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Systematic Review

Unveiling the burden of scrub typhus in acute febrile illness cases across India: A systematic review & meta-analysis

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***Background & objectives*: Scrub typhus is an emerging mite-borne zoonotic infection that has been overlooked, despite being one of the most widespread severe vector-borne diseases. With an estimated one billion people at risk worldwide and one million annual cases, it poses a significant public health concern. While various studies have investigated the prevalence of scrub typhus in different regions of India, a comprehensive regional systematic review and meta-analysis on the seropositivity of scrub typhus among acute febrile cases has been lacking. To address this gap, we conducted a systematic review and meta-analysis to compile information on the current seroprevalence of scrub typhus in acute febrile illness cases in India.**

***Methods*: A literature search of multiple databases on prevalence of scrub typhus in acute febrile illness in India, 60 eligible studies out of 573 studies. The prevalence of individual studies was double arcsine transformed, and the pooled prevalence was calculated using inverse variance method.**

***Results*: In total, these studies encompassed 34,492 febrile cases. The overall seroprevalence of scrub typhus among acute febrile illness cases in India was found to be 26.41 per cent [95% confidence interval (CI): 22.03-31.03]. Additionally, the pooled case fatality rate (based on data from six studies) among scrub typhus-positive cases yielded a case fatality rate of 7.69 per cent (95% CI: 4.37-11.72).**

***Interpretation & conclusions*: This meta-analysis shows that scrub typhus is a significant health threat in India. Preventive measures to control scrub typhus need to be given priority.**

Key words India - meta-analysis- *orientia tsutsugamushi* - prevalence- scrub typhus

Scrub typhus is an acute febrile bacterial disease that is caused by an intracellular pathogen *Orientia tsutsugamushi* belonging to the genus *Orientia* transmitted by the bite of mite larvae, is widespread in the western Pacific area also called *tsutsugamushi disease*[1]. Recently, the discovery of scrub typhus caused by newly identified Orientia species, such as *Candidatus Orientia chuto*[2], has triggered a major

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concern about the worldwide. Despite, scrub typhus disease's century-long existence, it remains highly neglected and has emerged as a significant public health problem in India. Originally, scrub typhus was confined to an area of more than 8 million km^2 named as the '*tsutsugamushi triangl*e', an area bounded to the north by northern Japan and the far east Russia, to the south by Australia, to the east by Japan and to the west by Pakistan, Afghanistan and India[3]. But, recent reports from South America and Middle East region have surfaced, making the scrub typhus a major public health concern, globally[4]. The pathogen is transmitted to humans and rodents through the bite of infected chiggers or mite larvae belonging to the genus *Leptotrombium*, with both humans and rodents serving as incidental hosts[5,6]. Chigger mites inhabit diverse vegetation areas, including scrub and primary forests, tall grasslands, plantations, agricultural fields and even beaches[7,8]. In India about 57 per cent of land is covered by crop cultivation. Farmers and agricultural workers are at highest risk of mite bite. Presently, an alarming one billion individuals in endemic areas are at risk of contracting scrub typhus, with approximately one million new infections occurring annually[3,9]. In Southeast Asia, scrub typhus accounts for up to 28 per cent of non-malarial fevers, and thus is a leading cause of death from any communicable disease[3,10].

The disease manifests itself six to 18 days after the bite of infected mite larvae (chiggers). These trombiculid mites carry the bacteria in their salivary glands and transmit it to the host during feeding. Unfortunately, the mite bite is painless, often going unnoticed as it causes intense itching after a few hours in individuals and mites are very small, and barely visible to the naked eye[10,11]. The infection presents with a sudden onset of fever, headache, and myalgia[11,12]. Within two to three days, a maculopapular rash typically appears, accompanied by an eschar at the bite site and enlargement of local lymph nodes. As the illness progresses, interstitial pneumonitis, generalised lymphadenopathy, and splenomegaly may arise. However, due to the delayed presentation of the characteristic eschar (pathognomic lesion) in most cases and the initial flu-like symptoms that are easily disregarded, severe complications and fatalities can occur among patients with scrub typhus[13]. Untreated cases can lead to multi-organ failure and death, underscoring the importance of early detection and prompt treatment for improved outcomes[14]. Environmental factors significantly influence the occurrence of scrub typhus, with practices like proximity to water bodies, outdoor cooking, pet ownership, and vegetation increasing the risk manifold[15,16].

In India, a rise in scrub typhus cases is observed during the rainy, post-monsoon and winter seasons, particularly in cooler months[17-20]. The disease is commonly seen in the rainy season due to increased exposure to trombiculid mites during harvesting and contact with newly growing vegetation[21,22]. *Orientia tsutsugamushi,* the bacterium responsible for scrub typhus, is transmitted through two main mechanisms: transovarial transmission (from infected female mites to their offspring *via* eggs) and transstadial transmission (passage from larval mite to nymph to adult). These modes of transmission fall under vertical transmission. No evidence has been reported thus far supporting horizontal transmission, where mites acquire Orientia from infected hosts and subsequently infect other host[23-25]. Scrub typhus is rapidly re-emerging in several regions of Micronesia, Maldives, and India, where it had previously been significantly neglected. Currently, multiple epidemics and sudden outbreaks of scrub typhus have been documented across various parts of India[26]. Limited diagnostic facilities, underreporting, inadequate case management, and insufficient vector control exacerbate the situation. In India, the management of scrub typhus lacks systematic case detection and appropriate measures for vector control. The combination of climate change and human expansion into previously uninhabited areas has increased the incidence and re-emergence of scrub typhus. Despite a growing awareness and the availability of reported articles, there remains a dearth of comprehensive, evidence-based data on the disease burden, prevalence, incidence, and geographic distribution. Such data are crucial for making informed decisions to implement effective prevention and control strategies. To address this knowledge gap, we conducted a systematic review and meta-analysis to estimate the burden of scrub typhus in India.

Material & Methods

Search strategy and selection criteria: The systematic review and meta-analysis followed JBI Evidence Synthesis Manual[27] and the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[28]. Various databases including Medline (PubMed), National Library of Medicine, Science Direct, Web of Science, and Google Scholar were searched for articles published until January 14, 2023. The search was conducted using keywords such

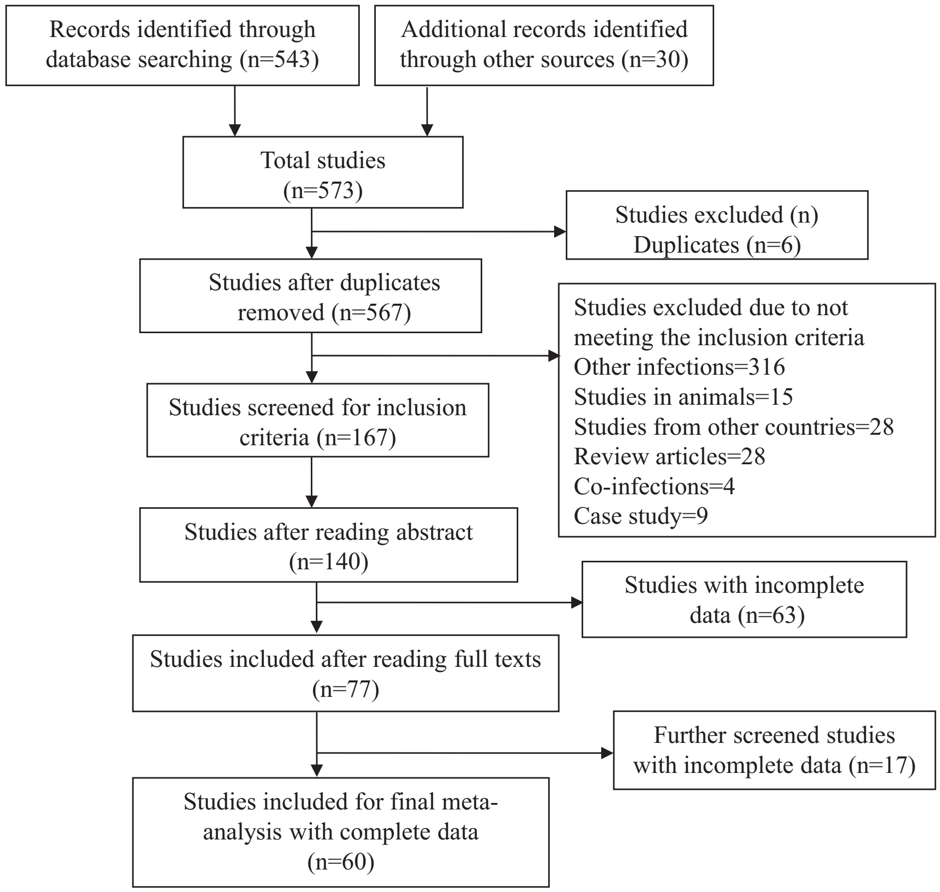

Fig. 1. PRISMA flowchart of studies selection. PRISMA indicates preferred reporting items for systematic reviews and meta-analyses. PRISMA, preferred reporting items for systematic reviews and meta-analysis.

as scrub typhus" OR "*Orientia tsutsugamushi*" OR "*O. tsutsugamushi*") AND (India) AND ("prevalence" OR "Incidence" OR "epidemiology" OR "survey" OR "distribution"). The Clarivate Analytics EndNote web version (*https://access.clarivate.com/login?app=endnote*) was used to manage duplicate records. Furthermore, a manual search of the references cited in the publications was performed. The complete search strategy is described in detail has shown in Figure 1.

Inclusion and exclusion criteria: The eligibility criteria for search strategy and studies selection were defined based on the condition, context and population framework as follows:

Population: Patients with acute undifferentiated febrile illness, pyrexia of unknown origin and acute febrile cases with fever <14 days with unknown aetiology were selected. Undifferentiated febrile illness was defined as fever of <14 days duration without any evidence of organ or system specific aetiology. Studies that focused on diseases other than undifferentiated febrile illness were excluded from this analysis.

Condition: Hospital based cross-sectional and epidemiological survey studies were included. Patients of all age groups and gender, admitted to the hospitals from different geographical regions of India were included. Outbreak studies and studies done on animals (chiggers and rodents) were excluded. Outbreak studies were not included as they can potentially lead to misinterpretation of prevalence data.

Context: Studies in which cases of scrub typhus were confirmed either by enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA) or PCR were included in this meta-analysis and those reporting scrub typhus positivity using alternative laboratory methods such as immune chromatography or Weil-Felix OX-K agglutination reaction were excluded. Weil-Felix test was initially used for the rapid sero-diagnosis of rickettsial infection in developing countries. It detects host immune response against different *Proteus* antigens such as OX19, OXK, and OX that cross-respond with rickettsiae. Due to cross-reactivity of the antigens there is very high chance of getting false results due to the use of non-rickettsial antigen resulted low sensitivity and specificity of the test. Currently, it has mostly been replaced by newer sensitive diagnosis tests. However, it is still in common use in resource limited settings for the primary screening of the scrub typhus that need further confirmation by the sensitive and specific tests. So, inclusion of those studies that used this test may not indicate the true positive cases of scrub typhus and may results the false prevalence of the disease. Studies describing the scrub typhus only on the basis of eschar sign were also excluded.

Initially, the selected studies underwent full abstract screening, and subsequently, the full texts of eligible studies were reviewed. To be considered eligible, the abstracts of the studies had to report the prevalence, incidence, number of reported cases, mortality, or burden of scrub typhus in any region of India. Duplicate articles, case control study, co-infection studies, animal and vector-based studies, review articles, studies involving other diseases, and studies carried out in other countries were excluded. All studies, showing the positivity and prevalence of scrub typhus among febrile patients with fever cases were selected for meta-analysis.

Data extraction: Three independent reviewers (GS, HVM and RK) used a pre-designed data extraction form to extract relevant information from the selected studies. The extracted data, including author names, publication year, study location, diagnostic test used, criteria for positivity, sample size (total number of acute febrile illness cases), fever onset, mortality, duration of sample collection, overall prevalence, gender-wise prevalence, and age groups, were recorded in a pre-designed template. The data were organised in Table I. In cases where there were discrepancies between the two reviewers, a fourth author (PS) was consulted to reach a consensus. Fever was found to be the most common symptom among scrub typhus patients. The primary outcomes of interest in this study were as follows: *(a)* the prevalence (proportion) of laboratory-confirmed scrub typhus infection among undifferentiated fever cases, *(b)* the case fatality ratio among laboratory-confirmed scrub typhus patients. The diagnosis of scrub typhus among undifferentiated fever cases and clinically suspected patients was based on commercially available serological test (IFA, IgM ELISA with a more than fourfold increase in antibody titers) or molecular laboratory assays like PCR targeting different genomic markers (*56kDa, 47kDa, groEL* genes). In studies where two or more tests were used for diagnosis or comparison of test efficacy, the prevalence was calculated based on the detection of Immunoglobulin M (IgM) antibodies against *O. tsutsugamushi* to ensure consistency. To calculate the case fatality ratio, the numerator included the reported number of deaths due to scrub typhus, while the denominator comprised laboratory-confirmed scrub typhus patients.

Statistical analysis: All statistical analyses were conducted using R Studio software (version 1.2.5042, "R core team 2017) with the "meta" package[29], following the guidelines of JBI guidelines for reporting systematic reviews of prevalence and incidence[27]. Individual studies prevalence was Freeman-Tukey Double Arcsine transformed, and Clopper-Pearson confidence interval method was used to calculate the confidence interval of individual study findings. Inverse variance method was used to calculate the pooled prevalence. Heterogeneity was tested using maximum likelihood ratio test. Baujat plot was plotted to detect the sources of heterogeneity[30]. Random effect model was used when there was high heterogeneity (I^2>50) in the studies.

Forest plots were constructed for display of overall prevalence and prevalence in different regions across India. Funnel plot with double arcsine transformed proportion on x-axis and standard errors on y-axis was plotted to see the symmetry of publications and the Egger's test using mixed-effects meta-regression model was used to evaluate publication bias. P<0.05 was considered statistically significant.

Results

Study characteristics: Among all databases screened, 573 studies were identified after the literature search. From PubMed, National Library of Medicine, Science Direct, and Google Scholar, 543 studies were identified, and an additional 30 records were added from other sources and cross references. After removing six duplicate records, 567 studies were found eligible for title and abstract screening. Out of these 567 studies, 400 were excluded as they didn't satisfy study inclusion criteria. These excluded studies were

Table I. Characteristics of the included studies

Author	Year	Area	City/ location	Total cases	Positive cases	Male	Female	Age (in yr)	Sample collection duration	Diagnosis test used	Fever
Abhilash *et al*[31]	2016	South India	Andhra Pradesh, Kerala, Tamil Nadu	1258	452	186	266			ELISA	
Ahmad *et al*[32]	2016	North India	Uttarakhand	233	65	30	35	18-65	Dec 2012-Dec 2013	ELISA	< 2 wk
Anitharaj *et al*[33]	2018	South India	Puducherry	220	134					ELISA	
Bahera *et al*[34]	2020	East India	Odisha	432	114			< 18	Jan- Dec 2017	ELISA	< 2 wk
Bal *et al*[35]	2019	East India	Odisha	413	201	124	77	< 15	June- Nov	ELISA	< 15 d
Bal *et al*[36]	2021	East India	Odisha	140	45					ELISA	
Bithu *et al*[37]	2014	North India	Rajasthan	271	133	80	53	40-46	Sep-Dec 2012	ELISA	
Chunchanur *et al*[38]	2019	South India	Bengaluru, Karnataka	100	22					ELISA	
Dave *et al*[39]	2022	West India	Udaipur, Rajasthan	3814	1340				Jan -Dec 2019	ELISA	
Giri *et al*[40]	2018	East India	Kolkata	861	97				March 2012- Dec 2015	ELISA	
Hussain *et al*[41]	2022	North India	Uttar Pradesh	1743	361					ELISA	
Jain *et al*[42]	2019	North India	Haryana	230	39					ELISA	
Kalal *et al*[43]	2016	South India	Bengaluru	103	53			< 18	Jan 2010- October 2012	ELISA	11 d
Karthikeyan *et al*[44]	2019	South India	Puducherry	50	37					ELISA	
Kavirayani *et al*[45]	2021	South India		214	32			< 18	Jan 2018 - June 2018	ELISA	
Khan *et al*[46]	2012	Northeast India	Assam, Nagaland	314	108						
Khan *et al*[47]	2015	North India	Dehradun	3540	412					ICT, ELISA	
Kolarul *et al*[48]	2018	South India	Manipal	1036	319	179	140	≥18		ELISA, IFA	
Kumar *et al*[49]	2014	North India	Chandigarh	201	49				Sep 2011 - Nov 2012	nPCR	
Lalrinkima *et al*[50]	2017	Northeast India	Mizoram	4081	283			21-30		ICT RDT kit, ELISA	
Mahajan *et al*[51]	2021	North India	Chandigarh	224	77			2 months - 14 yr	June 2013 - Dec 2017	ELISA	
Manjunathachar *et al*[52]	2021	Central India	Madhya Pradesh	144	48					ELISA, nPCR	

Contd...

Author	Year	Area	City/ location	Total cases	Positive cases	Male	Female	Age (in yr)	Sample collection duration	Diagnosis test used	Fever
Mittal *et al*[53]	2021	North India	Eastern Uttar Pradesh	125	25				Aug 2018- July 2019	ELISA	
Morch *et al*[54]	2017	Northeast India	Tezpur, Assam	295	75			≥5 - 60	April 2011-Nov 2012	ELISA	2-14 d
Morch *et al*[54]	2017	South India	Oddanchatram, Tamil Nadu	314	7			≥5 - 60	April 2011-Nov 2012	ELISA	2-14 d
Morch *et al*[54]	2017	South India	Ambur, Tamil Nadu	302	35			≥5 - 60	April 2011-Nov 2012	ELISA	2-14 d
Morch *et al*[54]	2017	Central India	Mungeli, Chhattisgarh	33	1			≥5 - 60	April 2011-Nov 2012	ELISA	2-14 d
Morch *et al*[54]	2017	South India	Anantpur, Andhra Pradesh	152	5			≥5 - 60	April 2011-Nov 2012	ELISA	2-14 d
Morch *et al*[54]	2017	West India	Ratnagiri, Maharashtra	237	20			≥5 - 60	April 2011-Nov 2012	ELISA	2-14 d
Morch *et al*[54]	2017	North India	Raxaul, Bihar	107	16			≥5 - 60	April 2011-Nov 2012	ELISA	2-14 d
Murhekar *et al*[55]	2016	North India	Uttar Pradesh	109	59				Sep-Oct 2015	ELISA, PCR	
Narvencar *et al*[56]	2012	West India	Goa	44	15				June 2009-Oct 2010	ELISA	5 d
Oberoi *et al*[57]	2014	North India	Ludhiyana, Punjab	772	98	60	37			ELISA	
Panigrahi *et al*[58]	2022	East India	Southern-Odisha	170	74					ELISA	
Parai *et al*[59]	2023	South India	Odisha	1840	523			18-45		ELISA	
Paulraj *et al*[60]	2021	South India	Nilgiri, Tamil Nadu	214	13	3	10		Oct 2014 - March 2016	ELISA	
Prakash *et al*[61]	2011	South India	Vellore, Tamil Nadu	87	51					WF, ELISA, nPCR	
Prakash *et al*[62]	2022	Central India	Chhattisgarh	221	83					ELISA, PCR	
Premraj *et al*[63]	2018	South India	Kanchipuram, Tamil Nadu	558	50	19	31	>60	June 2015 - May 2016	ELISA	
Raina *et al*[64]	2018	North India	Himachal Pradesh	1164	262					ELISA	
Ramyasree *et al*[65]	2015	South India	Andhra Pradesh	100	39					ELISA	
Rao *et al*[66]	2019	East India	Rourkela, Odisha	287	10			<15		ELISA	

Contd...

Author	Year	Area	City/ location	Total cases	Positive cases	Male	Female	Age (in yr)	Sample collection duration	Diagnosis test used	Fever
Rathi *et al*[67]	2011	Central India	Akola, Maharashtra	161	23						
Rauf *et al*[68]	2018	North India	Chandigarh	217	23			3-5			
Rawat *et al*[69]	2017	North India	Uttarakhand	281	158	62	106		Feb - Dec 2015	IFA, ELISA, RT PCR	
Rizvi *et al*[70]	2018	North India	Aligarh, Uttar Pradesh	357	91					RDT, ELISA	< 5 d
Roy *et al*[71]	2021	Central India	Wardha, Maharashtra	274	37					PCR,LAMP, ELISA	5 d
Rupa *et al*[72]	2015	South India	Puducherry	482	109			10 months - 80 yr	Jan 2012 - June 2015	WF, ELISA	
Sahu *et al*[73]	2015	East India	Eastern Odisha	150	50	33	17		April 2011 - Oct 2013	ELISA	
Sankhyan *et al*[74]	2014	North India	Chandigarh, Haryana, Himachal Pradesh, Punjab, Uttar Pradesh	35	15					ELISA	
Sarangi *et al*[75]	2016	East India	Odisha	71	26			<14	July 2015 - Dec 2015.	ELISA	
Shelke *et al*[76]	2017	Central India	Wardha, Maharashtra	270	127				Jan 2015 - Nov 2016	RDT, ELISA	
Shrinivasan *et al*[77]	2019	South India	Vellore, Tamil Nadu	103	70	30	40			PCR	
Tarai *et al*[78]	2022	North India	New Delhi	473	56					ELISA, PCR	
Thangraj *et al*[79]	2018	North India	Deoria and Gorakhpur, Uttar Pradesh	819	155					ELISA	
Thirunavukkarasu *et al*[80]	2021	South India	Puducherry	2710	660	350	310	< 12		ELISA	≥7 d
Usha *et al*[81]	2014	South India	Tirupati, Andhra Pradesh	280	158			25-65	April 2011 - Dec 2012	WF, ELISA	
Usha *et al*[82]	2016	South India	Andhra Pradesh	663	258					ELISA, nPCR	
Vikram *et al*[83]	2020	Central India	Chhattisgarh	169	35			16-30		ELISA	
Vivian *et al*[84]	2017	North India	Gorakhpur, Uttar Pradesh	224	40					ELISA	

ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription polymerase chain reaction; nPCR-nested polymerase chain reaction; WF, Weil-Felix test, RDT, rapid diagnostic test; IFT, immunofluorescence test; LAMP, loop-mediated isothermal amplification test; d, day(s); wk, week(s) review articles, book chapters, case reports, or studies performed on animals and vectors (mite) and co-infection studies, as mentioned above. All 167 studies fulfilling the inclusion criteria were reviewed from their abstract and then from their full text, which left us with 77 studies. Complete data was mentioned in only 60 studies (Fig. 1) which were found eligible for meta-analysis. The characteristics of studies included in the meta-analysis are depicted in Table I[31-84].

Characteristics of included studies: Sixty studies that reported scrub typhus positivity among acute undifferentiated febrile illness cases were analysed for estimating the prevalence. All the studies were published between the year 2006 and 2023. Most studies were conducted in south India (n=20, 33.3% of included studies) followed by the north India (n=19, 31.6%), eastern part of India (n=8, 13.3%), central India (n=7, 11.6%), north eastern region (n=3, 5%) and from west India (n=3, 5%). There was one study in which, samples were collected from south, north, north-east, west and central part of India. In total there were 34,492 cases from 60 studies which were analyzed; 10,786 cases were reported from the 20 studies conducted in southern region and 11,125 cases from 19 studies from the northern region, 4,690 cases from four studies from north-eastern region, 4,095 cases from three studies from western part, 2,524 cases from eastern part, and 1272 cases were from central part of India (Table I)[31-84].

Primary and secondary outcomes:

Sero-prevalence of scrub typhus: The meta-analysis of 34,492 cases from 60 studied revealed the overall sero-prevalence of scrub typhus among patients with undifferentiated fever cases (with unknown aetiology or undifferentiated febrile illness) as 26.41 per cent (95% CI: 22.03-31.03) in the random effect model (Fig. 2). The sub group analysis was performed for identifying the pooled prevalence in different regions across India. The highest prevalence of scrub typhus among febrile cases was found in the south India (30.23%, 95% CI: 20.56–40.86) followed by the east India (27.49%, 95% CI:16.82-39.63), whereas the lowest prevalence was reported from north-east India (20.62%, 95% CI: 8.55-36.22). However, there was no significant difference in sero-positivity across the regions [$\chi^2_5 = 1.68$, df = 5, (P= 0.89)].

Test of heterogeneity and sensitivity analysis: A significant degree of heterogeneity was observed in overall pooled data (I^2=98%, ζ2=0.0389). To identify the source of heterogeneity, Baujat plot was plotted. As shown in Figure 3, the studies performed by Dave *et al*[39], 2022; Khan *et al*[47], 2015; and Lalrinkima *et al*[50], 2017 contribute significantly to the heterogeneity.

To see the impact of each study on the strength of the analysis method used in the study and recheck the conclusions for the Freeman-Tukey double arcsine transformation, sensitivity analysis was performed by omitting the individual study and pooling the results of the other studies. As shown in Figure 4, study inference is not affected by omitting individual studies.

Publication bias: Egger's test (t=2.05, P=0.045) shows significant publication bias with an intercept of linear regression=3.65 (95% CI: 0.15-7.14). The publication bias is also evident from the funnel plot (Fig. 5). Regression test for funnel plot asymmetry using mixed-effect regression model also shows asymmetry [z=2.0713, P=0.0383, b=0.436 (95% CI: 0.326-0.547)] in the funnel plot. The publication bias may be due to variation in sample size in the included studies.

Critical appraisal/ quality assessment: A standardized JBI critical appraisal tool was used to evaluate the quality of the literature for selected studies in three categories- Yes (1), No (0) and Unclear (U). The JBI critical quality assessment was done by the authors (GS and MHV) and whenever there was confusion or discrepancy in decision making, the third author (RK) verified the decision. The methodological quality assessment of each study and the risk of bias for each aspect were reported in Table II. Out of 60 studies, three were found to be of low quality as the JBI quality score for those studies was less than six from the maximum score of nine.

Case fatality: Of the 60 studies, six studies had been identified in which authors have reported the mortality due to scrub typhus in undifferentiated febrile illness. Among these six studies two were from south India, one from north India, one from west India, one from north-central India and one from north-western India. A total of 743 cases were estimated from those six studies and mortality was reported of 51 cases. The pooled mortality rate in scrub typhus positive cases was estimated to be 7.69 (95% CI: 4.37-11.72) (Table III[85]). There was a low heterogeneity in the case fatality rate across the studies (I^2=67%, P<0.01) (Fig. 6).

The most common complication reported in these studies were acute respiratory distress syndrome. Multi-

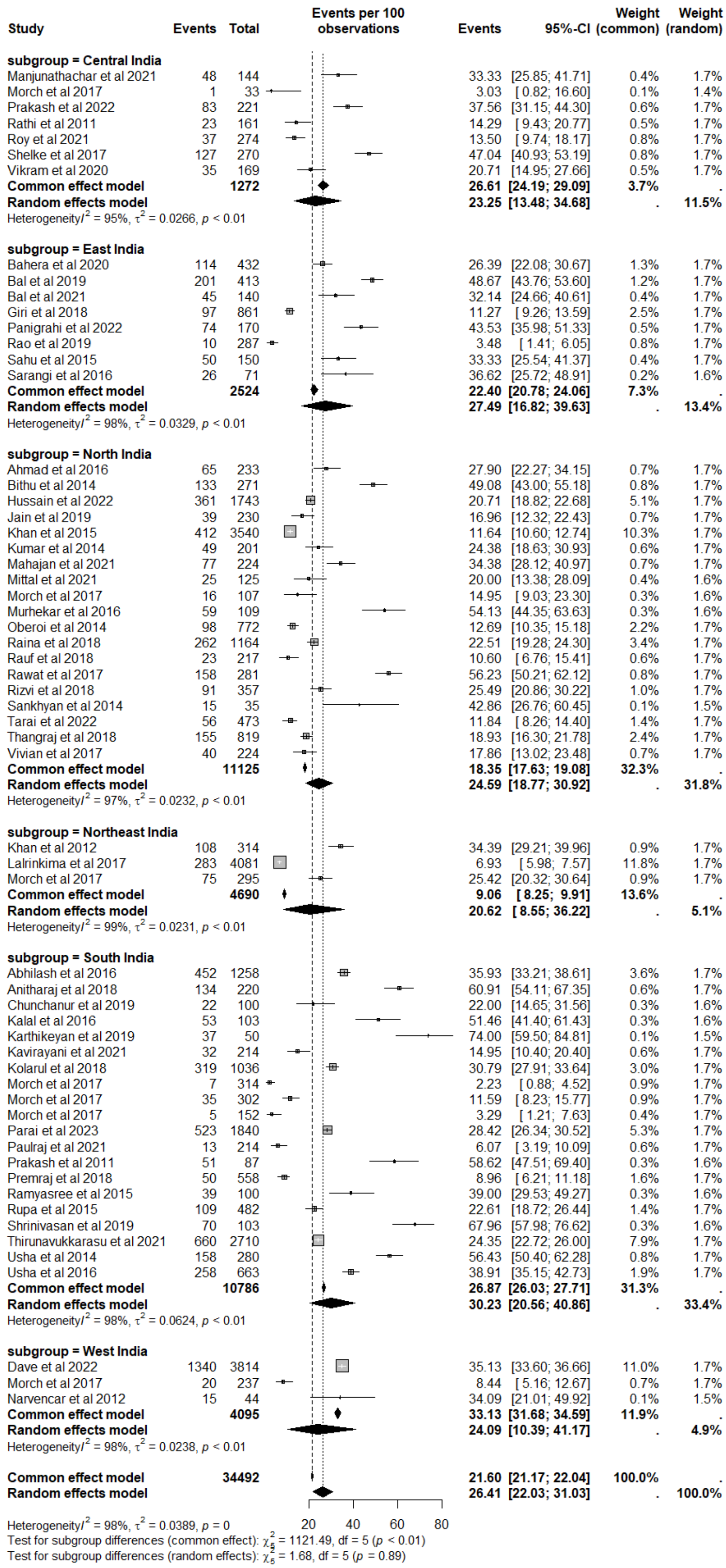

Fig. 2. Forest plot showing overall pooled prevalence and region-wise prevalence. The plot was generated using R software. CI, confidence interval.

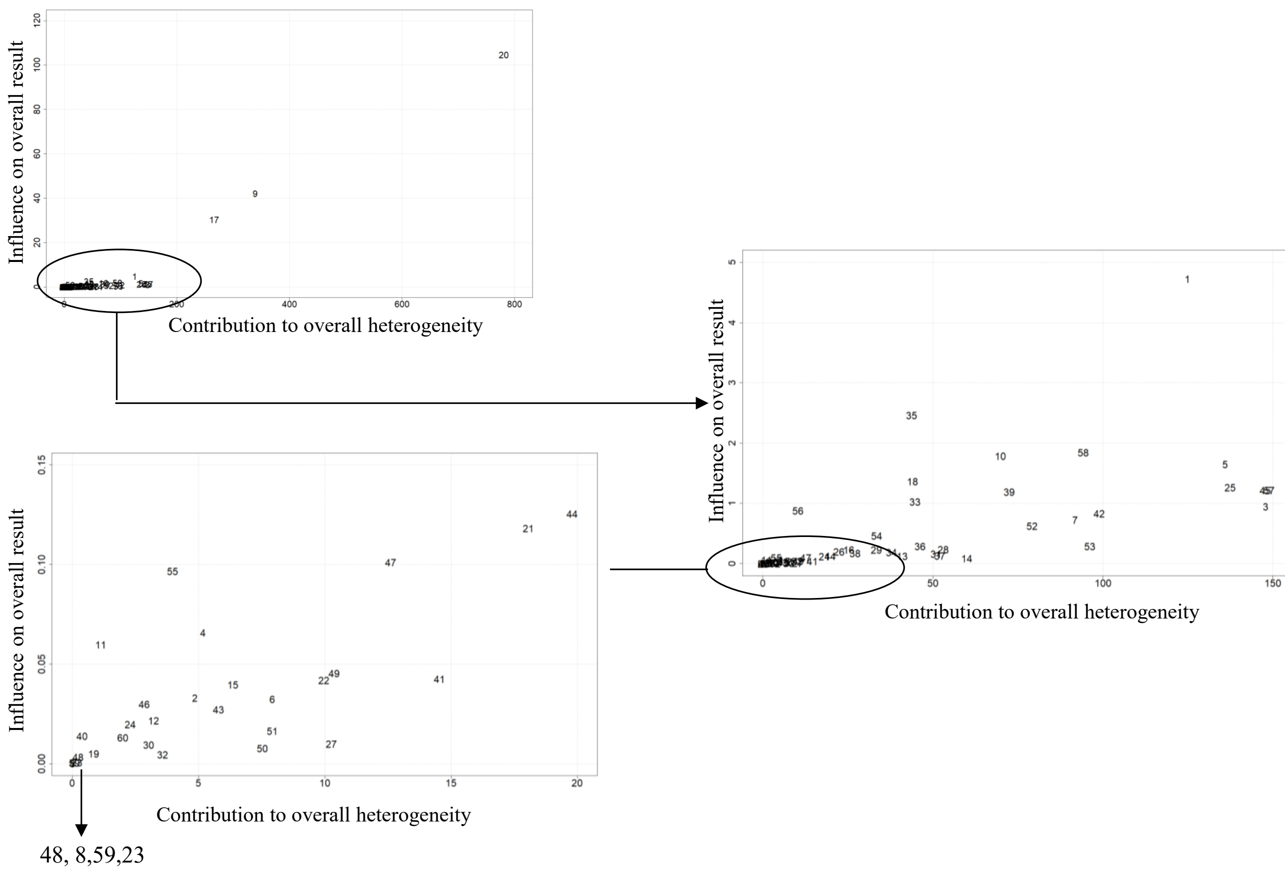

Fig. 3. Baujat Plot showing studies causing heterogeneity. The numbers in the plot corresponds to the study serial number as indicated in Table I. The plot was generated using R software.

organ distress syndrome associated with acute renal failure, meningoencephalitis, and liver dysfunction were reported among the cases who died. The highest case fatality was the outcome of severe complication due to delayed diagnosis and treatment. The results reflect the fact that the number of scrub typhus cases that actually occur are probably significantly higher than the numbers diagnosed and reported in this region. Yet the causes of this variation are unclear and can vary on host, region, and strain variables.

Discussion

Scrub typhus, a febrile illness, has experienced a surge in the number of cases in India, making it an escalating for public health. Nonetheless, the actual magnitude of the disease remains elusive due to under-diagnosis and under-reporting[86]. Henceforth, a systematic review and meta-analysis was undertaken to gather and analyse the published data from the past decade pertaining to the sero-prevalence of scrub typhus among patients with acute undifferentiated febrile illness, regional cut-off values or changes, and mortality rates across India. Our objective was to comprehensively understand the true prevalence of scrub typhus in the country. Following our set exclusion criteria, only hospital-based studies were included, and the estimated discovered overall sero-prevalence of scrub typhus among patients with acute undifferentiated febrile illness in India was 26.41 per cent. The study's findings highlighted the substantial differences in scrub typhus mortality. The findings of this systematic analysis show that the southern Indian states exhibit high prevalence (30.23%) and north-eastern part of the country has relatively lower prevalence (20.62%) of scrub typhus among undifferentiated fever cases. Furthermore, despite the low prevalence of the disease in the western part of India, it had the highest case fatality rate (33%) followed by north-west India (9.7%).

The burden of scrub typhus has increased due to excessive use of broad-spectrum antibiotics for other febrile infections, particularly as a response to

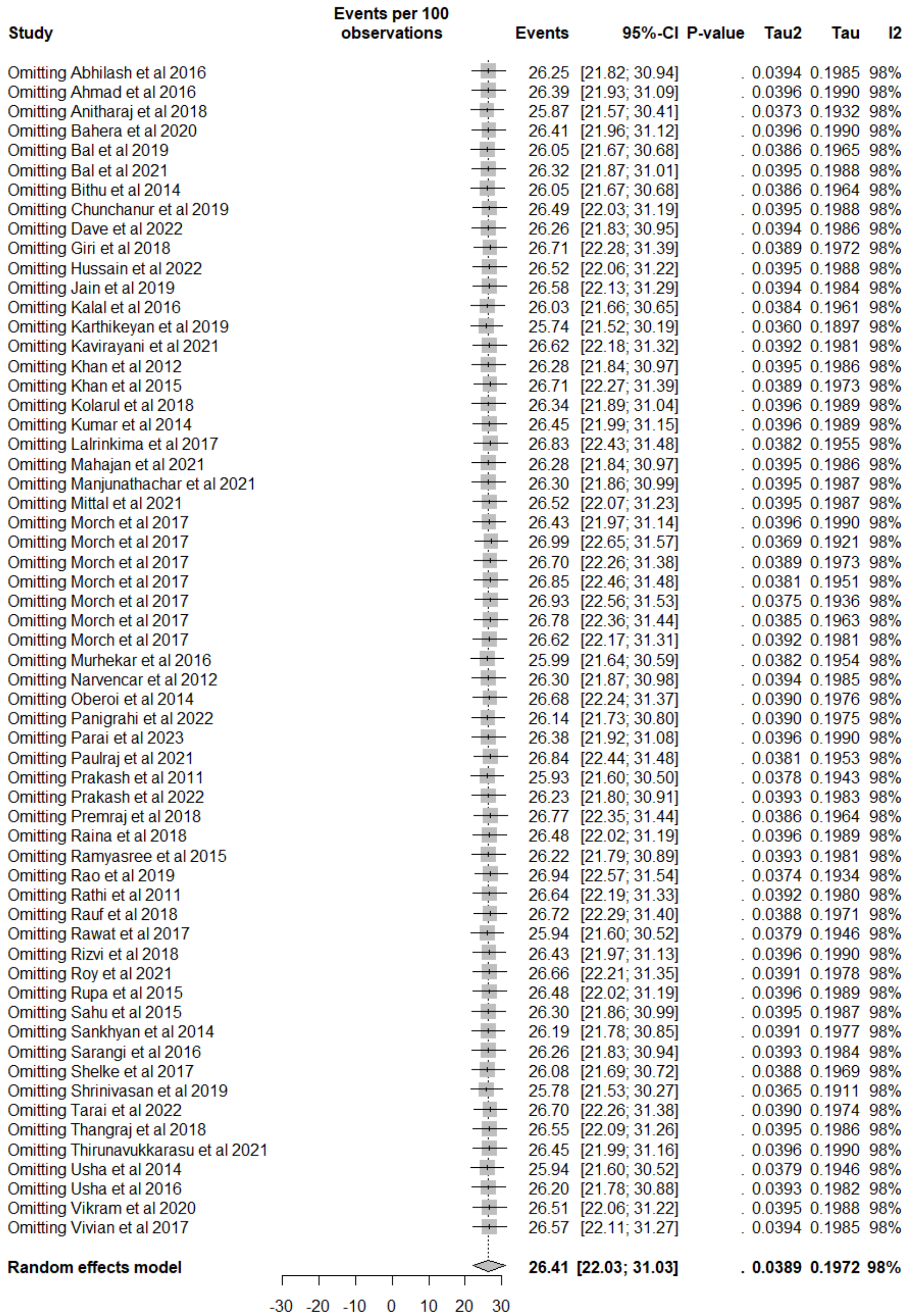

Fig. 4. Sensitivity analysis plot for prevalence and respective 95 % CI by omitting one study using R software.

associated complications and mortality. Tetracycline and chloramphenicol, once popular treatments for acute febrile illness caused by scrub typhus, have become less favored due to the emergence of antimicrobial resistance. This has led to an increase in the prevalence of scrub typhus and is likely to escalate the associated morbidity and mortality, as previously mentioned. Furthermore, in tropical regions, clinical signs of scrub typhus mimic those of other acute febrile illnesses such as leptospirosis, malaria, acute hepatitis and often results in inaccurate diagnosis or misdiagnosed as viral fever and left untreated relying on self-cure[87,88]. All these factors may have influenced the rise of scrub typhus in India over the past two decades.

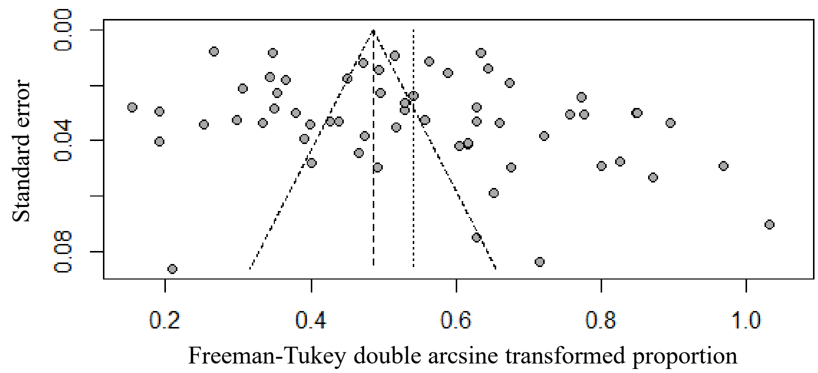

Fig. 5. Funnel plot showing publication bias using R software.

Our findings indicate that *Orientia tsutsugamushi* infection is widespread throughout the country. Notably, most of the studies reported were from the southern part of India due to increased awareness about the disease, heightened clinical suspicion among physicians following previously reported cases, and improved accessibility to diagnostic facilities in this region. These factors likely contributed to a greater number of studies being conducted in the southern part of the country.

A large heterogeneity among included studies was observed in present meta-analysis, which could be due to differences in time of sample collection (seasonal variation in positivity rate) or due to the use of different kits having different specificity and sensitivity. Since most of the studies had not reported the sensitivity and specificity of the method used for the diagnosis of the scrub typhus, the results of one study could not be compared with that of another. This is one the limitations of the analysis of the present investigation. Further, there were significant biases in included studies due to non-uniformity in sample size and effect size of included studies.

To effectively control and prevent the spread of scrub typhus, it is crucial to establish a robust surveillance system and continuously monitor cases in endemic regions using sensitive and early diagnostic assays. The utilization of sensitive diagnostic tests, such as scrub typhus IgM ELISA and Real time-PCR (RT-PCR) assays, has facilitated the detection of previously hidden or unidentified cases, resulting in an upsurge in reported numbers. Serological assays are primarily employed for the definitive diagnosis of scrub typhus in the absence of an eschar[89]. Consequently, the availability of improved tests requiring conventional and standardized laboratory facilities will likely result in a higher number of diagnosed cases. Therefore, the accessibility of sensitive and affordable point-of-care tests (POCTs) will be particularly beneficial in resource-limited settings, facilitating early disease detection and prompt treatment for scrub typhus.

In conclusion, to reduce the burden of scrub typhus, it is imperative to raise physician awareness about the disease from primary health care to tertiary health setup, implement point-of-care diagnosis, and ensure appropriate treatment with effective antibiotics. These essential measures, coupled with ongoing surveillance and enhanced diagnostic capabilities under one health aspect, will play a pivotal role in controlling the spread of scrub typhus and mitigating the impact of outbreaks of this neglected disease. In order to reduce the burden of the disease in the future, it is crucial to raise awareness about scrub typhus, implement point-of-care diagnosis, and ensure appropriate treatment.

Implication for practice: Scrub typhus is a seriously overlooked tropical disease, impacting mostly rural populations and increasing in urban regions. Our finding indicates the high burden of scrub typhus in India. When fever is the primary symptom of the illness, diagnosis becomes more difficult as other infections appear with same symptoms. This shows that a greater degree of clinical suspicion of scrub typhus should be applied to feverish patients and underlines the need for ensuring accessible and affordable diagnostic facilities in peripheral settings. Case fatality rates varied between geographical regions and states, mainly due to complication. Effective control and prevention of the spread and outbreaks of this neglected illness necessitate the implementation of early diagnostic procedures in endemic locations and a well-established surveillance system. These measures, combined with

Table II. JBI Quality score of selected studies (1-Yes, 0-No, U-uncertain)

Author, yr	Was the sample frame appropriate to address the target population?	Were study participants sampled in an appropriate way?	Was the sample size adequate?	Were the study subjects & the setting described in detail?	Was the data analysis conducted with sufficient coverage of the identified sample	Were valid methods used for the identification of the condition?	Was the condition measured in a standard, reliable way for all participants	Was there appropriate statistical analysis?	Was the response rate adequate, & if not, was the low response rate managed appropriately	JBI Quality score
Abhilash *et al*[31]	1	1	1	1	1	1	1	1	1	9
Ahmad *et al*[32]	1	1	1	1	1	1	1	1	1	9
Anitharaj *et al*[33]	1	0	1	1	0	1	1	1	1	7
Bahera *et al*[34]	1	1	1	1	U	1	1	1	1	8
Bal *et al*[35]	0	1	1	1	U	1	1	1	1	7
Bal *et al*[36]	1	1	1	1	1	1	1	1	1	9
Bithu *et al*[37]	1	1	1	1	1	1	1	1	1	9
Chunchanur *et al*[38]	U	1	0	0	1	1	1	0	1	5
Dave *et al*[39]	1	1	1	1	1	1	1	U	1	8
Giri *et al*[40]	0	1	1	1	U	1	1	1	1	7
Hussain *et al*[41]	U	1	1	0	1	1	U	0	1	5
Jain *et al*[42]	1	1	1	1	1	1	1	1	1	9
Kalal *et al*[43]	0	1	1	1	U	1	1	1	1	7
Karthikeyan *et al*[44]	1	1	0	1	1	1	1	U	1	7
Kavirayani *et al*[45]	0	1	1	1	U	1	1	1	1	7
Khan *et al*[46]	1	1	1	1	1	1	1	0	1	8
Khan *et al*[47]	1	1	1	1	1	1	1	U	1	8
Kolarul *et al*[48]	1	1	1	1	1	1	1	1	1	9
Kumar *et al*[49]	1	1	1	1	1	1	1	1	1	9
Lalrinkima *et al*[50]	1	1	1	1	1	1	1	0	1	8
Mahajan *et al*[51]	1	1	1	1	1	1	1	1	1	9
Manjunathachar *et al*[52]	1	1	1	1	1	1	1	1	1	9
Mittal *et al*[53]	1	1	1	1	1	1	1	1	1	9
Morch *et al*[54]	1	1	1	1	1	1	1	1	1	9
Morch *et al*[54]	1	1	1	1	1	1	1	1	1	9
Morch *et al*[54]	1	1	1	1	1	1	1	1	1	9
Morch *et al*[54]	1	1	1	1	1	1	1	1	1	9
Morch *et al*[54]	1	1	1	1	1	1	1	1	1	9
Morch *et al*[54]	1	1	1	1	1	1	1	1	1	9

Contd...

Author, yr	Was the sample frame appropriate to address the target population?	Were study participants sampled in an appropriate way?	Was the sample size adequate?	Were the study subjects & the setting described in detail?	Was the data analysis conducted with sufficient coverage of the identified sample	Were valid methods used for the identification of the condition?	Was the condition measured in a standard, reliable way for all participants	Was there appropriate statistical analysis?	Was the response rate adequate, & if not, was the low response rate managed appropriately	JBI Quality score
Morch *et al*[54]	1	1	1	1	1	1	1	1	1	9
Murhekar *et al*[55]	1	1	1	1	1	1	1	1	U	8
Narvencar *et al*[56]	1	1	0	1	1	1	1	1	0	7
Oberoi *et al*[57]	1	1	1	1	1	1	1	1	0	8
Panigrahi *et al*[58]	1	1	1	1	1	1	1	1	0	8
Parai *et al*[59]	1	1	1	1	1	1	1	1	1	9
Paulraj *et al*[60]	1	1	1	1	1	1	1	1	1	9
Prakash *et al*[61]	1	1	1	0	1	1	1	1	1	8
Prakash *et al*[62]	1	1	1	1	1	1	1	1	1	9
Premraj *et al*[63]	1	1	1	1	1	1	1	1	1	9
Raina *et al*[64]	1	1	1	1	1	1	1	1	1	9
Ramyasree *et al*[65]	1	1	0	1	U	1	1	1	U	6
Rao *et al*[66]	1	1	1	1	1	1	1	1	1	9
Rathi *et al*[67]	0	1	1	1	U	1	1	1	1	7
Rauf *et al*[68]	0	1	1	1	U	1	1	1	1	7
Rawat *et al*[69]	1	1	1	1	1	1	1	1	1	9
Rizvi *et al*[70]	1	1	1	1	1	1	1	1	1	9
Roy *et al*[71]	1	1	1	1	1	1	1	1	1	9
Rupa *et al*[72]	1	1	1	1	1	1	1	0	1	8
Sahu *et al*[73]	1	1	1	1	1	1	1	0	1	8
Sankhyan *et al*[74]	0	U	0	1	U	1	1	1	1	5
Sarangi *et al*[75]	0	1	1	1	U	1	1	0	1	6
Shelke *et al*[76]	1	1	1	1	1	1	1	1	1	9
Shrinivasan *et al*[77]	1	1	1	1	1	1	1	0	1	8
Tarai *et al*[78]	1	1	1	1	1	1	1	0	1	8
Thangraj *et al*[79]	0	1	1	1	U	1	1	1	U	6
Thirunavukkarasu *et al*[80]	0	1	1	1	U	1	1	1	1	7
Usha *et al*[81]	1	1	1	1	1	1	1	0	1	8
Usha *et al*[82]	1	1	1	1	1	1	1	1	1	9
Vikram *et al*[83]	1	1	1	1	1	1	1	1	1	9
Vivian *et al*[84]	1	1	1	1	1	1	1	1	1	9

Table III. Details of the studies reporting case fatality in positive scrub typhus cases

Author	Region	Total positive cases	Case fatality %
Ahmad *et al*[32]	North India	65	6 (9.2)
Bithu *et al*[37]	North western India	133	13 (9.7)
Chunchanur *et al*[38]	South India	22	1(4.5)
Narvencar *et al*[85]	West India	15	5 (33)
Tarai *et al*[78]	North-central India	56	5 (8.9)
Abhilash *et al*[31]	South-India	452	21(4.6)

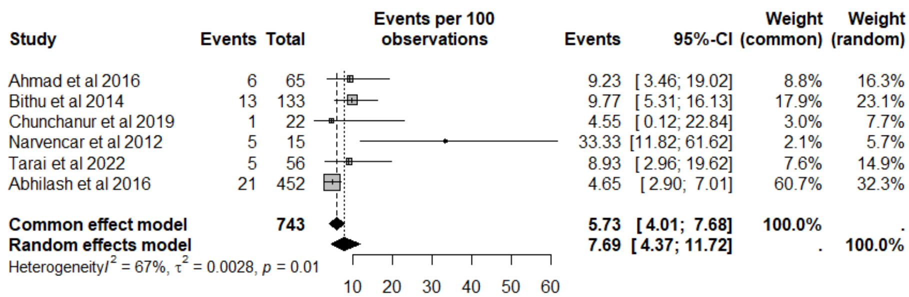

Fig. 6. Forest plot showing overall pooled prevalence of case fatality rate. The plot was generated using R software.

continuous surveillance and improved diagnostic capabilities, will play a vital role in controlling the spread of scrub typhus and mitigating the impact of outbreaks of this neglected disease.

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Conflicts of Interest: None.

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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