be tested with Chi-squared test. Association between continuous variables and scrub typhus seropositivity will be analysed using logistic regression.

The number of participants

Sample size per location i.e. 500 (1,000 participants for two sites in total) was calculated based on the lower limit of confidence interval for proportion in binomial distribution, to not miss 1% seroprevalence or higher (calculated on STATA 15.0). If no samples are

found to be seropositive, we can be 95% certain that the true seroprevalence in the population is not higher than 1%.

Data management

Access to data

Direct access will be granted to authorised representatives from the University of Oxford and any host institutions for monitoring and/or audit of the study to ensure compliance with regulations.

Data handling and record keeping

All study data will be entered on Excel spreadsheets. The participants will be identified by a unique study specific number and/or code in all databases. The name and any other identifying detail will NOT be included in any study data electronic file.

Quality control and quality assurance procedures

The study will be conducted in accordance with the current approved protocol, relevant regulations and standard operating procedures.

Ethical and regulatory considerations

Declaration of Helsinki

The Investigators will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material are submitted to the Eijkman Institute Ethics Committee and OXTREC for written approval.

The investigators will notify these ethics committees of any changes to the approved protocol. Those involving perceived or real increases of risk or discomfort to study subjects shall be reviewed and approved prior to implementation.

Participant confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only with participant ID numbers on all study documents and any electronic databases. All documents will be stored securely and only accessible by study staff and authorised personnel. In this study, the data will not be kept as identifiable data for longer than necessary for the purposes concerned.

Expenses and benefits

In addition to health screening linked to the research procedure, participants will be offered a nutritional supplement/hygiene package (e.g. powdered milk, toothbrush, toothpaste, soap).

Finance and insurance

Funding

This study will be funded by the Mahidol-Oxford Tropical Medicine Research Unit (MORU) and Oxford University Clinical Research Unit (OUCRU).

Role of funders

The funders have no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Insurance

This research will be appropriately covered through the University of Oxford's legal liability insurances.

Publication policy

In addition to including the study protocol in this thesis, I also plan to publish the protocol. The Investigators will be involved in reviewing drafts of the manuscripts,

abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by MORU and OUCRU. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. All results from this study will be publicly available in the supporting information of any published materials.

Competing interests

All investigators declared no competing interests.

Discussion

This clinical study was planned to start in the third quarter of 2020. However, due to the COVID-19 pandemic in Indonesia, the start of the study was delayed and therefore I was unable to include the results of the study in this thesis. There were several challenges associated with the pandemic that has contributed to the delay. First, as face-to-face research is discouraged, we needed to put a hold on the project. Due to the nature of this research, where we need to take venous blood samples, face-to-face meeting is unavoidable. The recruitment process we plan might also attract crowds.

Second, our site collaborator requested to postpone the study. Our site collaborator was occupied by Covid-19 response efforts, especially in the early months of the pandemic. Third, as the Eijkman Institute was one of the national referrals Covid-19 testing centres,

the laboratory resources were diverted to Covid-19 diagnosis and control. I also volunteered to help with the diagnostic efforts in the Eijkman Institute for a few months.

Fourth, although we have been granted ethics approval from Oxford Tropical Research Ethics Committee (OXTREC). The local ethics committee i.e. the Eijkman Institute Research Ethics Committee (EIREC) halted activities for a few months in the beginning of pandemic. In addition, the committee prioritised Covid-19 ethics applications. We have submitted our application on March 4th 2020 and granted ethical clearance on September 3rd 2021. We have also amended our protocol and ethics application to incorporate Covid-19 prevention measures.

The pandemic also forced us to apply Covid-19 prevention and mitigation steps to ensure the health and safety. This requires amendment of protocol and adaptation in the field. We need to place additional budget for personal protective equipment and disinfectant, as well as organise people flow to prevent crowding.

We will continuously monitor the Covid-19 situation in Indonesia to determine the feasibility of this project. Contingent on Covid-19 situation on site, we plan to start the study in late 2022.

Chapter 9. General discussion and conclusion

In this chapter I outline the conclusions of each results chapter and discuss some general themes that run through the thesis. I elaborate on the epidemiological, clinical, and policy consequences of the thesis findings, discuss research questions arising from them, and outline potential future research projects which may address these questions.

Scrub typhus has been observed in Indonesia since 1902. It was continuously documented during the colonial times. However, although the presence of scrub typhus has been noted since the early 1900s, up to this day we still do not have a good idea of the distribution of scrub typhus in Indonesia. Scrub typhus is indeed an understudied and neglected disease, and on the basis of my findings this is true more so in Indonesia than in many other Southeast Asian and Asia-Pacific countries.

Many factors influenced the lack of data on scrub typhus in Indonesia. Through *Chapter 3. History of scrub typhus in Indonesia*, I demonstrated how the 'macro-environment' affected when and where knowledge on scrub typhus was generated. In the past research was concentrated around colonial economic and military interests e.g. on plantations and during World War II. Political and economic stability is essential for a productive research ecosystem. Furthermore, political interests can also push consequential research agenda to be prioritised e.g. the advances in prevention of scrub

typhus achieved during World War II. Currently awareness is low in Indonesia, as is diagnostic capacity. This is not helped by the existing decentralised health administrative infrastructure. In addition to political will I conclude that Indonesia needs a sustainable and continuous research funding mechanism and national research infrastructure that ensures research on diseases with public health importance is not neglected.

In the case of scrub typhus, this lack of data, including almost no information on DALYs and economic cost, makes it difficult for policy makers to appreciate the impact of scrub typhus. We are therefore presented with a Catch-22 situation. Funding schemes focussed on neglected tropical diseases would be invaluable to solve this conundrum.

Looking more closely at the history of scrub typhus and identifying wider factors influencing scrub typhus research in Indonesia would be helpful to understand the driving forces. This would also help identify barriers and help develop a future roadmap, not only for scrub typhus, but also for other NTDs research.

In *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*, despite the limitations in data, we can see that scrub typhus exists and is widespread within the Indonesian archipelago. I have identified areas with best evidence for the presence of infective vectors i.e. North Sumatra and Biak, as well as

areas with unknown risk (no data). Scrub typhus vector research also faces challenges, such as the lack of expertise to identify trombiculid mite species. Likewise, research on scrub typhus in humans is hindered by the absence of laboratory diagnostic capacity reliable and widely available point-of-care tests. Diagnostic challenges are the main obstacle to scrub typhus research.

In *Chapter 7. Scrub typhus point-of-care testing: A systematic review and meta-analysis*, I explored the diagnostic value of point-of-care tests (POCTs) for scrub typhus. The currently available POCTs were mostly in the form of immunochromatographic test (ICT). The meta-analysis of the limited data on ICTs showed good specificity – indicating their potential use for ruling in diagnosis, however, the sensitivity is low. ICTs are serology-based; therefore, they also have the inherent weakness of serological tests, that is it often cannot be used to confirm the diagnosis and thus direct treatment in the beginning of the natural history because the antibody response takes time to develop. This highlights the need to develop antigen- or molecular-based POCTs that are accurate, affordable, and accessible.

Sufficiently accurate and widely available POCTs can revolutionise disease treatment and control. In the case of malaria 'rapid diagnostic tests' (RDT) play an important role, particularly in malaria-endemic areas. Most of these areas are in low- and middle-income settings where resources such as light microscopy and competent laboratory staff are often not readily available. Though they are not a full replacement for malaria

microscopy, these ICTs provide rapid results i.e. in 10-15 minutes, require no electricity or expensive machines, and are relatively easy to use. These qualities enable prompt and effective treatment of acute malaria cases.

In the absence of reliable and widely available supporting POCT or laboratory diagnostics, a more thorough physical examination is essential to identify any eschars. Clinicians' awareness of scrub typhus and familiarity with its clinical manifestations are therefore essential to inform the differential diagnosis.

The development of accurate, affordable, and accessible diagnostic methods takes time. It might not be possible to have one method/kit with all the desired qualities. Meanwhile, in addition to increasing awareness on scrub typhus, it is also important to understand its distribution to inform empiric treatment guidelines. Studies on vector (and infected vector) distribution and niche modelling to fill in the gaps would help to inform potential areas of active transmission and of absence. In addition, serosurveys to determine the range of scrub typhus transmission and inclusion of scrub typhus tests in acute febrile illness diagnostic panels are needed to understand where scrub typhus is a public health concern.

Based on the findings of *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*, the priority areas for further study are North Sumatra,

South Sumatra, Riau, Central Borneo, Nusa Tenggara Barat, Nusa Tenggara Timur, and Maluku. North Sumatra showed the highest number of data point – however, much of the evidence was dated. Riau and Central Borneo lacks evidence despite being surrounded by areas with scrub typhus vector or *O. tsutsugamushi* presence. Serosurveys and acute febrile illness studies using modern diagnostic methods would update our understanding in these three areas. Nusa Tenggara Barat, Nusa Tenggara Timur, and Maluku did not have any evidence on scrub typhus. Starting with rodent trapping to identify vector presence would be a good starting point in these areas. The studies done must have enough samples and therefore statistical power. Sample size is also important in areas with no evidence or of 'low risk of transmission', as sufficient power is necessary to define absence.

In *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples*, archived sera samples from acute febrile illness studies conducted in three sites (Jambi, Denpasar, Tabanan) were analysed using in-house ELISA. Overall, close to one-tenth of the samples were seropositive. We can conclude that there is active transmission of scrub typhus in these locations. There is no established ELISA cut-off for these three sites or for Indonesia in general. I could not get access to 'presumably unexposed' or 'negative control' populations to generate an evidence-based cut-off. Therefore, I explored three techniques to determine the cut-off in this situation: visual inflection point, change-point analysis, and finite mixture modelling. These methods could also be applied to serological testing for other diseases

when samples from 'presumably unexposed' population are unavailable. For acute case detection it is important to establish a geographically appropriate cut-off that takes into account the local background immunity. The archived sera only consisted of acute samples; therefore, we could not tease out and confirm if some of the seropositive patients were acutely ill with scrub typhus. Collecting acute and convalescent samples when utilising serological diagnostic methods in acute febrile illness studies is essential. In the future, community-based studies with appropriate sampling and sufficient sample size are crucial to determine seroprevalence. Sampling donated blood could be a good starting point to investigate community seroprevalence.

I planned to carry out a comparative seroprevalence study, the protocol for which is outlined in *Chapter 8. Serological investigation of risk of exposure to scrub typhus in residence at an oil palm plantation in North Sumatra relative to urban dwellers at Medan, North Sumatra (SEROSCRUB): A study protocol*. This study aims to investigate the seropositivity for scrub typhus among healthy adults who live and work in oil palm plantations in northern Sumatra and compare that rate with urban residents of Medan, a city in the same region. I also planned to examine factors associated with exposure to scrub typhus. We planned to start the study in 2020; however, unfortunately the Covid-19 pandemic has put this plan on hold. This cross-sectional seroprevalence survey would be the first step in obtaining data to understand the prevalence of scrub typhus in this historically endemic area in Indonesia.

The studies included in *Chapter 7. Scrub typhus point-of-care testing: A systematic review and meta-analysis* showed inconsistent reporting and methodological heterogeneity. Reporting guidelines exist, but their implementation is still lacking. A mechanism to incentivise complete reporting is needed, for example, by setting a minimum score on the reporting checklist for manuscript submission. Capacity building in research methodology and funding schemes for scrub typhus diagnostic accuracy studies would increase high-quality research output.

Likewise, in addition to the limited number of interventional studies found, the articles included in *Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform* also showed heterogeneity in methods. This heterogeneity illustrates the absence of consensus on treatment and research methodologies. Defining a set of data and design standards for RCTs and observational studies can facilitate comparison across studies and data aggregation. Key variables, study design, diagnostic methods, and endpoints should be standardised. The Clinical Data Interchange Standards Consortium (CDISC) could provide a framework for collecting, organising, analysing, and sharing data. This framework, complemented by consensus on outcomes, could ensure comparability and interoperability of data. The COMET (Core Outcome Measures in Effectiveness Trials) Initiative can guide the development and application of agreed core outcome sets (COS) to be measured and reported in clinical trials [154]. Consistent implementation of

reporting guidelines would help readers appraise articles and synthesise the data. To achieve these desired aims, joint efforts from stakeholders are needed.

There is still considerable uncertainty about the optimal treatment for scrub typhus. This is demonstrated by the highly variable treatment regimens used in the studies reviewed in *Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform*. There were a limited number of scrub typhus treatment trials in the literature. Ideally, more trials on treatment should be performed. However, this is expensive and takes time. There is likely a reasonable amount of data available to address this uncertainty. However, methodological heterogeneities and incomplete published datasets present challenges to combine the currently available published data without access to the underlying IPD. An IPD platform would facilitate curation, standardisation, and pooling of currently scattered data. This would enable in-depth investigation of priority research questions such as determining the optimal treatment dose and duration for children and pregnant women. Stakeholder engagement and cooperation will be crucial to establish a scrub typhus IPD platform.

In the future, further quality assessments of longitudinal studies may be useful to identify the source of heterogeneity and identify any barriers to develop more standardised methods and reporting. Bibliographic analysis of the relevant papers

would also be helpful to identify the researchers and groups/centres with substantial IPD datasets, which would be useful to inform and direct stakeholder engagement.

As previously touched upon, increasing the awareness of scrub typhus is key to providing prompt treatment, improving understanding, and ultimately controlling the disease. Advocating for the recognition of scrub typhus as an NTD by the WHO would help in increasing awareness. Hopefully, this in turn will lead to more research and development on diagnosis, treatment, and control strategies. In addition, including scrub typhus in medical school curricula and compulsory competencies are ways to increase awareness within medical communities. This should be done in parallel with research efforts, otherwise it would be difficult to break this chicken-and-egg situation of lack of both awareness and data.

Scrub typhus is indeed globally neglected and understudied. This is particularly the case with scrub typhus research in Indonesia. The absence of widely available and reliable diagnostics is a major obstacle in scrub typhus research and control. The work in this thesis has demonstrated that there is active transmission of scrub typhus in Indonesia. However, there are still many gaps in the understanding of its distribution across the archipelago. To strengthen the evidence, further studies such as vector distribution research, serosurveys, and acute febrile illness studies are needed. Pragmatic treatment trials are desirable; however, this requires substantial funding, expertise, and time. IPD meta-analyses would be one way to address knowledge gaps on treatment. There is a

role for an IPD platform in standardising and pooling of currently available treatment study data. Increasing awareness of this disease and officially recognising it as a neglected tropical disease are vital. Engagement with and the collaboration of stakeholders are essential if we are to further our understanding of scrub typhus in Indonesia and globally.

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Appendices

Appendix 1. Search strategy for evidence mapping

Databases

1. International databases:
   a. Books@Ovid
   b. Journals@Ovid Full Text
   c. CAB Abstracts <1990 to 2021 Week 23>
   d. CAB Abstracts <1910 to 1989>
   e. Embase 1974 to present
   f. Forest Science <1939 to 2021 Week 23>
   g. Global Health <1973 to 2021 Week 23>
   h. Medline (Ovid MEDLINE® Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE®) 1946 to present
   i. Zoological Record <1978 to 2008>
   j. Zoological Record Archive <1864 to 1977>
   k. Zoological Record <2009>
   l. Scopus
   m. IMSEAR database
2. Indonesian databases:
   a. https://garuda.ristekbrin.go.id/
   b. https://www.neliti.com/id/
   c. ejournal.litbang.depkes.go.id
3. Dutch journals:
   a. https://www.ntvg.nl/
   b. http://www.ariez.nl/artikelen.1206.lynkx?tijdschrift=tvi&event=showform
   c. https://dare.uva.nl/
4. Dutch museums:
   a. Natuurhistorisch Museum Rotterdam (hetnatuurhistorisch.nl/)
   b. Naturalis Biodiversity Center (bioportal.naturalis.nl)
5. Libraries:
   a. Eijkman Institute library
6. Local universities and research centres:
   a. Institute of Tropical Disease, Universitas Airlangga
   b. Pusat Kedokteran Tropis (Center of Tropical Medicine), Universitas Gajah Mada

c. Center for Research and Integrated Development of Tropical Health and Infectious Diseases (CRID-TROPHID), Universitas Indonesia
d. Others based on the Ministry of Research, Technology, and Higher Education (Kemenristekdikti) list (http://www.dikti.go.id/perguruan-tinggi/)

7. Ministry of Health networks:
   a. Perpustakaan Departemen Kesehatan (Central Health Library)
   b. Perpustakaan Sekretariat Badan Litbangkes (Indonesia NIHRD Secretariat Library)
   c. Litbangkes cabang (Indonesia NIHRD branch):
      a) Balai Besar Litbang Vektor dan Reservoir Penyakit (B2P2VRP) Salatiga
      b) Balai Litbang Pemberantasan Penyakit Bersumber Binatang (P2B2) Donggala
      c) Balai Litbang P2B2 Tanahbumbu
      d) Balai Litbang P2B2 Banjarnegara
      e) Loka Litbang P2B2 Baturaja
      f) Loka Litbang P2B2 Ciamis
      g) Loka Litbang P2B2 Waikabubak

For searches in libraries, local universities, and Ministry of Health networks, search in their websites first (if any). If there is no website, find a contact person.

Search language

1. English
2. Dutch: For pre-independence (1945) articles

Search in Indonesian was not done since pilot search has shown that there were many duplicates surfaced as results. All of the relevant articles in Indonesian found during piloting always use/mention “scrub typhus” in the text.

Search and cataloguing protocol

1. Login to databases
2. Type the search terms in the search box (refer to specific databases below)
3. Export the search results to EndNote
4. Remove duplicates
5. Screen the title and abstract
6. Make groups in the EndNote library:
   - ‘Relevant’: for papers fitting the eligibility criteria
   - ‘Useful’: for papers not fitting the eligibility criteria, but providing useful information (e.g. review)
   - ‘Excluded’: for papers completely irrelevant
7. Group into: ‘relevant’, ‘useful’, ‘excluded’
8. Retrieve the full text through EndNote

9. Manually search if not found
10. Request inaccessible full text through Bodleian Library, Oxford
11. Review full text
12. Catalogue the relevant papers in EndNote

Search protocol Ovid search

1. Select 'Advanced Search'
2. Run the search in the following databases:
   a. Books@Ovid
   b. Journals@Ovid Full Text
   c. CAB Abstracts <1990 to 2021 Week 23>
   d. CAB Abstracts <1910 to 1989>
   e. Embase 1974 to present
   f. Forest Science <1939 to 2021 Week 23>
   g. Global Health <1973 to 2021 Week 23>
   h. Medline (Ovid MEDLINE® Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE® Daily and Ovid MEDLINE®) 1946 to present
   i. Zoological Record <1978 to 2008>
   j. Zoological Record Archive <1864 to 1977>
   k. Zoological Record <2009>
3. Search strategy:
   1 scrub typhus/
   2 Orientia tsutsugamushi/
   3 "scrub typhus".ti,ab.
   4 tsutsugamushi.ti,ab.
   5 scrubtyphus.mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
   6 "mite typhus".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
   7 "japanese river fever".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
   8 "kedani fever".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
   9 "chigger borne rickettsia".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
   10 "chigger borne rickettsiosis".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
   11 "chigger borne typhus".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]

12 "mite fever".mp. [mp=tx, bt, ti, ab, ct, sh, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, an, ui, ds, on, sy]
13 "mite borne rickettsia".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
14 "mite borne rickettsiosis".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
15 "mite borne typhus".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
16 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15
17 trombiculid*.mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
18 leptotrombidium.mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
19 chigger.mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
20 17 or 18 or 19
21 indonesia.mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
22 "Netherlands East Indies".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
23 "Dutch East Indies".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
24 "Malay archipelago".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
25 "Dutch New Guinea".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
26 "Netherlands New Guinea".mp. [mp=tx, bt, ti, ab, ct, ot, hw, id, cc, tn, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, ui, sy]
27 21 or 22 or 23 or 24 or 25 or 26
28 16 or 20
29 27 and 28

4. Export to EndNote

Search protocol for Global Index Medicus

1. Search strategy:
   tw:((("scrub typhus" OR tsutsugamushi OR scrubtyphus OR "mite typhus" OR "japanese river fever" OR "kedani fever" OR "chigger borne rickettsia" OR "chigger borne rickettsiosis" OR "chigger borne typhus" OR "mite fever" OR "mite borne rickettsia" OR "mite borne rickettsiosis" OR "mite borne typhus") OR (trombiculid* OR leptotrombidium OR chigger)) AND (indonesia OR "Netherlands East Indies" OR "Dutch East Indies" OR "Malay archipelago" OR

"Dutch New Guinea" OR "Netherlands New Guinea")) AND (instance:"ghl") AND (db:("IMSEAR"))

2. Select 'IMSEAR (SEARO)' under the 'Sources' drop down list

Search protocol for Scopus

1. Search strategy:
   (((TITLE-ABS-KEY ("scrub typhus") OR TITLE-ABS-KEY (tsutsugamushi) OR TITLE-ABS-KEY (scrubtyphus) OR TITLE-ABS-KEY ("mite typhus") OR TITLE-ABS-KEY ("japanese river fever") OR TITLE-ABS-KEY ("kedani fever") OR TITLE-ABS-KEY ("chigger borne rickettsia") OR TITLE-ABS-KEY ("chigger borne rickettsiosis") OR TITLE-ABS-KEY ("chigger borne typhus") OR TITLE-ABS-KEY ("mite fever") OR TITLE-ABS-KEY ("mite borne rickettsia") OR TITLE-ABS-KEY ("mite borne rickettsiosis") OR TITLE-ABS-KEY ("mite borne typhus"))) OR ((TITLE-ABS-KEY (trombiculid*) OR TITLE-ABS-KEY (leptotrombidium) OR TITLE-ABS-KEY (chigger)))) AND ((TITLE-ABS-KEY (indonesia) OR TITLE-ABS-KEY ("Netherlands East Indies") OR TITLE-ABS-KEY ("Dutch East Indies") OR TITLE-ABS-KEY ("Malay archipelago") OR TITLE-ABS-KEY ("Dutch New Guinea") OR TITLE-ABS-KEY ("Netherlands New Guinea")))

Search protocol for Indonesian websites

1. Login if search information can be saved
2. Type the search terms in the search box:
   - "scrub typhus"
   - tsutsugamushi
   - leptotrombidium
3. If possible, export the search results (citation and abstract) to EndNote. If not, download the pdf and enter manually into EndNote.

Search protocol for Dutch websites

1. The listed Dutch websites does not seem to return relevant search results with long search string.
2. Type the following search terms:
   - "scrub typhus"
   - scrubtyfus
   - "scrub tyfus"
   - mijtekoorts
   - tsutsugamushi
   - trombiculid
   - leptotrombidium

- chigger

3. Screen for relevant articles

Appendix 2. List of studies included in evidence mapping

1	Schüffner W. Pseudotyphoid Fever in Deli, Sumatra (a variety of Japanese Kedani Fever). Philippine Journal of Science 1915;10(5):345-53.
2	Walch E, Keukenschrijver NC. Eenige opmerkingen aangaande de Epidemiologie van de Pseudotyphus. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1924;64(2):247-76.
3	Van Driel BM. A Remarkable Case of " Mite Fever". Geneeskundig Tijdschrift voor Nederlandsche Indie. 1927;67(5):667-74.
4	Walch EW. Trombiculae and Belated Mites from Dutch East Indies. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1927;67(6):922-32.
5	Wolff JW. Tropical Typhus, a Disease resembling Typhus Fever. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1929;69:429-58.
6	Van Steenis PB. A Case of Pseudotyphus (Schuffner) in Java. The Differential Diagnosis of Exanthematic Fever. Nederlands Tidjschrift voor Geneeskunde. 1931;31(5):495-502.
7	Wolff JW. Observations in Tsutsugamushi Fever and Tropical Typhus. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1931;71(1):35-45.
8	Emanuels BJ. Research on Tropical Typhus and (or) Mite Fever in Atjeh. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1932;72(4):196-205.
9	Bessem N. A Case of Mite Fever in the Western Division of Borneo. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1935;75(22):1909-10.
10	Dinger J, Lentjes L. Een geval van Mijtekoorts. Diagnose door middel der dierproef. Geneeskundig Tijdschrift voor Nederlandsch-Indië. 1935;75:1951-4.
11	Kuypers C. Een tweetal gevallen van Tropical Typhus. Geneeskundig Tijdschrift voor Nederlandsch-Indië. 1935;75(22):1896-903.
12	Van Waardenburg DA. Four Cases of Riekettsia Infection in Benkoelen Residency. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1936;76(12):712-21.
13	Wolff JW, Kouwenaar W. On Rickettsia Diseases in Sumatra. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1936;76(5):272-88.
14	Heinemann H. Reinfection with Pseudotyphus (Mite Fever). Geneeskundig Tijdschrift voor Nederlandsche Indie. 1938;78(48):3037-40.
15	Wolff JW, De Graaf W. Two New Cases of Mite Fever in Java. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1939;79(41):2577-88.
16	Kotter GF. Rickett-siosis among Troops in Atjeh. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1940;80(2):66-79.
17	Van Der Schroeff JP. An Epidemic of Mite Fever and Tropical Typhus in Atjeh and Dependencies. Geneeskundig Tijdschrift voor Nederlandsche Indie. 1941;81(20):1103-22.
18	Griffiths JT, Jr. A Scrub Typhus (Tsutsugamushi) Outbreak in Dutch New Guinea. Journal of Parasitology. 1945;31(5):341-50.
19	Griffiths JT, Jr. A Further Account of Tsutsugamushi Fever at Sansapor, Dutch New Guinea. Journal of Parasitology. 1947;33(4):367-73.

20	Gispen R. The Role of Rats and Mites in the Transmission of Epidemic Scrub Typhus in Batavia. Documenta Neerlandica et Indonesica de Morbis Tropicis. 1950;2(1):23-35.
21	Plooij M. Scrub Typhus. Nederlands Tidjschrift voor Geneeskunde. 1950;94(ii):1490-98.
22	Hoogerheide C, Ensink GJ. Two Small Explosions of Scrubtyphus in Netherlands New-Guinea. Documenta de Medicina Geographica et Tropica. 1957;9(1):94-6.
23	Liat LB, Hadi TR, Nalim S, Gandahusada S. Preliminary survey of rodents in two transmigration schemes in South Sumatra, Indonesia. Buletin Penelitian Kesehatan. 1978;6(1):15-23.
24	Hadi TR, Nalim S, Sukaeri, Dennis D. Scrub typhus survey of Biak and Owi islands: ectoparasites of small mammals and rickettsial isolations. Southeast Asian Journal of Tropical Medicine and Public Health. 1980;11(2):220-6.
25	Liat LB, Hadi TR, Sustriayu N. Rodent and scrub typhus survey in a ricefield at Kramat Tunngak area, Tanjung Priok, Jakarta, Indonesia. Southeast Asian Journal of Tropical Medicine and Public Health. 1980;11(2):232-9.
26	Liat LB, Sustriayu N, Hadi TR, Bang YH. A study of small mammals in the Ciloto field station area, West Java, Indonesia, with special reference to vectors of plague and scrub typhus. Southeast Asian Journal of Tropical Medicine and Public Health. 1980;11(1):71-80.
27	Dennis DT, Hadi TR, Brown RJ, Sukaeri S, Leksana B, Cholid R. A survey of scrub and murine typhus in the Ancol section of Jakarta, Indonesia. Southeast Asian Journal of Tropical Medicine and Public Health. 1981;12(4):574-80.
28	Gandahusada S, Dennis D, Stafford EE, Hartono T, Soepadijo, Rasidi C, Irving GS. Infectious disease risks to transmigrant communities in Indonesia: A survey in Lampung province, Sumatra. Buletin Penelitian Kesehatan. 1981;9(1):15-24.
29	Hadi TR, Sarbini S, Dennis DT. Survey of small mammal ectoparasites in West Sumatra, Indonesia. Southeast Asian Journal of Tropical Medicine & Public Health. 1981;12(2):275-7.
30	Hadi T, Nalim S, Wasito S, Purnomo. A survey on small mammals and their parasites in Batam Island, Riau, Indonesia. Buletin Penelitian Kesehatan. 1982;10(1):1-6.
31	Hadi T, Nalim S. Fauna binatang mengerat dan parasitnya di beberapa daerah transmigrasi di Indonesia, serta hubungannya dengan penyakit Rickettsiosis yang ditularkan oleh vektor. Buletin Penelitian Kesehatan. 1983;11(2):44-9.
32	Hadi TR, Sarbini S, Brown RJ. Small mammalian ectoparasites from Mt. Bromo area, East Java, Indonesia. Southeast Asian Journal of Tropical Medicine and Public Health. 1983;14(3):422-5.
33	Hadi TR, Supalin, Nalim S, Chang A. Ekologi penyakit scrub typhus di Unit Desa Transmigrasi Mulyorejo, Way Abung III, Lampung Utara. Buletin Penelitian Kesehatan. 1984;12(2):11-8.
34	Hadi TR, Nalim S. Survai vector dan reservoir penyakit zoonotik yang ditularkan oleh arthropoda di Desa Basi, Kecamatan Dondo, Kabupaten Buol - Tolitoli, Sulawesi Tengah, Indonesia. Buletin Penelitian Kesehatan. 1985;13(1):50-8.

35	Hadi TR, Sarbini S. Trombiculid mites of Sunter, North Jakarta Indonesia. The Southeast Asian journal of tropical medicine and public health. 1985;16(1):126-7.
36	Goff ML, Durden LA. A new species and new records of chiggers (Acari: Trombiculidae) from North Sulawesi, Indonesia. International Journal of Acarology. 1987;13(3):209-11.
37	Whitaker JO, Jr., Durden LA. Some ectoparasitic mites from mammals from Sulawesi Utara, Indonesia. Entomological News. 1987;98(1):26-30.
38	Nurisa I, Hadi TR, Nurindriani N. A study on bionomics of scrub typhus vector and its host species in a transmigration scheme of Kumpeh, Jambi, Sumatera. [Indonesian]. Bulletin of Health Studies in Indonesia. 1988;16(2):35-47.
39	Durden LA, Page BF. Ectoparasites of commensal rodents in Sulawesi Utara, Indonesia, with notes on species of medical importance. Medical and veterinary entomology. 1991;5(1):1-7.
40	Corwin AL, Soeprapto W, Widodo PS, Rahardjo E, Kelly DJ, Dasch GA, Olson JG, Sie A, Larasati RP, Richards AL. Surveillance of rickettsial infections in Indonesian military personnel during peace keeping operations in Cambodia. American Journal of Tropical Medicine and Hygiene. 1997;57(5):569-70.
41	Richards AL, Soeatmadji DW, Widodo MA, Sardjono TW, Yanuwiadi B, Hernowati TE, Baskoro AD, Roebiyoso, Hakim L, Soendoro M, Rahardjo E, Putri MP, Saragih JM, Strickman D, Kelly DJ, Dasch GA, Olson JG, Church CJ, Corwin AL. Seroepidemiologic evidence for murine and scrub typhus in Malang, Indonesia. American Journal of Tropical Medicine and Hygiene. 1997;57(1):91-5.
42	Richards AL, Rahardjo E, Rusjdi AF, Kelly DJ, Dasch GA, Church CJ, Bangs MJ. Evidence of Rickettsia typhi and the potential for murine typhus in Jayapura, Irian Jaya, Indonesia. American Journal of Tropical Medicine and Hygiene. 2002;66(4):431-4.
43	Richards AL, Ratiwayanto S, Rahardjo E, Kelly DJ, Dasch GA, Fryauff DJ, Bangs MJ. Serologic evidence of infection with ehrlichiae and spotted fever group rickettsiae among residents of Gag Island, Indonesia. American Journal of Tropical Medicine and Hygiene. 2003;68(4):480-4.
44	Ristiyanto, Boewono D, Handayani F, Notosoedarmo S. Keanekaragaman ektoparasit pada tikus rumah Rattus tanezumi dan tikus Polinesia Rattus exulans di daerah enzootik pes lereng Gunung Merapi, Jawa Tengah. Jurnal Ekologi Kesehatan. 2004;3(2).
45	Ristiyanto, Mulyono A, Agustina M, Yuliadi B, Muhidin. Indeks keragaman ektoparasit pada tikus rumah Rattus tanezumi temminck, 1844 dan tikus Polinesia R. exulans (Peal, 1848) di daerah enzootik pes lereng Gunung Merapi, Jawa Tengah. Vektora: Jurnal Vektor dan Reservoir Penyakit. 2009;1(2):73-86.
46	Punjabi NH, Taylor WRJ, Murphy GS, Purwaningsih S, Picarima H, Sisson J, Olson JG, Baso S, Wangsasaputra F, Lesmana M, Oyofo BA, Simanjuntak CH, Subekti D, Corwin AL, Richie TL. Etiology of acute, non-malaria, febrile illnesses in Jayapura, Northeastern Papua, Indonesia. American Journal of Tropical Medicine and Hygiene. 2012;86(1):46-51.
47	Ristiyanto, Handayani F, Mulyono A, Yuliadi B. Ektoparasit tungau trombikulid dan inangnya serta peluang penularan scrub typhus di beberapa daerah Pulau Jawa. Vektora. 2016;8(1):41-52.

48	Widjaja S, Williams M, Winoto I, Farzeli A, Stoops CA, Barbara KA, Richards AL, Blair PJ. Geographical Assessment of Rickettsioses in Indonesia. Vector borne and zoonotic diseases (Larchmont, NY). 2016;16(1):20-5.
49	Lokida D, Hadi U, Lau C-Y, Kosasih H, Liang CJ, Rusli M, Sudarmono P, Lukman N, Laras K, Asdie RH, Murniati D, Utama IMS, Mubin RH, Karyana M, Gasem MH, Alisjahbana B. Underdiagnoses of Rickettsia in patients hospitalized with acute fever in Indonesia: observational study results. BMC Infectious Diseases. 2020;20(1):364.

Appendix 3. PRISMA checklist for the systematic review of treatment landscape

Section/topic	#	Checklist item	Reported on
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract (manuscript)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction par. 5, 6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction par. 6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Methods par. 1
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods par. 1-4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods par. 1
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Methods par. 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods par. 2-4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods par. 5-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods par. 5-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Not done, justification in Methods par. 4-5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Methods par. 5-7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Methods par. 7

Section/topic	#	Checklist item	Reported on
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Not done, justification in Methods par. 4
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Results par.1, Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Results par. 2-10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Not done, justification in Methods par. 4.
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Not done, justification in Methods par. 4.
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion par. 1
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Discussion par. 4
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Discussion par. 3, 6-11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Funding

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Appendix 4. Search strategy for the systematic review of treatment landscape

Ovid (Embase)

Database: Embase

Search Strategy:

---

1 scrub typhus/

2 Orientia tsutsugamushi/

3 "scrub typhus".ti,ab.

4 tsutsugamushi.ti,ab.

5 scrubtyphus.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

6 "mite typhus".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

7 "japanese river fever".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

8 "kedani fever".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

9 "chigger borne rickettsia".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

10 "chigger borne rickettsiosis".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

11 "chigger borne typhus".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

12 mijtekoorts.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

13 "mite borne rickettsia".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

14 "mite borne rickettsiosis".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

15 "mite borne typhus".mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

16 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15

17 limit 16 to humans

18 limit 17 to yr="1998 -Current"

19 limit 18 to editorial

20 limit 18 to letter

21 limit 18 to "review"

22 limit 18 to "systematic review"

23 limit 18 to meta analysis

24 19 or 20 or 21 or 22 or 23

25 18 not 24

Ovid (Medline)

Database: Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE and Versions(R)

Search Strategy:

---

1 scrub typhus/

2 Orientia tsutsugamushi/

3 "scrub typhus".ti,ab.

4 tsutsugamushi.ti,ab.

5 scrubtyphus.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

6 "mite typhus".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

7 "japanese river fever".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

8 "kedani fever".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

9 "chigger borne rickettsia".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

10 "chigger borne rickettsiosis".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

11 "chigger borne typhus".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

12 mijtekoorts.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

13 "mite borne rickettsia".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

14 "mite borne rickettsiosis".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

15 "mite borne typhus".mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

16 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15

17 limit 16 to human

18 limit 17 to yr="1998 -Current"

19 limit 18 to systematic reviews

20 limit 18 to meta analysis

21 limit 18 to editorial

22 limit 18 to letter

23 limit 18 to "review"

24 19 or 20 or 21 or 22 or 23

25 18 not 24

Ovid (Global Health)

Database: Global Health

Search Strategy:

---

1 scrub typhus/

2 Orientia tsutsugamushi/

3 "scrub typhus".ti,ab.

4 tsutsugamushi.ti,ab.

5 scrubtyphus.mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

6 "mite typhus".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

7 "japanese river fever".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

8 "kedani fever".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

9 "chigger borne rickettsia".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

10 "chigger borne rickettsiosis".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

11 "chigger borne typhus".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

12 mijtekoorts.mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

13 "mite borne rickettsia".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

14 "mite borne rickettsiosis".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

15 "mite borne typhus".mp. [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

16 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15

17 "systematic review".mp. and 16 [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

18 ("meta analysis" or "meta-analysis").mp. and 16 [mp=abstract, title, original title, broad terms, heading words, identifiers, cabicodes]

19 limit 16 to yr="1998 -Current"

20 limit 17 to yr="1998 -Current"

21 limit 19 to yr="1998 -Current"

Cochrane library

"scrub typhus" OR tsutsugamushi OR scrubtyphus OR "mite typhus" OR "japanese river fever" OR "kedani fever" OR "chigger borne rickettsia" OR "chigger borne rickettsiosis" OR "chigger borne typhus" OR mijtekoorts OR "mite borne rickettsia" OR "mite borne rickettsiosis" OR "mite borne typhus"

Publication Year from 1998 to 2018

Scopus

(TITLE-ABS-KEY ("scrub typhus") OR TITLE-ABS-KEY (tsutsugamushi) OR ALL (scrubtyphus) OR ALL ("mite typhus") OR ALL ("japanese river fever") OR ALL ("kedani fever") OR ALL ("chigger borne rickettsia") OR ALL ("chigger borne rickettsiosis") OR ALL ("chigger borne typhus") OR ALL (mijtekoorts) OR ALL ("mite borne rickettsia") OR ALL ("mite borne rickettsiosis") OR ALL ("mite borne typhus")) AND PUBYEAR > 1997 AND (LIMIT-TO (DOCTYPE , "ar") OR LIMIT-TO (DOCTYPE , "cp") OR LIMIT-TO (DOCTYPE , "ip"))

Global Index Medicus

tw:("scrub typhus" OR tsutsugamushi OR scrubtyphus OR "mite typhus" OR "japanese river fever" OR "kedani fever" OR "chigger borne rickettsia" OR "chigger borne rickettsiosis" OR "chigger borne typhus" OR mijtekoorts OR "mite borne rickettsia" OR "mite borne rickettsiosis" OR "mite borne typhus") AND (instance:"ghl") AND (db:("WPRIM") AND year_cluster:(“1998”or “1999”or “2000”or “2001”or “2002”or “2003”or “2004”or “2005”or “2006”or “2007”or “2008”or “2009”or “2010”or “2011”or “2012”or “2013”or “2014”or “2015”or “2016”or “2017”or “2018”))

tw:("scrub typhus" OR tsutsugamushi OR scrubtyphus OR "mite typhus" OR "japanese river fever" OR "kedani fever" OR "chigger borne rickettsia" OR "chigger borne rickettsiosis" OR "chigger borne typhus" OR mijtekoorts OR "mite borne rickettsia" OR "mite borne rickettsiosis" OR "mite borne typhus") AND (instance:"ghl") AND (db:("IMSEAR") AND year_cluster:(“1998”or “1999”or “2000”or “2001”or “2002”or “2003”or “2004”or “2005”or “2006”or “2007”or “2008”or “2009”or “2010”or “2011”or “2012”or “2013”or “2014”or “2015”or “2016”or “2017”or “2018”))

tw:("scrub typhus" OR tsutsugamushi OR scrubtyphus OR "mite typhus" OR "japanese river fever" OR "kedani fever" OR "chigger borne rickettsia" OR "chigger borne rickettsiosis" OR "chigger borne typhus" OR mijtekoorts OR "mite borne rickettsia" OR "mite borne rickettsiosis" OR "mite borne typhus") AND (instance:"ghl") AND (db:("LILACS") AND year_cluster:(“1998”or “1999”or “2000”or “2001”or “2002”or “2003”or “2004”or “2005”or “2006”or “2007”or “2008”or “2009”or “2010”or “2011”or “2012”or “2013”or “2014”or “2015”or “2016”or “2017”or “2018”))

tw:("scrub typhus" OR tsutsugamushi OR scrubtyphus OR "mite typhus" OR "japanese river fever" OR "kedani fever" OR "chigger borne rickettsia" OR "chigger borne rickettsiosis" OR "chigger borne typhus" OR mijtekoorts OR "mite borne rickettsia" OR "mite borne rickettsiosis" OR "mite borne typhus") AND (instance:"ghl") AND (db:("IMEMR") AND year_cluster:(“1998”or “1999”or “2000”or “2001”or “2002”or “2003”or “2004”or “2005”or “2006”or “2007”or “2008”or “2009”or “2010”or “2011”or “2012”or “2013”or “2014”or “2015”or “2016”or “2017”or “2018”))

ClinicalTrials.gov

“scrub typhus”

WHO ICTRP

“scrub typhus”

Appendix 5. Variable dictionary for the systematic review of treatment landscape

STUDY

- **Study identification and publication details**

st_study_title	Free text: Short Hand Reference (first author, publication year) and, *if applicable*, Scientific title of the study/trial, as stated at registration. E.g. 'Botoni2007' or 'Morillo2017; STOPCHAGAS'
st_study_num	Text box: Assign study number to the paper, use 3 digits e.g. 001, 025, 103 Define this as the secondary unique field to use it as secondary identifier and have it displayed across forms
st_pub_date	Date of publication (**DD/MM/YYYY**) (earliest known, i.e. chose 'ePub ahead of print', 'Published online' date if available), or of presentation at a conference. If an exact publication date is unknown (online or published version), if possible, chose 'manuscript accepted' date and add 1 month; estimate it from the journal; or, arbitrarily DD=15 and MM=01 and year of publication.
st_author	Free text: details of the author or contact for correspondence, i.e., name and institution
st_author_contact	Email address for the author or contact for correspondence

- **Cohort**
- Number of cohorts within single study

st_num_cohort	Number of cohorts within this single 'study' reported in this article/abstract (see Notes for details).
st_coh_div_reas	Checkbox variable: describing the Reason for the division of study cohorts: 1, "**Several countries**" | 2, "**Different participant groups**" | 3, "**Different protocols per group** "
st_coh_div_desc	Free text: further justification for counting a single 'study' reported in this article/abstract as several 'cohorts' = set of participants sharing the same study meta-data characteristics (see Notes for details).

- **Setting**
- Where was the study conducted/cohort recruited?
- The number of participants included in each cohort

Following variables completed for each cohort: 1,2,3 etc.	
st_coh1_country	Dropdown variable: Name of the country of study. Country name based on UN classification: http://unstats.un.org/unsd/methods/m49/m49regin.htm Taiwan and unknown options added.
st_coh1_site	Free text: most precise name and address of each and every site of recruitment, including longitude and latitude GPS coordinates, if available.

st_coh1_region	Dropdown variable: World region of the country of study. Use UN classification : http://unstats.un.org/unsd/methods/m49/m49regin.htm
st_coh1_income	Dropdown variable: describing the income group of the country; **"Low" "Lower middle" "Upper middle" "High"**: as per World Bank: http://data.worldbank.org/country
st_coh1_site_cat	Checkbox variable: describing the category of the site chosen as a recruitment centre of target participants: "**hospital/health-centre**"; "**community**" (village/city, households, etc.); "**other**". Select "**unknown**" otherwise.
st_coh1_setting	Dropdown variable: describing the setting where the study was done: "**rural**"; "**semi-urban**"; "**urban**". Use Google Earth if unclear in full text. **Urban** if the area around the point, within 5x5 km square, is dominated by commercial buildings (e.g. offices, malls, apartments, high rise buildings). **Suburban** if the area around the point, within 5x5 km square, is dominated by housing. **Rural** if the area around the point, within 5x5 km square, is dominated by agricultural land/forest. Several options may apply. Enter "**unknown**" otherwise.
st_coh1_pt_setting	Checkbox variable: describing the setting where recruited participants live: "**rural**"; "**semi-urban**"; "**urban**". Several options may apply. If not mentioned or unclear, enter "**unknown**".
st_coh1_num_patients	Integer: Total number of participants in the initial cohort; i.e. who were *INCLUDED* into the study *and received* the intended intervention (drug(s), control, placebo, no-treatment, etc.), with the intention to follow them up for outcome assessment.
st_coh1_num_scrub	Integer: Total number of participants with scrub typhus in the initial cohort; i.e. who were *INCLUDED* into the study *and received* the intended intervention (drug(s), control, placebo, no-treatment, etc.), with the intention to follow them up for outcome assessment.

- **Study Characteristics**
- What study design was used and was data collected prospectively or retrospectively?
- Was the study comparative/were multiple arms involved in the study?
- Were patients exposed to a treatment or intervention during the study and if so, how many arms did the study involve, including control-arms?

st_type	Dropdown variable: detailing the study type. Discrete choice: 1, "**Interventional**" | 2, "**Observational**" *See* Notes *for further description of categories*
st_design	Dropdown variable: detailing the study type/design. Discrete choice: Dropdown variable: detailing the study design. Discrete choice: If st_type is "**Interventional**" then select one of the following study designs.

	1, "**RCT**" | 2, "**Quasi-randomised**" | 3, "**Non-randomised**" Select 3 "non-randomised" for single arm interventional study arms. If st_type is "**Observational"** then select one of the following study designs: | 4, "**Cohort-study**" | 5, "**Case report**" | 6 "**Case series"** | 7, **"Case-control**" | 8, "**Diagnostic test accuracy**" | 9, "**Other**" *See* Notes *for further description of categories*
st_pros_retro	Dropdown variable: describing whether data collected for the study was performed prospective or retrospectively. Discrete choice: 0, "**Prospective**" | 1, "**Retrospective**"
st_intv_arms	Integer: Number of study arms Study arms: an arm/group of patients receiving a specific treatment regimen or intervention. I.e., also referred to as number of treatment arms, comparative arms, treatment groups, intervention groups

- When was the study conducted and for how long were patients followed-up?

st_start_date	Start date of study (**DD/MM/YYYY**). If the exact date is unknown, then, arbitrarily, DD=15 and MM=01. If the year of study start date is unknown, refer to [**st_end_date]** and estimate the start year by subtracting the trial and follow-up duration from the [**st_end_date]** year. If follow-up duration is unknown, subtract one year from study end date.
st_end_date	End date of study (**DD/MM/YYYY**). If the exact date is unknown, if possible, estimate it from start date and duration of follow-up, or, arbitrarily DD=15 and MM=12. If the year of study end is unknown, arbitrarily select the year before the publication was submitted to or accepted for publication where provided or alternatively the year before the publication date.

PARTICIPANTS

- **Diagnostic inclusion**
- What analyses were performed to diagnose scrub typhus?

st_diag_meth	Checkbox variable: describing the types of diagnostic tests used to confirm diagnosis of participants at baseline/study inclusion or during study if observational. Several options may apply: 1, "**STIC**" | 2, "**Cell culture isolate**" | 3, "**IFA**" | 4, "**IIP**" | 5, "**PCR**" | 6, "**ELISA**" | 7, "**ICT**" | 8, "**Eschar presence**" | 9, "**Weil-Felix**" | 10, "**History and clinical manifestation**" | 11, "**Other**" | 99, "**Unknown**" *See* Notes *for further description of categories*
st_diag_meth_oth	If [**st_diag_meth**] = "11" → "**Other**" Textbox: Mention other diagnostic methods used
st_diag_meth_detail	Free text: Further details of diagnostic method and technique used to confirm diagnosis and/or confirm eligibility for study inclusion.

- **Other Inclusion Criteria**
- What is the age range and gender split of included patients?

pa_age_min	Minimum age of included participants, that is the youngest participant included in the study, in years. If minimum is not reported, use lower

	limit of eligible age range. If no eligible age range reported for adult participants, arbitrarily enter "**18**". Validation changed from integer to number to accommodate decimal numbers (i.e. if age is given in months).
pa_age_max	Maximum age of included participants, that is the eldest participant included in the study, in years. If maximum is not reported, use upper limit of eligible age range. If no upper age range and no maximum age reported enter "**99**". Validation changed from integer to number to accommodate decimal numbers (i.e. if age is given in months).
pa_age_mean	Integer: The mean age of included participants in a study. Calculate if the mean is not available, but the age of all participants is known (e.g. described in the supplement). Enter "-99" if unknown. Validation changed from integer to number to accommodate decimal numbers.
pa_age_median	Integer: The median age of included participants in a study. Calculate if the median is not available, but the age of all participants is known (e.g. described in the supplement). Enter "-99" if unknown. Validation changed from integer to number to accommodate decimal numbers.
pa_female	Integer: Total number of female participants included in the study. If not reported and a ratio of M:F is provided, calculate the number of females by applying the ratio to the total number of included participants. Enter "**-99**" if unknown.
pa_male	Integer: Total number of male participant included in the study. If not reported and a ratio of M:F is provided, calculate the number of males by applying the ratio to the total number of included participants. Enter "**-99**" if unknown.

- Were immunocompromised patients or pregnant women included?

me_preg_yn	Dropdown variable: were pregnant women included in the study? Discrete choice: 1, "**Included**" | 2, "**Excluded**" | 99, "**Unknown**" Assumption made that pregnant women were "**Excluded**" in paediatric studies where age of participants was ≤10years. Enter "**Unknown**" otherwise.
pa_preg_desc	If [**me_preg_yn**] = 1, "**Included**" Free text: further details on the eligibility criteria of pregnant participants

- Other specific inclusion criteria

pa_oth_incl	Free text: other inclusion criteria not falling into any of the previous categories.

- **Exclusion criteria**

pa_excl_scrub	Free text: description of any scrub typhus specific criteria that were used to determine ineligibility/exclusion from the study.
pa_excl_oth	Free text: list of severe illnesses or systematic diseases/health conditions leading to exclusion.
pa_oth_excl	Free text: Other exclusion criteria not falling into any previous categories

STUDY ARM

NOTE: IN REDCap the 'Study Arm' Form is now a repeatable instrument that requires a new 'study arm' form to be created for each arm – This is not automatically created for you. This replaces the old 'Intervention' form that would populate variables based on branch logic of the number of how many 'treatment arms' you entered.

The reason for these changes is to accurately capture instances where patients in a single study arm are exposed to multiple therapies/treatments/interventions.

The below variables need to be completed for each study arm in a separate 'study arm' form which is a repeatable instrument in REDCap.

- Details of the intervention/treatment regimens for each study arm and corresponding number of participants per study arm

Following variables completed for each treatment arm 1,2,3 etc.	
sa_target	Dropdown variable: What was the treatments/interventions given? Discrete choice: 1, "**Antibiotic treatment**" | 2, "**Symptomatic treatment**" | 3, "**Other"** | 99, "**Unknown**"
sa_num_intv	Integer: How many treatments/interventions were patients in this treatment arm exposed to? Notes: To capture total number interventions in a single study arm – i.e., if combination therapy, multiple interventions or drug therapy plus another intervention(s) For observational study, use this variable to capture antibiotic treatment administered for scrub typhus. E.g. if combination treatment of drug A plus drug B was administered in a single study arm enter '2'
sa_num_tx	Integer: Number of scrub typhus infected patients treated or exposed to the intervention. That is, the total number of scrub typhus patients in the study arm.
sa_num_followup	Integer: The reported number of patients followed-up in the study arm. If not reported, the maximum number of scrub typhus patients with at least one follow-up measurement post-baseline (i.e., during the treatment period or post-treatment follow-up) in the study arm. Enter "-1" if not applicable (e.g. retrospective studies or when 'provide samples at follow-up' is an inclusion criterion, meaning that the study cohort was defined *a posteriori*, based on compliance); and "-99" if unknown.
Branch logic – following variables to be completed for each intervention administered within the single study/treatment arm - corresponding to **[sa_num_intv]**	
sa_intv1_desc	Free text: Name of treatment(s) or intervention specific to the study arm of interest.
sa_intv1_drug	If **[sa_num_intv]** >= 1 Dropdown variable: Name of drug administered / active pharmaceutical ingredient (API) as per INN. Discrete Choice

	1, "**Doxycycline**" |2, "**Minocycline**" | 3, "**Chloramphenicol"** | 4, "**Rifampicin**" | 5, "**Azithromycin**" | 6, "**Telithromycin**" | 7, "**Levofloxacin**" | 8, "**Other**" | 99, "**Unknown**" When the comparative arm is no treatment, select "**other**". *Controlled vocabulary: International Nonproprietary Names (INN) should be used to identify pharmaceutical substances or active pharmaceutical ingredients.* If a fixed-dose combination (FDC) – in this variable select one of the APIs and the second API that makes up the single dosage form in the next variable [**in_intv1_drug_b]**. If there are more than 2 APIs in a single dose formulation – detail further in free text of **[in_intv1_reg_detail]**.
sa_intv1_drug_oth	If **[sa_intv1_drug]** = "8", "**Other**" Name of other medicine administered
sa_intv1_reg_yn	Dropdown: Are regimen details of the intervention or exposure available? Discrete choice: 0, "**No**" | 1, "**Yes**"
sa_intv1_drug_b	If **[sa_fixed]** = 1, "**fixed-dose**" Free text: name of second drug/API that makes up the single dosage form.
sa_intv1_route	If [**sa_intv1_reg_yn**] = 1, yes Dropdown variable: What was the route of administration? 1, "**Oral**" | 2, "**Intravenous**" | 3, "**Intramuscular**" | 4, "**Intralymphatic**" | 5, "**Subcutaneous**" | 7, "**Other**" | 99, "**Unknown**" *EMA Controlled Vocabulary for Route of administrations:* *http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002730.pdf*
sa_intv1_route_dur	If **[sa_intv1_route]** = 2, "Intravenous" Integer: Duration of infusion (in below units)
sa_intv1_dur_un	If **[sa_intv1_route_dur]** >= "1", not "Unknown" Dropdown variable: Unit of infusion duration time. Discrete choice: 1, "**Minutes**" | 2, "**Hours**" | 3, "**Days**" | 4, "**Other**" | 99, "**Unknown**"
sa_intv1_route_other	If **[sa_intv1_route]** =7, "Other" Free text: Description of 'Other' route of administration
sa_intv1_dose_method	If [**sa_intv1_reg_yn**] = 1, yes Dropdown variable: Method of dosing calculation. Discrete choice: 1, "**Target mg/kg**" | 2, "**Weight-band**" | 3, "**Age-band**" | 4, "**other**" | 99, "**unknown**"
sa_intv1_other_det	If [**sa_intv1_dose_method**] = 2, Weight-band, 3, Age-band, 4, Other Free text: dose used per administration Enter with unit
sa_intv1_dose_mgkg	If **[sa_intv1_dose_method]** = 1 "Target mg/kg" Free text: Targeted dosing or range in mg/kg
sa_intv1_freq_yn	If [**sa_intv1_reg_yn**] = 1, yes Dropdown: Are details of the frequency of administration of the drug(s)/treatment/intervention available? Discrete choice: 0, "**No**" | 1, "**Yes**" | 99, "**Unknown**"

	Note: If the frequency is reported as a range (eg. 1-2 times a day), select 'no'. Enter any information provided to the 'Detailed regimen' box.
sa_intv1_freq_con_yn	If [**sa_intv1_freq_yn**] = 1, yes Dropdown: Is the frequency of the regimen consistently applied ie., if there is a loading dose or changes in frequency over time, select 'No' Discrete choice: 0, "**No**" | 1, "**Yes**"
sa_intv1_freq	If [**sa_intv1_freq_yn**] = 1, yes Integer: frequency that the drug(s)/treatment/intervention was administered for a given time period -99 if unknown e.g. Twice daily (Frequency = 2; Time period units = days; time period magnitude = 1) e.g. Once every two days (Frequency = 1; time period units = days; time period magnitude = 2) e.g. Once every three months (Frequency = 1; time period units = months; time period magnitude = 3)
sa_intv1_freq_time_un	If [**sa_intv1_freq_yn**] = 1, yes Dropdown variable: Units of given time period 1, "**Hours**" | 2, "**Days**" | 3, "**Weeks**" | 4 "**Months**" | 5, "**Years**" | 6, "**Other**" 99, "**Unknown**" e.g. Twice daily (Frequency = 2; Time period units = days; time period magnitude = 1) e.g. Once every two days (Frequency = 1; time period units = days; time period magnitude = 2) e.g. Once every three months (Frequency = 1; time period units = months; time period magnitude = 3)
sa_intv1_freq_time_un_oth	If [**sa_intv1_freq_time_un] = 6, other** If [**sa_intv1_freq_time_un**] = 6, other **Free text: Other unit of time.**
sa_intv1_freq_time_mag	If [**sa_intv1_freq_yn**] = 1, yes Integer: Magnitude of given time period -99 if unknown e.g. Twice daily (Frequency = 2; Time period units = days; time period magnitude = 1) e.g. Once every two days (Frequency = 1; time period units = days; time period magnitude = 2) e.g. Once every three months (Frequency = 1; time period units = months; time period magnitude = 3)
sa_intv1_dura_fix_mag	If [**sa_intv1_reg_yn**] = 1, yes Integer: Duration of the **intended** (in the protocol or in guidelines if applicable) length of treatment or time the intervention was administered. Enter "**-99**" if unknown. If a range is given i.e., 7-14 days, please enter "**-1**" and provide the details in free text for the variable below **sa_intv1_reg_detail**
sa_intv1_dura_fix_un	If [**sa_intv1_dura_fix_mag**] **>**= "1", not "Unknown" Dropdown variable: Units for duration. Discrete choice: **1, Days | 2, Weeks | 3, Months | 4, Years | 9, Other**
sa_intv1_dura_fix_un_oth	If [**sa_intv1_dura_fix_un**] = 9, Other Free text: Other units for duration

sa_intv1_reg_detail	Free text: Further details and description of the treatment or intervention regimen specific to the study arm of interest, including the dose description and details of frequency Include details of how multiple therapies administered if **[In_intv1_combi]** = 3 "**other**"

OUTCOMES

- **Primary Treatment Related Outcomes**
- Were treatment/intervention related outcomes assessed at any time during the study post-baseline (during the treatment period or follow-up)?

ou_assessed	Dropdown variable: Were treatment/intervention related outcomes assessed at any time during the study post-baseline (during the treatment period or follow-up)? Discrete choice: 0, "**No**" | 1, "**Yes**"

- How was treatment response expressed as an outcome, how was it measured and how often was it measured?

ou_fevclr	Dropdown variable: Were fever clearance assessed at any time during the study post-baseline (during the treatment period or follow-up)? Discrete choice: 0, "**No**" | 1, "**Yes**" | 99, "**Unknown**"
ou_fevclr_measure	If **[ou_fevclr]** = 1, "Yes" Checkbox variable: How were fever clearance measured? Several options may apply: 1, "**Oral**" | 2, "**Axillary**" | 3, "**Core**" | 4, "**Aural**" | 99, "**Unknown"**
ou_fevclr_def	If **[ou_fevclr]** = 1, "Yes" Free text: description of how the fever clearance was expressed i.e. definition of fever clearance.
ou_fevclr_endpoints	If **[ou_fevclr]** = 1, "Yes" Integer: How often were participants followed-up for fever clearance assessments at any time during the study post-baseline (during the treatment period or follow-up), i.e. total number of fever clearance related measurements. Enter "**-99**" if unknown.
ou_fevclr_length	If **[ou_fevclr]** = 1, "Yes" Integer: the time in days, for fever clearance follow-up endpoint, counted from day of first treatment/intervention/start of study. Enter "**-99**" if unknown. For calculations in days; assumptions 1 month=30 days, 1 year 365 days.
ou_fevclr_time	Dropdown variable: Were fever clearance time/time to defervescence assessed at any time during the study post-baseline (after treatment was given or during follow-up period)? Discrete choice: 0, "**No**" | 1, "**Yes**" | 99, "**Unknown**"
ou_fevclr_time_details	If **[ou_fevclr_time]** = 1, "Yes"

	Free text: Further details on fever clearance time measurements.
ou_mort	Dropdown variable: Were mortality assessed at any time during the study post-baseline (during the treatment period or follow-up)? Discrete choice: 0, "**No**" | 1, "**Yes**" | 99, "**Unknown**" Mortality is considered to be assessed if: (1) it is explicitly mentioned i.e. mortality is an outcome of interest that will be specifically recorded), (2) mortality reported in the results, (3) adverse events were recorded.
ou_mort_length	If **[ou_mort]** = 1, "Yes" Integer: the time in days, for mortality follow-up endpoint, counted from day of first treatment/intervention/start of study. Enter "**-99**" if unknown. For calculations in days; assumptions 1 month=30 days, 1 year 365 days.
ou_clinres	Dropdown variable: Were clinical response/cure/recovery assessed at any time during the study post-baseline (during the treatment period or follow-up)? Discrete choice: 0, "**No**" | 1, "**Yes**" | 99, "**Unknown**"
ou_clinres_feverclr	If **[ou_ clinres]** = 1, "Yes" Dropdown variable: Were clinical response/cure defined as fever clearance? Discrete choice: 0, "**No**" | 1, "**Yes**" | 99, "**Unknown**"
ou_clinres_def	If **[ou_ clinres_feverclr]** = 0, "No" Free text: description of how clinical response to treatment was expressed i.e. definition of clinical response.
ou_clinres_endpoints	If **[ou_ clinres_feverclr]** = 0, "No" Integer: How often were participants followed-up for clinical response/cure at any time during the study post-baseline (during the treatment period or follow-up), i.e. total number of fever clearance related measurements. Enter "**-99**" if unknown.
ou_clinres_length	If **[ou_ clinres_feverclr]** = 0, "No" Integer: the time in days, for clinical response/cure follow-up endpoint, counted from day of first treatment/intervention/start of study. Enter "**-99**" if unknown. For calculations in days; assumptions 1 month=30 days, 1 year 365 days.
ou_scrcomp	Dropdown variable: Were scrub typhus complications assessed at any time during the study post-baseline (during the treatment period or follow-up)? Discrete choice: 0, "**No**" | 1, "**Yes**" | 99, "**Unknown**"
ou_scrcomp_measure	If **[ou_scrcomp]** = 1, "Yes" Checkbox variable: How were scrub typhus complication measured during follow up?
ou_scrcomp_def	If **[ou_scrcomp]** = 1, "Yes" Free text: description of how scrub typhus complications were expressed i.e. definition of complications.
ou_scrcomp_type	If **[ou_scrcomp]** = 1, "Yes" Checkbox variable: What type(s) of scrub typhus complications were noted?

	1, "**Respiratory**" | 2, "**Neurological**" | 3, "**Haematological**" | 4 "**Hepatological**" | 5, "**Nephrological**" | 6, "**Other**" | 99, "**Unknown**"
ou_scrcomp_type_other	If **[ou_scrcomp_type]** = 6, "Other" Free text: Description of other types of scrub typhus complications
ou_scrcomp_type_details	If **[ou_scrcomp]** = 1, "Yes" Free text: Further description of diagnosis and conditions counted as scrub typhus complications e.g. noting acute respiratory disease syndrome (ARDS) if **[ou_scrcomp_type]** → 1, "Respiratory".
ou_ scrcomp _endpoints	If **[ou_ scrcomp]** = 1, "Yes" Integer: How often were participants followed-up for scrub typhus complications assessments at any time during the study post-baseline (during the treatment period or follow-up), i.e. total number of fever clearance related measurements. Enter "**-99**" if unknown.
ou_ scrcomp _length	If **[ou_ scrcomp]** = 1, "Yes" Integer: the time in days, for scrub typhus complications follow-up endpoint, counted from day of first treatment/intervention/start of study. Enter "**-99**" if unknown. For calculations in days; assumptions 1 month=30 days, 1 year 365 days.

- **Safety and Other Patient Related Outcomes**
- Were adverse events / side effects / safety variables assessed any time during the study post-baseline (during the treatment period or follow-up)?
- Details of other patient related outcome measurements not captured above

ou_ae	Dropdown variable: Were adverse events / side effects / safety variables assessed any time during the study post-baseline (during the treatment period or follow-up)? Discrete choice: 0, "**No**" | 1, "**Yes**"
ou_ae_type	If [**ou_ae**] = 1, "Yes" Dropdown variable: Were adverse events assessed consisted of mortality and/or complications only? 0, "**No**" | 1, "**Yes, mortality only**" | 2, "**Yes, complication only**" | 3, "**Yes, mortality and complication**"
ou_pt_outcome	Checkbox variable: Were any of the following outcome measures assessed at any time during the study post-baseline (during the treatment period or follow-up)? Several options may apply: 1, "**Relapse**" | 2, "**Hospital stay**" | 3, "**Imaging parameters improvement**" | 4, "**Pharmacokinetics**"
ou_pt_outcome_details	Free text: Further details on the above outcome measures, including: definition, how it was measured.
ou_other_outcome	Free text: Details of any other patient related outcomes measured/assessed at any time during the study post-baseline (during the treatment period or follow-up) that are not captured in above categories.

Notes

- **Reasons for considering a single study as several cohorts**

A study is to be divided accordingly into different cohorts if and only if at least one of the three following conditions apply:

1. Multi-centric study, with centres in several countries → 1 cohort per country
2. The study clearly differentiates a priori between different categories of participants → 1 cohort per participant group
3. The protocol is otherwise amended depending on site/group (e.g. different diagnostic approach, different follow-up time-points) → 1 cohort per protocol

- **Detailed explanation of study type and definitions**

Use the following clinicaltrials.gov definitions of study type:
Interventional (experimental) Study: A type of clinical study in which participants are assigned to groups that receive one or more intervention/treatment (or no intervention) so that researchers can evaluate the effects of the interventions on biomedical or health-related outcomes. The assignments are determined by the study's protocol. Participants may receive diagnostic, therapeutic, or other types of interventions.

Observational Study: The general design of the strategy for identifying and following up with participants during an observational study. Types of observational study models include cohort, case-control, case-only, case-cross-over, ecologic or community studies, family-based, and other.

- **Detailed explanation of study design categories**

Definitions of study designs 1-5 are as per the Cochrane Consumers & Communication Review Group
Study Design Guide. For Review Authors. June 2013.
https://cccrg.cochrane.org/sites/cccrg.cochrane.org/files/public/uploads/Study_design_guide2013.pdf

Definitions of study design 6, Diagnostic Test Accuracy is as per The Joanna Briggs Institute Reviewers' Manual 2015. The systematic review of studies of diagnostic test accuracy.
https://joannabriggs.org/assets/docs/sumari/Reviewers-Manual_The-systematic-review-of-studies-of-diagnostic-test-accuracy.pdf

Interventional (experimental) Study Designs

1, "**RCT**"
RANDOMISED CONTROLLED TRIALS
In RCTs the investigator randomly assigns people to groups that will receive (intervention group) or not receive (control group) one or more interventions. The outcomes measured are then compared between the groups.

2, "**Quasi-randomised**"
QUASI-RANDOMISED CONTROLLED TRIALS
Trials that attempt to randomly assign participants to groups but use an inadequate approach to generate the random sequence are designated as quasi-randomised controlled trials. Such trials do attempt to randomly allocate participants with the intent of producing equivalent groups, but the randomisation methods used are not adequate because in practice they are relatively easy to

manipulate or predict. Inadequate randomisation approaches: Alternation, Case record numbers, Birth dates, Week days or month of the year.

3, "**Non-randomised**"
Either a single arm interventional study or a multi-arm study that does not apply randomisation when assigning individuals to treatment.

Observational Study Designs

4, "**Cohort-study**"
COHORT STUDY (SYNONYM: FOLLOW-UP, INCIDENCE, LONGITUDINAL, PROSPECTIVE STUDY)
An observational study in which a defined group of people (the cohort) is followed over time. This also includes non-comparative studies e.g. "prospective observational" that followed only one cohort. The outcomes of people in subsets of this cohort are compared, to examine for example people who were exposed or not exposed (or exposed at different levels) to a particular intervention or other factor of interest. A cohort can be assembled in the present and followed into the future (this would be a prospective study or a "concurrent cohort study"), or the cohort could be identified from past records and followed from the time of those records to the present (this would be a retrospective study or a "historical cohort study"). Because random allocation is not used, matching or statistical adjustment at the analysis stage must be used to minimise the influence of factors other than the intervention or factor of interest.

5, "**Case-control**"
CASE-CONTROL STUDY (SYNONYMS: CASE REFERENT STUDY, RETROSPECTIVE STUDY)
A study that starts with identification of people with the disease or outcome of interest (cases) and a suitable control group without the disease or outcome. The relationship of an attribute (intervention, exposure or risk factor) to the outcome of interest is examined by comparing the frequency or level of the attribute in the cases and controls. For example, to determine whether thalidomide caused birth defects, a group of children with birth defects (cases) could be compared to a group of children without birth defects (controls). The groups would then be compared with respect to the proportion exposed to thalidomide through their mothers taking the tablets. Case-control studies are sometimes described as being retrospective as they are always performed looking back in time.

6 & 7, "**Case-report or series**"
CASE STUDY (SYNONYMS: ANECDOTE, CASE HISTORY, SINGLE CASE REPORT)
An uncontrolled observational study involving an intervention and outcome for a single person (or other unit).
CASE SERIES
An uncontrolled observational study involving an intervention and outcome for more than one person.

8, "**Diagnostic test accuracy**"
Diagnostic test accuracy studies compare a diagnostic test of interest (the 'index test') to an existing diagnostic test (the 'reference test'), which is known to be the best test currently available for accurately identifying the presence or absence of the condition of interest. The outcomes of the two tests are then compared with one another in order to evaluate the accuracy of the index test. There are two main types of studies of DTA. The first is the diagnostic case-control design, also sometimes called the 'two gate design'. In this study design people with the condition (cases) come from one population (i.e. a health care centre for people known to have the condition), while people without the condition come from another. Although this design gives an indication of the

maximum accuracy of the test, the results will generally give an exaggerated indication of the test's accuracy in practice. The second study design is cross-sectional, and involves all patients suspected of having the condition of interest undergoing the index test and the reference test. Those who test positive for the condition by the reference test can be considered to be the cases, whereas those who test negative are the controls.

9, "**Other**" Any other study design not captured in categories 1-8.

- **Definition of confirmed scrub typhus diagnosis**

Definition of confirmed cases: history (i.e. went to endemic area or known foci of infection), clinical signs and symptoms (fever and other flu-like symptoms, eschar, organ dysfunction), and laboratory confirmation (PCR/IFA/IIP/ELISA/ICT/Weil-Felix).

- **Detailed explanation of diagnostic methods**

1, "**STIC**"
SCRUB TYPHUS INFECTION CRITERIA (STIC) have been proposed as a more robust method to diagnose scrub typhus with more confidence, by including a panel of parameters with high specificity: (i) isolation of *O. tsutsugamushi*, (ii) at least two positive out of the three PCR assays targeting the 56kDa, 47kDa, and groEL genes, (iii) admission IFA IgM titre of ≥1:12,800, (iv) 4-fold rise in IgM titre from paired samples. At least one of these criteria needs to be fulfilled for a positive scrub typhus diagnosis.

2, "***O. tsutsugamushi* isolation**"
Isolation of *O. tsutsugamushi* from cell culture

3, "**IFA**"
INDIRECT FLUORESCENCE ASSAY/INDIRECT FLUORESCENT ANTIBODY TEST/INDIRECT IMMUNOFLUORESCENCE ASSAY/INDIRECT IMMUNOFLUORESCENCE ANTIBODY TEST (IFA) detects antibody to scrub typhus in blood using immunofluorescent staining. The samples need to be assessed under fluorescence microscope. Often considered as a gold standard of scrub typhus diagnosis, despite the unideal sensitivity.

4, "**IIP**"
INDIRECT IMMUNOPEROXIDASE TEST detects antibody to scrub typhus in blood using an enzyme called peroxidase that will produce a change in colour. The samples can be assessed using light microscope.

5, "**PCR**"
POLYMERASE CHAIN REACTION (PCR)
The test is based on the detection of *O. tsutsugamushi* DNA sequences in patients' body fluid or tissues (e.g. eschar biopsy). PCR technique relies on amplification of DNA (Deoxyribonucleic acid) target sequences. The typical target are the 47kDa, 56 kDa, and groEL genes. The different PCR techniques for detecting *O. tsutsugamushi* DNA in patients' blood samples are: "Nested-PCR" (two step amplification often used for very low amount of DNA targets); "Real-Time PCR" or "Quantitative PCR" is based on the polymerase chain reaction technology, used to amplify and simultaneously quantify a targeted DNA molecule.

6, "**ELISA**"
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) is a method to detect antibody against scrub typhus in blood. It is less subjective than IFA. There are commercial test kits available.

7, “**ICT**”
IMMUNOCHROMATOGRAPHY TEST (ICT) is a method to detect antibody against scrub typhus in patient’s blood. It is relatively simpler to perform and results can be interpreted in about 15 minutes. Hence, it is often used as rapid point-of-care test.

8, “**Eschar presence**”
Diagnosis based on eschar presence, concordant history and clinical manifestation (including fever, other flu-like symptoms, rash, organ dysfunction). Eschar is necrotic skin lesion at the site of mite inoculation. It is a pathognomonic sign of scrub typhus.

9, “**Weil-Felix**”
Weil-Felix test is an agglutination test based on cross-reactions between antibodies against rickettsial infections with antigens of OX (OX 19, OX 2, and OXK) strains of Proteus species. Antibody against *O. tsutsugamushi* reacts with *P. mirabilis* OXK. It has low sensitivity and specificity.

10, “**History and clinical manifestation**”
Diagnosis based on history of travel to endemic area or foci of infection (“mite islands”) and clinical manifestation aside from eschar presence (including fever, other flu-like symptoms, rash, organ dysfunction).

11, “**Other**”

12, “**Unknown**”

Appendix 6. List of studies included in the systematic review of treatment landscape

1	Abhilash K, Mannam PR, Rajendran K, John RA, Ramasami P. Chest radiographic manifestations of scrub typhus. Journal of Postgraduate Medicine. 2016;62(4):235-8.
2	Abhilash K, Jeevan J, Mitra S, Paul N, Murugan T, Rangaraj A, David S, Hansdak S, Prakash J, Abraham A, Ramasami P, Sathyendra S, Sudarsanam T, Varghese G. Acute undifferentiated febrile illness in patients presenting to a Tertiary Care Hospital in South India: Clinical spectrum and outcome. Journal of Global Infectious Diseases. 2016;8(4):147-54.
3	Aggarwal HK, Deepak J, Vipin K, Anshul M, Sachin Y, Abhishek G. Emergence of scrub typhus in Northern India: experience from tertiary care hospital. Klimik Dergisi. 2014;27(1):06-Nov.
4	Ankita N, Yatendra S, Makrand S, Saxena SR. A study of acute febrile illness with thrombocytopenia from tertiary care centre of Uttarakhand. Annals of International Medical and Dental Research. 2018;4(3).
5	Bhargava A, Kaushik R, Kaushik RM, Sharma A, Ahmad S, Dhar M, Mittal G, Khanduri S, Pant P, Kakkar R. Scrub typhus in Uttarakhand & adjoining Uttar Pradesh: seasonality, clinical presentations & predictors of mortality. Indian Journal of Medical Research. 2016;144(6):901-9.
6	Attur RP, Kuppasamy S, Bairy M, Nagaraju SP, Pammidi NR, Kamath V, Kamath A, Rao L, Bairy I. Acute kidney injury in scrub typhus. Clinical and Experimental Nephrology. 2013;17(5):725-9.
7	Bal M, Mohanta MP, Sahu S, Dwibedi B, Pati S, Ranjit M. Profile of Pediatric Scrub Typhus in Odisha, India. Indian Pediatrics. 2019;56(4):304-6.
8	Balasubramanian P, Sharma N, Biswal M, Bhalla A, Kumar S, Kumar V. Critical illness scoring systems: Sequential organ failure assessment, Acute Physiology and Chronic Health Evaluation II, and quick sequential organ failure assessment to predict the clinical outcomes in scrub typhus patients with organ dysfunctions. Indian Journal of Critical Care Medicine. 2018;22(10):706-10.
9	Basu S, Saha A, Sarkar S, Sinha MK, Das MK, Datta R, Hazra A, Datta Kanjilal S, Datta S. Clinical profile and therapeutic response of scrub typhus in children: A recent trend from Eastern India. Journal of Tropical Pediatrics. 2019;65(2):139-46.
10	Behera B, Biswal M, Das R, Dey A, Jena J, Dhal S, Mohanty S, Mishra B, Praharaj A. Clinico-epidemiological analysis of scrub typhus in hospitalised patients presenting with acute undifferentiated febrile illness: A hospital-based study from Eastern India. Indian Journal of Medical Microbiology. 2019;37(2):278-80.
11	Bhat NK, Dhar M, Mittal G, Chandra H, Rawat A, Chandar V. Scrub typhus: A common rickettsial disease emerging in a new geographical region of north India. Journal of Pediatric Infectious Diseases. 2014;9(2):93-9.
12	Bithu R, Kanodia V, Maheshwari RK. Possibility of scrub typhus in fever of unknown origin (FUO) cases: An experience from Rajasthan. Indian Journal of Medical Microbiology. 2014;32(4):387-90.

13	Chansamouth V, Thammasack S, Phetsouvanh R, Keoluangkot V, Moore CE, Blacksell SD, Castonguay-Vanier J, Dubot-Peres A, Tangkhabuanbutra J, Tongyoo N, Souphaphonh P, Sengvilaipaseuth O, Vongsouvath M, Phommasone K, Sengdethka D, Seurbsanith A, Craig SB, Hermann L, Strobel M, Newton PN. The Aetiologies and Impact of Fever in Pregnant Inpatients in Vientiane, Laos. PLoS Neglected Tropical Diseases. 2016;10 (4) (no pagination)(e0004577).
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Appendix 7. The definitions of fever clearance time within studies

Stated definition of fever clearance time*	Temperature cut-off	Treatment administration	Antibiotic administration	Antipyretic administration	24-hour window	48-hour window	Twice temperature measurement	Number of studies
Not specified clearly								39
“Time to defervescence was defined as the interval between the time at which the first dose of antibiotic was administered and the time at which the body temperature was less than 37.3°C and persisted for more than 48 hours. Temperature was checked every 4 hours.”	37.3	0	0	0	0	1	0	1
“The primary endpoint was the fever clearance time; this was defined as the interval between the time at which the first dose of antibiotic was administered and the time at which the oral temperature first fell below 37.3°C and then remained below this threshold without antipyretics for a minimum of 48 h.”	37.3	0	0	1	0	1	0	1
“The time to fever clearance was defined as the period from the initial drug administration until the body temperature decreased to 37.3°C or lower and remained below this temperature for at least 48 hours without the use of an antipyretic.”	37.3	1	0	1	0	1	0	2
“Fever clearance time was defined as the time, from onset of treatment, to the first time the aural temperature dropped below 37.5°C and stayed at or below 37.5°C for 24 hours.”	37.5	1	0	0	1	0	0	1

“Time to defervescence, which was defined as the interval between the time at which the first dose of the study drug was administered and the time at which the oral temperature first returned to ≤37.5°C and was maintained for two consecutive measurements without antipyretics.”	37.5	1	0	0	0	0	1	1
“The fever clearance time was the interval between the first dose of antibiotic and the time when the patient's oral temperature first fell below 37·3º C, and remained below this threshold without antipyretics for a minimum of 48 h.”	37.3	0	1	1	0	1	0	1
“The fever clearance time was the number of hours between the first dose of medication and the first time when the morning oral temperature was less than 37·2ºC in the absence of antipyretic medication.”	37.2	1	0	1	0	0	0	1
								47

*Below is further description of each definition components:

Temperature cut-off: The different cut-off temperature to define fever in degree Celsius

Treatment administration: Mention of treatment or drug administration

Antibiotic administration: Mention of antibiotic administration

Antipyretic administration: Mention of antipyretic administration

24-hour window: Desired temperature maintained for 24 hours to declare fever clearance

48-hour window: Desired temperature maintained for 48 hours to declare fever clearance

Twice temperature measurement: Mention of two consecutive measurement in declaring fever clearance

0 and 1 symbolises ‘no’ and ‘yes’ respectively.

Appendix 8. PRISMA checklist for POCT systematic review and meta-analysis

Section/topic	#	Checklist item	Reported on
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract (manuscript)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction, par. 3-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction, par. 7
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Methods, par. 5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods, par. 1-2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods, par. 2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Methods, box 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods, par. 1-2
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods, par. 4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods, par. 4-5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Methods, par. 3, 5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Methods, par. 5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Methods, par. 5

Section/topic	#	Checklist item	Reported on
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Not assessed specifically
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Methods, par. 5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Results, par. 1; Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Figure 2, Results par. 3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Results par. 4-9, Figure 3-5, Table 2-6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Results par. 9, Figure 5
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Not assessed specifically
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Results par. 9
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion par. 1-2
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Discussion par. 7
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Discussion par. 11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Financial disclosure

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Appendix 9. QUADAS-2 scores

id	author	year_pub	Could the selection of patients have introduced bias?	Are there concerns that the included patients do not match the review question?	Could the conduct or interpretation of the index test have introduced bias?	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Could the reference standard, its conduct, or its interpretation have introduced bias?	Are there concerns that the target condition as defined by the reference standard does not match the review question?	Could the patient flow have introduced bias?	Score
1	Blacksell SD et al	2010	Low	Low	Low	Low	Unclear	Low	Low	6
2	Blacksell SD et al	2010	High	Low	Low	Low	Unclear	Low	Low	5
3	Blacksell SD et al	2012	Low	Low	Unclear	Low	Low	Low	Low	6
4	Cao et al	2007	High	High	Unclear	Low	Unclear	Unclear	Low	2
6	Ching et al	2001	High	Low	Unclear	Low	Unclear	High	Low	3
7	Chinprasatsak	2001	Low	Low	Low	Low	Unclear	Low	Low	6
8	Coleman et al	2002	Low	Low	Low	Low	Low	Low	Low	7
9	Kim et al	2016	High	High	Unclear	Low	Unclear	Low	Low	3
10	Kingston et al	2015	High	Low	Low	Low	Low	Low	Low	6
11	Lee et al	2014	High	Low	Unclear	Low	Unclear	Low	Low	4
13	Paris et al	2008	High	Low	Low	Low	Low	Low	Low	6

14	Ramyasree et al	2015	Low	Low	Unclear	Low	Unclear	Low	Low	5
15	Rodkvamtook et al	2015	Low	Low	Unclear	Low	Unclear	Low	Low	5
16	Silpasakorn et al	2012	High	Low	Unclear	Low	Unclear	Low	Low	4
17	Silpasakorn et al	2012	High	Low	Unclear	Low	Unclear	Low	High	3
18	Stephen et al	2015	Low	Low	Unclear	Low	Unclear	Low	Low	5
19	Stephen et al	2016	High	Low	Low	Low	Low	Low	High	5
20	Watt et al	1998	Low	Low	Unclear	Low	Unclear	Low	Low	5
21	Watthanaworawit et al	2015	Low	Low	Unclear	Low	Unclear	Low	Low	5
22	Wilkinson et al	2003	High	Unclear	Unclear	Low	Unclear	Unclear	Low	2
23	Wongchotigul et al	2005	High	Low	Unclear	Low	Unclear	Low	Low	4
24	Zhang et al	2011	High	Low	Unclear	Low	Unclear	Low	Low	4
25	Huber et al	2012	High	Unclear	Unclear	Low	High	Low	Low	3
26	Kim et al	2013	High	Low	Unclear	Low	Unclear	Unclear	Unclear	2
27	Paris et al	2011	Low	Low	Low	Low	Low	Low	Low	7
28	Pradutkanchana et al	1997	High	Low	Unclear	Low	Unclear	Low	Low	4
29	Prakash et al	2006	Low	Low	High	Low	High	High	High	3
30	Weddle et al	1995	High	Low	Unclear	Low	Unclear	Low	Low	4
31	Anitharaj et al	2016	Low	Low	Unclear	Low	Unclear	Low	Low	5
32	Kim et al	1993	High	Unclear	Unclear	Low	Unclear	Low	Low	3
33	Koay et al	1995	High	Unclear	Unclear	Low	Unclear	Low	Low	3

Appendix 10. SEROSCRUB questionnaire

In English:

Screening checklist

To be done before informed consent process. Eligible participants must fulfil **ALL** criteria below.

- ☐ At least 17 years old
- ☐ Resident at site for at least 3 years without an absence >6 months in the past 3 years
- ☐ Free of signs of acute febrile illness

Questionnaire

Informed consent must be obtained before this questionnaire is administered.

Demographic data

1. Age	
2. Sex	
3. Level of education	
4. Address (up to village level)	
5. Income per month	
6. Occupation	
7. Does your work involve working outdoor? - If yes, how many hours do you spend outdoor in a day? - Do you wear any protective clothing?	
8. Does your work involve being in proximity with animals?	

- If yes, how many hours do you in proximity to animals in a day? 9. Do you wear any protective clothing?	

Housing condition

1. Is there toilet available in the house?	
2. How many people live in the house?	
3. How many people (max) sleep in one room?	
4. How far is the house from plantation/forest/park?	
5. How far is the house from river?	

Past medical history

1. Have you been hospitalised in the past?	
2. Are there any medicines you take regularly?	

Travel history

1. Travel history in the past 6 months	

In Indonesian:

Checklist skrining

Harus dilakukan sebelum proses informed consent. Peserta yang memenuhi syarat harus memenuhi **SEMUA** kriteria di bawah ini.

- ☐ Paling tidak berusia 17 tahun
- ☐ Tinggal di lokasi setidaknya 3 tahun tanpa bepergian >6 bulan dalam 3 tahun terakhir
- ☐ Bebas dari tanda-tanda penyakit demam akut

Kuesioner

Informed consent harus diperoleh sebelum kuesioner ini diberikan.

Data demografik

1. Usia	
2. Jenis kelamin	
3. Pendidikan terakhir	
4. Alamat (hingga tingkat desa/kelurahan)	
5. Pendapatan per bulan	
6. Pekerjaan	
7. Apakah pekerjaan Anda melibatkan bekerja di luar ruangan? - Bila ya, berapa jam dalam sehari Anda berada di luar ruangani? - Apakah Anda menggunakan pakaian pelindung?	
8. Apakah pekerjaan Anda melibatkan bekerja di sekitar binatang?	

- Bila ya, berapa jam dalam sehari Anda berada di sekitar binatang? - Apakah Anda menggunakan pakaian pelindung?	

Kondisi rumah

1. Apakah ada toilet di dalam rumah Anda?	
2. Berapa jumlah orang yang tinggal di rumah Anda?	
3. Berapakah jumlah orang (maksimum) yang tidur dalam satu ruangan?	
4. Seberapa jauhkah rumah Anda dari perkebunan/hutan/taman?	
5. Seberapa jauhkah rumah Anda dari sungai?	

Riwayat kesehatan

1. Apakah Anda pernah dirawat di rumah sakit sebelumnya?	
2. Apakah Anda meminum obat tertentu secara teratur?	

Riwayat bepergian

1. Riwayat bepergian dalan 6 bulan terakhir	

Appendix 11. SEROSCRUB patient information sheet and informed consent form

In English:

PARTICIPANT INFORMATION SHEET

You are invited to take part in a study entitled "Scrub typhus antibodies in North Sumatra" conducted by the Eijkman-Oxford Clinical Research Unit. You are one of the 1000 voluntary participants that will be included in this study. This form contains explanation of why this research was conducted, what will take place in this study, the possible benefits and risks that you might get, and other important information.

What is the purpose of this research?

Scrub typhus is a fever caused by bacteria. These bacteria are spread by mite bites. The symptoms include: fever, headache, nausea, vomiting, rash, scab on the site of mite bite, and hearing loss. If not treated quickly, this disease can cause death.

The purpose of this research is to determine the number of residents who have been exposed to bacteria that cause scrub typhus, by looking at the presence or absence of immunity (antibodies) against these bacteria. In addition, things that increase the risk of exposure will also be explored. The information obtained from this study will be useful

for developing prevention measures for this disease. This research is not for commercial use.

Why have I been invited to take part?

You have been invited because you are of at least 17 years, free of signs of acute febrile illness, and residing in this area for at least 3 years without leaving the area for more than 6 months in the past 3 years.

Research procedure

For the study participants who agreed to take part, about a half teaspoon of blood would be taken for the examination of typhus scrub antibodies.

Possible risks

There would be no side effects from this study other than the sensation like being bitten by ants during blood taking.

Benefits

There are no direct benefits from your participation in this study. The study results will be useful for developing prevention and treatment measures for this disease. However, you will get health examination for blood sugar, hypertension and nutritional status

linked to screening process. Participants will be offered a nutritional supplement/hygiene package (including powdered milk, toothbrush, toothpaste, soap).

Cost

You will not be asked for payment for the examination that you will receive.

Confidentiality of information

The confidentiality of your examination results is maintained because only the principal investigator can see your data. The information obtained in this study, if published in a scientific journal or presented at a scientific meeting, will not reveal your identity. Eijkman Institute and University of Oxford will be responsible for ensuring the safe and proper use (solely for research purposes) of any personal information you provide.

Your participation

Your participation in this research is entirely voluntary. You can withdraw at any time without penalty.

If you understand and agree with the explanation we have provided, and you agree to participate in this research, please sign/stamp your thumbprint on the consent form for participation in this study, as a sign of agreement that about half a teaspoon of your

blood will be taken, your data will be recorded and research described above performed on your blood sample.

If in the future you have questions about this research that you are taking part in, you can contact:

Study team: dr. Kartika Saraswati, MSc, DTMH

Eijkman-Oxford Clinical Research Unit, Lembaga Biologi Molekuler Eijkman, Jl. P. Diponegoro No. 69, Jakarta

Tel: 021 3910414

Eijkman Institute Research Ethics Committee (EIREC)

Lembaga Biologi Molekuler Eijkman, Jl. P. Diponegoro No. 69, Jakarta

Tel: (021) 3917131

In Indonesian:

LEMBAR PENJELASAN UNTUK PESERTA

Saudara diajak untuk ikut serta dalam penelitian dengan judul "Antibodi scrub typhus di Sumatra Utara" yang dilakukan oleh Eijkman-Oxford Clinical Research Unit (Lembaga Penelitian Klinis Eijkman-Oxford). Saudara adalah salah satu dari 1000 orang peserta sukarela yang akan disertakan dalam penelitian ini. Formulir ini berisi penjelasan tentang mengapa penelitian ini dilakukan, apa yang akan berlangsung dalam penelitian ini, kemungkinan manfaat dan risiko yang akan Saudara dapatkan, dan informasi penting lainnya.

Apa tujuan penelitian ini?

Scrub typhus adalah penyakit demam yang disebabkan oleh bakteri. Bakteri ini disebarkan melalui gigitan tungau. Gejala-gejalanya berupa: demam, sakit kepala, mual muntah, ruam, keropeng pada tempat gigitan tungau, dan gangguan pendengaran. Bila tidak cepat ditangani, penyakit ini dapat menyebabkan kematian.

Tujuan dilakukannya penelitian ini adalah untuk menentukan jumlah penduduk yang terpapar pernah bakteri penyebab penyakit *scrub typhus*, dengan cara melihat ada tidaknya kekebalan (antibodi) terhadap bakteri ini. Kemudian, hal-hal yang meningkatkan risiko paparan juga akan ditelusuri. Informasi yang didapat dari penelitian

ini akan berguna untuk menyusun tindakan pencegahan penyakit ini. Penelitian ini bukan untuk kepentingan komersial.

Mengapa saya diajak untuk berpartisipasi?

Anda diundang karena Anda berusia setidaknya 17 tahun, bebas dari tanda-tanda penyakit demam akut, dan tinggal di daerah ini selama minimal 3 tahun tanpa meninggalkan daerah ini selama lebih dari 6 bulan dalam 3 tahun terakhir.

Prosedur penelitian

Kepada peserta penelitian yang setuju untuk ikut serta akan dilakukan pengambilan darah sebanyak kira-kira setengah sendok teh untuk pemeriksaan antibodi *scrub typhus*.

Kemungkinan risiko

Tidak akan ada efek samping dari penelitian ini selain seperti digigit semut pada saat pengambilan darah.

Manfaat

Tidak ada manfaat langsung dari partisipasi Anda dalam penelitian ini. Hasil penelitian ini akan berguna untuk mengembangkan pengobatan dan pencegahan untuk penyakit ini. Tapi, Anda akan mendapatkan pemeriksaan kesehatan peserta untuk gula darah,

tekanan darah, dan status gizi, yang terkait dengan proses penjaringan. Peserta akan ditawari suplemen gizi/paket higienis (termasuk susu bubuk, sikat gigi, pasta gigi, sabun).

Biaya

Saudara tidak akan dimintai bayaran untuk pemeriksaan yang akan Saudara terima.

Kerahasiaan informasi

Kerahasiaan hasil pemeriksaan Saudara terjaga karena hanya peneliti utama yang dapat melihat data Saudara. Informasi yang didapatkan dalam penelitian ini, jika dipublikasikan dalam jurnal ilmiah atau dipresentasikan dalam pertemuan ilmiah, tidak akan mengungkapkan identitas Saudara. Lembaga Eijkman dan University of Oxford akan bertanggung jawab untuk memastikan penggunaan (hanya untuk tujuan penelitian) yang aman dan baik atas informasi pribadi yang telah Anda berikan.

Keikutsertaan Saudara

Keikutsertaan Saudara dalam peneltian ini sepenuhnya bersifat sukarela. Saudara dapat mengundurkan diri kapan saja tanpa dikenakan denda.

Bila Saudara telah mengerti dan menyetujui penjelasan yang kami berikan, dan Saudara setuju untuk turut serta dalam penelitian ini, Saudara dipersilahkan menandatangani / memberi cap jempol kiri pada formulir persetujuan keikutsertaan dalam penelitian ini sebagai tanda persetujuan bahwa darah Saudara diambil sebanyak kira-kira setengah sendok teh, data Saudara dicatat dan dari darah Saudara dilakukan penelitian yang telah dijelaskan di atas.

Jika ke depannya Saudara memiliki pertanyaan mengenai penelitian yang Saudara ikuti ini, Saudara dapat menghubungi:

Study team: dr. Kartika Saraswati, MSc, DTMH

Eijkman-Oxford Clinical Research Unit, Lembaga Biologi Molekuler Eijkman, Jl. P. Diponegoro No. 69, Jakarta

Tel: 021 3910414

Eijkman Institute Research Ethics Committee (EIREC)

Lembaga Biologi Molekuler Eijkman, Jl. P. Diponegoro No. 69, Jakarta

Tel: (021) 3917131

In English:

INFORMED CONSENT FORM

You are invited to take part in a study entitled "Scrub typhus antibodies in North Sumatra". The purpose of this research is to determine the number of residents who have been exposed to bacteria that cause scrub typhus, by looking at the presence or absence of immunity (antibodies) against these bacteria. In addition, things that increase the risk of exposure will also be explored.

1. I have read (or being read out to) the information in this form and understand what is explained in this consent form. I am at least 17 years, and using my freedom to choose, hereby give my consent to be included as a participant in the research "Scrub typhus antibodies in North Sumatra ".
2. I understand that my personal medical data will be recorded and about half a teaspoon of my blood will be taken for this study.
3. I understand that all my data I provide for this research will be kept confidential by the research team, and my identity will be kept confidential.
4. I understand that the data and blood that I have provided will be used for research purposes and there is no commercial interest in the work or results of this research.
5. I understand that in the future my blood can be used for further research with the approval of the ethics committee.
6. I will apply the required health protocols to prevent COVID-19 transmission within the study site premises.

_____	_____	_____
Name of participant	Date	Signature/thumbprint

_____	_____	_____	_____
Name of witness	Relationship to participant	Date	Signature

_____	_____	_____
Name of person taking consent	Date	Signature/thumbprint

In Indonesian:

FORMULIR PERSETUJUAN SETELAH PENJELASAN (PSP)

Saudara diajak untuk ikut serta dalam penelitian "**Antibodi *scrub typhus* di Sumatra Utara**". Tujuan dilakukannya penelitian ini adalah untuk menentukan jumlah penduduk yang terpapar pernah bakteri penyebab penyakit *scrub typhus*, dengan cara melihat ada tidaknya kekebalan (antibodi) terhadap bakteri ini. Kemudian, hal-hal yang meningkatkan risiko paparan juga akan ditelusuri.

1. Saya telah membaca (atau dibacakan) informasi dalam formulir ini dan memahami apa yang dijelaskan pada formulir persetujuan ini. Saya berusia 17 tahun ke atas, dan menggunakan kebebasan saya untuk memilih, dengan ini memberikan persetujuan saya untuk dimasukkan sebagai peserta dalam penelitian "**Antibodi *scrub typhus* di Sumatra Utara**".
2. Saya mengerti data medik pribadi saya akan dicatat dan kira-kira setengah sendok teh darah saya akan diambil untuk penelitian ini.
3. Saya mengerti bahwa semua data saya yang saya berikan untuk penelitian ini akan disimpan secara rahasia oleh tim peneliti, dan identitas saya akan dijaga kerahasiaannya.
4. Saya paham bahwa data dan darah yang saya berikan akan digunakan untuk tujuan penelitian dan tidak ada kepentingan komersial dalam pengerjaan atau dari hasil penelitian ini.
5. Saya mengerti bahwa dikemudian hari darah saya dapat digunakan untuk penelitian lebih lanjut dengan persetujuan komite etik.
6. Saya akan menerapkan protokol kesehatan untuk mencegah penularan COVID-19 di lokasi penelitian.

_____	_____	_____
Nama peserta	Tanggal	Tanda tangan/cap jempol

_____	_____	_____	_____
Nama saksi	Hubungan dengan peserta	Tanggal	Tanda tangan

_____	_____	_____
Yang mengambil persetujuan	Tanggal	Tanda tangan/cap jempol

Appendix 12. Covid-19 risk mitigation SOP

Standard Operating Procedure
SEROSCRUB COVID-19 Risk Mitigation

I. OBJECTIVE

This standard operating procedure (SOP) provides instructions and guidelines of COVID-19 risks mitigation for the study **Serological Investigation of Risk of Exposure to Scrub Typhus in Residence at an Oil Palm Plantation in North Sumatra Relative to Urban Dwellers at Medan, North Sumatra (SEROSCRUB)** [1]. This SOP aims to minimise the risk of COVID-19 transmission and mitigate potential disruption during the conduct of the study.

This SOP was made based on the guidelines and recommendation from WHO, US CDC and Indonesian national health authority, and should be read as an extension to the EOCRU-wide SOP "**COVID-19 in the workplace: prevention, managing index case, and contact tracing**" [2].

II. SCOPE OF WORK

This SOP must be followed by all study personnel, and extended to participants and others present at study sites as appropriate. Information about the SOP will be disseminated through posters and flyers on site. Study personnel is also responsible to remind others. This SOP applies through the study period until there is further development that warrants changes.

III. PRECAUTIONS AND SAFETY

The risk of acquiring COVID-19 for individuals in the study site cannot be completely eliminated, but control measures will be taken to reduce the risk as much as possible. The safety of both study personnel and study participants must be prioritised. The study governance will monitor the development of COVID-19 pandemic in the area and response accordingly in collaboration with the district health office (DHO). The study activities will only proceed with the permission and directives of the district health office (DHO) in concordance with national policy.

IV. MATERIALS AND EQUIPMENT

Materials

- Medical mask
- N95 mask
- Gloves
- Soap
- Alcohol 70% disinfectant
- Bleach
- Alcohol-based hand rub
- Non-medical and medical waste bag and container
- Gown
- Face shield
- Plastic screen
- Education posters

Equipment

- Sink

V. PROCEDURES

1) General precaution

a) **Everyone must wash their hands** using running water and soap, or alcohol-based hand rub in accordance with the WHO guidelines (see annex 1) [3]. Posters of the said practice will be put in places where they can be easily visible. Hand-washing must be performed:
- Before entering and leaving study site
- Before and after touching surfaces
- To maintain personal hygiene (e.g. when hand is visibly dirty, before eating/drinking, after doffing PPE)

b) **Everyone should avoid touching the eyes, nose, and mouth area.** Should that is unavoidable, wash your hands after and/or before the occurrence as appropriate.

c) **Everyone must wear a medical mask properly,** covering both mouth and nose at all times except when having foods, medicines, and drinks; or if required to open their masks for physical examination. Masks will be provided by the study team in case a participant or others do not bring a mask.

d) **Everyone must maintain physical distance for at least 1 meter** between individuals unless there is a necessity to make a close contact in a study procedure.

e) **Everyone must practice cough and sneeze etiquette.** In a situation where one does not wear a mask, they must cover their mouth and nose when sneezing or coughing, preferably with tissue/paper towel, or with inner elbow. The used tissue/paper towel must be immediately disposed and hand hygiene needs to be performed afterwards.

f) **Study team should follow additional specific precautions** as outlined in section V point 2.

g) **Study personnel and study participants must adhere to the minimum safety requirement according to the healthcare facility's policy** where the study takes place, in addition to this SOP.

h) **Regular cleaning and disinfection of the environment and frequently touched surfaces** will be done according to WHO recommendation (see section V point 4a).

2) Study team

a) **Physicians or nurses** who perform physical examination and medical care must wear a medical mask, gown, face shield, and gloves [4-6].

b) **Phlebotomists** who perform sample collection must wear a medical mask, gown, face shield, and gloves [4-6].

c) **Laboratory staff** who perform blood examination must wear laboratory gown, medical mask, gloves and goggle. The blood processing must be performed in the biosafety cabinet [4-6].

d) **Disposable PPE that has been used must be properly removed and discarded** into the appropriate waste container, i.e.: biomedical waste container. **Reusable PPE** must be collected in the **designated containers** to be cleaned and disinfected.

e) **Study personnel should wash their hands** in these situations**:**
- Before touching a study participant
- Before aseptic procedure
- After exposure or suspected exposure to body fluid
- After touching a study participant

f) **Study personnel must be trained on this SOP prior to getting involved in study conduct**, and has the responsibility to explain and reiterate these measures to study participants**.** This training will be documented.

3) Dispense of compensation

The compensation given to each participant will be wrapped in separate bags. The parcels pick up for the participants will be regulated with a queue number to minimise contact with each other.

4) Environmental control

a) **Often-touched surfaces such as desks, chairs, door handles, and workstations, laboratory and office area will be cleaned regularly.** Cleaning will also be done any time the surfaces are visibly dirty, or are suspected to be contaminated (e.g., by sneezing or coughing without a mask). Cleaning process will be done according to these recommendations [7]:
- Alcohol 70% will be used to disinfect the surfaces.
- In case of being visibly dirty, the surface must be always first cleaned with water and soap or detergent to remove dirt and then followed by disinfection.
- Cleaning should always start from the cleanest to dirtiest areas. Surfaces will be sprayed with alcohol 70% 5-10 cm above the surfaces, and then wiped clean.
- Hands must be washed after the procedure.

b) **Plastic screens** have been installed in the study site as a physical barrier where appropriate and possible. Where plastic screen is not possible to be installed, face shield should be used by the study personnel during any procedures that need in-person interaction.

c) Where applicable, **clean instruments** before and after each use.

d) **Study personnel can only eat their meals in the designated area**. The table should be cleaned before and after having a meal.

e) **Environmental hygiene in lab** will comply with lab biosafety procedures.

f) The study activities will be done **in open space/outdoor** as much as possible.

5) Management of individuals who meet COVID-19 case definition and close contact

a) COVID-19 case definition (according to WHO) [8-9]:

Suspect case	1. A person who meets the clinical **AND** epidemiological criteria **Clinical criteria:** • Acute onset of fever AND cough; OR • Acute onset of ANY THREE OR MORE of the following signs or symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, anorexia/nausea/vomiting, diarrhea, altered mental status **Epidemiological criteria:** • Residing or working in an area with high risk of transmission of virus: closed residential settings, humanitarian settings such as camp and camp-like settings for displaced persons; anytime within the 14 days prior to symptom onset; OR • Residing or travel to an area with community transmission anytime within the 14 days prior to symptom onset; OR • Working in any health care setting, including within health facilities or within the community; anytime within the 14 days prior to symptom onset 2. A patient with severe acute respiratory illness (SARI: acute respiratory infection with history of fever or measured fever of ≥ 38^{o}C; and cough; with onset within the last 10 days; and requires hospitalization) 3. Asymptomatic person not meeting epidemiologic criteria with a positive SARS-CoV-2 Antigen-RDT
Probable	1. A patient who meets the clinical criteria of suspect case

case	AND is a contact of a probable or confirmed case, or linked to a COVID-19 cluster 2. A suspect case with chest imaging showing findings suggestive of COVID-19 disease: • Chest radiography: hazy opacities, often rounded in morphology, with peripheral and lower lung distribution; • Chest CT: multiple bilateral ground glass opacities, often rounded in morphology, with peripheral and lower lung distribution; • Lung ultrasound: thickened pleural lines, B lines (multifocal, discrete, or confluent), consolidative patterns with or without air bronchograms 3. A person with recent onset of anosmia (loss of smell) or ageusia (loss of taste) in the absence of any other identified cause 4. Death, not otherwise explained, in an adult with respiratory distress preceding death AND was a contact of a probable or confirmed case or linked to a COVID-19 cluster
Confirmed case	1. A person with a positive Nucleic Acid Amplification Test (NAAT) 2. A person with a positive SARS-CoV-2 Antigen-RDT AND meeting the probable case definition or suspect criteria 1 or 2 3. An asymptomatic person with a positive SARS-CoV-2 Antigen-RDT who is a contact of a probable or confirmed case
Contact	A close contact is defined as anyone with the following exposures to a COVID-19 case, from 2 days before to 14 days after the case's onset of illness: • Being within 1 meter of a COVID-19 case for >15 minutes; • Direct physical contact with a COVID-19 case; • Providing direct care for patients with COVID-19 disease

	without using proper personal protective equipment (PPE); • Other definitions, as indicated by local risk assessments If confirmed cases are asymptomatic, contacts should be managed in the same way as for a symptomatic case with an exposure period from 2 days before the case was sampled, to 14 days after.

b) **Should a study personnel or a study participant meets the COVID-19 case definition above**, a communication will be made between the study team and the local primary health care centre (PHC) and DHO to decide on the necessary steps, complying with the regulation.

c) **The study team will be available to support and help** to find any medical help should it be needed. The health management of sick study personnel will be given to an authorised medical team and done according to the protocol, clinical judgement, and local health authority and PHC's regulation. Health and safety of both study personnel and study participants is our utmost priority.

6) Notification and reporting

a) **Principal investigator (PI) and other co-investigators will be immediately notified** in case of a COVID-related incidence in the study site.

b) **An initial written report will be made and submitted** to PI and co-investigators following the notification. The report will be submitted in less than 24 hours.

c) **The identity of the index case and contacts will be treated confidentially** and will be disclosed only internally to directly involved persons (if we must do our own contact tracing) for the purpose of conducting a complete tracing (unless forced by legal authorities).

d) **Authorized PHC's staff will be notified of the occurrence immediately**. Study team will cooperate to take any action required by the PHC following the notification.

VI. QUALITY CONTROL PROCEDURES

1) Supervision

In order to ensure every procedure is done appropriately, there will be supervision under authority of the investigators and physicians on site. Regular checking of the availability of PPE and other supplies for cleanliness and disinfectants will be done regularly.

2) Training

The following training is mandatory for all study personnel:

a) **Training on this SOP** prior to involvement in the study conduct. Every study personnel will sign on a training documentation form as a proof that they have taken the training.

b) **PPE hands-on training.** We will carry out the PPE donning and doffing procedure workshop according to WHO best practice. Every relevant study personnel will sign on a training documentation form as a proof that they have taken the training.

Retraining for study personnel will be considered should there be any incident happens.

3) Reiteration study participants and personnel

Study personnel will inform study participants about the IPC guidelines (physical distancing, hand hygiene, mask, and cough and sneeze etiquette).

VII. REFERENCES

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2) EOCRU. SOP-EOCRU/HS.302/09.2020/814: COVID-19 in the workplace: prevention, managing index case, and contact tracing. Version 2.0. EOCRU. 2020.
3) WHO. Coronavirus disease (COVID-19) advice for the public [Internet]. WHO. 2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public
4) WHO. Rational use of personal protective equipment for coronavirus disease (COVID-19) and considerations during severe shortages: Interim guidance 6 April 2020 [Internet]. https://www.who.int/publications/i/item/rational-use-of-personal-protective-equipment-for-coronavirus-disease-(Covid-19)-and-considerations-during-severe-shortages
5) CDC. Using Personal Protective Equipment (PPE) [Internet]. CDC. 2020. Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/using-ppe.html
6) Gugus Tugas Percepatan Penanganan Covid 19. Rekomendasi standar penggunaan APD untuk Penanganan COVID-19 di Indonesia [Internet]. Revisi 1. Jakarta; 2020. 1–25 p. Available from: https://covid19.go.id/p/protokol/rekomendasi-standar-penggunaan-apd-untuk-penanganan-Covid-19-di-indonesia-revisi-1https://www.who.int/publications/i/item/cleaning-and-disinfection-of-environmental-surfaces-inthe-context-of-Covid-19
7) World Health Organization. Cleaning and disinfection of environmental surfaces in the context of COVID-19 16 May 2020 [Internet]. WHO. 2020. Available from: https://www.who.int/publications/i/item/cleaning-and-disinfection-of-environmental-surfaces-inthe-context-of-Covid-19

8) WHO. WHO COVID-19 Case definition. 16 December 2020. Available from: WHO COVID-19 Case definition
9) WHO. Contact Tracing in the Context of COVID-19. 10 May 2020. Available from: https://www.who.int/publications/i/item/contact-tracing-in-the-context-of-Covid-19
10) WHO. Criteria for Releasing COVID-19 Patients from Isolation. 17 June 2020. Available from: https://www.who.int/news-room/commentaries/detail/criteria-for-releasing-Covid-19-patients-from-isolation

VIII. STATEMENT

I have read and fully understand on the standard operating procedures above

No.	Name	Title	Initial
1			
2			
3			
4			
5			

Annex 1

HOW TO HANDWASH?

WASH HANDS WHEN VISIBLY SOILED! OTHERWISE, USE HANDRUB

Duration of the entire procedure: 40-60 seconds

0



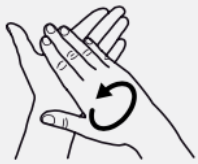
Wet hands with water;

1



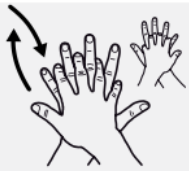
Apply enough soap to cover all hand surfaces;

2



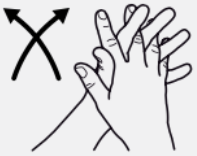
Rub hands palm to palm;

3



Right palm over left dorsum with interlaced fingers and vice versa;

4



Palm to palm with fingers interlaced;

5



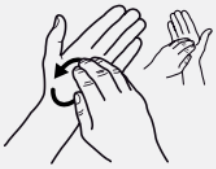
Backs of fingers to opposing palms with fingers interlocked;

6



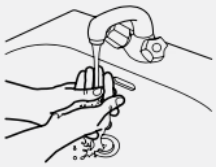
Rotational rubbing of left thumb clasped in right palm and vice versa;

7



Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

8



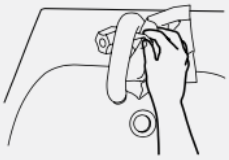
Rinse hands with water;

9



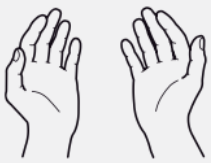
Dry hands thoroughly with a single use towel;

10



Use towel to turn off faucet;

11



Your hands are now safe.

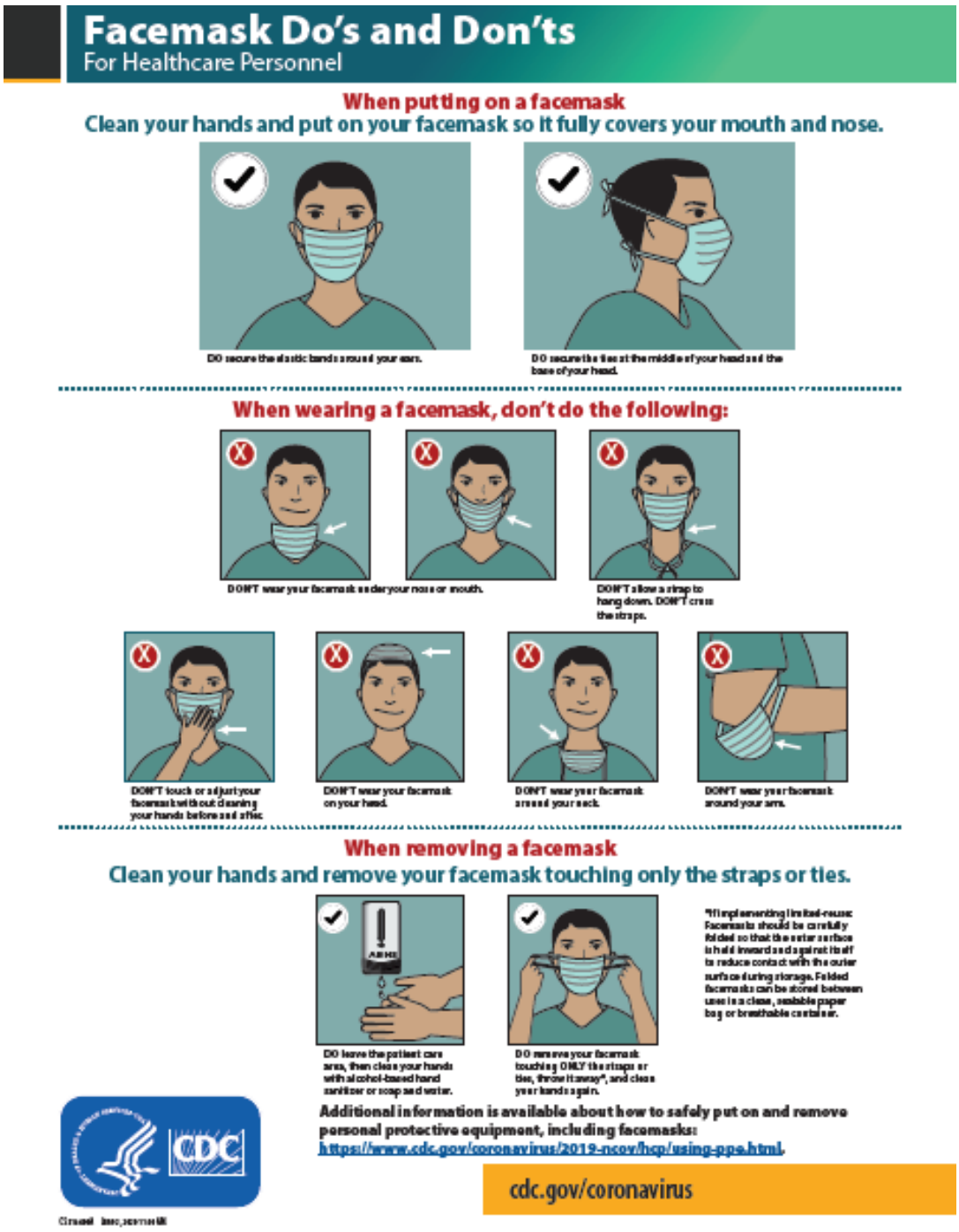
Source: WHO, 2009

HOW TO HANDRUB?



Source: WHO, 2009

Annex 2



Source: CDC, 2020

Annex 3

How to don and doff PPEs, inclusively gown (education and training will be conducted prior to start of study)



Source: WHO, 2015

Steps to take off personal protective equipment (PPE) including gown

1 Always remove PPE under the guidance and supervision of a trained observer (colleague). Ensure that infectious waste containers are available in the doffing area for safe disposal of PPE. Separate containers should be available for reusable items.

2 Perform hand hygiene on gloved hands.[1]

3 Remove apron leaning forward and taking care to avoid contaminating your hands. When removing disposable apron, tear it off at the neck and roll it down without touching the front area. Then untie the back and roll the apron forward.



4 Perform hand hygiene on gloved hands.

5 Remove outer pair of gloves and dispose of them safely.
Use the technique shown in Step 17

6 Perform hand hygiene on gloved hands.

7 Remove head and neck covering taking care to avoid contaminating your face by starting from the bottom of the hood in the back and rolling from back to front and from inside to outside, and dispose of it safely.



OR



8 Perform hand hygiene on gloved hands.

9 Remove the gown by untying the knot first, then pulling from back to front rolling it from inside to outside and dispose of it safely.



10 Perform hand hygiene on gloved hands.

11 Remove eye protection by pulling the string from behind the head and dispose of it safely.



OR



12 Perform hand hygiene on gloved hands.

13 Remove the mask from behind the head by first untying the bottom string above the head and leaving it hanging in front; and then the top string next from behind head and dispose of it safely.



14 Perform hand hygiene on gloved hands.

15 Remove rubber boots without touching them (or overshoes if wearing shoes). If the same boots are to be used outside of the high-risk zone, keep them on but clean and decontaminate appropriately before leaving the doffing area.[2]

16 Perform hand hygiene on gloved hands.

17 Remove gloves carefully with appropriate technique and dispose of them safely.



18 Perform hand hygiene.

1 While working in the patient care area, outer gloves should be changed between patients and prior to exiting (change after seeing the last patient)

2 Appropriate decontamination of boots includes stepping into a footbath with 0.5% chlorine solution (and removing dirt with toilet brush if heavily soiled with mud and/or organic materials) and then wiping all sides with 0.5% chlorine solution. At least once a day boots should be disinfected by soaking in a 0.5% chlorine solution for 30 min, then rinsed and dried.

World Health Organization

Source: WHO, 2015

Supplements

Supplement 1. Evidence mapping full dataset

S1_evidence_maping_dat.xlsx

A document containing the full dataset analysed in *Chapter 4. The geographical distribution of scrub typhus in Indonesia: Evidence mapping*. There are two sheets: (1) 'scrub typhus data' containing the full data set; and (2) 'variable dictionary' containing list of variable names and definitions.

Supplement 2. Scoring spreadsheet for evidence mapping

S2_scoring_spreadsheet.xlsx

A spreadsheet to calculate the score of each subnational units based on certainty of evidence.

Supplement 3. Serosurvey full dataset

S3_serosurvey_dat.xlsx

A document containing the full dataset analysed in *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples*. There are two sheets: (1) 'serosurvey data' containing the full data set; and (2) 'dictionary' containing list of variable names and definitions.

Supplement 4. Stata commands for serosurvey analysis

S3_serosurvey_analysis.do

A .do file containing Stata commands used in the data analysis for *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples*.

Supplement 5. R codes for serosurvey analysis

S4_serosurvey_analysis.R

An R script detailing the R codes used in the data analysis for *Chapter 5. Serosurvey of scrub typhus in Indonesia: A cross-sectional analysis of archived fever studies samples*.

Supplement 6. Data dictionary for the systematic review of treatment landscape

S5_data_dictionary.xlsx

A spreadsheet of data dictionary used in the REDCap database for the systematic review of the scrub typhus treatment landscape.

Supplement 7. List of studies for the systematic review of treatment landscape

S6_list_of_studies_st_iddo.xlsx

Spreadsheets listing studies screened in Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform. These spreadsheets include the irrelevant, excluded, and included studies.

Supplement 8. Full dataset of the systematic review of treatment landscape

S7_st_iddo_dat.xlsx

Spreadsheets containing the full dataset analysed in Chapter 6. Systematic review of the scrub typhus treatment landscape: Assessing the feasibility of an individual participant-level data (IPD) platform. There are two sheets: (1) 'dat-lab' with labelled data; and (2) 'dat_nolab' with unlabelled data.

Supplement 9. QUADAS-2 sub-questions

S8_quadas_subq.xlsx

A spreadsheet detailing the results of each assessment question point of QUADAS-2.