

Genetic diversity and multiplicity of Plasmodium falciparum infection in Southeast Asia: New insights from a systematic review

Rengganis Praswitasari, Dominicus Husada, Nur Aini Hasan, I Made Dwi Mertha Adnyana

To enable PROSPERO to focus on COVID-19 submissions, this registration record has undergone basic automated checks for eligibility and is published exactly as submitted. PROSPERO has never provided peer review, and usual checking by the PROSPERO team does not endorse content. Therefore, automatically published records should be treated as any other PROSPERO registration. Further detail is provided [here](#).

Citation

Rengganis Praswitasari, Dominicus Husada, Nur Aini Hasan, I Made Dwi Mertha Adnyana. Genetic diversity and multiplicity of Plasmodium falciparum infection in Southeast Asia: New insights from a systematic review.

PROSPERO 2024 CRD42024606943. Available from <https://www.crd.york.ac.uk/PROSPERO/view/CRD42024606943>.

REVIEW TITLE AND BASIC DETAILS

Review title

Genetic diversity and multiplicity of Plasmodium falciparum infection in Southeast Asia: New insights from a systematic review

Original language title

Genetic diversity and infection multiplicity of Plasmodium falciparum in Southeast Asia based on msp-1 and msp-2: a systematic review and meta-analysis

Review objectives

- What is the geographical distribution of Plasmodium falciparum genetic diversity in Southeast Asia?
- What is the prevalence of Plasmodium falciparum polyclonal infections in Southeast Asia?
- What factors are associated with Plasmodium falciparum genetic diversity and multiplicity of infection (MOI) in Southeast Asia?

Keywords

Plasmodium falciparum; Southeast Asia; merozoite surface protein; genetic diversity; multiplicity of infection; molecular epidemiology; malaria surveillance

SEARCHING AND SCREENING

Searches

A systematic literature search was conducted in the Scopus, PubMed, ProQuest, and Google Scholar databases. The search was limited to articles published in English between 2014 and 2024.

Study design

Only randomized study types will be included.

Included

observational study design, including cross-sectional, case-control, cohort, and/or randomized controlled trials (RCTs)

Excluded

Studies were excluded if they did not provide numerical data on allele frequency or MOI values, used genotyping methods other than PCR electrophoresis, or did not include a validated DNA extraction protocol.

Link to search strategy

A full search strategy is available in the full protocol as described in the *Availability of full protocol* section

ELIGIBILITY CRITERIA

Condition or domain being studied

In Southeast Asia, *Plasmodium falciparum* causes most of the malaria cases. Despite its crucial roles in disease severity and drug resistance, comprehensive data on *Plasmodium falciparum* genetic diversity and multiplicity of infection (MOI) are sparse in Southeast Asia. This study summarizes available information on genetic diversity and MOI, focusing on key markers (*msp-1* and *msp-2*). The systematic review aimed to evaluate their influence on malaria transmission dynamics and offer insights for enhancing malaria control measures in Southeast Asia.

Population

children and adult in Southeast Asia

Intervention(s) or exposure(s)

Interventions/Exposures to be Reviewed

Genetic Diversity of *Plasmodium falciparum*:

- Merozoite Surface Proteins (*msp-1* and *msp-2*).
- MOI

*Inclusion Criteria

Language: Articles published in English.

Study Design: Observational studies (cross-sectional/survey, case-control, and cohort) or randomized clinical trials (RCTs).

Data Elements: Studies must provide minimum required data elements, including:

Country of study

Sample size

Calendar year(s) when the study was conducted

Detailed laboratory methods used to genotype markers for genetic diversity or MOI.

Methods: Studies must include detailed methods for determining *Plasmodium falciparum* genetic diversity and MOI, such as:

Mean expected heterozygosity

Allele frequencies

Mean MOI or percentage of multiple infections.

*Exclusion Criteria

Absence of Key Terms: Studies that did not include the key terms '*Plasmodium falciparum* genetic diversity and/or MOI' in the title and/or abstract.

Experimental Animals: Studies using experimental animals rather than human subjects.

Review Articles: Case reports, case series, or editorials that do not present original research data.

Inappropriate Laboratory Methods: Studies that employed inappropriate laboratory molecular methods, such as inadequate DNA extraction, PCR, or fragment analysis.

This structured approach ensures that the review captures relevant studies that contribute to understanding the genetic diversity and multiplicity of infection of *Plasmodium falciparum*, which is crucial for malaria control strategies in Southeast Asia.

Comparator(s) or control(s)

Alternatives for Comparison

Control Groups with Low Genetic Diversity or MOI:

Studies from regions with low *Plasmodium falciparum* genetic diversity or MOI will serve as control groups, helping to highlight the impact of high diversity and MOI on malaria transmission and treatment outcomes.

Geographic Regions:

Comparisons will be made between regions with varying malaria transmission intensities, including high, moderate, and low transmission settings.

Symptomatic vs. Asymptomatic Infections:

The review will compare genetic diversity and MOI between symptomatic and asymptomatic malaria cases to understand how these factors influence disease presentation and transmission dynamics.

Inclusion Criteria:

Geographic Variation: Studies from Southeast Asia reporting on *Plasmodium falciparum* genetic diversity and MOI.

Transmission Intensity: Studies categorizing populations based on malaria transmission levels.

Clinical Presentation: Studies comparing symptomatic and asymptomatic cases with relevant genetic diversity and MOI data.

Exclusion Criteria:

Lack of data on genetic diversity or MOI.

Studies limited to a single country without comparative data.

Non-human studies.

Studies using inadequate genotyping methods.

This approach aims to provide a clear understanding of *Plasmodium falciparum* genetic diversity and MOI in different contexts, aiding malaria control strategies in Southeast Asia.

Context

Inclusion Criteria:

Geographic Variation: Studies from Southeast Asia reporting on *Plasmodium falciparum* genetic diversity and MOI.

Transmission Intensity: Studies categorizing populations based on malaria transmission levels.

Clinical Presentation: Studies comparing symptomatic and asymptomatic cases with relevant genetic diversity and MOI data.

Exclusion Criteria:

Lack of data on genetic diversity or MOI.

Studies limited to a single country without comparative data.

Non-human studies.

Studies using inadequate genotyping methods.

This approach aims to provide a clear understanding of *Plasmodium falciparum* genetic diversity and MOI in different contexts, aiding malaria control strategies in Southeast Asia.

OUTCOMES TO BE ANALYSED

Main outcomes

the outcome: *Plasmodium falciparum* genetic diversity and multiplicity (MSP1&2) of infection (MOI), the outcomes of interest are critical for understanding the implications of genetic variation on malaria transmission, disease severity, and treatment efficacy. Below are the details of the outcomes to be assessed, including their definitions and measurement criteria.

Measures of effect

Effect Measures for Main Outcomes

Genetic Diversity of *Plasmodium falciparum*:

Mean Expected Heterozygosity: Reported as a mean value, comparing genetic diversity across populations or regions.

Allele Frequencies: Comparison of allele frequencies at genetic markers across groups to understand population structure.

Multiplicity of Infection (MOI):

Mean MOI: Average number of distinct genotypes per infected individual, compared between symptomatic and asymptomatic cases or transmission settings.

Odds Ratios (OR): Used to compare the likelihood of multiple infections (e.g., MOI > 1) between groups.

Clinical Outcomes:

Relative Risks (RR): Assessing the risk of treatment failure or adverse outcomes between groups with different levels of genetic diversity or MOI.

Risk Differences: Measuring the absolute difference in clinical outcome risks between groups with high vs. low genetic diversity or MOI.

Number Needed to Treat (NNT): Calculated to determine how many patients must be treated to prevent one adverse outcome in varying genetic diversity or MOI contexts.

These measures offer insights into the impact of *Plasmodium falciparum* genetic diversity and MOI on malaria transmission and treatment outcomes.

Additional outcomes

none

Measures of effect

none

DATA COLLECTION PROCESS

Data extraction (selection and coding)

Before data extraction, the articles were checked for duplication via Mendeley Reference Manager software. Two reviewers (RP and NAH) independently extracted data from the included articles via a predefined extraction form. This form included variables such as (1) author characteristics, including the first author's name, year of publication, country and location of the study, and sampling period; (2) study characteristics, including study design, sample size, and clinical and demographic characteristics of the population (age, sex, symptomatic/asymptomatic status); (3) transmission characteristics, including the endemicity or intensity of malaria transmission at the study site and the annual parasite index (API); (4) laboratory

methodology for confirming malaria cases/diagnoses, specimen types, and DNA extraction methods used; and (5) genetic diversity, including the frequency of msp-1 (K1, MAD20, RO33) and msp-2 (FC27, 3D7/IC) family alleles and multiplicity of infection (MOI) data, including the prevalence of polyclonal infection, number of genotypes, and/or average MOI value. Any discrepancies in the data extracted by the two reviewers were reverified by referring to the original article until a consensus was reached.

Risk of bias (quality) assessment

Methodological quality assessment was conducted via the Joanna Briggs Institute (JBI) Critical Appraisal Tools appropriate for the design of the studies analyzed. The three instruments used included (1) the JBI critical appraisal checklist for analytical cross-sectional studies with eight evaluation criteria for analytical cross-sectional studies; (2) the JBI critical appraisal checklist for case-control studies with ten evaluation criteria for case-control studies; and (3) the JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data with nine evaluation criteria for prevalence studies. Each study was evaluated independently by two reviewers (DH and IMDMA) using a four-category rating system: "yes" (green), "no" (red), "unclear" (yellow), and "not applicable" (blue).

PLANNED DATA SYNTHESIS

Strategy for data synthesis

The proposed methods for data synthesis in this systematic review are designed to rigorously analyze and summarize the available evidence on Plasmodium falciparum genetic diversity and MOI. By employing both qualitative and quantitative approaches, including meta-analysis with appropriate statistical models and heterogeneity assessments, the review aims to provide comprehensive insights that can inform malaria control strategies in southeast asia.

Analysis of subgroups or subsets

The planned subgroup investigations aim to provide a nuanced understanding of the factors influencing Plasmodium falciparum genetic diversity and MOI. By analyzing these subgroups, the review will identify specific patterns and trends that can inform targeted malaria control strategies in different contexts across southeast asia. Each subgroup analysis will utilize appropriate statistical methods to ensure robust and meaningful interpretations of the data.

REVIEW AFFILIATION, FUNDING AND PEER REVIEW

Review team members

Mrs Rengganis Praswitasari. ORCID: 0009-0001-7479-2093. Master Program of Tropical Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya 60286, Indonesia. Indonesia.

No conflict of interest declared.

Dr Dominicus Husada. ORCID: 0000-0001-9191-9546. Department of Pediatrics, Faculty of Medicine, Universitas Airlangga, Surabaya 60286, Indonesia. Indonesia.

No conflict of interest declared.

Mrs Nur Aini Hasan. ORCID: 0009-0001-2582-4853. Department of Anatomy and Histology, Faculty of Medicine, Universitas Muhammadiyah Sidoarjo, Sidoarjo, Indonesia. Indonesia.

No conflict of interest declared.

Mr I Made Dwi Mertha Adnyana (review guarantor). ORCID: 0000-0002-7167-7612. Department of Medical Professions, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, 36361 Indonesia; Associate Epidemiologists, Indonesian Society of Epidemiologists, Special Capital Region of Jakarta, Indonesia; Royal Society of Tropical Medicine and Hygiene, London, United Kingdom. Indonesia.

No conflict of interest declared.

Named contact

Dr Dominicus Husada (dominicushusada@yahoo.com). ORCID: 0000-0001-9191-9546. Department of Pediatrics, Faculty of Medicine, Universitas Airlangga, Surabaya 60286, Indonesia. Indonesia.

Review affiliation

Airlangga University

Funding source

Universitas Muhammadiyah Sidoarjo

TIMELINE OF THE REVIEW

Review timeline

Start date: 28 October 2024. End date: 31 March 2025

Date of first submission to PROSPERO

28 October 2024

Date of registration in PROSPERO

07 November 2024

CURRENT REVIEW STAGE

Publication of review results

Results of the review will be published in English.

Stage of the review at this submission

Review stage	Started	Completed
Pilot work	✓	✓
Formal searching/study identification	✓	✓
Screening search results against inclusion criteria	✓	✓
Data extraction or receipt of IPD	✓	✓
Risk of bias/quality assessment	✓	✓
Data synthesis	✓	✓

Review status

The review is completed.

ADDITIONAL INFORMATION

Additional information

none

PROSPERO version history

- [Version 1.1, published 07 Nov 2024](#)
- [Version 1.0, published 07 Nov 2024](#)

Review conflict of interest

None known

Country

Indonesia

Medical Subject Headings

Asia, Southeastern; Genetic Variation; Humans; Malaria, Falciparum; Merozoite Surface Protein 1; Plasmodium falciparum; Prevalence

Details of any existing review of the same topic by the same authors

none

Revision note

The study design, selection process, and data collection underwent modifications, and the entire review process was disclosed in detail and transparently. The stages of the process have been reported in accordance with the research objectives, and the manuscript is currently being submitted for publication. The following text is intended to provide a comprehensive overview of the subject matter.

Disclaimer

The content of this record displays the information provided by the review team. PROSPERO does not peer review registration records or endorse their content.

PROSPERO accepts and posts the information provided in good faith; responsibility for record content rests with the review team. The guarantor for this record has affirmed that the information provided is truthful and that they understand that deliberate provision of inaccurate information may be construed as scientific misconduct.

PROSPERO does not accept any liability for the content provided in this record or for its use. Readers use the information provided in this record at their own risk.

Any enquiries about the record should be referred to the named review contact