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RESEARCH ARTICLE

The prevalence of leptospirosis infections among humans in Malaysia: a systematic review and meta-analysis

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ABSTRACT

Leptospirosis is an infectious and zoonotic disease caused by pathogenic spirochetes of Leptospira. It affects global health issues, especially endemic in tropical and subtropical regions. This meta-analysis assessed the prevalence of leptospirosis in Malaysia and explored factors contributing to variability studies. A systematic review was conducted, which identified 301 records from six key databases. After eliminating duplicates and applying the inclusion criteria, 24 studies were selected for qualitative and quantitative analysis. Pooled prevalence and heterogeneity were calculated using a random-effects model. The pooled prevalence of leptospirosis was 26.7% (95% CI: 20.5–34.0%) with high heterogeneity (I^2 = 97.43%, $p < 0.001$). Thus, the reported prevalence decreased from 29.7% (2001–2010) to 18.1% (2011–2020). Additionally, cross-sectional studies reported a 27.4% prevalence, while prospective studies showed a higher rate (53.0%). Diagnostic methods affected the results, with MAT reporting 29.8% and combined PCR-MAT showing the highest prevalence at 31.9%. Leptospirosis remains common in Malaysia, demonstrating the need for better public health interventions, especially in flood-prone areas. Diagnostic techniques and surveillance must be improved and essential for better detecting and managing the disease.

Keywords: Zoonotic; leptospirosis; endemic; prevalence; risk factors.

INTRODUCTION

Leptospirosis is an infectious and zoonotic disease caused by pathogenic spirochetes of Leptospira. It affects global health issues, especially endemic in tropical and subtropical regions (Rafizah *et al*., 2013; Sahimin *et al*., 2019). Besides that, the World Health Organization (WHO) classified it as a neglected disease due to underreporting worldwide because of its widespread. Countries such as Malaysia, which has warm and humid climates, are prone to leptospirosis infection, and one of the main factors is this. On top of that, environmental and occupational factors contribute to the transmission of the disease (Philip & Ahmed, 2023).

Besides dengue and malaria, leptospirosis is endemic in Malaysia and ranks as one of the top causes of infectious disease-related mortality in Malaysia. It became a notifiable disease in 2010, and systematic reporting and associated mortality due to leptospirosis infection has improved the understanding of its epidemiology (Philip & Ahmed, 2023). Furthermore, this mandatory reporting has discovered the scale of the problem, with the incidence of leptospirosis increasing, specifically in rural and flood-prone areas (Benacer *et al*., 2016). Recently, a few news reports have reported a leptospirosis outbreak in Malaysia. For example, in November 2024, the Negeri Sembilan Health Department detected eight suspected cases of leptospirosis linked to the Ulu Bendul Recreational Park (Baharom *et al*., 2024). The park was temporarily closed until December 2024. Another report was in June 2024. Another report was that the Gunung Ledang National Park was temporarily closed for about two weeks due to reports of leptospirosis cases in the district (Lasim *et al*., 2024).

The disease is spread by multiple animal hosts, such as rodents, livestock, and domesticated animals, which initially carry the bacteria in their kidneys and excrete it through their urine. Humans can become infected via direct contact with contaminated animal urine or indirectly when exposed to contaminated water, soil, or vegetation (Thayaparan *et al*., 2013). Moreover, leptospirosis transmission is more common in areas with poor sanitation, high rodent populations, or in occupations that involve close contact with animals or environments where the bacteria can easily grow, such as agriculture and waste management (Soo *et al*., 2020). The most common route of infection is through mucous membranes or broken skin; it can also be transmitted by ingestion or inhalation of aerosolized leptospires (Thayaparan *et al*., 2013).

In 1925, about 32 cases of leptospirosis were diagnosed among rubber plantation workers and rural inhabitants in Malaysia, and it became the earliest recorded case in Malaysia at that time (Benacer *et al*., 2016). Since then, leptospirosis has been a persistent public health problem and issue, significantly affecting the agricultural sector because workers are regularly and constantly exposed to contaminated environments. Before this, the incidence of leptospirosis in Malaysia was reported to range from 1 to 10 cases

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per 100,000 people, but the reports needed to be more accurate due to underreporting and misdiagnosis (Benacer *et al*., 2016).

Leptospirosis can present from asymptomatic, mild symptoms to severe and life-threatening symptoms. Patients typically present with sudden onset of fever, chills, and headache. These signs and symptoms are nonspecific and occur with other causes of acute febrile syndrome, including influenza, dengue fever, or malaria. The headache is often severe and described as a bitemporal, frontal throbbing headache accompanied by retro-orbital pain and photophobia. Due to the same presentation with other acute febrile illnesses, it can lead to misdiagnosis and late diagnosis (Zainuddin *et al*., 2017). Severe leptospirosis is characterized by multiple organ failure and dysfunction including the liver, kidneys, lungs, and brain. The combination of multiple organ failure such as jaundice, acute kidney injury, and bleeding is called Weil disease, a type of severe form of leptospirosis. Progression to severe leptospirosis and circulatory collapse may be accompanied by acute respiratory distress syndrome (ARDS) and severe pulmonary haemorrhage syndrome (SPHS), which can cause high mortality rates, particularly in endemic regions like Malaysia (Philip *et al*., 2021).

In addition, the country's tropical climate, including Malaysia, heavy rainfall, and frequent flooding are well-known risk factors for leptospirosis transmission due to suitable conditions for the survival of Leptospira bacteria in the environment (Sara *et al*., 2020). Therefore, leptospirosis cases will increase significantly during the rainy season due to floods that spread contaminated water to nearby people. Flood-prone areas such as Kedah, Kelantan, and Sarawak have higher incidences of leptospirosis due to their vulnerability and susceptibility to seasonal flooding (Sara *et al*., 2020; Suut *et al*., 2016). This environmental factor and poor sanitation infrastructure in many rural areas make leptospirosis a recurring public health issue in these regions (Philip *et al*., 2021).

Besides environmental factors, high-risk occupation is one of the main factors in the transmission of leptospirosis in Malaysia. Leptospirosis is considered an occupational hazard for people who work in agriculture, animal husbandry, sewage and waste management, and other outdoor jobs due to frequent and regular exposure to potentially contaminated environments (Zin *et al*., 2019). Occupations such as farmers, market workers, garbage collectors, and sewage workers are particularly vulnerable and susceptible to infection because they are in regular and frequent contact with water, soil, or waste that may contain leptospires (Ridzuan *et al*., 2016a). On top of that, front-line workers in sanitation services in urban areas are at high risk of infection due to their exposure to drainage systems, sewers, and garbage collection sites, which often become contaminated with *Leptospira, particularly* during periods of heavy rainfall (Atil *et al*., 2020).

The diagnosis of leptospirosis is challenging due to its nonspecific symptoms, which overlap and are similar to other febrile illnesses common in tropical regions. Laboratory tests are essential for confirmation diagnosis. The most common diagnostic tests are the microscopic agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA), and molecular techniques like polymerase chain reaction (PCR) (Jeffree *et al*., 2020). MAT is considered the gold standard for serological diagnosis, but it is low sensitivity in detecting the early stages of infection (Samsudin *et al*., 2018). In addition, the most sensitive test for detecting *Leptospira* during the acute phase of the disease is PCR, which can detect early diagnosis and identification of specific *Leptospira* strains. Unfortunately, it requires high cost and specialized equipment (Neela *et al*., 2019).

Moreover, about 66 Leptospira species have been reported worldwide, making diagnosing and controlling leptospirosis challenging. There are several species endemic and found in Malaysia; the common species include *L. interrogans*, *L. borgpetersenii*, and *L. kirschneri*, as well as novel species such as *L. semungkisensis* and *L. fletcheri* (Pui *et al*., 2017; Rahman *et al*., 2018). Animals such as dogs, rodents, and cats, which act as reservoirs for bacteria, can transmit infection to humans. On top of that, multiple Leptospira species and the ability to live in our country's environment make the control and prevention of disease difficult.

Furthermore, there are multiple ways, such as improved sanitation, rodent control, public health education, and chemoprophylaxis, for high-risk groups to prevent and control leptospirosis in Malaysia effectively. Chemoprophylaxis, such as antibiotics such as doxycycline, can be used for people frequently exposed to contaminated environments and reduce the risk of leptospirosis infection in populations (Hanapi *et al*., 2021). In addition, early antibiotic usage is practical and helpful in preventing severe complications and outcomes, particularly in highly endemic parts of the country (Daud *et al*., 2018). However, the usage of chemoprophylaxis is limited and controlled due to antibiotic resistance (Loong *et al*., 2018).

This systematic review and meta-analysis aim to evaluate and identify leptospirosis' prevalence in Malaysia and the risk factors involved. Besides that, the study seeks to assess regional variations in prevalence across different states or regions and examine temporal trends, including seasonal patterns and outbreaks.

MATERIALS AND METHODS

Study Design and Protocol

This study is a systematic review and meta-analysis following the PRISMA 2020 guidelines. The study protocol was registered in the PROSPERO with registration number CRD42023494502. All study designs except systematic reviews, meta-analyses, review articles, case reports, and protocols will be included. The study focuses on research conducted in Malaysia.

Eligibility criteria

Studies involving human patients regardless of gender, ethnicity, and comorbidities were included.

Search strategies

Data for this study were extracted from research articles available on the following platforms: Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, Scopus, ScienceDirect, and Google Scholar. The search strategy was adapted for each database. We restricted publications to the English language only. The search was conducted using the descriptors "leptospira," "leptospirosis," "Malaysia," "prevalence," "seroprevalence," "infection," and "disease," along with the names of all states in Malaysia. Boolean operators "AND" and "OR" were utilized to refine the search. All search results were imported into EndNote 21.

Inclusion and exclusion criteria

We included only original research articles and studies conducted and published until December 2023, covering all states and regions in Malaysia. Studies involving populations, including both adult and paediatric groups residing in Malaysia, were included. Only studies published in English were considered. Studies were excluded if they contained insufficient information (e.g., lack of population size), were duplicates, did not report prevalence, or were review articles, conference abstracts, or case studies.

Data Selection and Extraction

The titles and abstracts of all retrieved citations were imported into EndNote. Two investigators independently screened titles and abstracts for eligibility based on predetermined criteria. Full texts of articles deemed potentially eligible were retrieved for further assessment. The investigators independently assessed the full text of each study for eligibility and reached consensus on the studies to include. Any discrepancies between the reviewers were resolved through discussion.

A blind title selection process was employed to minimize bias, followed by abstract screening to assess relevance and determine if the inclusion criteria were met. Full-text records of the selected abstracts were then retrieved. Duplicate records were removed, and data such as publication year, study location, detection method, prevalence of leptospirosis, and total population size were extracted. Besides, disagreements among reviewers were resolved through consensus. Studies that met exclusion criteria were identified and excluded.

Risk of Bias Assessment

The quality of all 24 featured studies was assessed using the Joanna Briggs Institute (JBI) critical appraisal checklist for prevalence data. The checklist assesses nine items: (1) appropriate sampling frame, (2) proper sampling technique, (3) adequate sample size, (4) description of study subjects and setting, (5) sufficient data analysis, (6) use of valid methods for the identified conditions, (7) valid measurement for all participants, (8) appropriate statistical analysis, and (9) adequate response rate. Each item was rated as "yes," "no," "unclear," or "not applicable." A score of 1 was assigned for a "yes" response, while "no" and "unclear" responses were scored as 0. The average score for each item was calculated, and studies scoring below or above the mean were classified as low or high quality, respectively. If two reviewers reached an agreement on a study's quality, it was included in the analysis.

Statistical analysis

OpenMeta software was used to calculate pooled prevalence and determine the random-effects model. Comprehensive Meta-Analysis software was employed to assess the bias of the included studies.

RESULTS

Study Selection

A total of 301 records were initially identified from various databases, including PubMed, Google Scholar, Scopus, Web of Science, Science Direct, and the Cochrane Library. After removing 97 duplicate records, 204 records remained for screening (Figure 1). Of these, 116 were excluded based on relevance or quality, leaving 88 reports sought for retrieval. However, 6 reports could not be retrieved, resulting in 82 reports being assessed for eligibility. Following the eligibility assessment, 58 reports were excluded for reasons such as not meeting inclusion criteria or lacking sufficient data. Ultimately, 24 studies were included in both the qualitative and quantitative synthesis (meta-analysis). There are few prevalence studies in my final inclusion research related to orang asli, plantation workers, high-risk occupations, the army, orang asli, refugee workers, and urban workers. Features of the included studies are presented in Table 1.

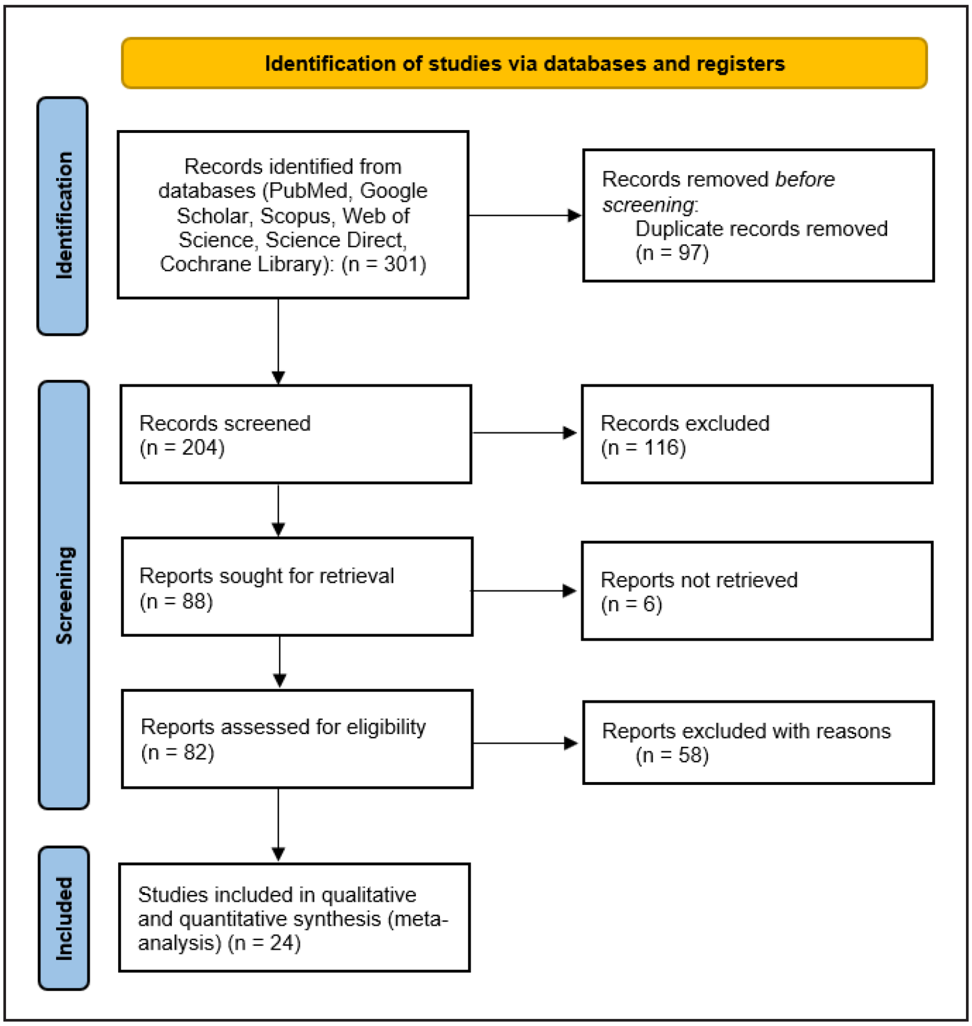

Figure 1. Summary of article search and selection process.

Table 1. Major characteristics of included studies

Study ID	Study period	Location	Study design	Method of diagnosis	Lepto positive cases	Patient background	Study Reference
Rafizah *et al*., 2013	2010-2011	Multiple cities	Cross-sectional	ELISA and MAT	84	High risk occupation	(Rafizah *et al*., 2013)
Ridzuan *et al*., 2016b	NR	Multiple cities	Cross-sectional	MAT	100	Plantation workers	(Ridzuan *et al*., 2016b)
Zainuddin *et al*., 2017	NR	Kelantan	Cross-sectional	ELISA and MAT	98	Town service workers	(Zainuddin *et al*., 2017)
Sara *et al*., 2020	NR	Multiple cities	Cross-sectional	ELISA and MAT	100	Army	(Sara *et al*., 2020)
Loong *et al*., 2018	2016-2018	Selangor	Cross-sectional	PCR and ELISA	46	Orang asli	(Loong *et al*., 2018)
Neela *et al*., 2019	2016	Multiple cities	Cross-sectional	ELISA and MAT	2	Military	(Neela *et al*., 2019)
Philip *et al*., 2020	2016-2017	Multiple cities	Prospective	PCR and MAT	92	NR	(Philip *et al*., 2020)
Atil *et al*., 2020	2017	Sabah	Cross-sectional	PCR and MAT	37	Urban service workers	(Atil *et al*., 2020)
Ridzuan *et al*., 2016a	2014	Multiple cities	Cross-sectional	MAT	100	Plantation workers	(Ridzuan *et al*., 2016a)
Daud *et al*., 2018	2016	Multiple cities	Cross-sectional	MAT	87	Cattle farmers	(Daud *et al*., 2018)
Rakesh *et al*., 2021	2014-2017	Kedah	Cross-sectional	MAT	108	NR	(Rakesh *et al*., 2021)
Rafizah *et al*., 2012	2010-2011	Multiple cities	Cross-sectional	MAT	84	NR	(Rafizah *et al*., 2012)
Rahman *et al*., 2018	2017-2017	Kelantan	Cross-sectional	MAT	78	Wet market workers	(Rahman *et al*., 2018)
Philip *et al*., 2021	2016-2017	Multiple cities	Prospective	MAT	83	NR	(Philip *et al*., 2021)
Hanapi *et al*., 2021	2019-2020	Kuala Lumpur	Cross-sectional	ELISA	107	Refugee	(Hanapi *et al*., 2021)
Suppiah *et al*., 2017	2014-2016	Selangor	Retrospective	MAT	11	NR	(Suppiah *et al*., 2017)
Hii *et al*., 2021	2019	Sarawak	Cross-sectional	PCR	55	NR	(Hii *et al*., 2021)
Jeffree *et al*., 2020	2017	Sabah	Cross-sectional	PCR and MAT	133	Urban sanitation workers	(Jeffree *et al*., 2020)
Thayaparan *et al*., 2015	2011-2012	Multiple cities	Cross-sectional	ELISA and MAT	71	NR	(Thayaparan *et al*., 2015)
Shafei *et al*., 2012	2008	Kelantan	Cross-sectional	MAT	73	Town service workers	(Shafei *et al*., 2012)
Suut *et al*., 2016	2011-2013	Sarawak	Cross-sectional	MAT	190	Rural areas	(Suut *et al*., 2016)
Sahimin *et al*., 2019	2017-2018	Multiple cities	Seroprevalence	ELISA	67	Urban poor communities	(Sahimin *et al*., 2019)
Samsudin *et al*., 2018	2016	Selangor	Cross-sectional	ELISA and MAT	107	Market workers	(Samsudin *et al*., 2018)
Zin *et al*., 2019	2015-2016	Selangor	Cross-sectional	ELISA and MAT	29	NR	(Zin *et al*., 2019)

NR: Not reported. NS: Not specified.

Pooled prevalence leptospirosis

The pooled prevalence of leptospirosis in Malaysia, based on 24 studies, was found to be 26.7% (95% CI: 20.5–34.0%), with significant variability among the studies (I^2 = 97.43%, $p < 0.001$), reflecting differences in populations, methodologies, and environmental conditions. This high prevalence underscores the serious public health challenge posed by leptospirosis, particularly given Malaysia's frequent floods and tropical climate that contribute to exposure to contaminated water (Figure 2).

Subgroup analysis

Over time, the prevalence decreased from 29.7% (95% CI: 22.4–38.3%) between 2001 and 2010 to 18.1% (95% CI: 7.1–38.8%) between 2011 and 2020 (Figure 3), which may indicate improvements in sanitation and awareness, though high heterogeneity (I^2 > 96%) suggests that other factors are also at play.

The Table 3 presents the prevalence of leptospirosis in Malaysia based on different periods, regions, study designs, and diagnostic methods. The overall prevalence is 26.7%, with a slight decrease in recent years (18.1% for 2011–2020) compared to 29.7% in 2001–2010, possibly due to improved disease control efforts.

The prevalence varies by region, with Kedah (54.0%) and Sarawak (37.4%) showing the highest rates, while Kuala Lumpur (24.7%) and Selangor (18.0%) report lower numbers. Environmental conditions, exposure risks, and healthcare accessibility may influence these differences.

Study design and diagnostic methods also affect reported prevalence. Prospective studies (53.0%) detect more cases than retrospective studies (4.1%), while PCR (37.4%) and MAT (29.8%) identify more infections compared to ELISA (17.9%). The high variability (I^2 > 95%) suggests differences in study populations and methodologies.

Most studies in the analysis were cross-sectional, showing a pooled prevalence of 27.4%, whereas prospective studies reported a higher prevalence of 53.0% (Figure 4). Retrospective and surveillance studies reported lower prevalence, potentially due to underreporting or missed cases in retrospective designs. This variation reflects how different study designs capture leptospirosis cases, with prospective studies possibly being more sensitive in tracking cases over time.

Regionally, prevalence varied, with Kelantan reporting 29.5%, Kedah at 54%, Sarawak at 37.4%, and Selangor at a lower 18% (Figure 5), likely due to differences in rurality, flooding frequency, and infrastructure.

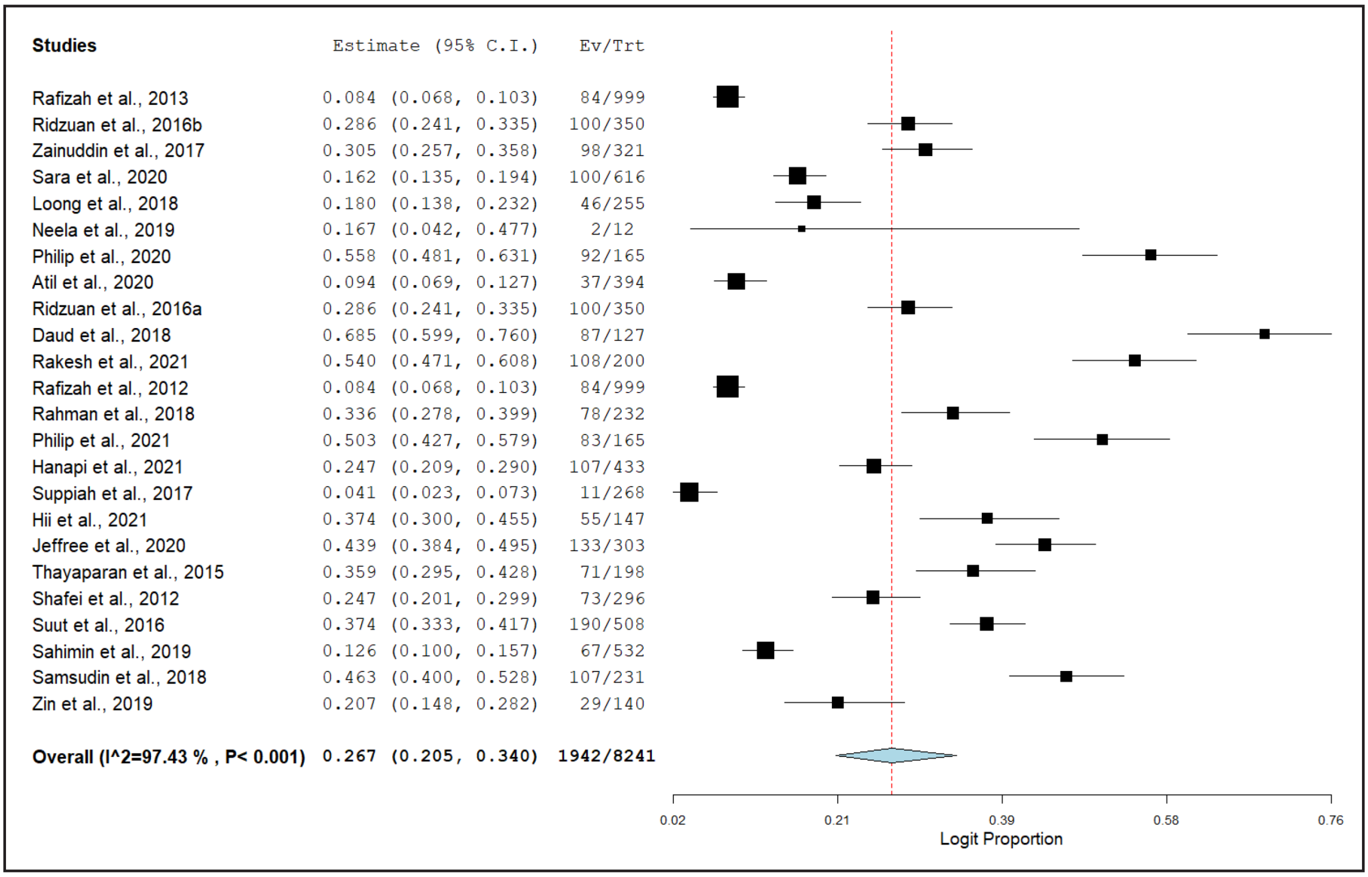

Figure 2. Forest plot of the pool prevalence of Leptospirosis in Malaysia.

Table 2. Demographical characteristics, overall dengue prevalence, dengue serotypes and reported symptoms among studied cases

Study ID	Gender		Age groups (Years)	Prevalence of Lepto		Symptoms	Study Reference
	Male	Female		Total studied cases	Lepto positive cases		
Rafizah *et al*., 2013	543	456	NS	999	84	Fever	(Rafizah *et al*., 2013)
Ridzuan *et al*., 2016b	296	54	NR	350	100	Asymptomatic	(Ridzuan *et al*., 2016b)
Zainuddin *et al*., 2017	309	12	NS	321	98	NR	(Zainuddin *et al*., 2017)
Sara *et al*., 2020	608	8	NS	616	100	Asymptomatic	(Sara *et al*., 2020)
Loong *et al*., 2018	NR	NR	NS	255	46	Fever	(Loong *et al*., 2018)
Neela *et al*., 2019	6	6	NR	12	2	Fever, headache, myalgia	(Neela *et al*., 2019)
Philip *et al*., 2020	60	32	14-77	165	92	Febrile illness, headache, myalgia, athralgia, meningeal irritation, jaundice	(Philip *et al*., 2020)
Atil *et al*., 2020	313	81	NS	394	37	NR	(Atil *et al*., 2020)
Ridzuan *et al*., 2016a	296	54	NR	350	100	NR	(Ridzuan *et al*., 2016a)
Daud *et al*., 2018	104	16	NR	127	87	Asymptomatic	(Daud *et al*., 2018)
Rakesh *et al*., 2021	71	37	1-60	200	108	Fever, vomiting, reduced appetite, cough, myalgia	(Rakesh *et al*., 2021)
Rafizah *et al*., 2012	543	456	NS	999	84	Fever	(Rafizah *et al*., 2012)
Rahman *et al*., 2018	83	149	18-79	232	78	Asymptomatic	(Rahman *et al*., 2018)
Philip *et al*., 2021	54	29	NR	165	83	Mild, sever	(Philip *et al*., 2021)
Hanapi *et al*., 2021	223	210	NS	433	107	NR	(Hanapi *et al*., 2021)
Suppiah *et al*., 2017			NR	268	11	Fever, abdominal pain, lethargic	(Suppiah *et al*., 2017)
Hii *et al*., 2021	88	59	2-78	147	55	Fever, myalgia, headache, rash, jaundice	(Hii *et al*., 2021)
Jeffree *et al*., 2020	222	78	23-60	303	133	NR	(Jeffree *et al*., 2020)
Thayaparan *et al*., 2015	72	126	NS	198	71	Fever	(Thayaparan *et al*., 2015)
Shafei *et al*., 2012	296		NS	296	73	Asymptomatic	(Shafei *et al*., 2012)
Suut *et al*., 2016	223	285	5-86	508	190	NR	(Suut *et al*., 2016)
Sahimin *et al*., 2019	206	326	NS	532	67	Headache, fever, myalgia, abdominal discomfort, jaundice	(Sahimin *et al*., 2019)
Samsudin *et al*., 2018	152	79	NS	231	107	Asymptomatic	(Samsudin *et al*., 2018)
Zin *et al*., 2019	NR	NR	NR	140	29	NR	(Zin *et al*., 2019)

NR: Not reported. NS: Not specified.

Table 3. Prevalence of leptospirosis infection in Malaysia according to the different subgroups

Subgroup	Number of studies	Prevalence (%)	95% CI	Heterogeneity test	
				I^2 (%)	p-value
Year					
2011-2020	4	18.1	7.1 – 38.8	98.69	<0.001
2001-2010	17	29.7	22.4 – 38.3	96.53	<0.001
Overall	21	27.0	19.9 – 35.6	97.66	<0.001
Region/Province					
Multiple cities	11	26.5	16.4 – 39.9	98.16	<0.001
Kelantan	3	29.5	24.7 – 34.8	62.73	0.068
Selangor	4	18.0	7.0 – 39.1	97.09	<0.001
Sabah	2	22.2	3.8 – 67.5	98.94	<0.001
Kedah	1	54.0	47.1 – 60.8	NA	NA
Kuala Lumpur	1	24.7	20.9 – 29.0	NA	NA
Sarawak	2	37.4	33.8 – 41.2	NA	0.998
Overall	24	26.7	20.5 – 34.0	97.43	<0.001
Study design					
Cross-sectional	20	27.4	20.9 – 35.0	97.25	<0.001
Prospective	2	53.0	47.6 – 58.4	NA	0.321
Retrospective	1	4.1	2.3 – 7.3	NA	NA
Surveillance	1	12.6	10.0 – 15.7	NA	NA
Overall	24	26.7	20.5 – 34.0	97.43	<0.001
Method of diagnosis					
ELISA and MAT	7	23.3	13.6 – 36.9	97.22	<0.001
MAT	10	29.8	19.4 – 42.7	97.81	<0.001
PCR and ELISA	1	18.0	13.8 – 23.2	NA	NA
PCR and MAT	3	31.9	10.9 – 64.2	98.47	<0.001
ELISA	2	17.9	8.9 – 32.8	95.65	<0.001
PCR	1	37.4	30.0 – 45.5	NA	NA
Overall	24	26.7	20.5 – 34.0	97.43	<0.001

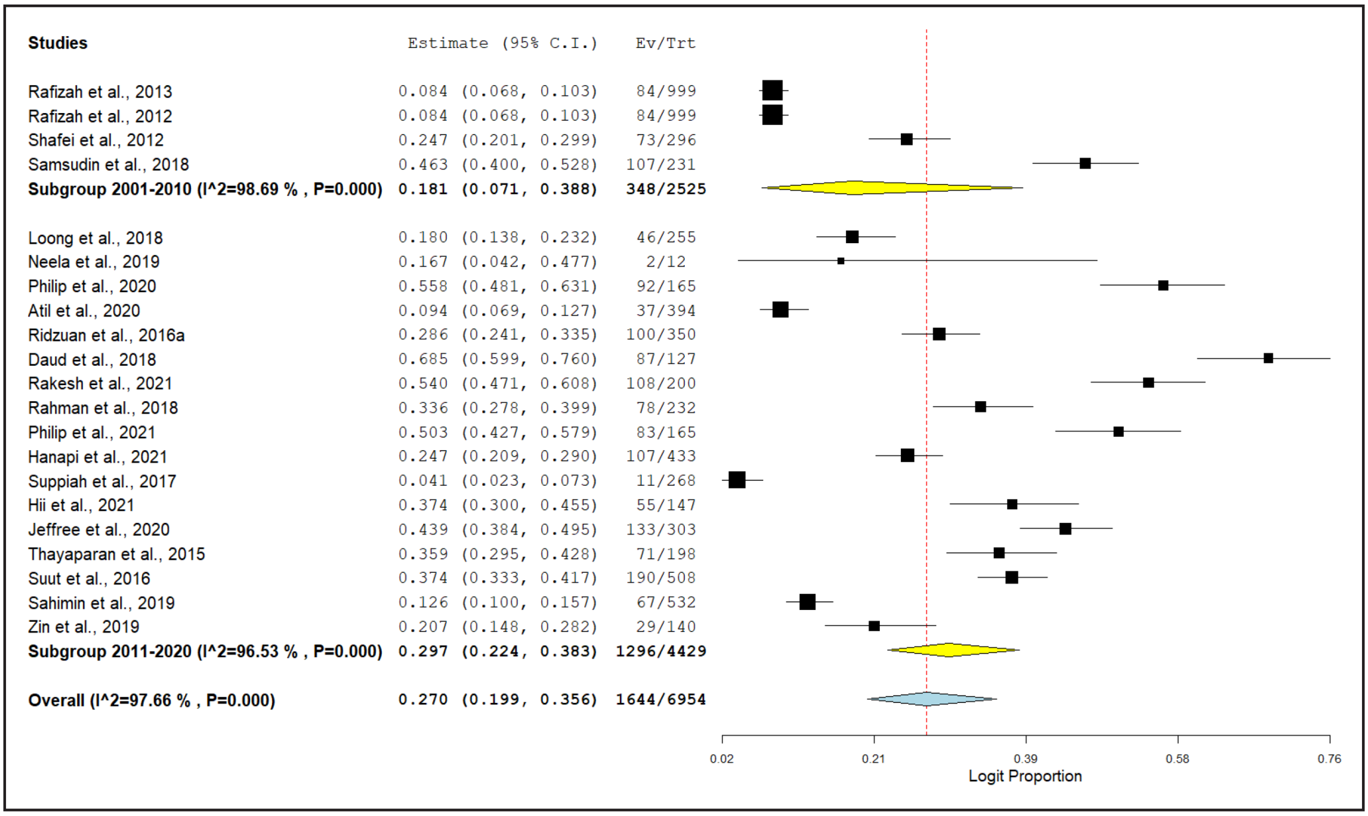

Figure 3. Forest plot of the pool prevalence of Leptospirosis in Malaysia (Year-wise).

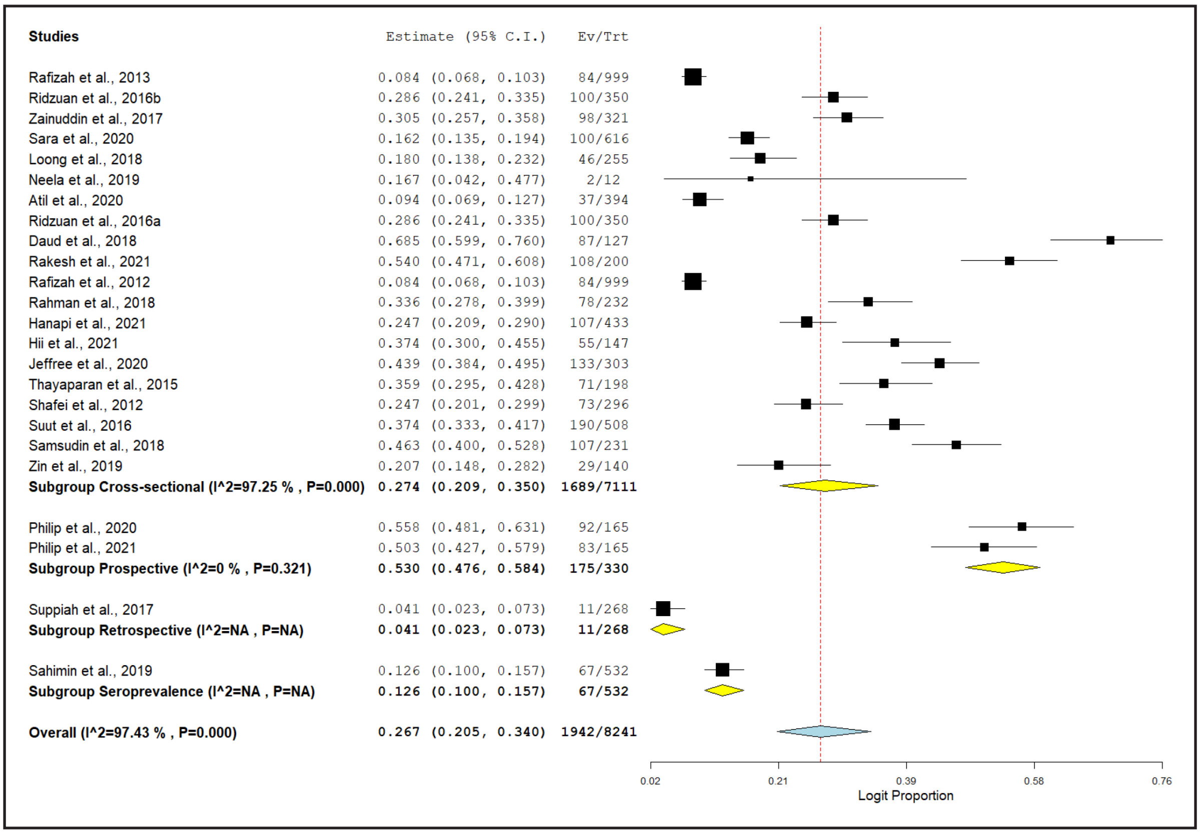

Figure 4. Forest plot of the pool prevalence of Leptospirosis in Malaysia (study design).

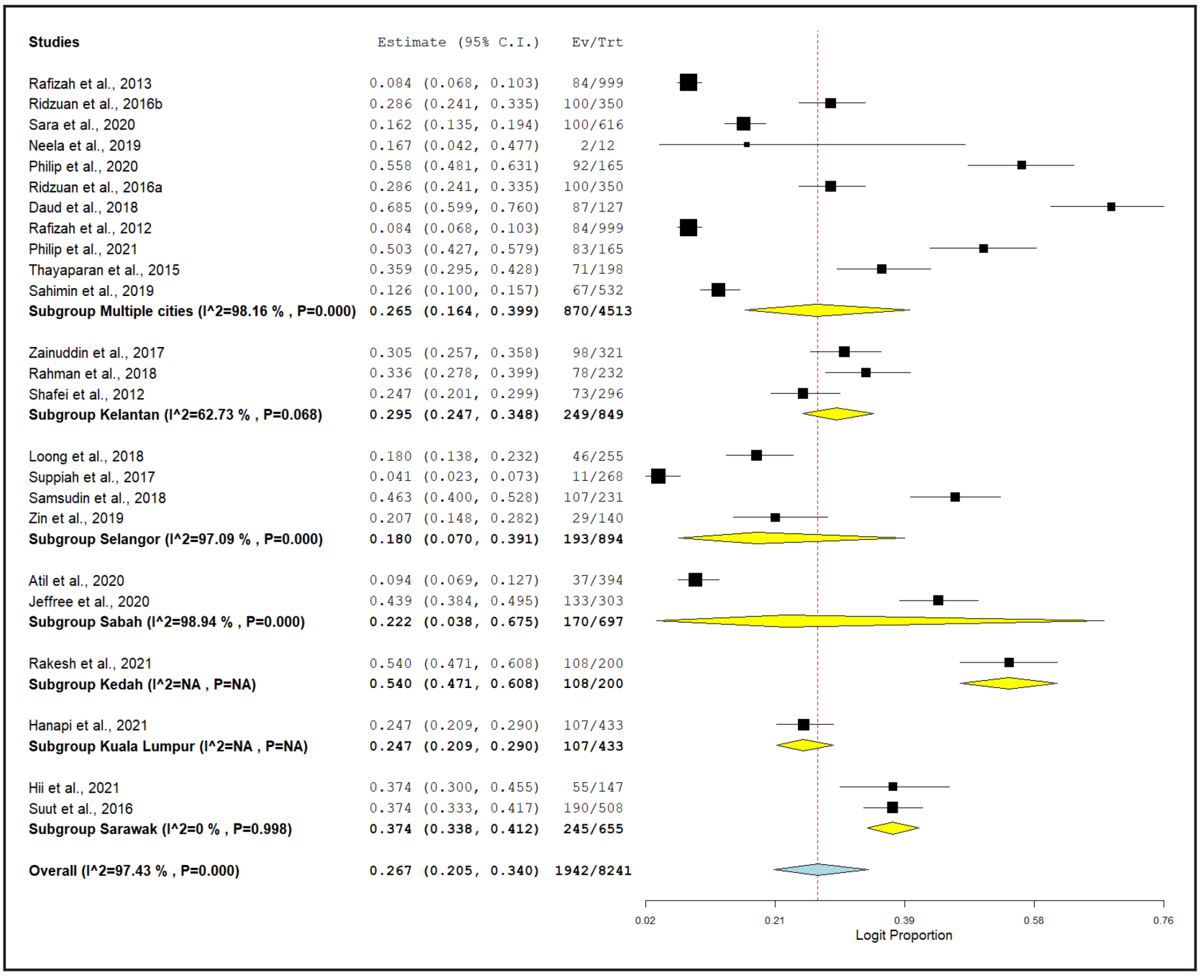

Figure 5. Forest plot of the pool prevalence of Leptospirosis in Malaysia (Study region).

The choice of diagnostic method significantly impacted reported prevalence (Figure 6). Studies using MAT, regarded as the gold standard, reported a prevalence of 29.8%, whereas ELISA showed a lower prevalence of 17.9%. Studies combining PCR and MAT yielded the highest prevalence at 31.9%, indicating that more sensitive diagnostic tools are necessary for accurate detection. The variability in diagnostic methods across studies is a key factor contributing to the heterogeneity in prevalence rates.

The quality assessment of studies has been shown in Figure 7. The funnel plot revealed some asymmetry, suggesting potential publication bias (Figure 8). The Egger test, used to assess this asymmetry, indicated the likelihood that smaller studies with higher prevalence rates were more likely to be published, whereas studies with lower prevalence rates may have been underreported. This publication bias could have an impact on the overall conclusions of the meta-analysis, leading to an overestimation of the pooled prevalence.

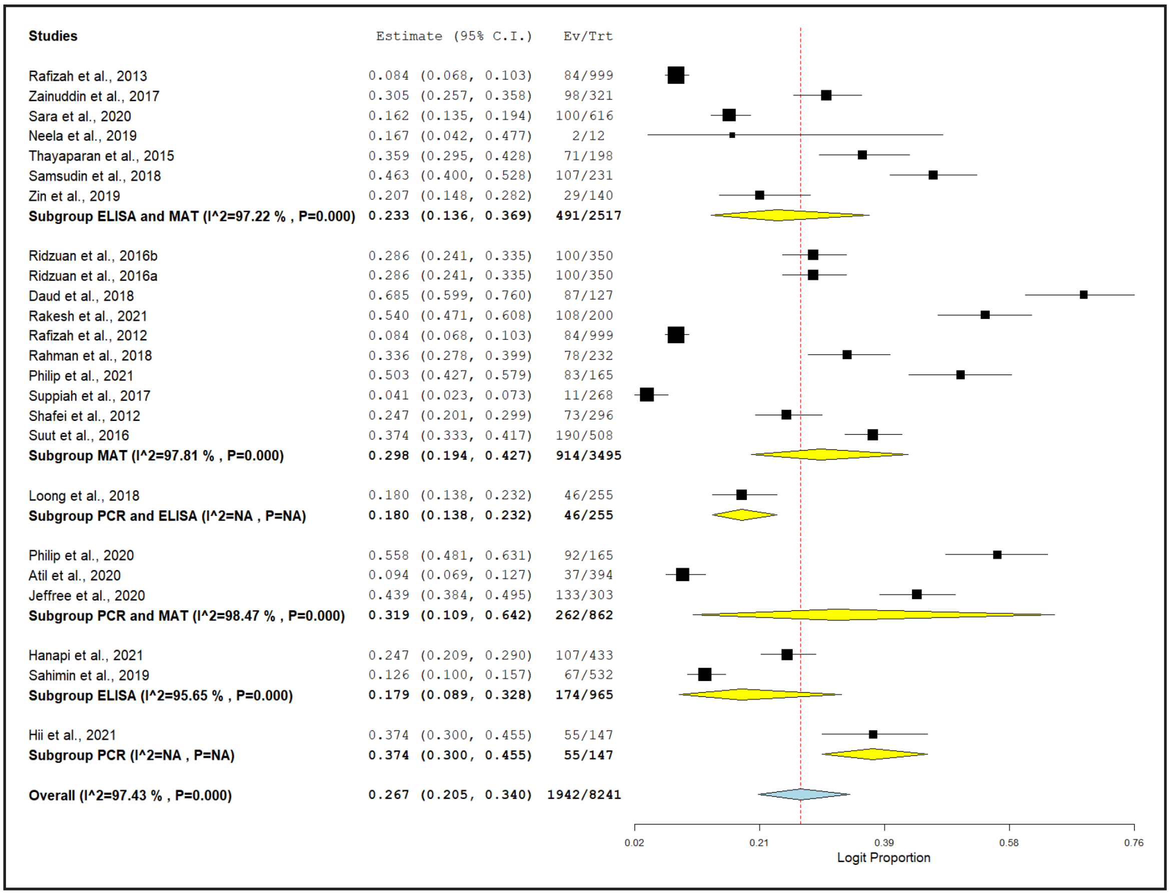

Figure 6. Forest plot of the pool prevalence of Leptospirosis in Malaysia (Method of diagnosis).

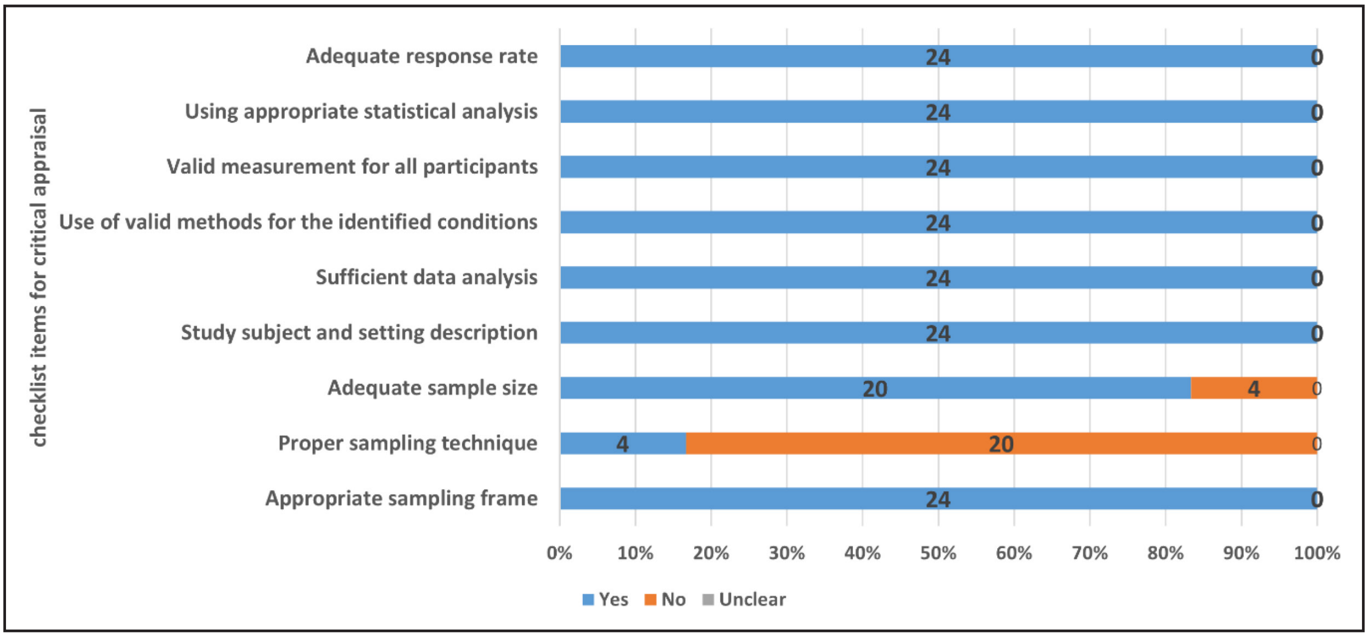

Figure 7. Quality assessment of included studies using JBI tool.

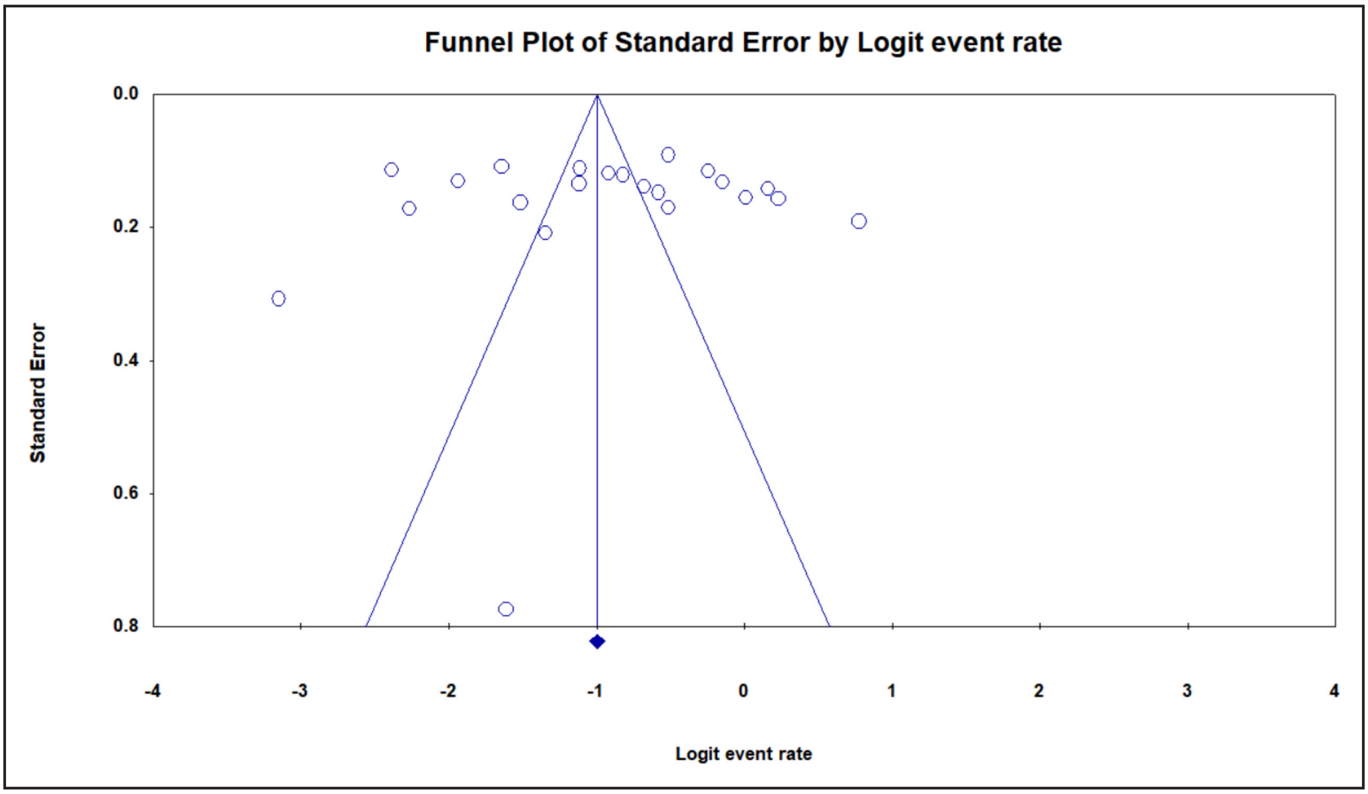

Figure 8. Funnel plot showing no significant publication bias for leptospirosis [Egger's test, p = 0.3943] estimates.

DISCUSSION

Leptospirosis has been endemic since years ago. Based on systemic review and meta-analysis, the final inclusion was 24 studies which estimated the prevalence of leptospirosis in Malaysia to be 26.7% (95% CI: 20.5–34.0%), showing a significant level of heterogeneity (I^2 = 97.43%, $p < 0.001$) across the studies. Differences largely influence this variability in the populations studied, diagnostic techniques employed, and environmental conditions.

Besides, leptospirosis is a zoonotic disease that is a public health challenge in Malaysia, particularly in rural areas and flood-prone states like Kedah and Kelantan. These areas experience higher risks due to increased exposure to contaminated water sources (Benacer *et al*., 2016). For example, a previous study in Sarawak recorded a high prevalence of 37.4% due to frequent contact with polluted water in the local population (Zainuddin *et al*., 2017). Similarly, another research study found a prevalence of 24.7% among public servants in Kelantan, where seasonal floods significantly contribute to leptospirosis outbreaks (Rafizah *et al*., 2013).

Forest plots show a wide variation in the prevalence of leptospirosis in Malaysia. For example, states like Kedah reported the highest prevalence, about 54 %, mainly due to frequent flooding and agricultural activities, which are easily exposed to transmission of Leptospira. As mentioned, urban states such as Kuala Lumpur and Selangor have better sanitation, lower exposure to contaminated water and fewer flooding events than rural areas, leading to lower prevalence of leptospirosis. Therefore, the forest plot demonstrated the influence of geographical, occupational and diagnostic factors on leptospirosis prevalence.

This study shows several diagnostic methods, such as ELISA, MAT and PCR. In several studies, a combination of ELISA and MAT was used to help with the diagnostic accuracy of the disease. In the early stage of diagnosis, ELISA, which detects IgM antibodies, can help detect infection. Besides, MAT is considered the gold standard for detecting antibodies in the later stages of disease. The pooled prevalence for studies using both ELISA and MAT was 23.3%, with significant heterogeneity (I^2 = 97.22%). This combination of ELISA and MAT helps cover different disease stages, improving diagnostic reliability and accuracy. However, differences in reported prevalence rates are likely influenced by the various diagnostic methods used across studies.

This meta-analysis study highlights that people who work in high-risk occupations, such as agriculture, waste management, and market-related jobs, exhibit markedly higher infection rates. A previous study reported a prevalence of 46.3% among market workers and food handlers in Malaysia, demonstrating the evidence occupational exposure is one of the leading risk factors for spreading leptospirosis (Samsudin *et al*., 2018). In addition, the higher infection rate is due to these workers being frequently exposed to contaminated water, food, and other materials, subsequently increasing their risk of infection. Another previous study identified a prevalence rate of 27.4% among garbage collectors in Kelantan, demonstrating the increased risks of working in waste management (Ridzuan *et al*., 2016b).

The forest plot also illustrates these results by showing higher prevalence rates for populations in high-risk occupations. Additionally, research on rural agricultural workers and urban service workers, including market handlers and garbage collectors, show higher prevalence rates than the general population's studies. This difference highlights the occupational risk factors correlating to leptospirosis transmission, especially in areas with poor sanitation and frequent flooding (Suppiah *et al*., 2017). Furthermore, it is essential to identify high-risk groups for implementing targeted public health interventions, and the forest plot demonstrated the disproportionate impact of leptospirosis on certain occupational and demographic groups. Therefore, people who work in agriculture, waste management, and market environment more commonly have a high risk of transmission of leptospirosis. Moreover, another study documented the prevalence of leptospirosis among garbage collectors in Kelantan at 27.4% due to frequently handling contaminated waste and being prone to exposure to floodwaters (Ridzuan *et al*., 2016b). Another supporting results from a study includes that 46.3% of market workers were reported as the highest within an urban occupation (Samsudin *et al*., 2018). This high prevalence is because they regularly come into contact with contaminated food and water supplies.

Besides occupational factors, environmental exposure is also a primary risk factor. Rural states like Kelantan, Kedah, and Sarawak consistently report higher prevalence rates due to frequent flooding and inadequate sanitation infrastructure (Sara *et al*., 2020; Zainuddin *et al*., 2017). During a flood, water contaminated with *Leptospira bacteria* transmits throughout the community and agricultural region, increasing human exposure to the infection. The forest plot also revealed that studies from flood-prone areas demonstrated much higher prevalence rates, further strongly showing evidence of the link between environmental exposure and leptospirosis transmission (Sara *et al*., 2020).

As mentioned before, the prevalence of leptospirosis varied significantly across different parts of Malaysia. According to studies, Kelantan reported a prevalence of 29.5%, while higher rates were reported in Kedah (54%) and Sarawak (37.4%). However, Selangor showed a low prevalence of 18 % compared to Kelantan and Kedah. Environmental factors such as rurality and frequent floods increase human exposure to leptospirosis infection. Besides, urbanized areas have better and more proper sanitation than rural areas, so they have a lower prevalence of leptospirosis.

Besides, the forest plot documented the significant result in leptospirosis prevalence in Malaysia. Based on a previous study results, in Sarawak 37.4% prevalence was noted. Several factors, such as dense forests, agricultural activities, and inadequate sanitation infrastructure, contribute to Sarawak's prevalence (Zainuddin *et al*., 2017). Similarly, another study documented that Kelantan public workers had a high seropositivity rate of 24.7 % due to frequent exposure to contaminated waste and floods (Rafizah *et al*., 2013). All studies illustrate that environmental factors are one of the main factors for leptospirosis outbreaks or becoming endemic in Malaysia (Suppiah *et al*., 2017).

Several studies show that urbanized states, including Kuala Lumpur and Selangor, had better infrastructure and equipment, proper sanitation, and fewer flood events than rural areas. All these factors contribute to lower prevalence rates (Atil *et al*., 2020). Besides, Forrest's plot consistently shows that rural and flood-prone states had higher prevalence rates than urban states. The geographic difference shows the essentials of implementing proper public health strategies.

Furthermore, according to the past two decades, the prevalence of leptospirosis in Malaysia has decreased. Based on analysis, the estimated prevalence from 2001 to 2010 was 29.7% (95% CI: 22.4–38.3%), whereas from 2011 to 2020, the prevalence decreased to 18.1% (95% CI: 7.1–38.8%). Furthermore, the decreasing trend is due to improved public health interventions, including better sanitation and public awareness. In addition, the high heterogeneity ($I^2 > 96\%$) means that other factors, such as differences in study methods or varying regional risks, are also one of the factors.

Leptospirosis cases in Malaysia are strongly linked with seasonal patterns; for example, during the rainy monsoon season, there is a significant increase in leptospirosis prevalence in Malaysia. In addition, a previous study showed a strong association between monsoon rains and leptospirosis outbreaks (Sara *et al*., 2020). During periods of heavy rainfall, rural areas with poor drainage systems will experience flooding more frequently. Floodwaters, usually contaminated with *Leptospira* bacteria, are a significant transmission of leptospirosis. Based on another study in Kelantan, which faces frequent and recurrent flooding, especially during the monsoon season, demonstrated reports of high rates of leptospirosis due to increased environmental exposure (Rafizah *et al*., 2013).

In addition, the forest plot illustrated higher prevalence rates in studies conducted during or immediately after the monsoon season, especially in flood-prone states like Kelantan and Kedah. During monsoon seasons, the importance of prevention public health interventions, such as flood management, public awareness campaigns, and the provision of protective equipment for high-risk populations (Benacer *et al*., 2016; Sara *et al*., 2020). Even though studies show a slight decline in the overall prevalence of leptospirosis in recent years, most likely due to improved public health measures, the forest plot shows that the country remains prone to leptospirosis infection during seasonal outbreaks, especially during the monsoon season (Suppiah *et al*., 2017).

Another risk factor for leptospirosis infections is age and comorbidities. Few studies have reported that older individuals, especially those over 34, are more easily prone to getting infections. Furthermore, people with underlying diseases such as diabetes or chronic kidney disease are more eligible for a higher risk of severe outcomes. In previous studies, it was reported that people with underlying diseases tend to get more severe forms of leptospirosis, such as renal failure and pulmonary haemorrhage (Neela *et al*., 2019; Philip *et al*., 2021).

This meta-analysis helps manage leptospirosis outbreaks and helps public health implement measures, especially in flood-prone rural areas like Kelantan and Kedah. Besides that, preventive measures such as public education campaigns, better and proper sanitation, and flood management are crucial to reducing exposure to leptospirosis infection (Suppiah *et al*., 2017). Besides that, the emergency department also plays a vital role in the early diagnosis and management of leptospirosis outbreaks. A previous study highlighted the importance of quick diagnosis and treatment in emergencies, especially during monsoon seasons when cases increase (Jeffree *et al*., 2020). Therefore, early identification and treatment of high-risk individuals, such as garbage collectors and market workers, is essential and can help to reduce morbidity and mortality as well as severe forms of leptospirosis.

LIMITATIONS OF THE STUDY

This study has a few limitations that can influence the prevalence results, such as information bias and lack of data in some research. More data on urban areas like Kuala Lumpur and Selangor must be collected. Most studies focused on rural states like Kelantan, Terengganu and Kedah. The analysis revealed significant inconsistencies ($I^2 = 97.43\%$) across the studies owing to many changes in the population, location, and diagnostic methods. Besides that, laboratory tests such as MAT, PCR and ELISA give inaccurate results, which influence the actual outcome and prevalence.

CONCLUSION

In conclusion, this systematic review and meta-analysis comprehensively assess leptospirosis prevalence and associated risk factors in Malaysia. Additionally, the pooled prevalence of 26.7% demonstrated the significant burden of the disease, particularly in rural and flood-prone areas like Kedah and Kelantan. The study also reported that occupational risks, such as agricultural workers, market handlers, and garbage collectors, and environmental factors, such as seasonal flooding and inadequate sanitation, cause leptospirosis transmission. Furthermore, a few prevention strategies include the use of protective equipment for high-risk workers, public health campaigns before the monsoon season, and chemoprophylaxis for high-exposure populations. However, the study has limitations, including high heterogeneity, variations in diagnostic methods, and potential publication bias. These factors should be considered when interpreting the findings. Further research is needed to balance regional data and standardize diagnostics for more accurate prevalence estimates. Moreover, leptospirosis remains a significant public health concern in Malaysia, requiring ongoing monitoring, improved prevention efforts, and reduced occupational risks to control future outbreaks.

Conflict of Interest Statement

The author declares that they have no conflict of interests.

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