



RESEARCH ARTICLE

Epidemiology of Human *Entamoeba histolytica* Intestinal Infection in Malaysia: A Systematic Review and Meta-Analysis

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ARTICLE HISTORY

Received: 7 October 2025

Revised: 10 December 2025

Accepted: 16 December 2025

Published: 31 March 2026

ABSTRACT

Entamoeba histolytica (*E. histolytica*) is a protozoan parasite that causes amoebiasis in humans. It is prevalent in developing countries, particularly in areas with inadequate sanitation and limited access to clean water. While some data on the infection in the Malaysian population is available, comprehensive data on the overall prevalence is lacking. Our study aimed to determine the prevalence of *E. histolytica* in Malaysia through systematic review and meta-analysis using data published up to 2025. Fourteen studies covering diverse population groups from various states in Malaysia, including rural and urban residents, schoolchildren, indigenous communities, and high-risk populations were reviewed. We found an overall pooled prevalence of 7% with high heterogeneity ($I^2 = 92.5\%$). Prevalence varied widely by state and population subgroup, with higher rates in Pahang (18%) and among aboriginal schoolchildren (16%). Lower prevalence was found among urban residents (2%) and patients with gastrointestinal disorders (2%). There was only a slight difference in prevalence between individuals with co-infections (8%) and those without (7%). Studies using microscopy showed higher prevalence (7%) than molecular methods (4%). This is likely due to the misidentification of non-pathogenic *Entamoeba* species as *E. histolytica* when using microscopy. These findings contribute to a better understanding of the epidemiology of *E. histolytica* intestinal infection in Malaysia. Although the overall prevalence is relatively low, the results highlight the need for continued surveillance and more accurate diagnostic approaches to support targeted control.

Keywords: *Entamoeba histolytica*; amoebiasis; meta-analysis; systematic review; Malaysia.

INTRODUCTION

Intestinal protozoan infections are a major public health concern, affecting approximately 3.5 billion people worldwide, with around 450 million currently infected and experiencing symptoms. *Entamoeba histolytica* (*E. histolytica*) is a pathogenic intestinal protozoan parasite and the causative agent of human amoebiasis. It is the only amoebic species known to be virulent and capable of harming the host, leading to both intestinal and extraintestinal complications, including amoebic colitis and amoebic liver abscess (Guillén, 2023; Haque *et al.*, 2003; Servián *et al.*, 2022). Annually, an estimated 50 million people are infected, resulting in over 100,000 deaths, classifying it as the third highest-mortality parasitic disease worldwide (Cui *et al.*, 2019; Lin *et al.*, 2022). While amoebiasis is distributed globally, it is more prevalent in developing countries located within tropical and subtropical regions including Malaysia (Servián *et al.*, 2022; Tengku & Norhayati, 2011). The primary mode of transmission for *E. histolytica* is the fecal-oral route. Infection

occurs when individuals ingest cysts through contaminated food or water (Hung *et al.*, 2012; CDC, 2024; CDC, 2019). Additional risk factors include residing in or travelling to areas with poor sanitation or having limited access to clean water (CDC, 2024). Infection also occurs through person-to-person contact, including sexual contact, particularly among men who have sex with men (Hung *et al.*, 2012). Approximately 10% of individuals infected with this parasite develop amoebiasis or other symptoms, while 90% remain asymptomatic (Kim *et al.*, 2013).

Intestinal protozoan infections are prevalent in Malaysia and continue to contribute to the epidemiology of infectious diseases in the country (Kumarasamy *et al.*, 2023). The prevalence of *Entamoeba* spp. infection in human ranges from 13.4% to 75% (Thomas & Sinniah, 1982; Tokijoh *et al.*, 2022). Among indigenous (Orang Asli) communities, reported prevalence ranged from 17.6% to 22.5% (Syazwan *et al.*, 2022). However, most of these studies relied on microscopic stool examination, which made it difficult to distinguish pathogenic *E. histolytica* from the non-pathogenic *E. dispar* and

E. moshkovskii (Tokijoh *et al.*, 2022). Earlier studies primarily relied on microscopy for the diagnosis of *E. histolytica*, a method that often led to overestimation of infection. Although there was a notable increase in the use of Polymerase Chain Reaction (PCR) and other molecular techniques in Malaysia from 2009 onwards, several subsequent studies continued to rely solely on microscopy (Sinniah *et al.*, 2012; Abd Ghani & Jeyaprakasam, 2021). As a result, the true prevalence of each *Entamoeba* species remains uncertain (Sinniah *et al.*, 2012; Abd Ghani & Jeyaprakasam, 2021).

To date, no comprehensive systematic review or meta-analysis is available that consolidates nationwide prevalence data of *E. histolytica* in Malaysia, along with key factors such as population demographics, geographical distribution, clinical presentations, and diagnostic methodologies. Such evidence is crucial for identifying vulnerable populations, guiding effective surveillance and understanding Malaysia's burden in comparison to other endemic regions worldwide. Our study aimed to fill this gap by conducting the first systematic review and meta-analysis of *E. histolytica* prevalence in Malaysia, covering published data up to year of 2025. Our study provides the overall prevalence of *E. histolytica* across various regions and populations in Malaysia.

MATERIALS AND METHODS

Search strategy

The review was conducted in accordance with the PRISMA guidelines (Moher *et al.*, 2009). The protocol was registered in PROSPERO (International Prospective Register of Systematic Reviews) under registration number CRD420251066440. The relevant articles were searched in the PubMed, Web of Science, Scopus, and ScienceDirect databases in March 2025. There were no limitations on the publication year for the search. All the search keywords were identified using the authors' knowledge and the keywords of published articles on the topic. The keywords were: Malaysia, epidemiology, prevalence, *Entamoeba histolytica*, *Entamoeba* spp., *Entamoeba* complex, *E. histolytica* and entamoebiasis.

Inclusion criteria

This review focuses on previously published studies on the prevalence of *E. histolytica* among various populations in Malaysia. Studies involving human populations in Malaysia were included regardless of participant characteristics or study settings. Included studies were those conducted using appropriate methodologies and reporting primary data relevant to the prevalence or distribution of *E. histolytica*.

Exclusion criteria

Articles that reported only the detection of *E. histolytica* without specific findings on prevalence were excluded. Conference proceedings, case reports, animal studies, and review papers were also excluded.

Data extraction

Data extracted from the included articles comprised the authors' names, year of publication, sampling methods, study setting, diagnostic methods used to detect *E. histolytica*, and primary results such as prevalence percentages and co-infection data. The authors' main conclusions and quality assessment scores were also recorded (Supplementary Table S1).

Identification

Figure 1 shows the PRISMA flowchart illustrating the selection of included studies. Initially, two authors developed a search strategy using various keywords and their synonyms. All search results were imported into EndNote 21 software, and 61 duplicate papers were removed. An additional 63 papers were excluded for various reasons, including non-research items such as indexed citations

and conference abstracts without full papers. One independent author reviewed the titles and abstracts of the remaining 324 papers. Subsequently, a total of 45 papers were chosen for full-text analysis. Another three authors evaluated the eligibility of the retained papers. After a thorough review of 45 papers, 19 were initially selected for qualitative analysis. Ultimately, 14 papers met all inclusion criteria and were included in the final analysis (Thomas & Sinniah, 1982; Rajeswari *et al.*, 1994; Jamaiah & Rohela, 2005; Basuni *et al.*, 2012; Ngui *et al.*, 2012; Sinniah *et al.*, 2012; Al-Harazi *et al.*, 2013; Lau *et al.*, 2013; Adli & Abd Ghani, 2020; Gee *et al.*, 2020; Ngui *et al.*, 2020; Abd Ghani & Jeyaprakasam, 2021; Syazwan *et al.*, 2022; Tokijoh *et al.*, 2022).

A meta-analysis random-effects model was applied to analyze the pooled prevalence with a 95% confidence interval (CI) of *E. histolytica* infection. The quality of all included papers was assessed using the BSA Medical Sociology Group survey-based studies. The tool consists of seven questions. Every question answered with a "yes" was given one point, and answered with a "no" was given zero points. Scores of 1-2 were considered low quality, 3-5 moderate quality, and scores between 6-7 were regarded as good quality. Only moderate and high-quality papers were included in the analysis.

RESULTS

This systematic review and meta-analysis included 14 studies carried out in Malaysia, published up to 2025 (Table 1). Most studies were conducted among Aboriginal populations, where the prevalence of *E. histolytica* infection ranged from 1.3% to 46.6%. Several studies combined laboratory diagnostic methods with questionnaire-based assessments to gather information on sociodemographic factors, hygiene practices, clinical symptoms, and risk exposures (e.g., water source, food handling, sanitation). This enabled a more comprehensive evaluation of *E. histolytica* infection in relation to

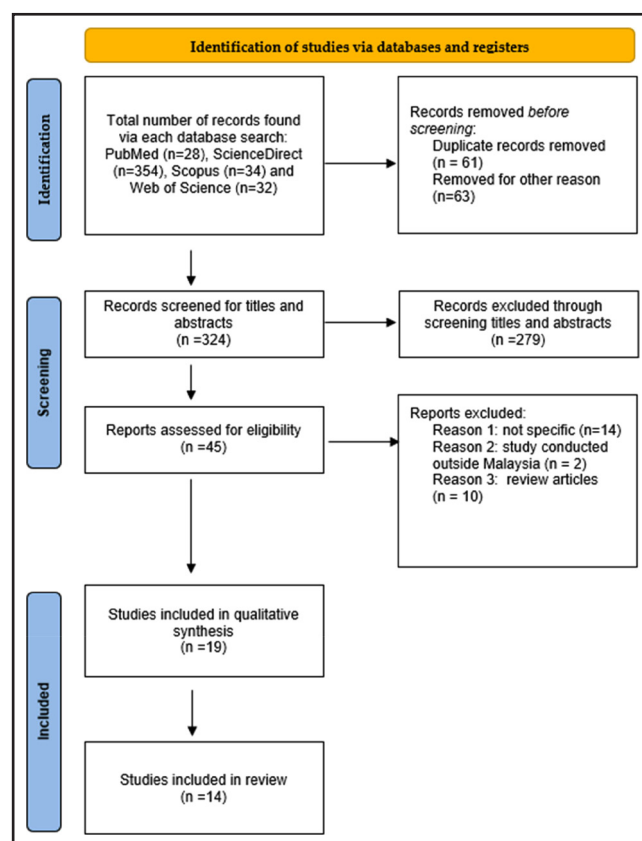


Figure 1. PRISMA flow chart of the included studies.

behavioral and environmental factors in their studies (Rajeswari *et al.*, 1994; Ngui *et al.*, 2012; Tokijoh *et al.*, 2022).

Across all studies, the pooled prevalence of *E. histolytica* infection was 7% (95% CI: 4%–12%) based on a random-effects model using generalized linear mixed models (GLMM). The prediction interval ranged from 1% to 47%. The studies were conducted in specific locations in various states, six were performed in Selangor,

four in Pahang, one in each Johor and Kelantan (Table 1). Subgroup analysis by state revealed marked differences in prevalence. The highest pooled prevalence was observed in Pahang at 18%, followed by Perak at 10%, Selangor at 5%, Johor at 3%, and Kelantan at 2% (Figure 2). A significant subgroup difference was detected ($p < 0.001$), indicating that geographical location contributed to the differences in prevalence (Figure 3).

Table 1. Studies on the prevalence of *E. histolytica* in Malaysia

Year of Study	States	Methods of Detection	Sample size (n)	Percentage (%)	Gastrointestinal Symptoms	Co-infection	Author/Year
Aborigine general population							
1982	Selangor	DM	34	5.88	Asymptomatic	No	Thomas & Sinniah, 1982
2009-2011	Selangor	PCR	107	1.9	Asymptomatic	Yes	Ngui <i>et al.</i> , 2012
2009-2011	Pahang	PCR	230	15.7	Asymptomatic	Yes	Ngui <i>et al.</i> , 2012
2009-2011	Johor	PCR	89	3.4	Asymptomatic	No	Ngui <i>et al.</i> , 2012
2010-2011	Selangor	PCR	207	12.6	Asymptomatic	Yes	Lau <i>et al.</i> , 2013
2010-2011	Pahang	PCR	29	6.9	Asymptomatic	No	Lau <i>et al.</i> , 2013
2010-2011	Perak	PCR	98	16.3	Asymptomatic	Yes	Lau <i>et al.</i> , 2013
2011	Perak	DM	77	1.3	Asymptomatic	Yes	Sinniah <i>et al.</i> , 2012
2014-2015	Selangor	PCR	411	5.1	Symptomatic & Asymptomatic	Yes	Ngui <i>et al.</i> , 2020
2018-2019	Perak	PCR	55	7.3	Asymptomatic	No	Syazwan <i>et al.</i> , 2022
Aborigine schoolchildren							
1982	Selangor	DM	50	4	Asymptomatic	No	Thomas & Sinniah, 1982
1994	Selangor	DM	78	15.4	Asymptomatic	No	Rajeswari <i>et al.</i> , 1994
2008	Pahang	DM	307	14.7	Asymptomatic	Yes	Al-Harazi <i>et al.</i> , 2013
2020	Perak	PCR	116	46.6	Asymptomatic	Yes	Gee <i>et al.</i> , 2020
2020	Pahang	DM	111	36	Asymptomatic	No	Adli & Abd Ghani, 2020
2020-2021	Perak	PCR	544	5	Symptomatic	Yes	Tokijoh <i>et al.</i> , 2022
2021	Perak	DM	139	14.4	Asymptomatic	No	Abd Ghani & Jeyaprakasam, 2021
Rural schoolchildren							
1994	Selangor	DM	357	9	Asymptomatic	No	Rajeswari <i>et al.</i> , 1994
Urban general population							
1982	Selangor	DM	271	4.8	Asymptomatic	No	Thomas & Sinniah, 1982
2005	Selangor	DM	246	0.004	Asymptomatic	No	Jamaiah & Rohela., 2005
Patients with Gastrointestinal disorder							
2008 - 2010	Kelantan	PCR	225	1.8	Symptomatic	Yes	Basuni <i>et al.</i> , 2012

DM: Direct microscopy.
PCR: Polymerase Chain Reaction.

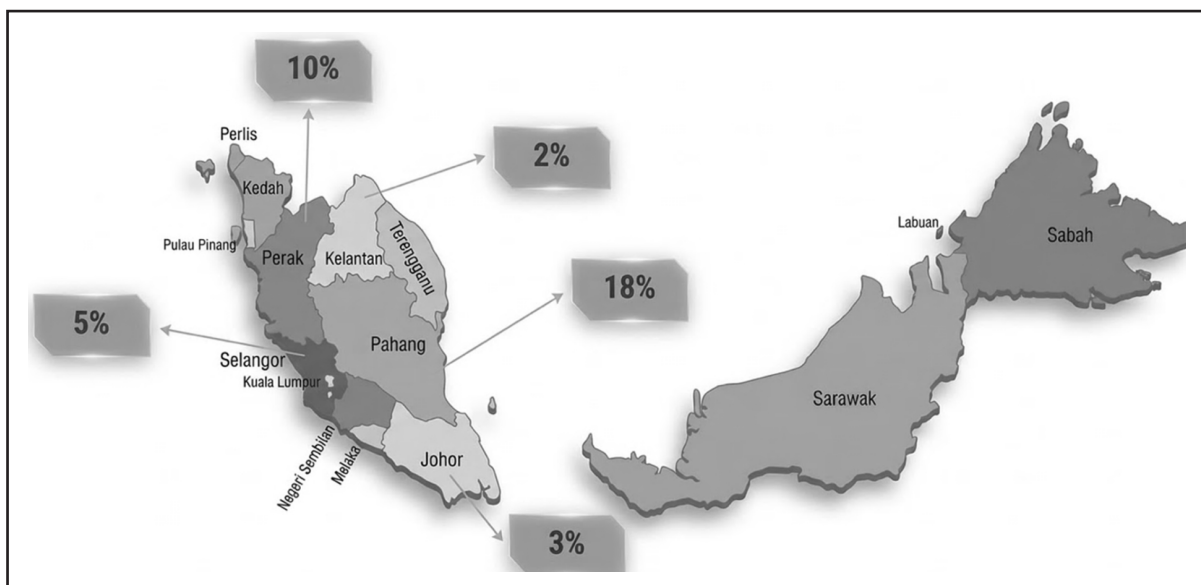


Figure 2. Distribution of *E. histolytica* across various states in Malaysia.

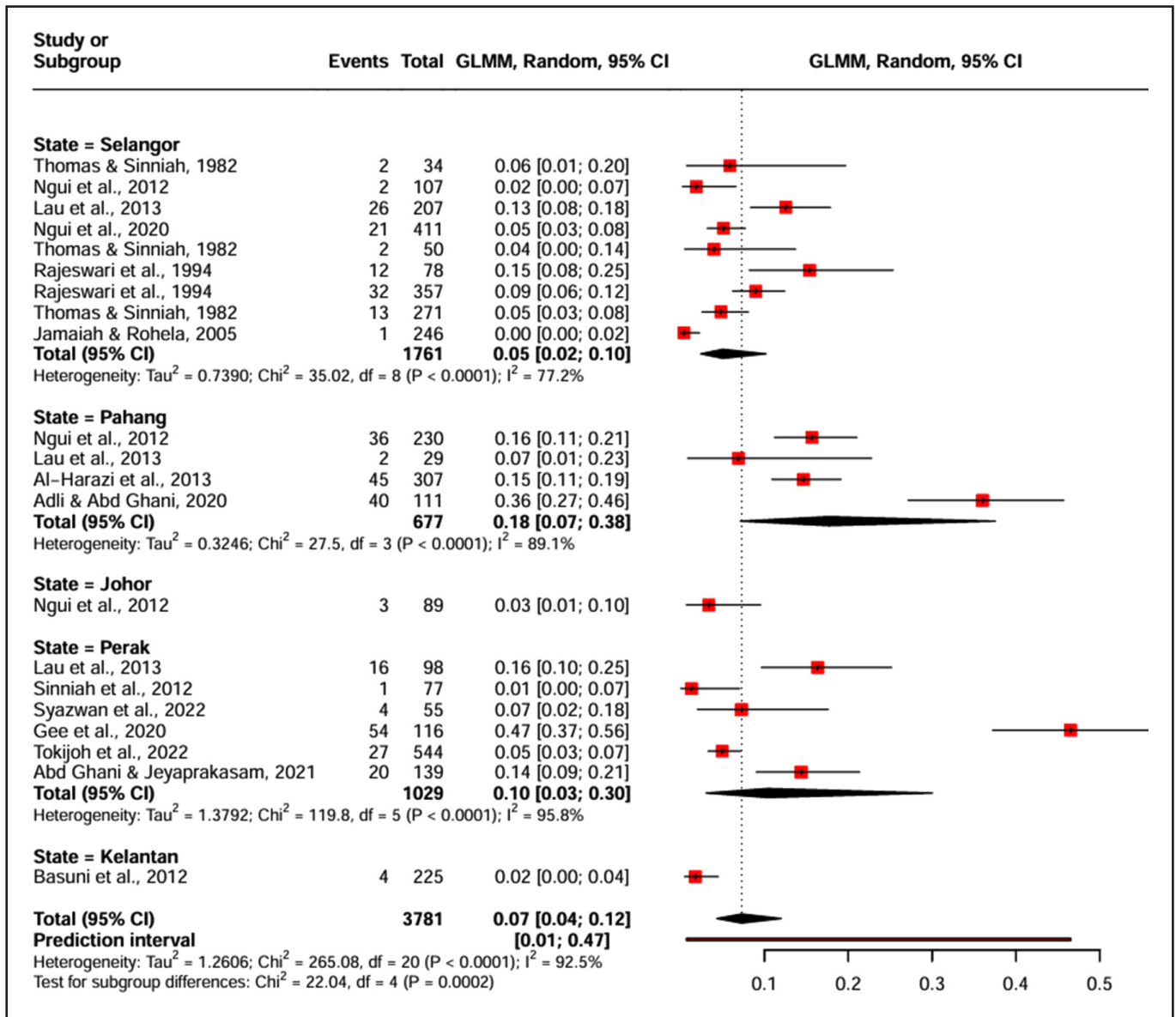


Figure 3. Forest plot of pooled prevalence of *E. histolytica* in states of Malaysia using a random-effects model with 95% CI.

Among the various population groups, the highest pooled prevalence was recorded among aboriginal schoolchildren at 16%, followed by the rural schoolchildren group at 9%, and aborigine general population at 7%. In contrast, lower prevalence was observed in both urban general population (2%) and patients with gastrointestinal disorders (2%). Subgroup differences were statistically significant ($p < 0.01$), indicating that the prevalence varied by population group (Figure 4).

Variations in prevalence were also associated with the detection methods used (Figure 5). Studies using direct microscopy (DM) reported a pooled prevalence of 7% while studies utilizing PCR yielded a slightly lower pooled prevalence of 4%. For individuals without co-infection, the pooled prevalence was 7%, with substantial heterogeneity ($I^2 = 89.1\%$, $p < 0.0001$). In contrast, individuals with co-infection showed a slightly higher pooled prevalence of 8%, also with high heterogeneity ($I^2 = 94.8\%$, $p < 0.0001$) (Figure 6). The asymptomatic individuals had a pooled prevalence of 8% with high heterogeneity ($I^2 = 90.8\%$, $p < 0.0001$), while the mixed group (comprising symptomatic and asymptomatic individuals) had a prevalence of 5%. Studies focusing exclusively on symptomatic individuals reported the lowest prevalence of 3% with moderate heterogeneity ($I^2 = 73.9\%$, $p = 0.05$). Although this estimate was

based on only two studies and characterized by high heterogeneity ($I^2 = 73.9\%$) (Figure 7).

DISCUSSION

E. histolytica remains an important parasitic pathogen, though its burden varies considerably worldwide. In Malaysia, the pooled prevalence up to 2025 was 7%, with considerable heterogeneity ($I^2 = 92.5\%$) and a wide prediction interval (1%–47%), suggesting notable variation across studies. This heterogeneity is influenced by multiple factors, including diagnostic approaches, study design, population characteristics, and geographical settings. Earlier studies relied heavily on microscopy, which cannot distinguish *E. histolytica* from the morphologically identical but non-pathogenic *E. dispar* and *E. moshkovskii*, potentially inflating prevalence estimates (Ngui et al., 2012; Calle-Pacheco et al., 2022).

Although more specific diagnostic tools such as PCR and serological assays (e.g., ELISA) demonstrate high sensitivity and specificity and are widely used for diagnosing extraintestinal amoebiasis and asymptomatic *Entamoeba* infections (Fotedar et al., 2007; Hooshyar & Rostamkhani, 2022), their application in Malaysia prevalence studies remains limited, further contributing

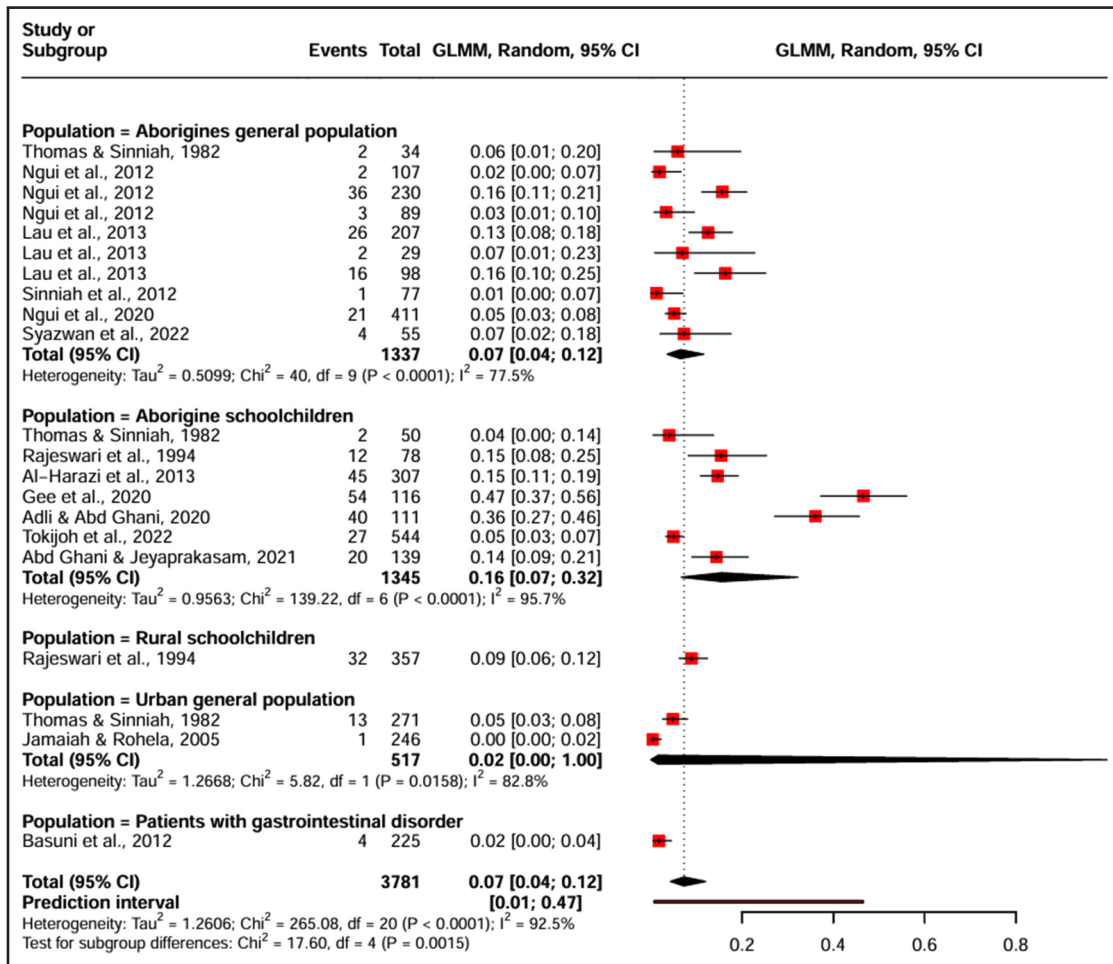


Figure 4. Forest plot of pooled prevalence of *E. histolytica* in various cohort in Malaysia using a random-effects model with 95% CI.

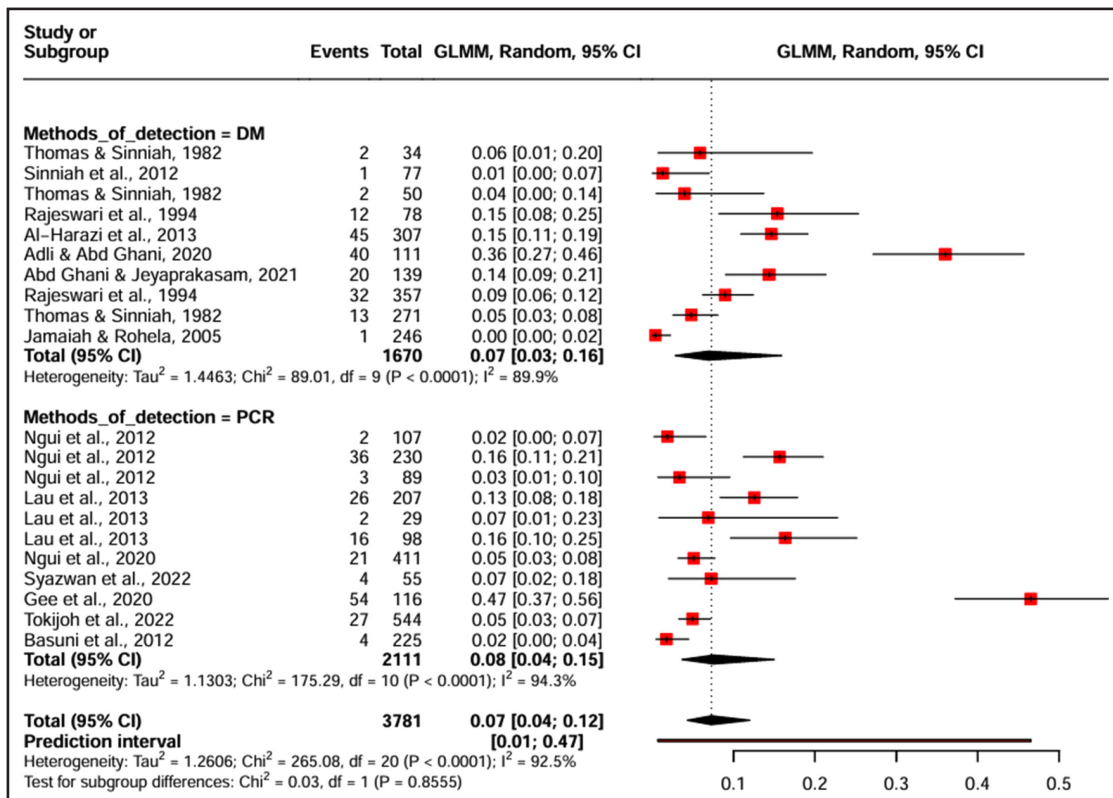


Figure 5. Forest plot of pooled prevalence of *E. histolytica* based on the method of diagnosis using a random-effects model with 95% CI.

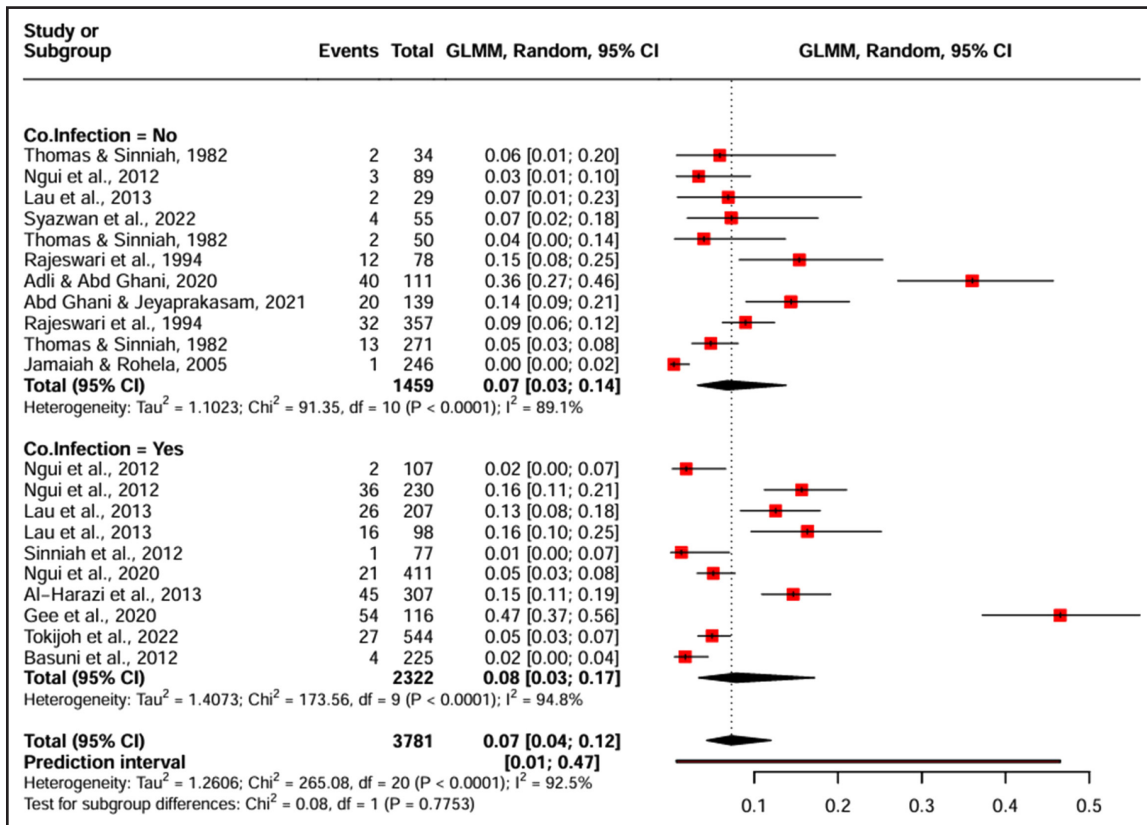


Figure 6. Forest plot of pooled prevalence of *E. histolytica* with the presence of co-infection using a random-effects model with 95% CI.

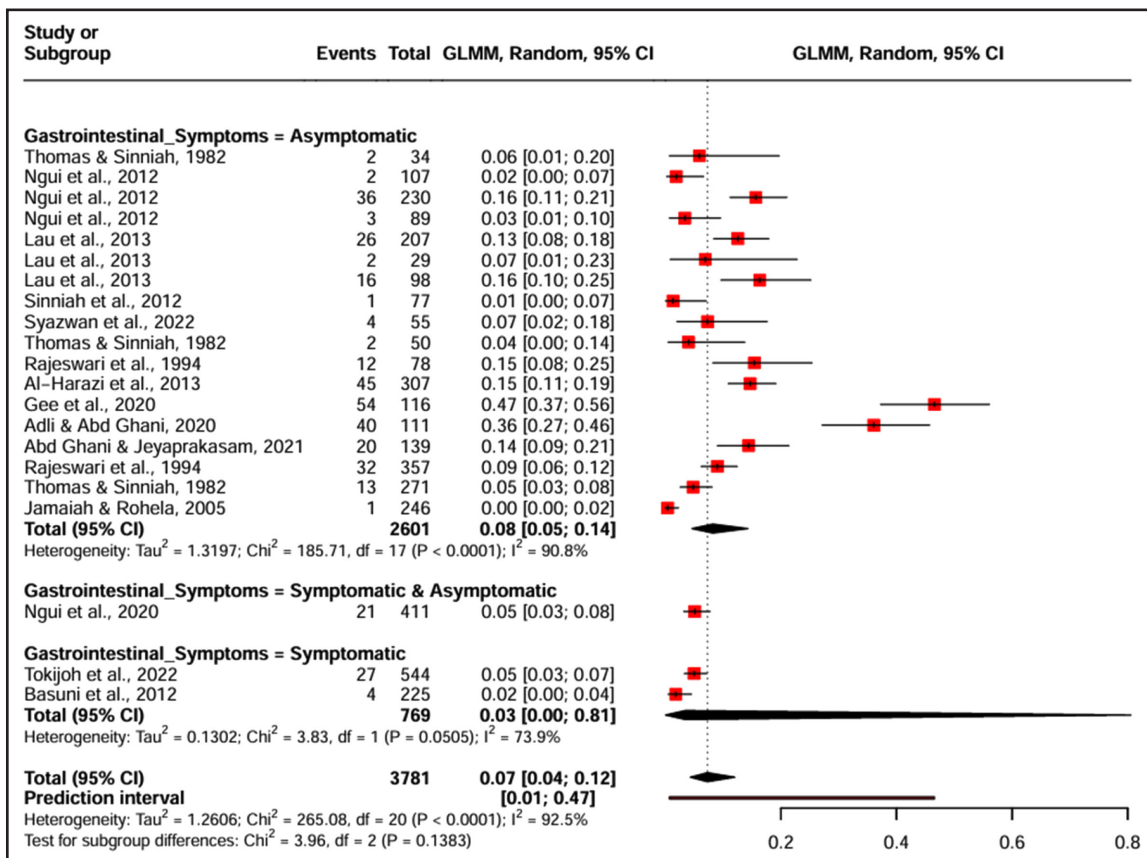


Figure 7. Forest plot of pooled prevalence of *E. histolytica* infection among symptomatic and asymptomatic individuals, using a random-effects model with 95% CI.

to uncertainty in true prevalence (Tokijoh *et al.*, 2022; Kawashima *et al.*, 2023; Chou & Austin, 2025). As a result, microscopy examination continues to be the primary diagnostic method in most community studies, which likely contributed to the heterogeneity observed in this study.

Geographical disparities were evident, with prevalence ranging from 18% in Pahang to 2% in Kelantan (Basuni *et al.*, 2012; Chin *et al.*, 2016). Higher prevalence was often associated with studies among indigenous communities, where reliance on untreated water sources, open defecation, and poor hand hygiene practices could have increased transmission risks (Chin *et al.*, 2016; Syazwan *et al.*, 2022). For example, a study carried out in Perak associated infections to gardening activities and direct soil contact (Syazwan *et al.*, 2022). In contrast, lower prevalence in urban populations (2%) likely reflects improved sanitation, piped water supply, and higher health literacy, though data were limited and mostly outdated (Thomas & Sinniah, 1982; Jamaiah & Rohela, 2005). Importantly, no eligible studies were identified from several states, including Terengganu, Kedah, Perlis, Melaka, Penang, Negeri Sembilan, Sabah, and Sarawak, highlighting significant gaps in surveillance.

When compared internationally, the prevalence in Malaysia (7%) is higher than reports from India (1.7%) (Parija & Khairnar, 2005), Nepal (0.7%) (Feng *et al.*, 2018), the Philippines (0.8%) (Rivera *et al.*, 2021), and Thailand (1.0%) (Suntaravitun & Dokmaikaw, 2018), but lower than Brazil (22%) (Dos *et al.*, 2021) and Ethiopia (15.2%) (Hailu *et al.*, 2020). Direct comparison should be interpreted with caution, however, given methodological differences and the limited number of studies in some regions.

Analysis by population group revealed clear disparities. Aboriginal schoolchildren showed the highest prevalence (16%), followed by the general aboriginal population (7%) and rural schoolchildren (9%) (Table 1). These findings are consistent with previous evidence that showed infection was shaped more by environmental and socioeconomic conditions than ethnicity itself (Kumarasamy *et al.*, 2023). Indigenous groups relying on untreated water and lacking sanitary facilities remain disproportionately affected (Thomas & Sinniah, 1982; Chin *et al.*, 2016; Sahimin *et al.*, 2024). By contrast, urban populations, where access to health services and public awareness is greater, exhibited the lowest prevalence (2%) (Thomas & Sinniah, 1982; Jamaiah & Rohela, 2005; Al-Areeqi *et al.*, 2017). Importantly, within these high-risk populations, children appeared to be particularly vulnerable, suggesting that age further modified exposure and susceptibility. Behavioral factors such as playing barefoot, hand-to-mouth activity, and inconsistent hygiene practices, combined with limited sanitation in schools, likely increased their risk of acquiring infection (Mesgarian *et al.*, 2017; Fauziah *et al.*, 2022; Syazwan *et al.*, 2022). Poor hand hygiene alone has been shown to nearly double the risk of parasitic infection in children (Ebrahimi *et al.*, 2007). Moreover, evidence from Dhaka indicated that children with *E. histolytica*-related diarrhoea were significantly more likely to be malnourished and stunted than non-infected children, highlighting the broader health impact of infection in this vulnerable group (Mondal *et al.*, 2006).

From the clinical perspective, most infections were asymptomatic. Globally, around 90% infected individuals remained without symptoms, with only 10% developing clinical disease (Walsh, 1986; Ravdin *et al.*, 1990; Ravdin, 1995). Our analysis reflected this pattern: the pooled prevalence of asymptomatic infection in Malaysia was 8%, compared to 3% for symptomatic cases (Figure 7) (Basuni *et al.*, 2012; Tokijoh *et al.*, 2022). Although often overlooked, asymptomatic carriers may have sustained transmission in the community and contributed to subclinical intestinal damage (Shirley *et al.*, 2018; Yanagawa *et al.*, 2021).

While most *E. histolytica* infections are limited to the intestine, the parasite sometimes caused invasive infection by disseminating the extraintestinal sites due to its potent virulence factors, including

cysteine proteases and amoebapores, which enable it to destroy host tissues and evade immune defenses (Begum *et al.*, 2015; Guillén, 2023). Amoebic liver abscess (ALA) represents the most prevalent extraintestinal infection, which are the leading form of invasive amebiasis and the most common liver abscess in tropical regions (Jindal *et al.*, 2021; Kumar *et al.*, 2024). In rare cases, the parasite may spread to the lungs or brain (He *et al.*, 2025). Although data from Malaysia are limited, extraintestinal amoebiasis contributes to clinical morbidity and highlights the importance of prompt diagnosis and treatment. Globally, 2–5% of patients with intestinal amebiasis progressed to liver abscess formation (Arellano-Aguilar *et al.*, 2017). However, in Malaysia, the prevalence of extraintestinal amoebiasis is remains unclear, as published data are scarce and suggest it is uncommon compared to intestinal infection (Tengku & Norhayati, 2011; Anuar *et al.*, 2013).

Treatment in Malaysia is guided by infection severity. Invasive infections, which may cause cramping, abdominal pain, diarrhea, weight loss, or extraintestinal symptoms such as liver abscess, are managed with metronidazole followed by a luminal agent such as paromomycin (Kantor *et al.*, 2018). Non-invasive intestinal infections, including mild or non-bloody diarrhea, are treated with nitazoxanide, an effective alternative (Marie & Petri, 2014). Asymptomatic cyst carriers remain a public health concern due to their role in transmission. Treatment with luminal agents such as paromomycin, iodoquinol, or diloxanide furoate may be considered to eliminate the carrier state (Blessmann & Tannich, 2002).

Finally, co-infection analysis showed little difference between studies reporting polyparasitism (8%) and those that did not (7%) (Figure 6), suggesting that concurrent infections did not substantially alter reported prevalence. This likely reflects the fact that most included studies were not designed to assess co-infections in depth, and microscopy-based diagnostics may have under-detected other species.

This study presents the first comprehensive systematic review and meta-analysis of the national prevalence of *E. histolytica* in Malaysia, drawing on data from diverse populations across multiple states and studies published up to 2025. The review focused on intestinal amoebiasis, as only a few studies address extraintestinal cases. Similarly, the review did not assess other *Entamoeba* species such as *E. dispar* and *E. moshkovskii*, primarily due to the limited use of molecular diagnostic techniques in the analyzed studies. As a result, the true distribution of *E. histolytica* in Malaysia may have been underestimated, not due to low prevalence, but because of diagnostic limitations and a lack of species-specific research. Current data reflect a broader trend in Malaysian research, which has shifted toward nematodes and *Blastocystis* sp. in intestinal parasitology. Therefore, the limited data on *E. histolytica* should be interpreted with caution, as it indicates gaps in surveillance and molecular investigation rather than the absence of the pathogen. Accurate species differentiation remains essential to define the disease burden and guide public health strategies.

Conflicts of Interest

The author declares that they have no conflict of interests.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2024/SKK10/UKM/02/14).

Availability of data and materials

The full data supporting this systematic review are available in the included studies in the references section. The analyzed data presented in this study are available in Supplementary Table S1.

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