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Asymptomatic/submicroscopic *Plasmodium vivax* infection: A systematic review and META-analysis on the hidden challenge for preventing *re*-establishment of malaria transmission

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ABSTRACT

Background: Plasmodium vivax is not only the second most prevalent cause of malaria worldwide, but also the second leading cause of imported malaria in China. This poses a significant threat to preventing the re-establishment of malaria transmission, as the competent vector (*Anopheles sinensis*) suitable for *vivax* malaria transmission is widely distributed in China. Particularly, the asymptomatic *P. vivax* infection as another important source of infection deserves further study, but it is rarely reported.

Methods: PubMed, CNKI and Wanfang databases were systematically searched for asymptomatic *P. vivax* infection relevant studies published between February 2014 and February 2024. I-squared statistics (I^2) was used to assess heterogeneity among included studies. The pooled prevalence and pooled odds ratio and their corresponding 95 % Confidence Interval were estimated using the random effects model in Review Manager 5.4 software.

Results: Seventy-one eligible studies were included in this analysis. Both study countries ($P < 0.001$, $I^2 = 95$ %) and diagnostic methods ($P = 0.001$, $I^2 = 95$ %) were the source of heterogeneity. The rates of asymptomatic malaria infection detected by the gold standard method of microscopy in the countries from Africa, Asia, Oceania and Americas were 9.2 %, 4.8 %, 15.6 % and 14.5 %, respectively. And the corresponding rates of asymptomatic *P. vivax* infection were 4.0 %, 2.1 %, 10.6 % and 13.0 %. In terms of diagnostic methods, the rate of asymptomatic *P. vivax* infection (5.6 %) detected by polymerase chain reaction in the population was the highest ($P < 0.001$).

Conclusion: According to the asymptomatic *P. vivax* infection worldwide, the countries with the higher rate of asymptomatic infection are the main source of *vivax* malaria cases imported into China, which indicates a potentially higher potential risk of importation of asymptomatic *P. vivax* infection. Therefore, it is necessary to develop more sensitive, easier to operate, and more cost-effective techniques to detect and screen asymptomatic malaria infections in a timely manner, so as to prevent re-establishment of malaria transmission.

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1. Introduction

Malaria is an ancient parasitic disease that remains a major public health problem worldwide. According to the latest World Malaria Report 2023, the total number of malaria cases reached 249 million globally in 2022, an increase of 5 million compared to 2021, and the number of *Plasmodium vivax* cases was estimated at 6.9 million (WHO, 2023a). *P. vivax*, one of the five malaria parasites that infect humans, has the widest geographic distribution, which is not only the second leading cause of malaria in the world, but also the main parasite responsible for malaria recurrence.

For countries or regions that have eliminated malaria, they continue to face the risk of re-establishment of malaria transmission caused by imported malaria. For example, China was certified as malaria-free by the World Health Organization (WHO) in June 2021, but there are still a large number of imported malaria cases reported every year. Particularly *P. vivax* was the second leading cause of imported malaria, and the suitable mosquito vector (*Anopheles sinensis*) for *vivax* malaria transmission is still widely distributed in China, thereby malaria reintroduction has been a big challenge of the consolidation of malaria elimination in China.

Asymptomatic malaria infection is defined as the presence of asexual parasites in the blood, but without symptoms of the disease (WHO, 2023b), which is usually not easily detected by microscopic examination. This can become a major challenge to further eliminating malaria in many endemic countries (Koepfli et al., 2017; Imwong et al., 2015), because asymptomatic malaria infections still carry gametic cells and can infect mosquitoes (Jiram et al., 2019; Vallejo et al., 2016; Kiattibutr et al., 2017). Although all the *Plasmodium* species can cause asymptomatic infections, *P. vivax* infection is characterized by triggering periodic asymptomatic infections or spontaneous activation of dormant cells in the liver (Krotoski, 1989). Studies had shown that asymptomatic or submicroscopic infections were more likely to occur in low-transmission settings, and it was estimated to cause 20–50 % of all transmitted cases (Okell et al., 2012), indicating the great importance of timely and accurate detection of all malaria infections including asymptomatic or submicroscopic infections in these settings for malaria control and elimination (Coura et al., 2006; Owusu-Agyei et al., 2001). However, malaria diagnosis is mainly relied on light microscopy, which is the gold standard and has a detection limit of between 50 and 100 parasites/μL in a thick blood film (Wongsrichanalai et al., 2007; WHO, 2015). Obviously, light microscopy cannot meet the requirements for the detection of asymptomatic infection, and more sensitive methods, such as molecular techniques, are needed (Adams et al., 2015).

In the present study, we aimed to understand the potential risk of the asymptomatic/submicroscopic *P. vivax* infections especially post elimination phase in China, published data on asymptomatic/submicroscopic *P. vivax* infections using different diagnostic methods worldwide in the past ten years was systematically reviewed and further analyzed.

2. Methods

2.1. Search strategy and selection criteria

In this systematic review and meta-analysis, we collected published data of asymptomatic/submicroscopic *P. vivax* infections between February 2014 and February 2024, using the following advanced search terms in title and/or abstract in one of the most commonly used database PubMed search engine as follows: ((“asymptomatic”[Title/Abstract] OR “sub-microscopic”[Title/Abstract] OR “sub-clinical”[Title/Abstract]) OR “low-density”[Title/Abstract] OR “sub-patent”[Title/Abstract] AND (“*Plasmodium vivax*”[Title/Abstract] OR “*P. vivax*”[Title/Abstract]) AND “2014/02”:”2024/02”[Date - Publication]). To ensure completeness, “sub-microscope”, “sub-clinical”, “low-density” and “sub-patent” were added to the search. Moreover, the CNKI and Wanfang database, the most commonly used Chinese databases in China, were added for searching.

2.2. Data extraction

Studies of asymptomatic/submicroscopic *P. vivax* infection in the past decade were screened and included in the current analysis if published studies met the following criteria: (1) *P. vivax* was detected by rapid diagnostic tests (RDTs), microscopy or polymerase chain reaction (PCR); (2) The prevalence of asymptomatic/submicroscopic *P. vivax* infection with clear case and population numbers were reported. Meanwhile, the literature about reviews, meta-analysis, duplicate literature, editorial, reports, books, documents, case studies, methods studies, modeling studies, genetic diversity studies, and non-*P. vivax* studies were excluded from this study. For all eligible articles included in the analysis, the following information was extracted: name of the authors, year of publication, the country of samples collected, year of the samples studied, diagnostic methods, the total number of participants, total number of asymptomatic malaria infection, the number of asymptomatic *P. vivax* infection.

2.3. Data analysis

Data collation was performed using the Microsoft Office Excel and a map showing the distribution of the countries studied was created in ArcGIS 10.8. The meta-analysis was conducted using Review Manager 5.4 software. The number of asymptomatic *P. vivax* infection compared to asymptomatic *P. falciparum* infection was analyzed according to different countries and different diagnostic methods, using the summary odds ratios (ORs) and 95 % Confidence Interval (CI). Random effects models as described previously (Tamhane et al., 2010; Kotepui et al., 2020) were used to produce summary ORs. The calculated I-squared statistics (I^2) were reported to determine whether there was heterogeneity among the included studies.

3. Results

3.1. The characteristics of included studies

As described in the flow chart, a total of 479 relevant literature were initially retrieved in PubMed (444), CNKI (24) and Wanfang (11) databases respectively in this study (Fig. 1). Among these, a total of 92 literature including reviews, meta-analysis, editorial, comments, reports, books, documents, duplicate literature, achievement and published erratum were removed. Then, the titles and abstracts of the remaining 387 literature were read and screened, and 190 studies were excluded, including case studies, methods studies, modeling studies, genetic diversity studies, and non-*P. vivax* studies. The remaining 198 literature were read for the full text, and finally 71 literature passed the review of inclusion and exclusion criteria, which including 68 literatures from PubMed and 3 from CNKI.

3.2. The prevalence of asymptomatic P. vivax infection in different countries

A high level of heterogeneity ($I^2 = 95$ %) among the included studies was found, thereby the summary ORs were calculated using random-effects models (Fig. 2). In terms of study countries, 29.6 % (21/71) of the studies were conducted in Africa (Biruksew et al., 2023; Solomon et al., 2020; Abagero et al., 2024; Subussa et al., 2021; Feleke et al., 2020; Nega et al., 2015; Bylicka-Szczepanowska and Korzeniewski, 2022; Tadesse et al., 2015; Tadesse et al., 2018; Alemu and Mama, 2018; Zhou et al., 2016; Getachew et al., 2023;

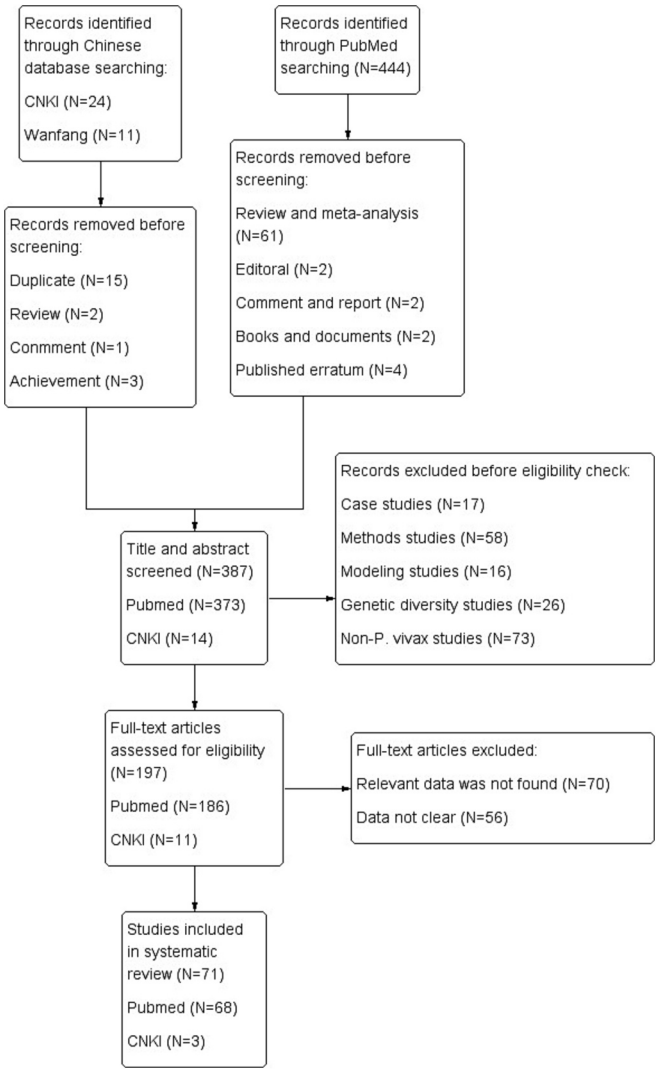

Fig. 1. Flow diagram of search strategy and selection criteria. The figure showed the number of manually screened and excluded literature identified in PubMed, CNKI and Wanfang database.

Fru-Cho et al., 2014; Golassa et al., 2015; Niang et al., 2018; Tilaye et al., 2022; Motshoge et al., 2016; Motshoge et al., 2021; Gemechu et al., 2023; Assefa et al., 2020; Howes et al., 2018), 46.5 % (33/71) in Asia (Imwong et al., 2015; Jiram et al., 2019; Ramaswamy et al., 2020; Parker et al., 2017; Doum et al., 2023; Ouma et al., 2020; Turki et al., 2015; Tu et al., 2022; Bhowmick et al., 2021; Sattabongkot et al., 2018; Barman et al., 2023; Phommasone et al., 2016; Iwagami et al., 2017; Zaw et al., 2017; Noordin et al., 2020; San et al., 2022; Longley et al., 2017; Tripura et al., 2016; Shimizu et al., 2020; Wangchuk et al., 2019; Pongvongsa et al., 2016; Nguitragool et al., 2017; Zhao et al., 2018; Hawash et al., 2019; Liu et al., 2019; Huwe et al., 2022; Peto et al., 2016; Huang et al., 2017; Thanh et al., 2015; Ahmed et al., 2015; Wang et al., 2020; Cang-lin et al., 2016; Zhou et al., 2023), 18.3 % (13/71) in the Americas (Rosas-Aguirre et al., 2017; Rosas-Aguirre et al., 2021; Almeida et al., 2018; Rodríguez Vásquez et al., 2018; Villasis et al., 2022; Mosnier et al., 2020; de Alencar et al., 2018; Carrasco-Escobar et al., 2017; Almeida et al., 2021; Vásquez-Jiménez et al., 2016; Maselli et al., 2014; Barros et al., 2022; Miguel et al., 2019) and 5.6 % (4/71) in Oceania (Koepfli et al., 2017; Waltmann et al., 2015; Unger et al., 2019; Quah et al., 2019). Moreover, a total of 133,384 samples were included in this study, of which 7015 (5.3 %) were asymptomatic *P. vivax* infections and 3508 (2.6 %) were asymptomatic *P. falciparum* infections.

In a subgroup analysis comparing the results of 71 studies from the four continents, there was a significant difference between asymptomatic *P. vivax* infection and asymptomatic *P. falciparum* infection (OR = 1.63, 95 % CI: 1.25–2.12). Moreover, a difference ($P < 0.001$, $I^2 = 91.7$ %) was also found in the analysis of heterogeneity, indicating that the place where the study conducted was an important factor in the present study (Fig. 2). And the test for overall effect of each continents had shown significant difference ($P < 0.001$).

Additionally, the rates of asymptomatic malaria infection detected by the gold standard method of microscopy in the countries from Africa, Asia, Oceania and Americas were 9.2 %, 4.8 %, 15.6 % and 14.5 %, and the rates of asymptomatic *P. vivax* infections were 4.0 %, 2.1 %, 10.6 % and 13.0 %, respectively (Table 1). In Asia, the asymptomatic *P. vivax* infection detected in the Thai-Myanmar border accounted for the highest proportion (6.9 %) of the total number of samples in this region compared to other countries or regions in Asia. The countries with a high proportion of asymptomatic *P. vivax* infections detected in Africa, Oceania and the Americas were Senegal (7.8 %), Papua New Guinea (14.0 %) and Peruvian Amazon (26.6 %), respectively (Table 1). Moreover, the majority of studies conducted in Africa were from Ethiopia (71.4 %) followed by Botswana (9.5 %), and studies in Asia involved 13 countries and regions with a wide distribution (Fig. 3). Among the 13 studies in the Americas, studies were mainly conducted in Colombia (15.4 %), followed by Peru (30.8 %) (Fig. 3). Fewer studies were conducted in Oceania, with two each from Solomon Islands and Papua New Guinea (Fig. 3).

3.3. Asymptomatic P. vivax infection by different diagnostic methods

Three different diagnostic methods of RDTs, microscopy and PCR were reported in these studies (Fig. 4), and there was a statistical difference in the detection of asymptomatic *P. vivax* infection and asymptomatic *P. falciparum* infection using different methods (OR = 1.27, 95 % CI 0.98–1.63). Meanwhile, diagnostic methods might be one source of heterogeneity ($P < 0.001$, $I^2 = 91.9$ %) in the present study. Furthermore, there were 159 (0.7 %), 1761 (4.8 %) and 5178 (5.6 %) asymptomatic *P. vivax* infections, which detected by RDTs, microscopy and PCR, respectively. The difference in the test for overall effect of each diagnostic method was statistically significant ($P < 0.001$).

4. Discussion

Imported malaria will be a long-term challenge in malaria-eliminating countries or regions as globalization continues and population mobility intensifies. In the present study, asymptomatic malaria infection as a potentially important source of infection, particularly the asymptomatic *P. vivax*, which has been reported in the China-Myanmar border (Huang et al., 2017; Wang et al., 2020; Cang-lin et al., 2016), the China-Laos border (Zhang et al., 2022) and the China-Vietnam border (Tu et al., 2022), threatening the consolidation of malaria elimination in China.

Studies have shown that *P. vivax* is mainly distributed in Southeast Asia, the Western Pacific, Central and South America and parts of Africa, of which Africa is dominated by the prevalence of *P. falciparum*, but the burden of *P. vivax* in Ethiopia is serious (Price et al., 2020). The imported *vivax* malaria cases reported in China from 2017 to 2023 were mainly from the countries of Asia and Africa (Zhang, L., 2024; Zhang, L. et al., 2023; Zhang, L. et al., 2022; Zhang, L. et al., 2021; Zhang, L. et al., 2020; Zhang, L. et al., 2019; Zhang, L. et al., 2018). According to statistics, the imported *P. vivax* infection cases in China from 2021 to 2023 were mainly from Myanmar, followed by Ethiopia (Zhang, L. and Xia, 2024; Yin et al., 2023). In this study, we collected data on asymptomatic *P. vivax* infections in Africa, Asia, Oceania and Americas, where happen to be the source of imported malaria cases in China. Thus, it is critical to detect asymptomatic malaria infection in a timely manner, not only asymptomatic individuals rarely seek healthcare, but also the density of malaria parasites is too low to be detected. More importantly, they can act as silent reservoirs of transmission to susceptible mosquitoes, leading to secondary transmission of malaria (Jiram et al., 2019; Tu et al., 2022). For travelers returning from these malaria-endemic countries, we should be on the lookout not only for reported cases of malaria, but also for potential asymptomatic infections, especially when an index case is present. Furthermore, China shares the longest land border in the world with 14 countries (9 countries are still malaria-endemic) (China's Land Border, 2016), and malaria prevention and control in border areas is an important global challenge (Wangdi et al., 2015). In fact, the imported malaria from neighboring malaria-endemic countries continues to threaten China to consolidate the malaria elimination achievement, particularly in Yunnan Province, which borders Myanmar, Laos and Vietnam, and asymptomatic malaria cases including *P. vivax* infection were found in these border areas (Tu et al., 2022; Zaw et al., 2017; Zhao et al., 2018; Huang et al., 2017; Wang et al., 2020; Cang-lin et al., 2016; Zhang et al., 2022). If these asymptomatic

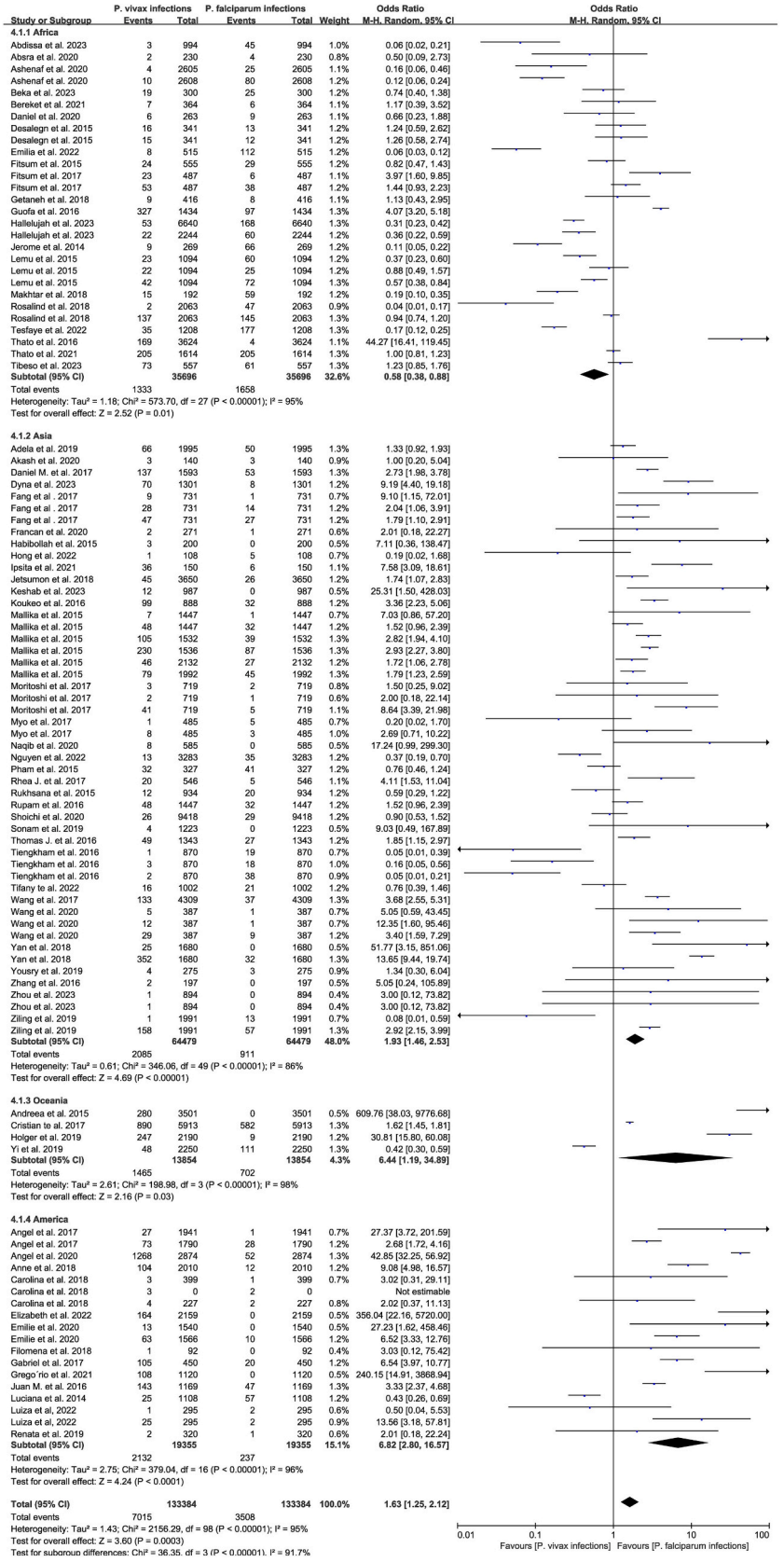

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Fig. 2. Subgroup analysis of the prevalence of asymptomatic *P. vivax* infections and asymptomatic *P. falciparum* infections in different continents.

Table 1
The number of asymptomatic malaria infections and asymptomatic *P. vivax* infections in different countries or regions.

Country/Region		Total number	Number of positives (%)*	Number of *P. vivax* infections (%)*
Africa	Ethiopia	17,488	1359 (7.8)	625 (3.6)
	Central African Republic	515	126 (24.5)	8 (1.6)
	Cameroon	269	87 (32.3)	9 (3.3)
	Senegal	192	74 (38.5)	15 (7.8)
	Botswana	5238	681 (13.0)	374 (7.1)
	Madagascar	2063	49 (2.4)	2 (0.1)
Subtotal		25,765	2376 (9.2)	1033 (4.0)
Asia	Malaysia	2580	164 (6.4)	74 (2.9)
	Cambodia	7131	1031 (14.4)	311 (4.4)
	India	1277	83 (6.5)	51 (4.0)
	Tailand	17,923	382 (2.1)	224 (1.3)
	Myanmar	5050	47 (0.9)	28 (0.6)
	Vietnam	5742	208 (3.6)	91 (1.6)
	Iran	471	6 (1.3)	5 (1.1)
	Laos	2477	199 (8.0)	104 (4.2)
	China-Vietnam border	108	6 (5.6)	1 (0.9)
	Myanmar-China border	1315	18 (1.4)	16 (1.2)
	Thailand–Myanmar border	1532	144 (9.4)	105 (6.9)
	Bhutan	1223	4 (0.3)	4 (0.3)
	Saudi Arabia	275	8 (2.9)	4 (1.5)
	Bangladesh	1002	42 (4.2)	16 (1.6)
	Indonesia	934	32 (3.4)	12 (1.3)
Subtotal		49,040	2374 (4.8)	1046 (2.1)
Oceania	Solomon Islands	5751	439 (7.6)	328 (5.7)
	Papua New Guinea	8103	1728 (21.3)	1137 (14.0)
Subtotal		13,854	2167 (15.6)	1465 (10.6)
America	Peru	2159	164 (7.6)	164 (7.6)
	Brazil	4945	333 (6.7)	241 (4.9)
	Colombia	1568	200 (12.8)	146 (9.3)
	French Guiana–Brazil	1566	73 (4.7)	63 (4.0)
	Peruvian Amazon	5265	1473 (28.0)	1400 (26.6)
Subtotal		15,503	2243 (14.5)	2014 (13.0)
Total		104,162	9160 (8.8)	5558 (5.3)

infections are not detected in time when they enter into China, it will be a certain risk of malaria re-introduction. Thus, malaria screening for these populations is an important measure for border malaria control, and the use of more sensitive testing techniques should be chosen instead of routine malaria RDTs, not to mention that the *P. vivax* RDT kits are not as readily available as *P. falciparum* RDTs.

However, the use of sensitive technologies for routine malaria screening still presents numerous challenges. First, microscopic examination using thick and thin blood smears is still the gold standard for malaria diagnosis that can diagnose and distinguish different species of parasites (WHO, 2023b; Moody and Chiodini, 2000), but it is not sensitive enough to detect the parasites at very low densities. Second, RDTs are extensively used due to its ease of operation and the absence of additional equipments, but the commercial RDTs shows the limit sensitive to 100 to 200 parasites /μL (WHO, n.d.), and RDTs for *P. vivax* detection are not specific enough. Third, the nucleic acid amplification testing such as PCR with higher sensitivity, typically detecting 5–10 parasites /μL, is very effective in cases of low parasite density below the threshold required for microscopy or RDTs (Ramaswamy et al., 2020; Okell et al., 2009), which is also presented in this study that PCR was easier to detect asymptomatic malaria infections (10.0 %) than microscopy (6.9 %) and RDTs (4.3 %) respectively. However, PCR is generally not suitable for large-scale field use in malaria-endemic areas, nor for routine diagnosis in endemic areas where a large proportion of the population may have low-density parasitaemia, due to the relatively high cost, and the need for specialized personnel and specialized equipment, as well as the lack of standardized methods (Ahmed et al., 2018; Snounou et al., 1993; Moreira et al., 2015). Therefore, there is a need to develop new technologies that are more sensitive, easier to operate and low coast to detect and screen asymptomatic malaria infections in a timely manner. In recent years, a study from Malaysia had shown that the loop-mediated isothermal amplification (LAMP) (Imai et al., 2017; Han et al., 2007) can detect 2 parasites/μL of *P. vivax*, with a high sensitivity comparable to PCR (Piera et al., 2017), and it has been proved to be a suitable test for national malaria control screening and can be promoted in the clinical field. Another study, in recent years, used recombinase-aided amplification (RAA) and a lateral-flow dipstick (LFD) (RAA-LFD) analysis for specific amplification of *P. vivax* and showed a sensitivity of 10 pg/mL to whole blood genomic DNA of malaria patients infected with *P. vivax* (Lin et al., *2022)*. It can be used for reliable field testing in the environment of scarce resources and limited laboratory capacity. Those isothermal amplification techniques can specifically amplify *P. vivax* genes and has the characteristics of simple operation, higher specificity, fast and convenient, which could be gradually popularized and applied in the future.

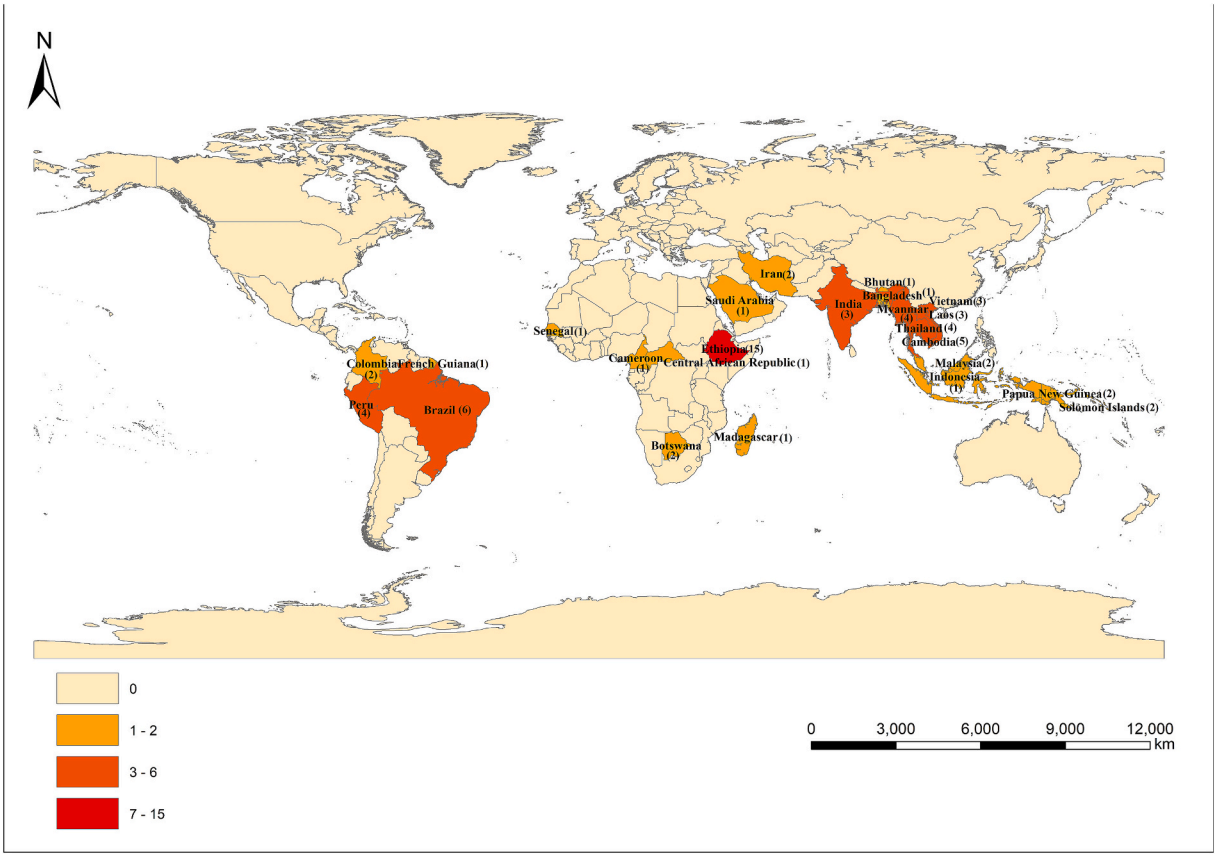

Fig. 3. Map of the distribution of countries included in this study. The information in parentheses after the country name indicates the number of included literature. The literature data of China-Vietnam border (1), Thai-Myanmar border (1) and Myanmar-China border (3) were not shown in the figure. * Map lines delineate study areas and do not necessarily depict accepted national boundaries.

There are several limitations to this study. First, only the literature published in the past ten years were included, and only three databases were used. Second, there was a time lag in publication compared with the actual study time. Third, not all studies used three testing techniques (microscopy, RDTs, and PCR) at the same time to detect asymptomatic infections.

5. Conclusion

The widespread distribution of *vivax* malaria and its unique biological characteristics make it an important obstacle to malaria eradication. In this study, we used a meta-analysis method to analyze the data of asymptomatic *P. vivax* infection from Africa, Asia, Oceania and Americas, where happen to be the source of imported malaria cases in China. Importantly, the countries with the higher rate of asymptomatic infection were the origins of the main source of *vivax* malaria cases imported into China, indicating a potentially higher potential risk of malaria re-introduction in China, if they are not detected in time. Therefore, it is required to develop more sensitive, easier to operate, and more cost-effective techniques to detect and screen asymptomatic malaria infections in post-elimination phase, so as to prevent re-establishment of malaria transmission.

CRediT authorship contribution statement

Siqi Wang: Writing – original draft, Formal analysis, Data curation. **He Yan:** Project administration, Formal analysis, Data curation. **Li Zhang:** Project administration, Formal analysis, Data curation. **Zhigui Xia:** Writing – review & editing, Supervision. **Jianhai Yin:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

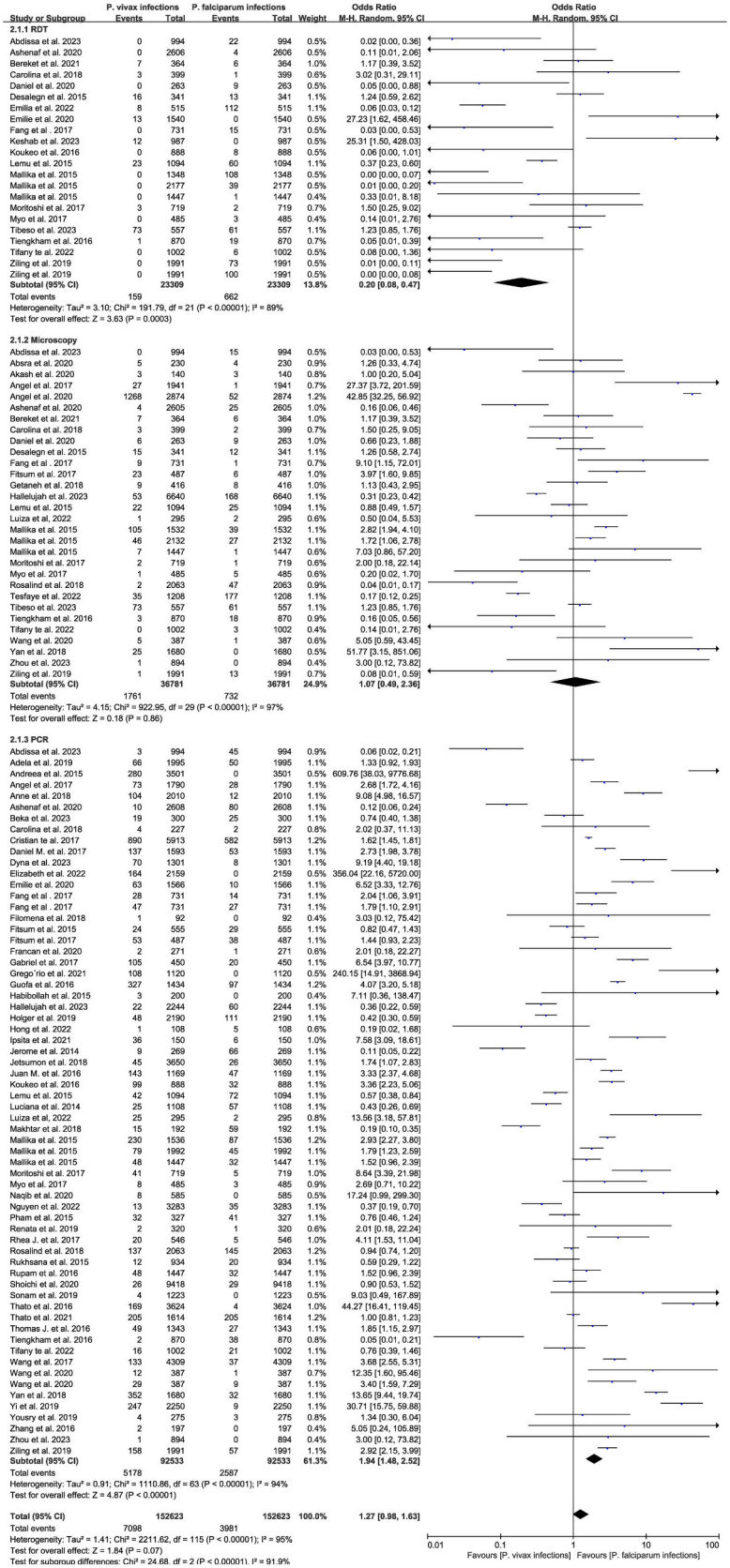

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Fig. 4. Subgroup analysis of asymptomatic *P. vivax* infections and asymptomatic *P. falciparum* infections under different diagnostic methods.

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Declaration of competing interest

The authors declare that they have no competing interests.

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