

REVIEW

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Prevalence and determinants of zoonotic malaria in the Greater Mekong Subregion (GMS) and Malaysia: systematic review and meta-analysis

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Abstract

Background Zoonotic malaria is a growing public health concern in Southeast Asia, with Malaysia and Thailand accounting for 95.2% of the 3290 global cases reported in 2023. It is caused by *Plasmodium* species primarily adapted to long- and pig-tailed macaques, transmitted to humans via certain *Anopheles* mosquitoes. This meta-analysis quantifies its prevalence and risk factors in the Greater Mekong Subregion and Malaysia.

Methods This meta-analysis was conducted following the PRISMA (Preferred Reporting Items for Systematic) guidelines. A comprehensive literature search was conducted in PubMed and Scopus databases (2000–2024) to identify studies on zoonotic malaria infection in humans and monkeys. Backward search was done using Google Scholar. Inclusion criteria were defined using the CoCoPop (Condition, Context, and Population) framework. Two reviewers independently extracted data, and study quality was assessed using appropriate risk-of-bias tools. A random-effects meta-analysis was conducted using the *metafor* package in R programme, with heterogeneity assessed via I^2 statistics and subgroup analyses. Meta-regression using a linear mixed-effects models estimated unadjusted odds ratios for transmission determinants.

Results The overall pooled prevalence of malaria infection was 8.6% in humans and 35.0% in monkeys. The pooled prevalence was higher in Malaysia (22.8%) than GMS (1.2%). In GMS, Myanmar with a pooled prevalence of 4.9% contributes a higher burden of human zoonotic malaria than Thailand (1.8%). Significantly high heterogeneity between studies was recorded for both human ($I^2 = 99.7%$, $P < 0.0001$) and monkey ($I^2 = 98.7%$, $P < 0.0001$) infections. The major risk factors assessed were gender (males: OR = 4.3), age (adults 21–40 years: OR = 5.6), mobility, misdiagnosis, and *Plasmodium knowlesi* infection. Most cases (4773; 89.6%) were initially misdiagnosed by microscopy as non-zoonotic. Prevalence in monkeys was highest in *Macaca nemestrina* (63.3%) followed by *Macaca fascicularis* (31.4%) with *Plasmodium inui* and *Plasmodium cynomolgi* as the most frequent parasites.

Conclusions This study contributes to the understanding of the transmission complexities of zoonotic malaria in the GMS countries and Malaysia, highlighting critical knowledge gaps and the need for a multidisciplinary approach to managing its further spread.

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Keywords Pooled prevalence, Zoonotic malaria, Heterogeneity, Risk factors, *Plasmodium knowlesi*, *Plasmodium inui*, *Plasmodium cynomolgi*

Background

Zoonotic malaria, primarily caused by *Plasmodium knowlesi*, has emerged as a substantial public health challenge in Southeast Asia, where ecological and socio-environmental conditions support ongoing malaria transmission [1, 2]. Natural human infection with *P. knowlesi* was first diagnosed in 2004 in Kapit division of Malaysia [3] and Bangkok, Thailand [4] followed by Myanmar in 2006 [5], Cambodia in 2007 [6]

Vietnam in 2009 [7], and Lao People’s Democratic Republic (Laos) [8]. With the exception of China that was certified malaria-free in 2021, there has been a rapid and sustained decline in malaria cases and deaths across the Greater Mekong Subregion (GMS) in the last decade [2, 9, 10] (Fig. 1). While Thailand, Cambodia, and Vietnam aim for malaria elimination by 2025, Lao PDR and Myanmar target 2030 [2, 9, 11]. The impressive progress has been a result of robust 1–3–7

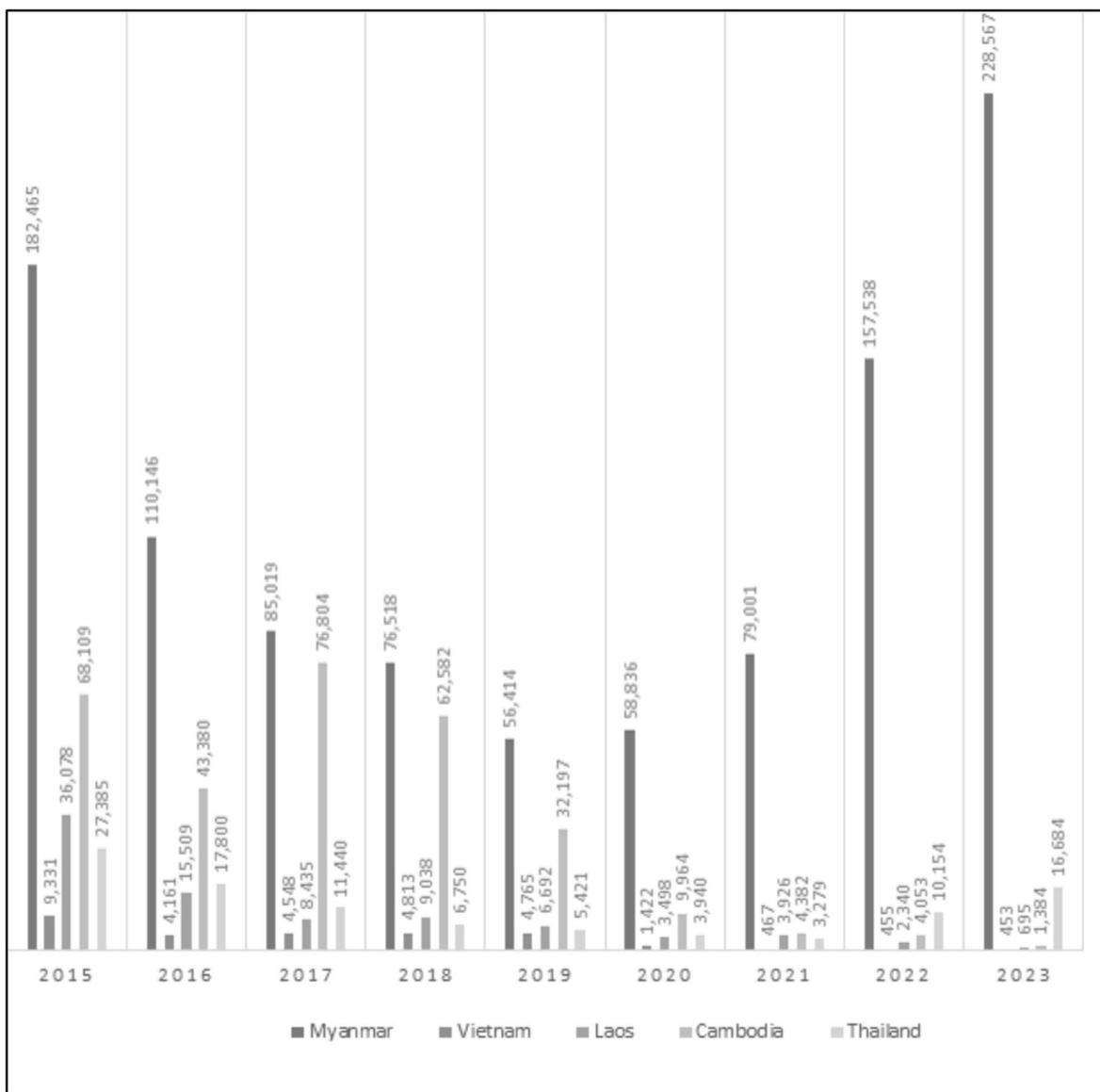


Fig. 1 Total confirmed malaria cases in the Greater Mekong Subregion (GMS), 2015–2023 (Data source: WHO Malaria report 2024)

surveillance and response activities, targeted or mass drug administration, vector control, containment of artemisinin-resistant falciparum malaria, community participation, political commitment, case management, and donor support. Despite significant regional progress, the ongoing political conflict in Myanmar has posed substantial challenges, including cross-border transmission with Thailand which underscores the complexity of addressing this issue effectively [2]. Compared to GMS, Malaysia serves as a blueprint for understanding the prevalence and transmission of zoonotic malaria in Southeast Asia. Although human malaria was eliminated by 2018, the continued rise in *P. knowlesi* cases has delayed Malaysia's eligibility for WHO malaria-free certification [12]. In 2023, all indigenous malaria deaths in Malaysia (n = 14) were attributed to *P. knowlesi*, while Cambodia (n = 11) and Thailand (n = 258) recorded a rise in the number of symptomatic cases [2]. These scenarios underscore the need to integrate zoonotic malaria into public health strategies, particularly by addressing the sustained transmission of *P. knowlesi* in areas where human–monkey interactions are common.

Zoonotic malaria results from the spillover of the malaria-causing pathogens from their wildlife reservoirs into human population. Studies have shown that monkeys act as reservoir hosts for zoonotic malaria parasites, with regions of high monkey prevalence overlapping with human infection hotspots [13, 14]. Recently, human infections with zoonotic malaria parasites, including *P. knowlesi*, *Plasmodium inui*, *Plasmodium cynomolgi*, *Plasmodium fieldi* and *Plasmodium coatneyi* have been reported [4, 15]. The genetic diversity and evolutionary patterns of zoonotic *Plasmodium* species suggest ongoing transmission between mosquito vectors, monkeys, and humans. Despite significant progress in controlling human malaria in GMS, zoonotic malaria persists due to its complex transmission dynamics involving monkeys as reservoirs and *Anopheles* mosquitoes as vectors. This complicates traditional malaria elimination efforts [16]. Variations in ecological, environmental, and socio-economic factors across the GMS further influence the transmission of zoonotic malaria [17]. Diagnosing zoonotic malaria is very challenging especially in areas where microscopy is the gold standard. According to the World Health Organization (WHO), *P. knowlesi* malaria must be confirmed by molecular techniques with a preference for polymerase chain reaction (PCR) [2]. This testing system requires expertise and equipment, not available in most malaria areas in Southeast Asia. In addition, malaria drug resistance is a worrying factor to the eliminations of malaria [18, 19], which must be clearly understood in regards to zoonotic malaria transmission.

Research in zoonotic malaria is relatively new, existing studies are fragmented, with inconsistent methodologies and limited regional synthesis.

To inform effective interventions and policy frameworks, a comprehensive understanding of the prevalence and determinants driving zoonotic malaria transmission in the GMS is essential. This necessitates a systematic meta-analysis to consolidate evidence, identify key determinants, and highlight knowledge gaps to guide targeted control strategies and research priorities. This study collated data specific to GMS and Malaysia and analysed the significant factors that are accelerating the transmission of zoonotic malaria. First, data showing the prevalence of human zoonotic malaria in the GMS and Malaysia were consolidated. This was followed by analysis of factors from the literature published from 2000 to 2024. The methods used to assess and incriminate (or failure to do so) these factors were scrutinized to reveal the gaps in research. Vectors of zoonotic malaria are presented in a separate review (in preparation); the present analysis focuses on zoonotic *Plasmodium* species infections in humans and wildlife reservoirs.

Methods

Systematic search strategy and protocol registration

The study protocol was registered as CRD42025643674 in the International Prospective Register of Systematic Reviews (PROSPERO). A systematic search following PRISMA guidelines [20] was conducted to identify relevant studies on zoonotic malaria in the GMS countries and Malaysia from 2000 to 2024. The search was performed in electronic databases PubMed and Scopus during August and October 2024. The keywords such as simian malaria, *Plasmodium knowlesi*, zoonotic malaria were all combined using boolean operators (“OR” and “AND”) with geographical scale of reference e.g. Thailand, the Greater Mekong Subregion, and Malaysia. Backward search was conducted in Google scholar and Google search. Search results were exported in Research Information Systems (RIS) format and managed using EndNote 21, with automated and manual deduplication. Full-text articles were retrieved for the selected studies, and screened based on predefined inclusion and exclusion criteria.

Inclusion and exclusion criteria

This systematic review and meta-analysis followed the CoCoPop framework (Condition, Co=Infection with any of the five zoonotic *Plasmodium*, Context, Co=geographical scope i.e. GMS and Malaysia, human socio-demographic characteristics and environment; Population, Pop=Humans and monkeys) [21]. The inclusion criteria were: (1) observational studies

(cross-sectional, case–control, cohort) conducted in the GMS countries or Malaysia with PCR-confirmed zoonotic malaria infections in humans and monkeys; (2) studies among all age-groups and gender of human populations or captive and free wildlife monkeys; (3) English language articles published in peer-reviewed journals not limited by year of study. However, case reports and laboratory studies on the biology of zoonotic malaria *Plasmodium* spp. and therapeutic drug efficacy, vector infectivity studies, including studies conducted out of the defined context and those that did not report zoonotic *Plasmodium* parasites, were excluded. Any disagreements were resolved through consensus or consultations amongst the reviewers.

Data extraction and quality assessment

Data extraction was performed using a standardized form designed for this review. The extracted data included: (1) Study characteristics or prevalence data: authors, publication year, study design, *Plasmodium* parasites prevalence, human hosts and macaque reservoirs; (2) *Plasmodium* parasites: species and method of detection; (3) Human hosts: demographic characteristics and evidence of zoonotic malaria infection; (4) Monkey reservoirs: Geographical distribution and infection with *Plasmodium* parasites.

The methodological quality of included studies was assessed using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist (Additional File 1: Figure S1) for studies reporting prevalence data. Each study was evaluated based on key domains such as selection bias, measurement bias, confounding control, and statistical appropriateness. Studies scoring $\geq 70\%$ were classified as high quality and included in the analysis. Studies scoring 50–69% were considered moderate quality and included with caution. Studies were excluded if they scored $< 50\%$, indicating high risk of bias. Disagreements in quality assessment were resolved by discussion among reviewers [22].

Outcome measurements

The primary outcome of this systematic review and meta-analysis is the pooled proportions (overall prevalence) of zoonotic malaria in humans and monkeys residing in GMS and Malaysia. The overall prevalence is calculated as a weighted average of individual study proportions [23] as follows:

$$P_t = \left(\sum w_i P_i \right) / \left(\sum w_i \right)$$

where: P_i = proportion (prevalence) in study i ; w_i = weight for study i

$$w_i = 1 / (v_i + \tau^2)$$

where: v_i = variance of P_i ; τ^2 = between-study variance (heterogeneity)

$$v_i = [P_i(1 - P_i)] / n_i$$

where: n_i = sample size for study i .

Subgroup meta-analysis was performed for country prevalence, regional prevalence, sample size, and year of publication. As well, subgroup meta-analysis on monkey studies was done for habitats.

The second outcome was determinants of zoonotic malaria among different age groups, gender, activities engaged in before infection (such as agriculture, travel, forest works), parasite species, and misdiagnosis of human zoonotic malaria, which were estimated by the pooled odds ratio with a 95% confidence interval using a random effects meta-analysis.

Statistical analysis

All statistical analyses were conducted in RStudio (version 2024.09.1 + 394) [24]. This meta-analysis utilized the random effects models to calculate prevalence [22]. Pooled prevalence estimates and their corresponding 95% confidence intervals (CIs) were calculated using the *metaprop* function from the *metafor* package [25], and results were visually presented using forest plots as percentage proportions. Subgroup meta-analysis was visually presented with bar plots using the *ggplot2* package [26] by extracting subgroup prevalence values from the forest plots.

Heterogeneity among studies was assessed using the I^2 statistic, which quantifies the percentage of variability in effect estimates that is due to heterogeneity rather than chance. Higher I^2 values indicate greater heterogeneity across studies [27].

Publication bias was assessed through funnel plots, further evaluated using Egger's test [28]. To assess the robustness of the findings, a sensitivity analysis was performed by systematically excluding studies one at a time.

A meta-regression analysis was conducted to evaluate the association between different variables (*Plasmodium* species, age, monkey interaction, sex, mobility, and misdiagnosis) with species and zoonotic malaria prevalence. Prior to model fitting, multicollinearity among the variables was assessed using Variance Inflation Factors (VIF), with a threshold of 10. Studies lacking sufficient data on individual covariates were excluded from the respective analyses, but no covariate was removed entirely from the model due to missingness. The odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using a mixed-effects model and calculated using the *rma* function [25].

Results

Literature review and publication characteristics

A total of 2088 studies were retrieved from the electronic databases. Only 57 articles were included based on the predefined inclusion and exclusion criteria. The final set included 47 peer-reviewed articles, 5 dispatches, 4 reports, and 1 symposium abstract. All included articles were evaluated in the meta-analysis and data synthesis (Fig. 2, Additional File 2).

This study reviewed the prevalence of zoonotic malaria in both monkeys and humans. Out of the 57 articles, 42 studies [3, 6–8, 29–66], reported human infections, 11

studies reported on monkey infections [67–77], and 4 studies reported on both [78–81] (Table 1, Additional File 2: Table S1). Among the studies reporting human zoonotic malaria, 29 were from Malaysia (Peninsular Malaysia: n=5; East Malaysia: n=21, both: n=3) and 17 from the GMS (Thailand: n=8; Cambodia: n=3; Vietnam: n=2, Myanmar: n=2; Laos: n=1, and Laos/Vietnam: n=1). The data collection years of the included studies range from 1996 [29, 56] to 2021 [43, 78] (Additional File 2: Table S1). A total of 8392 (27.97%) zoonotic malaria human cases from a population sample of 79,090 were confirmed by PCR from 46 studies. *Plasmodium*

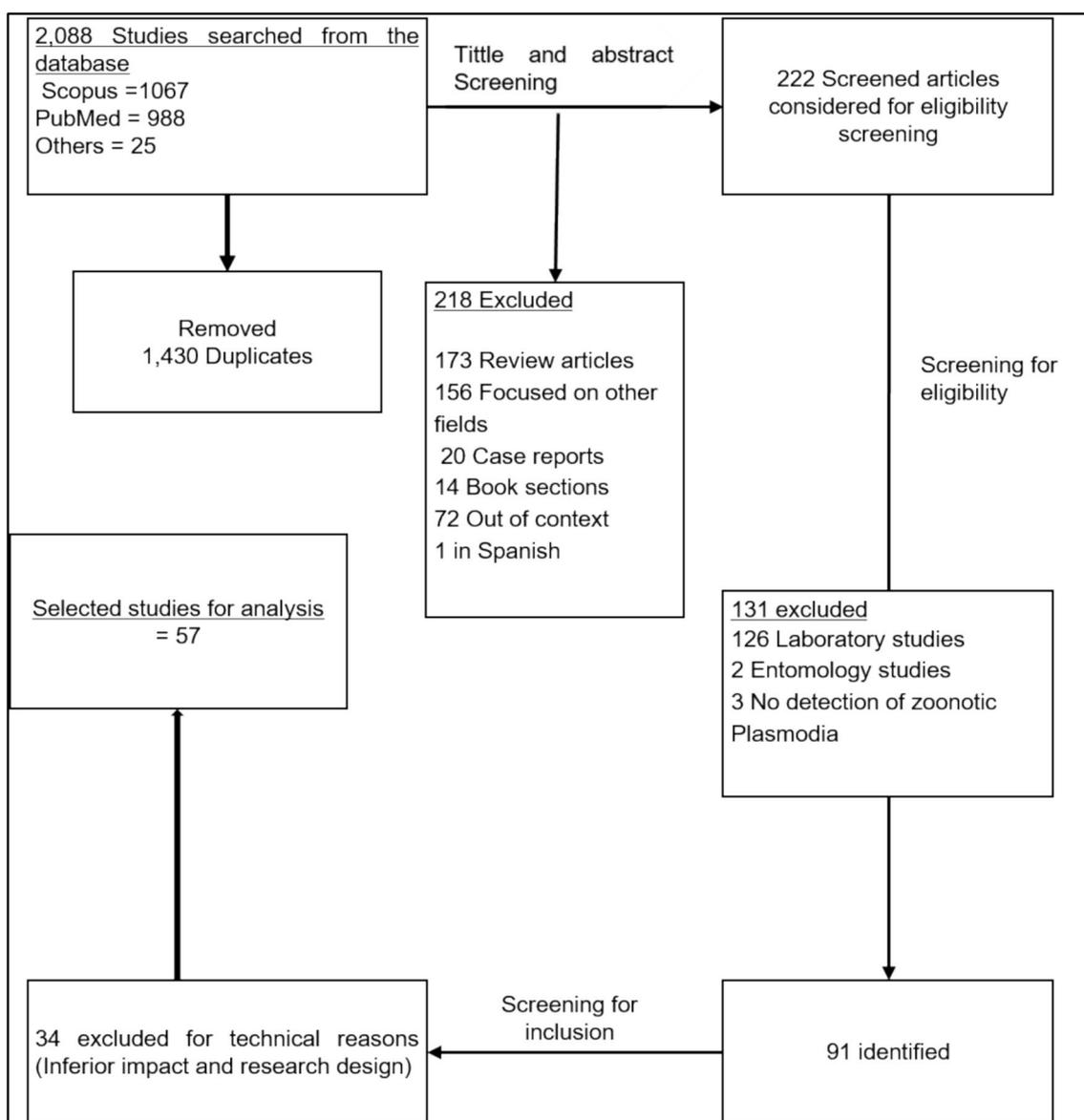


Fig. 2 The PRISMA flowchart for the selection of the included studies. An independent reviewer and two authors (AA and JH) screened the titles, abstracts. Full texts were screened by AA while JH reviewed the included articles

Table 1 Characteristics of the studies included in the review for zoonotic *Plasmodium* spp. detected in humans

No.	Study design	Country	Province	Cases	Total	Year (s) of study	Plasmodium species		Author
							Pk	Others	
1	Retrospective	Thailand	Songkhla	24	3980	2008–09	24	na	[29]
2	Cross-sectional	Thailand	Tak, Chanthaburi, Yala, and Narathiwat	2	419	2008	2	na	[32]
3	Cross-sectional	Thailand	Ranong	15	15	2021	15	na	[78]
4	Prospective	Thailand	Chanthaburi, Tak, Narathiwat, Yala, and Prachuap Khiri Khan	10	1874	2006–07	10	na	[30]
5	Prospective	Thailand	Tak, Chanthaburi, Ubon Ratchathani, Yala, and Narathiwat	68	5271	2007–11; 2016	30	Pcy (21), Pct (0), Pin (19), Pflid (3)	[79]
6	Prospective	Thailand	Tak, Chanthaburi, Ubon Ratchathani, Yala, and Narathiwat	27	1359	2007–18	18	9	[31]
7	Cross-sectional	Thailand	Trat, Prachuap Khiri Khan, Surin, Chanthaburi, Chumphon, Surat Thani, and Phang-nga	31	966	2018–20	31	na	[35]
8	Cross-sectional	Thailand	Surat Thani	3	9418	2019	3	na	[34]
9	Cross-sectional	Laos and Vietnam	Savannakhet and Quang Tri	12	3059	2010	12	na	[66]
10	Prospective	Laos	Attapeu	1	2698	2016	1	na	[8]
11	Cross-sectional	Vietnam	Ninh Thuan	3	95	2004	3	na	[7]
12	Prospective	Vietnam	Khan Hoa	32	305	2009–10	32	na	[33]
13	Cross-sectional	Myanmar	Bago	16	1638	2013	16	na	[36]
14	Cross-sectional	Myanmar		32	146	2008	32	na	[38]
15	Cross-sectional	Cambodia	Pailin and Battambang	21	4573	2013–16	8	Pcy (13)	[37]
16	Cross-sectional	Cambodia	Pailin and Kratié	2	1475	2010	2	0	[6]
17	Retrospective	Cambodia	Battambang	6	966	2021–22	34	na	[39]
18	Cross-sectional	Malaysia	Sabah	266	744	2020	6	na	[43]
19	Retrospective	Malaysia	Sabah	191	220	2009	191	na	[42]
20	Prospective	Malaysia	Sabah	130	295	2010–11	130	na	[41]
21	Prospective	Malaysia	Sabah	392	18,993	2009–11	392	na	[40]
22	Case–control	Malaysia	Sabah	3263	3867	2020–21	266	na	[43]
23	Retrospective	Malaysia	Sarawak	122	188	2015–17	3572	na	[44]
24	Prospective	Malaysia	Kelantan	7	1954	2006–08	107	na	[45]
25	Cross-sectional	Malaysia	Sabah	20	1147	2019	7	na	[46]
26	Cross-sectional	Malaysia	Sabah	4	1957	2014–15	4	na	[48]
27	Retrospective	Malaysia	Sabah	42	189	2008–11	42	na	[49]
28	Case–Control	Malaysia	Sabah	229	1367	2012–15	229	na	[50]
29	Cross-sectional	Malaysia	Sabah	5	876	2015	3	Pcy (2)	[51]
30	Cross-sectional	Malaysia	Johor, Pahang, Kelantan, Melaka N.Sembilan, Perak, Pulau Pinang, Selangor, Kuala Lumpur, Terengganu	164	200	2008–15	164	na	[80]
31	Cross-sectional	Malaysia	Sarawak	12	1995	na	36	na	[52]
32	Cross-sectional	Malaysia	Sarawak	23	306	2013–14	12	na	[54]
33	Prospective	Malaysia	Sabah	65	243	2010	65	na	[55]
34	Prospective	Malaysia	Pulau Pinang, Kuala Lumpur, Pahang, Terengganu, Sarawak and Sabah	57	112	2015–16	57	na	[55]
35	Retrospective	Malaysia	Sarawak	36	47	1996	35	na	[56]
36	Prospective	Malaysia	Sabah	154	207	2012–13	154	na	[57]

Table 1 (continued)

No.	Study design	Country	Province	Cases	Total	Year (s) of study	Plasmodium species		Author
							Pk	Others	
37	Cross-sectional	Malaysia	Sarawak	845	1047	2013–17	845	Pcy (6)	[65]
38	Cross-sectional	Malaysia	Johor, Pahang, Kelantan, and Selangor	10	585	2019–20	10	na	[58]
39	Retrospective	Malaysia	Johor	189	516	2011–19	189	na	[59]
40	Cross-sectional	Malaysia	Sarwak	7	555	2014	7	0	[60]
41	Cross-sectional	Malaysia	Sarawak	120	208	2000–02	120	0	[3]
42	Retrospective	Malaysia	Sabah	1018	1366	2011–13	1018	na	[61]
43	Cross-sectional	Malaysia	Pahang, Kedah, Kelantan, Selanger, Malacca, Perak, Pulau Penang	77	111	2005–08	77	0	[81]
44	Retrospective	Malaysia	Pahang, Kelantan, Selangor, Perak, Negeri Sembilan, Melaka, and Sarawak	55	645	2011–14	40	Pcy (9), Pct (3), Pin (3)	[62]
45	Cross-sectional	Malaysia	Sarawak	317	436	2018–19	317	0	[63]
46	Cross-sectional	Malaysia	Pahang, Kelantan, Selangor, Negeri Sembilan, Melaka, Sabah, Johor, Kedah, Terengganu and Sarawak	267	457	2012–13	267	na	[64]

Pcy, *Plasmodium cynomolgi*; Pk, *P. knowlesi*; Pct, *P. coatneyi*; Pin, *P. inui*; Pfld, *P. fieldi*; Na, not assessed in that study

knowlesi infections (n=8304) were observed in all the 46 studies, while only six studies reported other zoonotic *Plasmodium* spp. infections (n=88) (Table 1). Among the studies reporting on monkeys (n=15), eight were conducted in Malaysia (East Malaysia: n=2, Peninsular Malaysia: n=5; both: n=1) and seven were conducted in GMS (Thailand: n=5, Thailand/Vietnam/Myanmar: n=1; Cambodia/Laos: n=1), as shown in Table 2. A total of 1695 infected monkeys (out of 5061) were reported in 15 studies. All studies on monkeys were conducted within the year range of 2003 [72] to 2022 [75]. *Macaca fascicularis* (n=863 out of 3276) was the most infected macaque species observed in 13 studies, followed by *Macaca nemestrina* (n=113 out of 508) observed in four studies and *Macaca leonina* (n=5 out of 25), *Macaca arctoides* (n=16 out of 32), and *Semnopithecus obscurus* (n=1 out of 7) with an occurrence in 1 study each. Two studies [79, 81] did not specify the infected monkey species but reported overall 697 infections out of 1160 samples (Additional File 2: Table S2).

Prevalence of zoonotic malaria among humans in GMS and Malaysia

A total of 46 studies were included in the meta-analysis for estimating the prevalence of zoonotic malaria in humans across the GMS and Malaysia. The individual study prevalence estimates ranged from 0.032 to 100%. Fourteen studies reported high prevalence estimates (50–100%) among which one study reported more than 87%. The remaining studies (n=32) reported prevalence

rates below 50% (Fig. 3). The pooled prevalence, obtained using the random-effects model, was estimated at 8.46% (95% CI 3.66–18.35%). The forest plot illustrates the variation in the estimates for individual studies, with wider confidence intervals in studies with smaller sample sizes. Heterogeneity was substantial ($I^2=99.7\%$, $p<0.001$), suggesting considerable variability among studies. The prediction interval ranged from 0.022 to 97.51%, indicating potential prevalence variability in future studies.

Publication bias was assessed using Egger's regression test (Table 3) and the funnel plot (Fig. 4) which showed asymmetry, suggesting potential small-study effects or publication bias. Egger's test statistic showed a regression intercept of -10.73 (95% CI -18.68 to -2.77) and a significant p -value of 0.011, indicating no statistically significant effect from small studies. To further assess the impact of bias, a trim-and-fill analysis was performed, which adjusted the pooled prevalence of malaria to 33.06% (95% CI 14.83–58.35), suggesting that publication bias may have slightly influenced the overall estimate. Despite the observed heterogeneity, no significant outliers were detected, and all studies overlapped within the pooled estimate's confidence interval. This suggests that, while prevalence varies across studies, the overall estimate is a reliable summary measure.

Subgroup meta-analysis on prevalence of zoonotic malaria in humans

Subgroup analysis was conducted to explore potential sources of heterogeneity in prevalence estimates,

Table 2 Characteristics of the studies included in the review for zoonotic *Plasmodium* spp. detected in monkeys from GMS and Malaysia

No.	Country	Province	M. fascicularis		M. nemestrina		Others		Year (s) of study				Plasmodium species				Author		
			n	N	n	N	n	N	Pk	Pcy	Pct	Pin	Pfld	Pk	Pcy	Pct		Pin	Pfld
1	Thailand	Songkhla	40	580	0	0	0	0	0	2018–21	2	6	0	32	0	[78]			
2	Thailand	Pattalung, Pattani, Yala and Narathiwat	10	195	72	449	1	7	2008–09	6	6	5	69	1	[73]				
3	Thailand	Tak, Chanthaburi, Ubon Ratchathani, Yala, and Narathiwat	687	1015	0	0	0	0	2007–09	97	172	91	236	91	[79]				
4	Thailand	Ranong and Prachuap Khiri Khan	14	105	0	0	0	0	2006	0	0	1	11	0	[74]				
5	Thailand	Chachoengsao, Prachuap Khiri Khan, Nakhon Ratchasima and Ranong	6	36	0	0	21	57	2017–19	1	8	5	14	8	[69]				
6	Thailand/Myanmar/Vietnam	Magway, Ca Mau, Bueng Khan, Nakhon Phanom, Kanchanaburi, Ratchaburi, Nakhon Sri Thammarat, Phang-nga, Phatthalung, Saraburi, Chainat, Nakhon Sawan, and Pichit	98	690	0	0	0	82	–	0	45	16	38	0	[70]				
7	Cambodia/Laos	–	70	98	0	0	0	0	2011–13	1	55	17	12	2	[77]				
8	Malaysia	Selangor	35	70	0	0	0	0	2014	21	18	16	23	1	[67]				
9	Malaysia	Pahang, Perak, and Johor	59	98	5	5	0	0	2016	11	42	14	42	4	[68]				
10	Malaysia	Selangor, Kuala Lumpur, Kedah, Pahang, Terengganu, Kelantan and Pinang	48	415	0	0	0	0	2008–15	48	–	–	–	–	[80]				
11	Malaysia	Sabah	80	82	21	26	0	0	2004–08	84	61	71	89	4	[71]				
12	Malaysia	Sarawak	23	45	15	28	0	0	2003–12	11	14	17	25	0	[72]				
13	Malaysia	Johor, Selangor, Pahang, Melaka, Kelantan, Kedah, Perak, Perlis, Negeri Sembilan, Terengganu, Putrajaya, Kuala Lumpur	204	410	0	0	0	0	2019–22	119	128	127	152	135	[75]				
14	Malaysia	Kedah, Kelantan, Terengganu, Pahang, Selangor, Sabah, Putrajaya, and Kuala Lumpur	176	419	0	0	0	0	2016–19	68	116	67	32	33	[76]				
15	Malaysia	Kuala Lumpur, Selangor and Pahang	10	145	0	0	0	0	2007	10	–	–	–	–	[81]				

Pcy, *P. cynomolgi*; Pk, *P. knowlesi*; Pct, *P. coatneyi*; Pin, *P. inui*; Pfld, *P. fieldi*

N total number of monkeys in the study; n total number of the infected

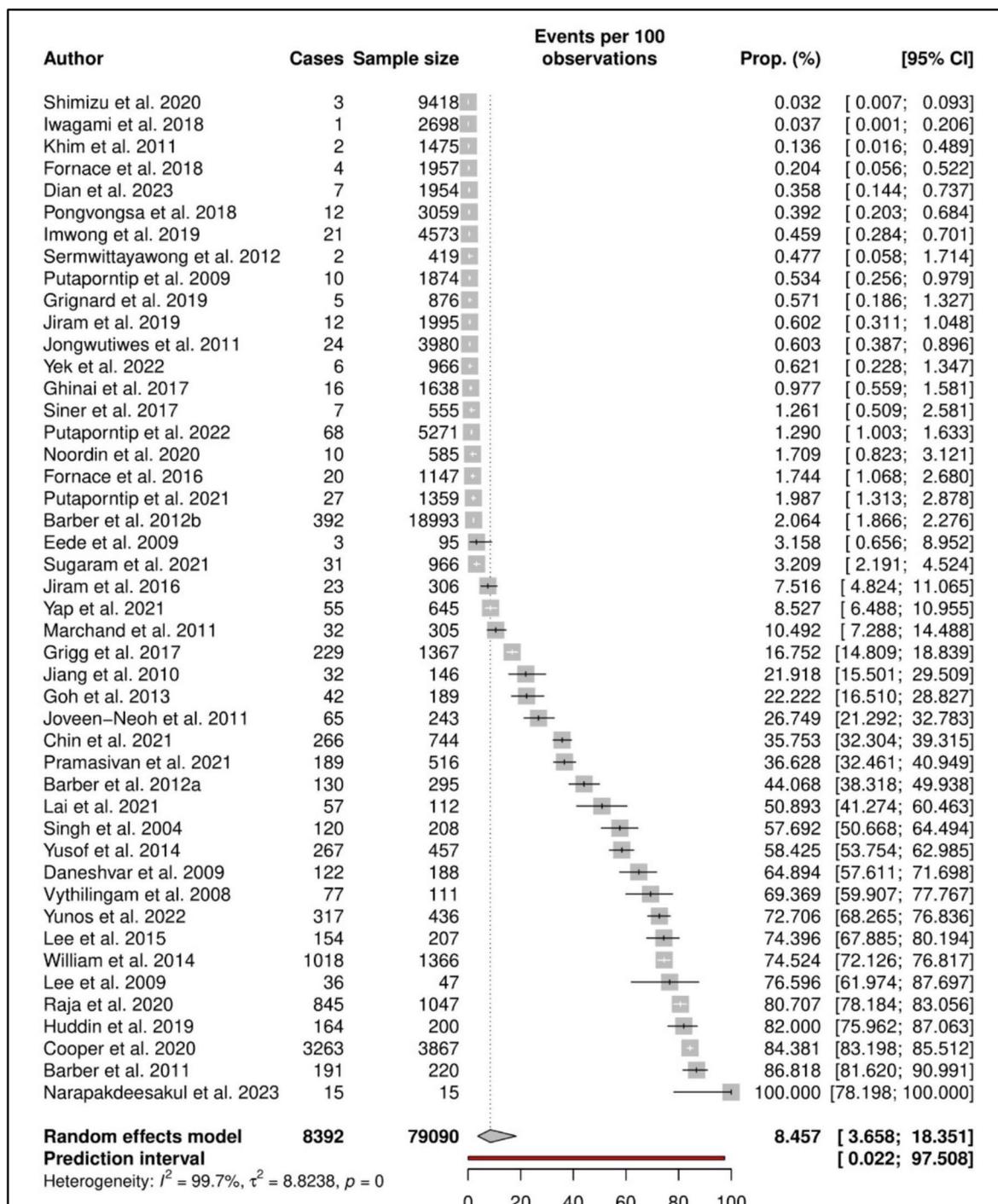


Fig. 3 Forest plot of prevalence estimates 46 studies reporting zoonotic malaria in humans across the Greater Mekong Subregion (GMS) and Malaysia (MYS)

stratified by GMS *versus* Malaysia and among countries within GMS (Fig. 5), and between regions within Malaysia (Fig. 6), sample sizes (Fig. 7a), and publication period (Fig. 7b). The pooled prevalence varied across

subgroups (Table 4). With the exception of studies conducted in Cambodia ($I^2 = 43.2$, $p = 0.1717$) and studies conducted in Malaysia during 2000–2009 ($I^2 = 63.2$, $p = 0.0430$), the analysis showed that within group

Table 3 Egger’s test on the prevalence of zoonotic malaria in humans and monkeys from the Greater Mekong Subregion and Malaysia

Egger’s test						
Population	Intercept	df	SE	t	p> t	95% CI
Human	-10.73	44	4.06	-2.64	0.011	-18.68--2.77
Monkey	-4.84	13	5.14	-0.94	0.364	-14.94--5.24

df degrees of freedom; SE standard error, t Egger’s test statistic

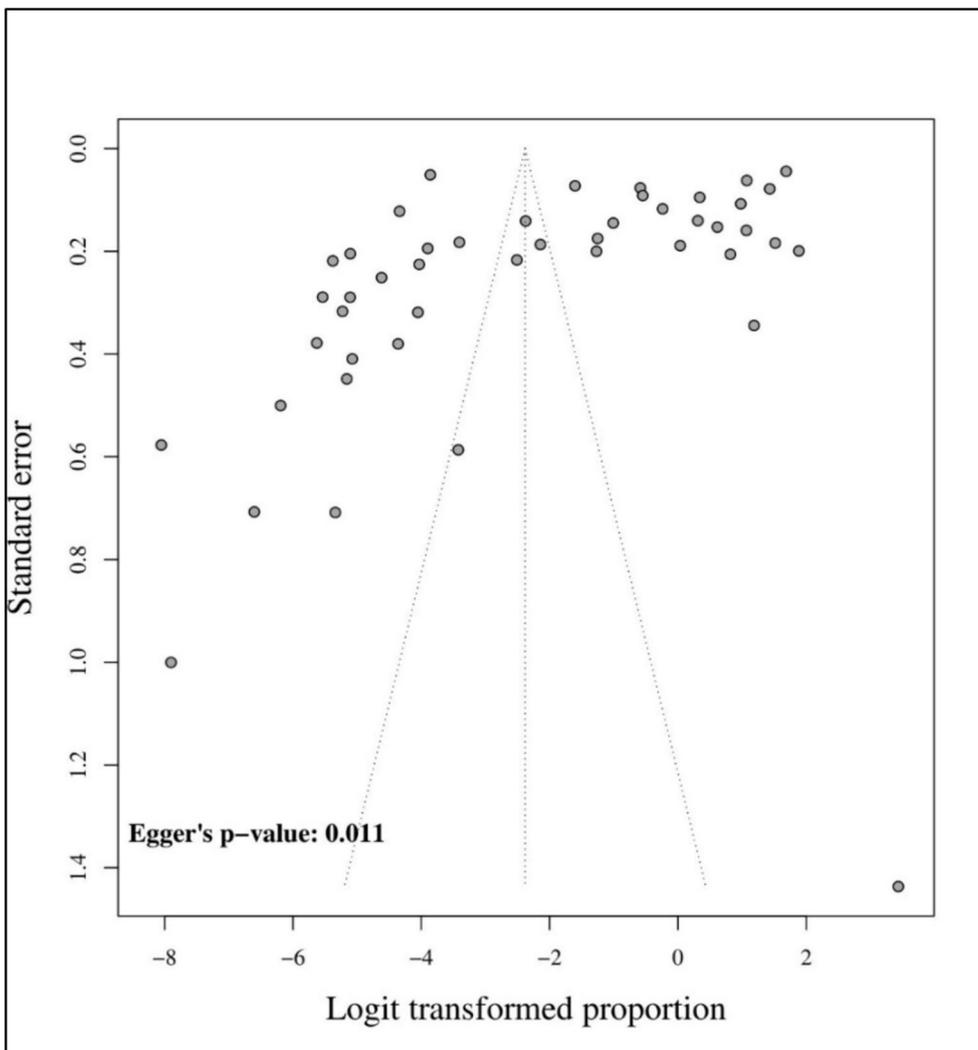


Fig. 4 Funnel plot assessing publication bias indicating Egger’s p-value

heterogeneity was significantly high suggesting that samples from the same geographical locations with similar sample size may contribute to prevalence variability. Among geographic locations, mixed results were observed. There was significant difference between

groups by region (GMS vs. Malaysia) ($p < 0.0001$), however, subgroup analysis for countries within the GMS region, and subregions of Malaysia were not significantly different. These results suggest that different geographic locations may have no effect on the prevalence variability.

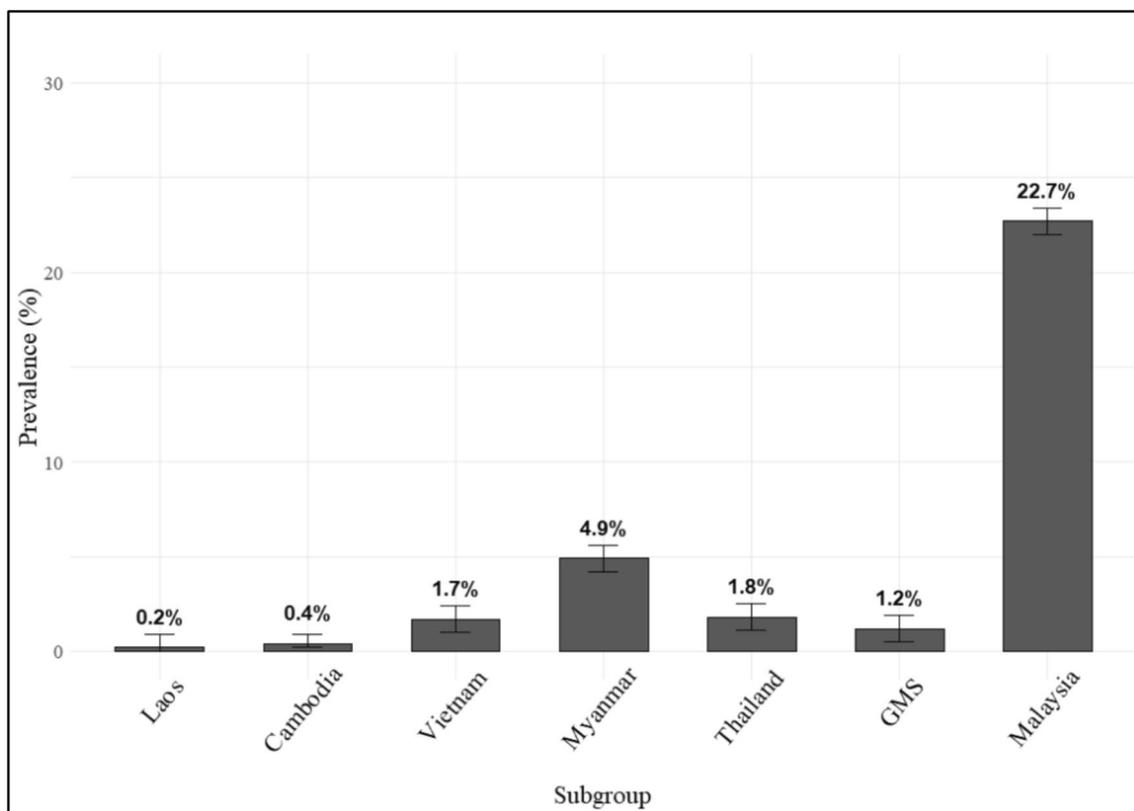


Fig. 5 Subgroup meta-analysis showing the prevalence of zoonotic malaria in the Greater Mekong Subregion (GMS) and Malaysia. The error bars indicate the confidence intervals, while the values on each bar are pooled prevalence for each

The determinants of zoonotic malaria transmission in humans

The factors associated with zoonotic malaria transmission in GMS and Malaysia are presented in Table 5. A total of 46 studies were included for parasite-level analysis since all studies reported at least one uniform parasite. *Plasmodium knowlesi* was significantly associated with a higher prevalence of zoonotic malaria (OR=24.09, 95% CI 3.135–185.0, $p=0.0022$), indicating that infections caused by this species were 24 times more likely compared to the other zoonotic *Plasmodium* species. The high OR and wide confidence interval suggest both a strong association and some variability among studies. Misdiagnosis of *Plasmodium* species was assessed in a subset of 22 studies. Prevalence due to misdiagnosis of zoonotic malaria was different depending on the *Plasmodium* species it was anticipated to be. Prevalence was only statistically significantly low when negative cases were analysed (OR=0.047, 95% CI 0.010–0.227, $p=0.0001$). Misdiagnosis as *P. falciparum* (reference) compared to *Plasmodium vivax* or *Plasmodium malariae* did not differ significantly in their impact on prevalence of zoonotic malaria. The results suggest that certain

misidentifications could contribute to over- or underestimation of zoonotic malaria prevalence. Individuals under 60 years of age had significantly higher odds of infection than those over 60, with the 21–40 age group showing the strongest association (OR=5.61, 95% CI 1.202–26.20, $p=0.0282$). Among factors related to mobility, no significant differences were observed. However, the prevalence was 3.5 times higher in cases that had moved to forests and 1.1 higher in government personnel compared to those doing agriculture activities. The results indicate that males had significantly higher odds of infection compared to females (OR=4.297, 95% CI 1.095–16.86, $p=0.0366$). This suggests that males were approximately 4.3 times more likely to be infected with zoonotic malaria than females.

Prevalence of zoonotic malaria in monkeys

The meta-analysis was conducted on 15 studies estimating the prevalence of zoonotic malaria in monkeys across the GMS and Malaysia where individual study prevalence estimates ranged from 6.9 to 93.5%. The pooled prevalence, obtained using the random-effects model, was estimated at 34.77% (95% CI 19.12–54.59). Heterogeneity

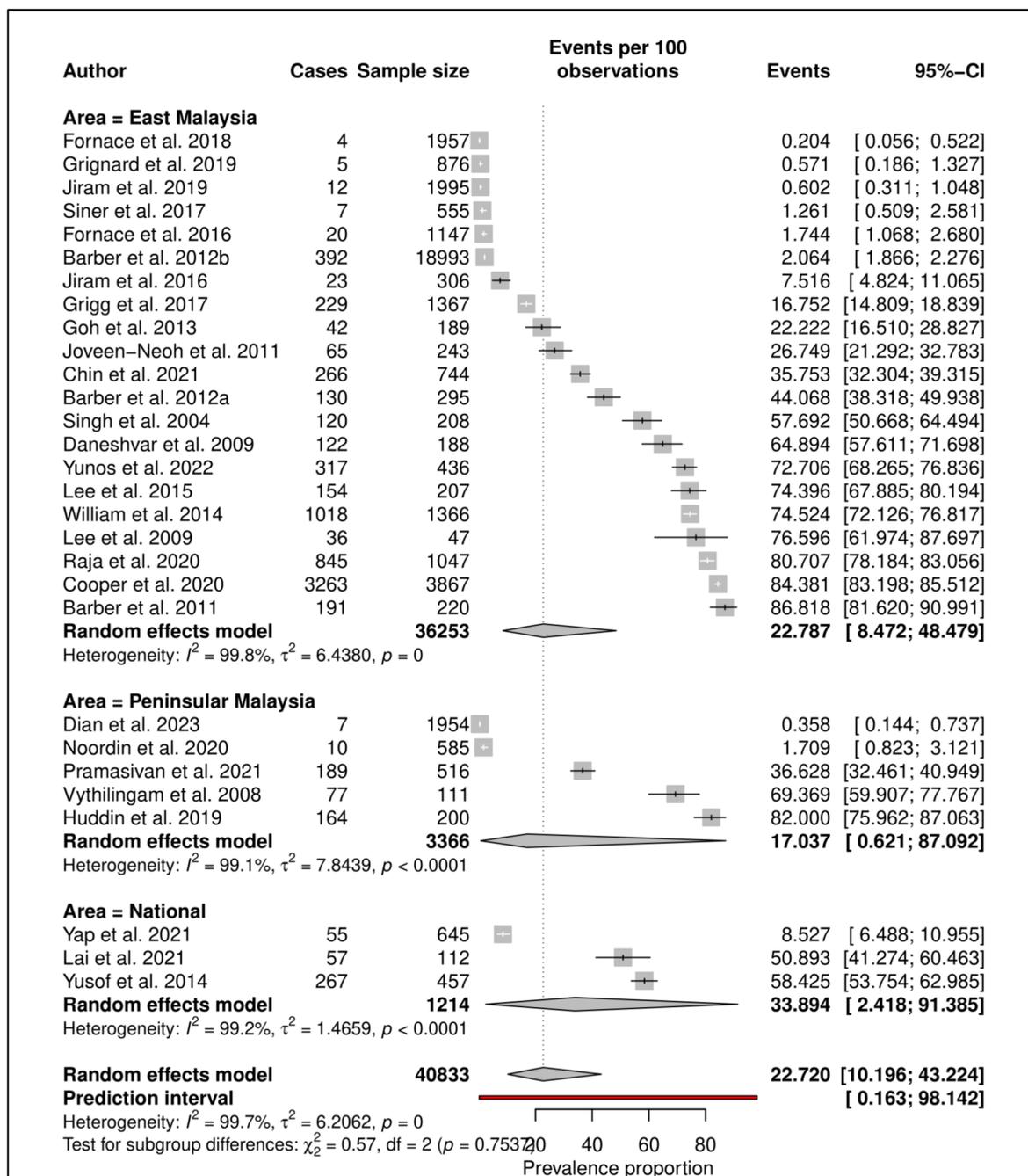


Fig. 6 Subgroup meta-analysis showing the prevalence of zoonotic malaria in humans in Malaysia

was significantly high ($I^2 = 98.8\%$, $p < 0.0001$), suggesting considerable variability among studies. The prediction interval ranged from 2.10 to 92.80%, indicating potential prevalence variability in future studies (Fig. 8).

Publication bias was assessed using a funnel plot (Fig. 9) and Egger’s regression test (Table 3). The funnel

plot showed asymmetry, suggesting potential small-study effects or publication bias. Egger’s test for publication bias showed a regression intercept of -4.84 (95% CI -14.94 to -5.24 , $p = 0.364$), indicating no publication bias. This suggests that, while the studies showed significant heterogeneity, the overall estimate is a reliable summary measure.

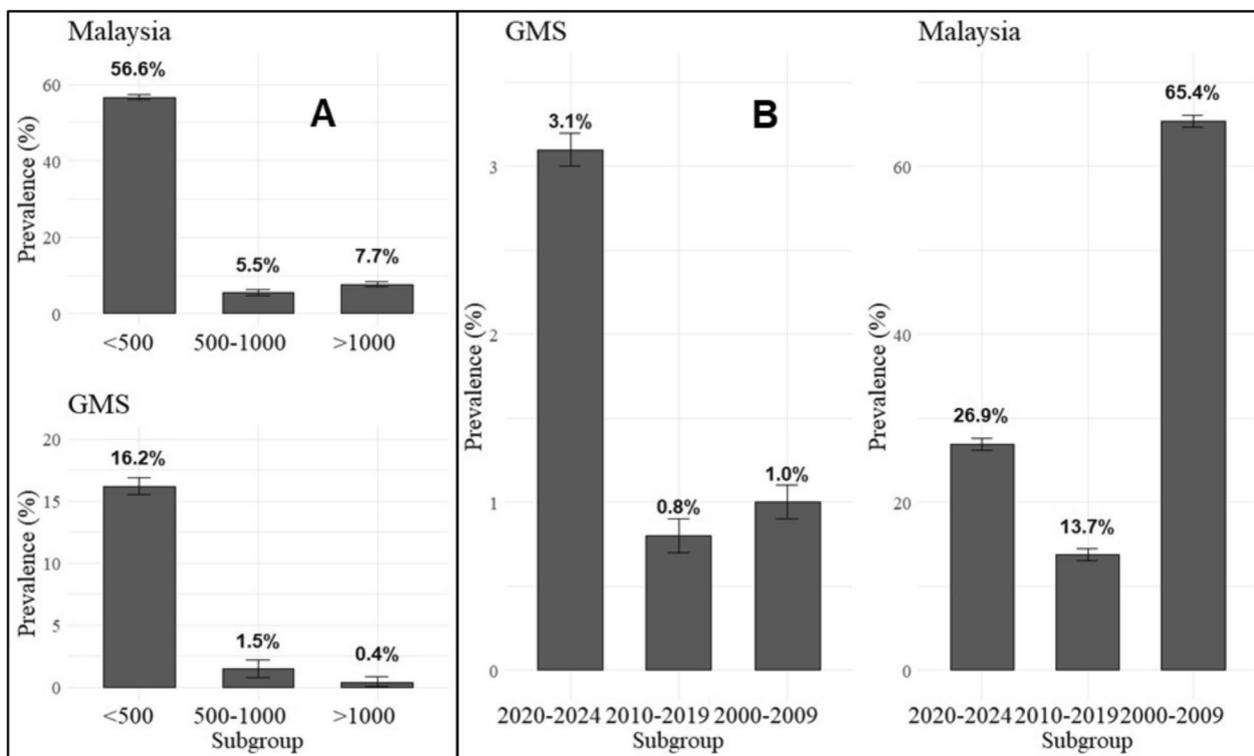


Fig. 7 Subgroup meta-analysis showing the prevalence of zoonotic malaria in humans in the Greater Mekong Subregion (GMS) and Malaysia stratified by sample size (a) and year of publication (b). The error bars indicate the confidence intervals, while the values on each bar are pooled prevalence of each subgroup

Subgroup meta-analysis on prevalence of zoonotic malaria in monkeys

Subgroup analysis was conducted to explore potential sources of heterogeneity in prevalence estimates, stratified by *Plasmodium* species (Fig. 10), monkey species (Fig. 11) and monkey habitat during the study (Fig. 12). All subgroups in each meta-analysis were not significantly different from each other ($p > 0.05$). However, significant study-level heterogeneity was observed (I^2 range: 94.2–98.5%; $p < 0.0001$).

Discussion

The majority of emerging infectious diseases are zoonotic, and with the most recent pandemic, COVID-19, as an explicit example, are driven by zoonotic pathogens [82]. Following the first natural human infection with *P. knowlesi* in 1965 [83], years of research and speculation culminated in 2004 with the reporting of more than 100 cases of monkey malaria in Malaysia [3] and one case in Thailand [4]. Since then, numerous cases have been reported continuously, mostly in Malaysia when compared to GMS. With a pooled prevalence of 8.5 and 35.5% among human and monkey populations, respectively, the current study offers a comprehensive

assessment of the prevalence of zoonotic malaria in the GMS and Malaysia.

The current study investigated critical risk factors associated with zoonotic malaria transmission among humans such as age, gender, mobility, interaction with monkeys, infecting *Plasmodium* spp. and diagnosis. Age was revealed to be a major influence, supporting the hypothesis that different age groups are more exposed or susceptible to infection [84]. However, age alone is insufficient to draw firm conclusions. Seasonality may attenuate the impact of *P. falciparum* malaria stratification by age as demonstrated by Carneiro et al. [84]. Randall et al. [82] demonstrated that age-structured malaria may further be influenced by lymphotoxin-alpha (LT- α), an immune regulating cytotoxin protein [85]. Gender analysis in the current meta-analysis showed a fourfold higher risk for males than females, which could be related to occupational and behavioural differences, particularly in areas where outdoor activities such as farming, rubber tapping, logging, and military service are largely male-dominated [2, 86, 87]. The odds ratio reported here is similar to that reported by Grigg et al. (Odds ratio = 4.20) who retrospectively investigated 222 cases along with 683 matched controls in Sabah, Malaysia [50]. However,

Table 4 Subgroup meta-analysis for the prevalence of zoonotic malaria in humans from the Greater Mekong Subregion (GMS) and Malaysia

Subgroup		P (%)	95% CI	I ²	P-value1*	χ ²	P-value2*
GMS		1.199	0.322–4.359	96.9	<0.0001	16.78	<0.0001
Malaysia		22.72	10.20–43.22	99.7	<0.0001		
Thailand		1.781	0.117–21.90	92.7	<0.0001	8.20	0.0846
Laos		0.175	0.000–99.96	87.4	0.0049		
Cambodia		0.413	0.186–0.916	43.2	0.1717		
Vietnam		1.734	0.018–63.26	96.1	<0.0001		
Myanmar		4.933	0.000–100	99.1	<0.0001		
East Malaysia		22.79	8.472–48.48	99.8	<0.0001	0.57	0.7532
Peninsular Malaysia		17.04	0.621–87.09	99.1	<0.0001		
East/Peninsular Malaysia		33.89	2.418–91.39	99.2	<0.0001		
GMS							
Year of publication	2020–2024	3.057	0.052–65.56	93.0	<0.0001	0.68	0.7106
	2010–2019	0.756	0.166–3.374	98.1	<0.0001		
	2000–2009	0.994	0.000–97.52	86.3	0.0068		
Sample size	< 500	16.180	0.266–93.31	90.4	<0.0001	8.60	0.0136
	500–1000	1.468	0.001–96.96	92.8	0.0002		
	> 1000	0.368	0.145–0.928	91.1	<0.0001		
Malaysia							
Year of publication	2020–2024	26.86	5.538–69.70	99.6	<0.0001	17.41	0.0002
	2010–2019	13.68	3.812–38.79	99.7	<0.0001		
	2000–2009	65.42	54.42–74.99	63.2	0.0430		
Sample size	< 500	56.64	40.16–71.78	97.6	0.0001	19.44	<0.0001
	500–1000	5.495	0.832–28.71	98.8	<0.0001		
	> 1000	7.741	0.813–46.206	99.9	<0.0001		

P, Pooled prevalence; P-value1, p-value (within group); P-value2, p-value (between group); χ², Q-test statistic for the groups; I², Heterogeneity test within groups

contrary to expectations, interaction with monkeys did not show a significant association with increased risk. This indicates that factors beyond direct primate exposure, such as vector ecology and environmental changes, may drive transmission [50, 88] and may involve other factors such as vector dynamics [89, 90], environmental conditions [47, 91], and human behaviour or movement patterns [50], which determines exposure risk. Although mobile migrant populations (MMPs), such as seasonal forest workers, agriculturalists, government personnel, and travellers are strongly associated with malaria transmission [92, 93], the current meta-analysis did not find a significant difference between the compared groups. Forest workers exhibited a 3.5-fold increased risk, although this was not statistically significant. Whilst several reports have suggested a high correlation between forest-related activities and zoonotic malaria infection [50], it is important to interpret these findings carefully, particularly when considering vector habitats [90] given that land use modifications affect vector bio-ecology and host adaptations [91]. Deforestation, afforestation with plantation forests or agricultural activity modify not only vector

habitats but also mosquito populations, with logged areas having a higher abundance of zoonotic malaria vectors than in primary thick forests [91]. Furthermore, deforestation has significantly reduced the species diversity of monkeys and increased the human-monkey contact [17, 91], raising the risk of transmission. With the certification of 46 malaria-free provinces, the Department of Disease Control, Ministry of Public Health, Thailand [94, 95] faces the residual burden along the international borders [94] where there is high incidence of MMPs and a high risk of developing active foci [96]. The main challenge with MMPs is that most of these people are undocumented, unwilling to access designated health care centres or are unable to do so due to financial constraints, and are unable or unwilling to adhere to the mosquito preventive measures such as sleeping under insecticide-treated nets (ITNs) and applying mosquito repellents [93].

This meta-analysis showed that *P. knowlesi* is the primary cause of zoonotic malaria in human populations (Odds ratio = 24.09) compared to other zoonotic *Plasmodium* species, while sporadic cases of *P. cynomolgi* have

Table 5 Factors associated with the prevalence of zoonotic malaria in humans from the selected studies in GMS and Malaysia

Variables	n	N	Prevalence (OR ± SE)*	95% CI	p-value
Parasite (S=46)					
Others	88	79,090	Intercept		
<i>Plasmodium knowlesi</i>	8304		24.09 ± 1.040	3.135–185.0	0.0022
Misdiagnosis (S=22)					
<i>Plasmodium falciparum</i>	133		Intercept		
<i>Plasmodium vivax</i>	148		0.114 ± 0.788	0.024–0.536	0.006
<i>Plasmodium malariae</i>	4436		0.307 ± 0.792	0.065–1.454	0.1368
Negative cases	56		0.047 ± 0.801	0.010–0.227	0.0001
Age (S=20)					
>60	27		Intercept		
1–20	83	37,760	2.107 ± 0.800	0.439–10.10	0.3517
21–40	331		5.61 ± 0.786	1.202–26.20	0.0282
41–60	165		2.579 ± 0.799	0.539–12.35	0.2356
Gender (S=30)					
Female	1025		Intercept		
Male	4235	67,386	4.297 ± 0.698	1.095–16.86	0.0366
Monkey interaction (S=11)					
Near	867	32,659	Intercept		
Not seen	284		0.276 ± 1.263	0.023–3.278	0.3077
Mobility (S=21)					
Agriculture	279		Intercept		
Forest works	712	28,645	3.447 ± 0.808	0.707–16.71	0.1257
Personnel	72		1.046 ± 1.172	0.105–10.40	0.9697
Travel	99		0.246 ± 0.841	0.047–1.278	0.0952

N, Total number people included in the study; n, total number PCR-positive cases; OR, odds ratio; SE, standard error, S, total number of selected studies, CI, confidence interval; Statistically significant results are shown in bold

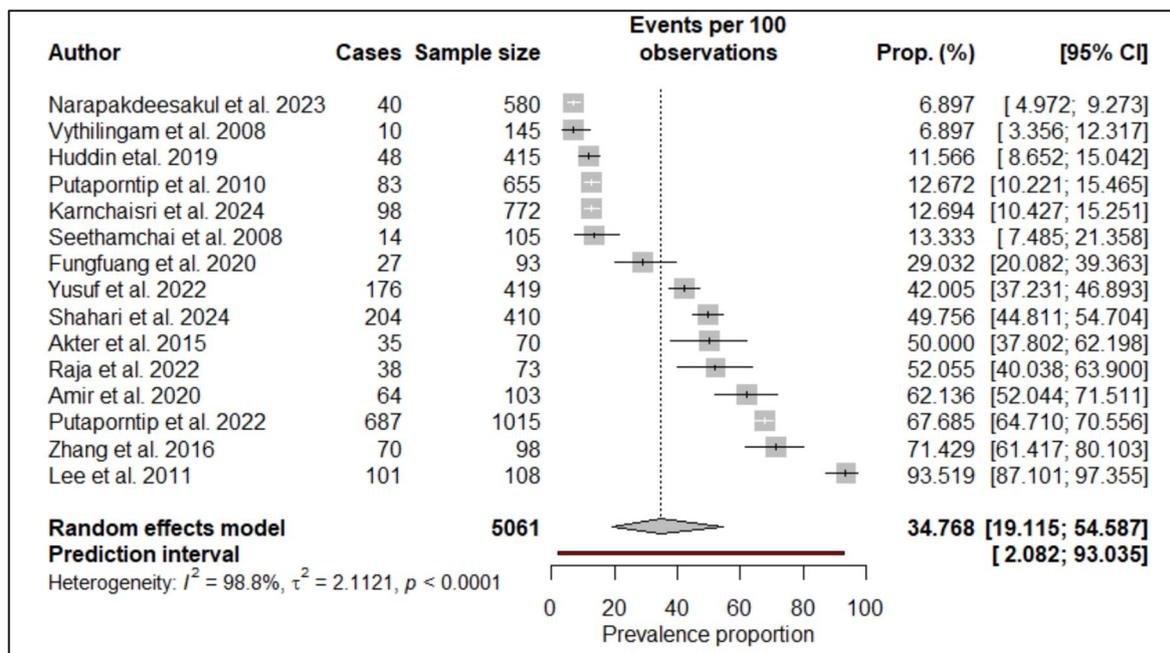


Fig. 8 Forest plot of prevalence estimates on 15 studies reporting zoonotic *Plasmodium* in monkeys across GMS and Malaysia

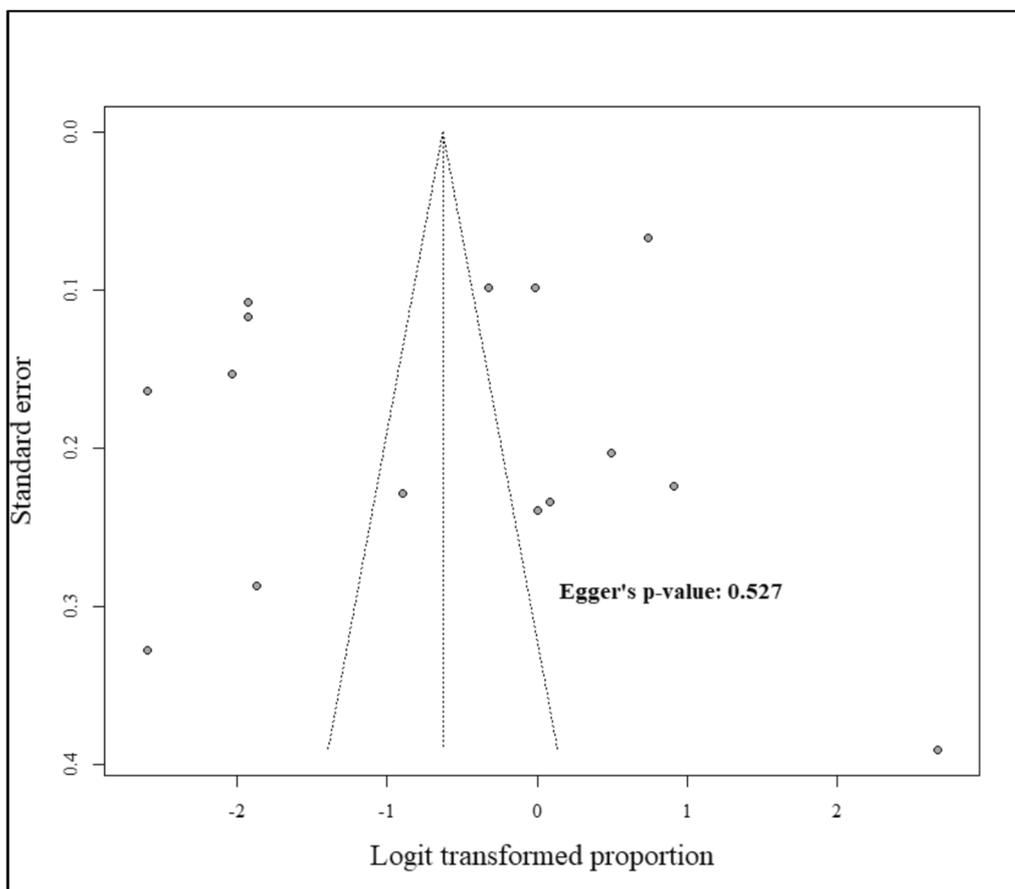


Fig. 9 Funnel plot assessing publication bias indicating Egger's p -value

been reported in Cambodia [37], and mixed infection in Thailand, e.g. with *P. inui* and *P. fieldi* [79], and Malaysia, e.g. with *P. inui* and *P. coatneyi* [51, 62, 65]. *Plasmodium knowlesi* may be considered the fifth causative agent of malaria in humans [97] and is endemic across Southeast Asia [98] where the spectrum of clinical disease ranges from asymptomatic infection [99], to severe malaria [100, 101] and death [102]. The parasite is well adapted in humans, monkeys, and *Anopheles* mosquito vectors [14]. Several reports have mentioned a submicroscopic and cryptic *P. knowlesi* [37, 46, 52], which indicates its danger and requirement for more tailored diagnosis. The schizogonic cycle of red blood cells is as short as 24 h compared to *P. falciparum*, *P. vivax*, *Plasmodium ovale*, which takes 48 h and *P. malariae* that takes approximately 76 h. Drivers of *P. knowlesi* transmission operating at these micro-scales relate to pathogen exposure, immunity, and health status of the human hosts [103]. It can be microscopically misdiagnosed as *P. falciparum*, *P. vivax*, *P. malariae* or can go undetected [29, 61]. Febrile disease due to *P. cynomolgi* and *P. inui* have been reported in addition to the multiple infection with different zoonotic plasmodia in

humans [62]. In the GMS, results of malaria epidemiology are still reported from microscopic examinations and rapid diagnostic tests (RDTs) [104]. Misdiagnosis is possible across all countries where microscopy is the routine means of malaria surveillance [7, 31, 105] coupled with inadequate external quality assurances and competency assessment of malaria microscopists involved in malaria parasite detection, species identification, and parasite count [106]. However, even PCR may fail to detect the parasite [107]. It has been observed that *P. knowlesi* primers randomly cross-react with genomic DNA from other *Plasmodium* spp. [106], which complicates identification in cases of mixed infections [7].

Notwithstanding, it should be mentioned that PCR diagnosis failures are rare, even though quality assessment needs a lot of attention. Diagnosis failures have been frequent in studies reporting asymptomatic cases [33, 37] and mixed infections [62, 79]. Consequently, treatment may be challenging, as completion of schizogony for *P. knowlesi* takes 24 h compared to 48 h for *P. vivax* or *P. falciparum* [108].

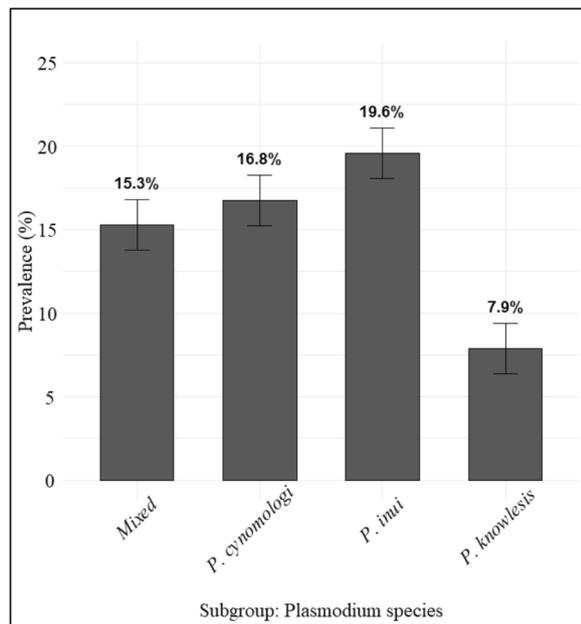


Fig. 10 Subgroup meta-analysis showing the prevalence of zoonotic malaria by *Plasmodium* species in monkeys among 15 studies from GMS and MYS. The error bars indicate the confidence intervals, while the values on each bar are pooled prevalence of each subgroup. Mixed species included only *Plasmodium coatneyi* and *P. fieldi*

Among monkeys, the pooled prevalence of *P. inui* (19.6%) was the highest, followed by *P. cynomolgi* (16.8%), and *P. knowlesi* (7.9%) (Fig. 10). The high prevalence of plasmodia other than *P. knowlesi* presents more challenges as these have been found to infect humans [62, 79]. The overall pooled prevalence for all parasites was high in long-tailed monkeys, *M. fascicularis* (31.4%) and pig-tailed monkeys, *M. nemestrina* (63.3%) (Fig. 11). Despite only four studies examining zoonotic malaria infections in *M. nemestrina* (compared to 13 on *M. fascicularis*), these findings underscore the critical role of pig-tailed monkeys in zoonotic malaria transmission. Other species found infected include *M. leonina* (20.0%), *M. arcoides* (50.0%), and *S. obscurus* (14.3%). The increased number of infected monkey species and their proximity to human populations increases the risk human infections [69, 74]. This meta-analysis did not find a significant difference ($p=0.9214$) in prevalence of zoonotic malaria infection between wild and non-wild (captive and peridomestic) monkeys (Fig. 12). This result further asserts that zoonotic malaria transmission in monkeys is influenced by various other factors but does not rule out the significance of non-wild monkeys in exposing humans to infection risk due to the close contact [90]. The primary limitation observed in monkey-related studies is the lack of detailed reporting on monkey habitats and mobility

behaviours. While several authors have noted that deforestation is driving increased contact between monkeys and humans [13, 109], the 15 studies included in this meta-analysis did not record any environmental changes observed during data collections. Consequently, it remains unclear whether the movement of monkeys into human residences [70, 71] or the visitation of humans to monkey habitats [15, 110] serves as the primary driver of transmission.

The present meta-analysis revealed a high study-level heterogeneity (Fig. 3). Additionally, heterogeneity was high in subgroup meta-analysis stratifying by region (GMS vs. Malaysia), sample size, and year of publication (only Malaysia) showing significant differences between groups (Table 4). These biases imply that making solid conclusions should be carefully done. However, no significant outliers were detected, and all studies overlapped within the pooled estimate's confidence interval. This suggests that, while prevalence varies across studies, the overall estimate is a reliable summary measure. Variations in study designs, geographical landscapes, and sampling efforts could have contributed to these variations [13, 111]. For example, using Malaysia as a reference due to a higher burden of zoonotic malaria than in GMS [2], more robust study designs and diagnostic procedures could have been installed in place [49, 57].

Evolutionary history and molecular studies of *P. knowlesi* in GMS have indicated both geographic and species genetic diversity. Jongwutwesi et al. [29] conducted a sequencing of complete Merozoite Surface Protein 1 Gene of *P. knowlesi* (Pkmsp-1). The authors found that 6 (in pairs of 2) of the surveyed patients obtained the parasites from the same source. The results further showed that the isolates exhibited genetic diversity between human and monkey infections [29]. The same gene was exploited by Putaporntip et al. [29] to confirm the relationship of two isolates that were collected from the same endemic area and were identical by sequencing results of the block 7 of the small subunit ribosomal ribonucleic acid (SSU rRNA) locus. The results showed that the two sequences were polymorphic [30]. In Ranong Province, western Thailand, two *P. knowlesi* patients were found with identical sequence of the circumsporozoite protein gene. Moreover, this was also identical to human and monkey isolates from Thailand and Malaysia [32]. Analysis of mitochondrial encoded cytochrome C oxidase 1 (mtCOX1) sequences of 10 *P. knowlesi* cases from Ubon Ratchathani Province also revealed diversity even where compared to a previous cases of natural human infection [31]. In other zoonotic *Plasmodium* spp, analysis of the mtCOX1 sequences of *P. cynomolgi* among 6 patients showed that all isolates possessed different genetic sequences, suggesting that several strains or clones of this

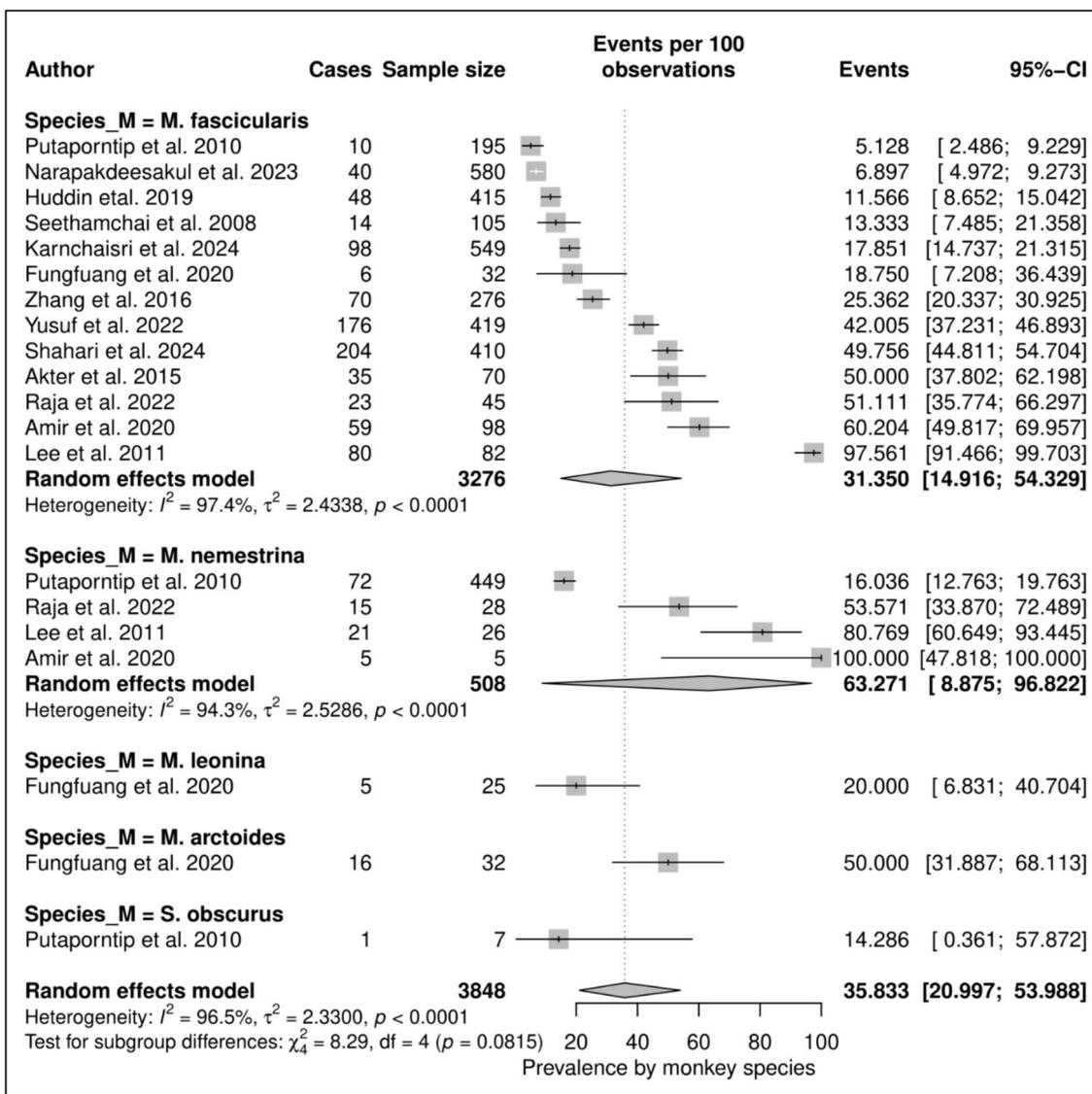


Fig. 11 Subgroup meta-analysis showing the prevalence of zoonotic malaria infection in monkeys from Great Mekong Subregion (GMS) and Malaysia (MYS) stratified by monkey species. The data is displayed as proportion (%) for each study, which were used to calculate the subgroup pooled prevalence

zoonotic parasite are capable of cross-transmission from macaques to humans [31]. *Plasmodium inui* isolates from the Yala Province in southern Thailand were found to be genetically different, showing different strains circulating at the time in humans [79]. In the study of Narapakdeesakul et al. [78], monkey isolates with *P. inui* from southern Thailand were clustered in three distinct clades based on the phylogenetic analysis of the *cytb* gene sequences. Four haplotypes were clustered with *M. nemestrina* and *Anopheles leucosphyrus* isolates from Peninsular Malaysia, while other two haplotypes were closely associated with *P. inui* isolates from macaques, *M. fascicularis*, *M.*

mulatta, *Macaca cyclopsis*, and *Anopheles hackeri*, in Malaysia, Taiwan, and South China [78]. These studies highlight that zoonotic malaria transmission in GMS and Malaysia is complex and involves multiple host species and geographic regions. The clustering of *P. inui* isolates into distinct clades indicates genetic diversity and potential adaptation to different host species and regions. The association of certain haplotypes with specific macaque species and mosquito vectors across various locations highlights the interconnectedness of malaria transmission dynamics in the region. This implies that efforts to control zoonotic malaria must consider the diverse host

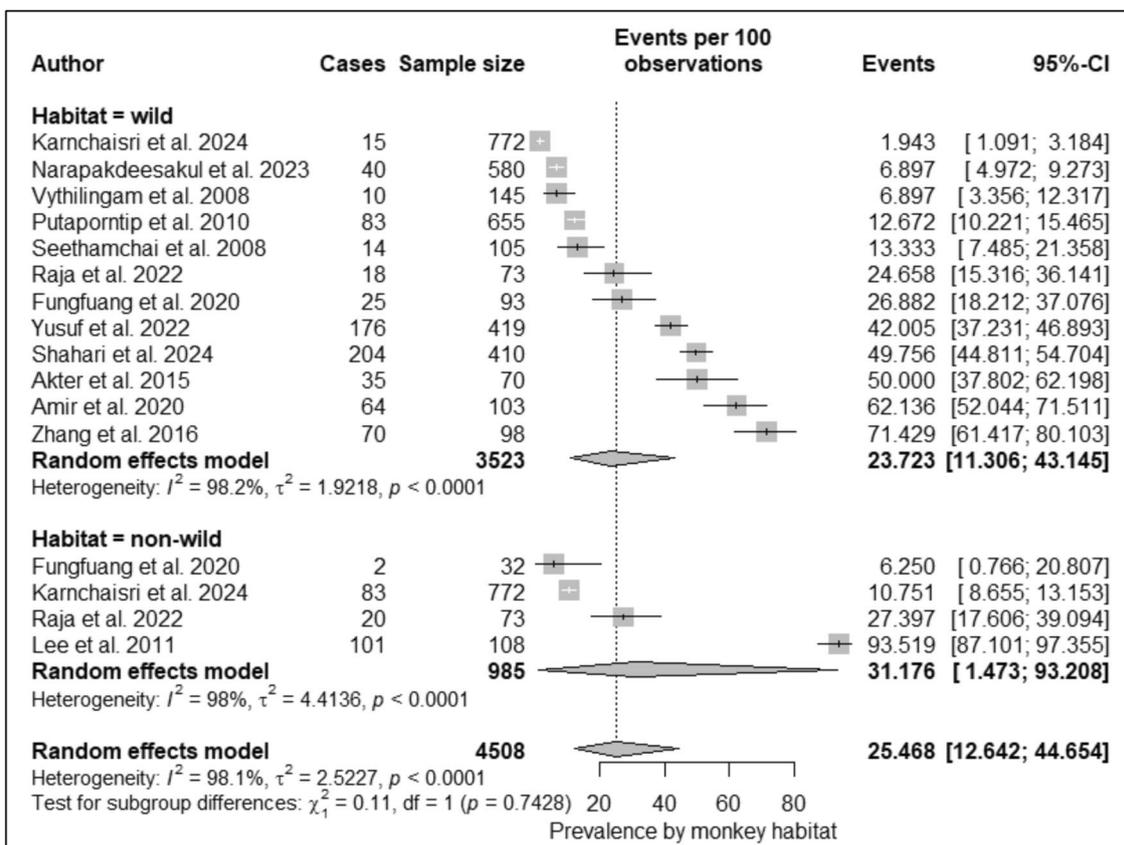


Fig. 12 Subgroup meta-analysis showing the prevalence of zoonotic malaria infection in monkeys from Great Mekong Subregion (GMS) and Malaysia (MYS) stratified by monkey habitat. The data is displayed as proportion (%) for each study, which were used to calculate the subgroup pooled prevalence

and vector species involved, as well as the geographic spread of these interactions.

Conclusion

Zoonotic malaria is increasingly becoming a public health issue in Southeast Asia with Malaysia and Thailand bearing the biggest recorded burden. The current meta-analysis revealed a high study-level heterogeneity implying that there are significant differences between different studies. The study also highlights that there are a number of factors that influence the transmission of zoonotic malaria in GMS and Malaysia ranging from age of humans, gender, mobility, infecting *Plasmodium* species to monkey species and their distribution. There is a need for refined research strategies. Comprehensive research involving the hosts and vectors across different micro-spatial and temporal scales is necessary. Although information regarding monkey-to-human interaction is critically important in transmission of zoonotic malaria, there is limited record of this data. Controlling zoonotic malaria is challenging because it involves managing both wildlife reservoirs and mosquito vectors, thus, requires

an integrated approach involving public health, wildlife management, and environmental conservation. Integration of One Health approaches that consider the interactions between human health, animal health, and the environment is crucial. This includes collaborative surveillance networks, interdisciplinary research, and the development of targeted interventions and control measures. Addressing these research gaps and implementing evidence-based strategies may enhance the capacity to prevent and control zoonotic malaria transmission, thereby reducing the burden of zoonotic malaria infections in human populations.

Summary of key findings

1. There is a high heterogeneity among studies reporting zoonotic malaria in GMS and Malaysia.
2. Pooled prevalence of zoonotic malaria was higher in Malaysia (22.8%) than GMS (1.2%).
3. In GMS, Myanmar showed the highest pooled prevalence (4.9%) followed by Thailand (1.8%).

4. Zoonotic malaria misdiagnosis is prevalent (89.6% false negative) when microscopy is used as the gold standard.
5. Overall, *P. knowlesi* is the most frequently reported zoonotic malaria parasite in humans, while *P. inui* is most prevalent in monkeys.
6. There are multiple circulating zoonotic *Plasmodium* species (*P. cynomolgi*, *P. inui*, *P. fieldi*, *P. coatneyi*, and *P. knowlesi*) in humans and monkeys in GMS and Malaysia.
7. With a pooled prevalence of 63.3%, *Macaca nemestrina* are the riskiest monkey species in transmitting zoonotic malaria in GMS and Malaysia
8. Forest-related activities showed a high risk of zoonotic malaria transmission.
9. Being male, and adult in the age group 21–40, are high risk factors for zoonotic malaria infection as well as transmission.
10. There is limited information on the socio-ecological, behavioral and climatic determinants of zoonotic malaria transmission.

Recommendations for future research and public health interventions

1. Study designs need to be refined to achieve comprehensive approaches for understanding zoonotic malaria transmission particularly, the description of monkey-to-human interaction behaviours.
2. Cambodia has made progress by administering intermittent preventive treatment for forest goers [92]. Such interventions should be implemented across all nations of GMS and Malaysia.
3. Further research and implementation of drug administration, surveillance, and vector control in regions with high mobile and migrant populations (MMP) are needed. The adoption of One Health principles will be crucial in the control and prevention of zoonotic malaria.
4. Unraveling the parasite's genetic diversity and transmission dynamics should be approached at a micro-spatial scale.
5. Robust diagnostic tools are critically necessary. However, training and sensitization of clinicians and technical personnel engaged in diagnosis are extremely important.

Abbreviations

GMS	The Greater Mekong Subregion
WHO	World Health Organization
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews

JBI	Joanna Briggs Institute
CoCoPoP	Condition-Context-Population
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
cytB	Cytochrome b gene
Pkmsp-1	<i>Plasmodium knowlesi</i> Merozoite surface protein-1
SSU rRNA	Small Subunit ribosomal RNA
OR	Odds ratio
CI	Confidence interval
MMPs	Mobile migrant populations
ITN	Insecticide-treated nets

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12936-025-05464-w>.

Additional file 1

Additional file 2

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Author contribution

A.A., J.H., and T.C. conceptualized the research question and designed the study methodology. A.A. conducted the systematic literature search and screened the studies for inclusion, performed the statistical analysis, and created the meta-analysis models, and drafted the manuscript. J.H. contributed to data extraction, statistical analysis, and quality assessment of the included studies. U.A., S.M., C.S., and R. N. assisted in critically reviewing the manuscript. T.C. supervised the project, provided funding, and ensured the overall integrity of the work.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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