# THE LANCET **Global Health**

# **Supplementary appendix**

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: van Eijk AM, Stepniewska K, Hill J, et al. Prevalence of and risk factors for microscopic and submicroscopic malaria infections in pregnancy: a systematic review and meta-analysis. *Lancet Glob Health* 2023; published online June 2. https://doi.org/10.1016/S2214-109X(23)00194-8.

# **Supplement to: van Eijk et al., Prevalence of and risk factors for microscopic and submicroscopic malaria infections in pregnancy: a systematic review and meta-analyses**

### Authors

Anna Maria van Eijk, Kasia Stepniewska,Jenny Hill, Professor Steve M. Taylor, Professor Stephen J. Rogerson, Gilles Cottrell, R. Matthew Chico, Julie R. Gutman, Halidou Tinto, Holger W. Unger, Professor Stephanie K. Yanow, Manfred Accrombessi, Professor Ayola A. Adegnika, Rukhsana Ahmed, , Eliana María Arango Flórez, Professor Myriam Arévalo-Herrera, Emmanual Arinaitwe, Paulo Arnaldo, Professor Per Ashorn, Ulla Ashorn, Azucena Bardaji, Inoni Betuela, Praveen K. Bharti, Francis Bohissou, Professor Camila Bôtto-Menezes, Vera Braun, Valerie Briand, Jessica Briggs, Maria Eugenia Castellanos, Professor Daniel Chandramohan, Enesia Banda Chaponda, Professor Chetan E. Chitnis, Laura Cohee, Michel Cot, Professor Umberto d'Alessandro, Lise Denoeud-Ndam, Meghna Desai, Professor Alassane Dicko, Xavier Ding, Professor Grant Dorsey, Patrick E. Duffy, Professor Maha A. Elbadry, Sonia M. Enosse, Yue-Mei Fan, Nadine Fievet, Michal Fried, Professor Blaise Genton, Professor Raquel Gonzalez, Professor Brian Greenwood, Linda Kalilani, Johanna H. Kattenberg, Professor Kassoum Kayentao, Carole Khairallah, Professor Christopher L. King, Professor Dhanpat Kumar Kochar, Professor Swati Kochar, Felix Koukouikila-Koussounda, Sarah H. Landis, Professor Miriam K. Laufer, Professor Rose F. G. Leke, Eusebio Macete, Sonia Maculuve, Mwayiwawo Madanitsa, [Almahamoudou](https://pubmed.ncbi.nlm.nih.gov/?term=Mahamar+A&cauthor_id=34432975) Mahamar, Professor Ken Maleta, Indu Malhotra, Rella Zoleko Manego, Professor Flor Ernestina Martínez-Espinosa, Achille Massougbodji, Professor Don Mathanga, Michela Menegon, Professor Clara Menendez, Petra Mens, Professor Martin Meremikwu, Professor Frank P. Mockenhaupt, Professor Ghyslain Mombo-Ngoma, Dominic Mosha, Professor Ivo Müeller, Alain Nahum†, Paul Natureeba, Professor Nicaise Ndam, Professor Francine Ntoumi, Professor Olabisi A. Oduwole, Professor Bernard A. Okech, Maria Ome-Kaius, Kephas Otieno, Norma Padilla, Professor Michael Ramharter, Professor Rosemary Rochford, Professor Anna Rosanas-Urgell, Maria Ruperez, Katherine R. Sabourin, Sergi Sanz, Henk D. Schallig, Susana Scott, Professor Esperanca Sevene, Carlo Severini, Professor Harry Tagbor, Professor Diane Wallace Taylor, Professor Maminata Traore Coulibaly, Professor Ana-Maria Vasquez, Annie Walker-Abbey, Blair J. Wylie, Professor Djimon M. Zannou, Professor Steven R. Meshnick†, Professor Feiko O. ter Kuile, Professor Alfredo Mayor †Deceased

### Affiliations

Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, Liverpool L3 5QA, UK (A M van Eijk PhD, J Hill PhD, Prof F O ter Kuile PhD, C Khairallah MSc, H W Unger PhD); Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK (K Stepniewska PhD); Division of Infectious Diseases and Duke Global Health Institute, Duke University, Durham, NC, USA (S M Taylor MD); Department of Infectious Diseases, Doherty Institute, The University of Melbourne, Melbourne, Australia (Prof S J Rogerson PhD); Université de Paris, IRD, MERIT, F-75006 Paris, France (G Cottrell PhD, M Cot MD, N Fievet PhD, Prof N Ndam PhD); London School of Hygiene & Tropical Medicine, London, UK (R M Chico PhD, Prof D Chandramohan PhD, Prof B Greenwood MD, Prof U d'Alessandro PhD, S Scott PhD); Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA (J R Gutman MD); Institut de Recherche en Sciences de la Santé - Unité de Recherche Clinique de Nanoro, Burkina Faso (Prof H Tinto PhD, Prof M Traore PhD); Menzies School

of Health Research, Charles Darwin University, Darwin, Australia (H W Unger PhD); School of Public Health, Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada (Prof S K Yanow PhD); Institut de Recherche Clinique du Benin, Abomey-Calavi, Benin (M Accrombessi MD); Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon (Prof A A Adegnika PhD, R Z Manego MD, Prof G Mombo-Ngoma PhD); Eijkman Institute for Molecular Biology, Jakarta, Indonesia (R Ahmed PhD); Grupo Salud y Comunidad, Grupo Malaria Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia (E M Arango-Flórez PhD, Prof A-M Vasquez PhD); Caucaseco Scientific Research Center/Universidad del Valle, Cali, Colombia (Prof Myriam Arévalo-Herrera PhD); Infectious Diseases Research Collaboration, Kampala, Uganda (E Arinaitwe PhD); Instituto Nacional de Saúde, Maputo, Mozambique (P Arnaldo PhD, S M Enosse PhD); Center for Child, Adolescent and Maternal Health Research, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland (Prof P Ashorn PhD, U Ashorn PhD, Y Fan PhD); ISGlobal, Barcelona Institute for Global Health, Hospital Clínic-Universitat de Barcelona, Barcelona, Spain (A Bardaji PhD, Prof A Mayor PhD, Prof R Gonzalez PhD, Prof C Menendez PhD, M Ruperez Phd, S Sanz MSc); Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea (I Betuela MD, M Ome-Kaius PhD); ICMR-National Institute of Malaria Research, New Delhi, India (P K Bharti PhD); Centre de Recherche Entomologique de Cotonou, Cotonou, Benin (F Bohissou MD, A Nahum PhD); Tropical Medicine Foundation Dr. Heitor Vieira Dourado, Manaus, Brazil (Prof C Bôtto-Menezes PhD, F E Martinez-Espinosa); Institute of Tropical Medicine and International Health, Charité-University Medicine Berlin, Berlin, Germany (V Braun PhD, Prof F P Mockenhaupt MD); IRD, Inserm, Université de Bordeaux, GHiGS team, UMR 1219, Bordeaux, France (V Briand MD); Department of Medicine, University of California, San Francisco, CA, USA (J Briggs MD, Prof G Dorsey MD); Public Health and Tropical Medicine, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia (M E Castellanos PhD); Department of Biological Sciences, University of Zambia, Lusaka, Zambia (E B Chaponda PhD); Malaria Parasite Biology and Vaccines, Department of Parasites & Insect Vectors, Institut Pasteur, Paris, France (Prof C Chitnis PhD); Center for Vaccine Development and Global Health, University of Maryland School of Medcine, Baltimore, MD USA (L Cohee MD, Prof M K Laufer MD); Epicentre, Paris, France (L Denoeud-Ndam MD); U.S. Centers for Disease Control and Prevention, New Delhi, India (M Desai PhD); Malaria Research & Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Sciences Techniques and Technologies of Bamako, Bamako, Mali (Prof A Dicko MD, A Mahamar PhD); FIND, Geneva, Switzerland (X Ding PhD); National Institute of Allergy and Infectious Diseases, Bethesda, United States (P E Duffy MD, M Fried PhD, A Walker-Abbey PhD); Department of Environmental and Global Health and Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA (Prof M A Elbadry PhD, Prof B A Okech PhD); Swiss Tropical and Public Health Institute, Basel, Switzerland (Prof B Genton PhD); College of Medicine, University of Malawi, Blantyre, Malawi (L Kalilani PhD); Department of Biomedical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium (J H Kattenberg PhD, Prof A Rosanas-Urgell PhD); University of Sciences, Techniques, and Technologies of Bamako, Bamako, Mali (Prof K Kayentao PhD); Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA (Prof C L King PhD, I Malhotra PhD); Sardar Patel Medical College and Associated Group of Hospitals, Bikaner, Rajasthan, India (Prof D K Kochar MD, Prof S Kochar MD); Fondation Congolaise pour la Recherche Médicale, Brazzaville, République du Congo (F Koukouikila-Koussounda PhD); Epidemiology and Real World Evidence, Biomarin, London, UK (M K Landis PhD); Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Yaounde, Cameroon (Prof R F G Leke PhD); Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique (E Macete PhD, S Maculuve MD, Prof E Sevene PhD, Prof A Mayor PhD); Department of Clinical Sciences, Malawi University of Science and Technology, Thyolo, Malawi (M Madanitsa PhD);

School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi (Prof K Maleta PhD, Prof D Mathanga PhD); Leônidas & Maria Deane Institute FIOCRUZ, Manaus, Brazil (Prof F E Martinez-Espinosa PhD); Faculté des Sciences de la Santé, Université d'Aboméy Calavi, Cotonou, Benin (A Massougbodji PhD); Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy (M Menegon MSc, C Severini PhD); Department of Medical Microbiology and Infection Prevention, Amsterdam University Medical Centers, Amsterdam, the Netherlands (P Mens PhD, H D Schallig PhD); Department of Paediatrics, University of Calabar, Calabar, Nigeria (Prof M Meremikwu MD); Ifakara Health Institute, Ifakara, Tanzania (D Mosha PhD); Population Health & Immunity Division, Walter and Eliza Hall Institute, Melbourne, Australia (Prof I Mueller PhD); Makerere University-John Hopkins University collaboration, Kampala, Uganda (P Natureeba MD); Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana (Prof N Ndam PhD); Institute for Tropical Medicine, University of Tübingen, Germany (Prof F Ntoumi PhD, Prof A A Adegnika PhD); Department of Medical Laboraory Science, Achievers University Owo, Nigeria (Prof O A Oduwole PhD); Malaria Branch, KEMRI/Centre for Global Health Research, Kisumu, Kenya (K Otieno MSc); Universidad del Valle de Guatemala, Guatemala City, Guatemala (N Padilla PhD); Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & University Medical Center Hamburg-Eppendorf, Hamburg, Germany (Prof M Ramharter MD, R Z Manego, Prof G Mombo-Ngoma); Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA (Prof R Rochford PhD, K R Sabourin PhD), University of Health and Allied Sciences, Ho, Ghana (Prof H Tagbor); Department of Tropical Medicine, Medical Microbiology & Pharmacology, John A. Burns School of Medicine University of Hawaii, Honolulu, Hawaii, USA (Prof D W Taylor PhD); Columbia University Irving Medical Center, New York, United States (B J Wylie MD); Faculté des sciences de la santé de Cotonou, Université d'Abomey-Calavi, Bénin (Prof D M Zannou MD); Institute for Global Health and Infectious Diseases, School of Medicine, University of North Carolina, Chapel Hill, NC, USA (Prof S R Meshnick PhD).

# <span id="page-4-0"></span>Table of Contents Supplement





# <span id="page-6-0"></span>Supplemental Methods

### <span id="page-6-1"></span>Supplement 1: Abbreviation list (alphabetical order)



### <span id="page-6-2"></span>Supplement 2: Supplemental information on search

The first mention of PCR for malaria identification in the Malaria in Pregnancy library was in 1998; we used 1997 to detect additional materials that might have been missed in the library using the terms "Polymerase chain reaction" OR PCR OR subpatent OR submicroscopic OR sub-patent OR submicroscopic OR Lamp OR "loop-mediated isothermal amplification". The search was repeated in Pubmed (450 results), Google Scholar (446 results) and the Global Health database with "AND pregnan\* AND malaria" added to the search terms. No new studies were identified relative to the search using the Malaria in Pregnancy Library database. We also searched reference lists and review articles identified in the primary search. We contacted researchers with relevant publications to identify any further, unpublished studies. Note that in some studies, PCR was used on samples that were preserved from older studies, with the inclusion of studies conducted before 1998 as a result. The last search was conducted on November 10, 2021. An update of the search using the Malaria in Pregnancy Library and PubMed from November 10, 2021, to 26 November 2022, resulted in 36 and 33 entries, respectively; after deduplication of the combined file, 48 entries from 40 studies remained and five new studies with potential information on submicroscopic malaria were identified. There was insufficient information in these five studies for data-extraction. These five studies were not included in the analyses described in this paper.

This study was registered in Prospero-CRD42015027342 as a systematic review and not as an individual participant data analysis. This will be corrected at a later stage*.*

Pubmed search: ("Polymerase chain reaction" OR PCR OR subpatent OR submicroscopic OR subpatent OR sub-microscopic OR Lamp OR "loop-mediated isothermal amplification") AND pregnan\* AND Malaria AND 1997/01/01:2021/10/01[dp]



## <span id="page-7-0"></span>Supplement 3: Requested variables for IPD analysis

We emailed authors of eligible studies to inform about the study and inquire about their interest. Authors were emailed at least three times before deciding not to include the study as IPD, unless they informed us about their interest. When authors were not interested to take part, or could not be contacted, or for other reasons did not take part, an attempt was made to extract the data needed. The following variables were requested from IPD studies: Study identity number, date of visit, malaria test (microscopy, PCR, cRDT, LAMP), malaria test result, species and country where available, source of blood for malaria test at delivery (maternal or placental), gravidity, age, history of fever, body temperature, history of recent antimalarial treatment, gestational age, net use, ITN use, IPTp use or other type of malaria prevention, haemoglobin, and treatment arm if part of a trial. If at delivery: singleton, birth outcome (live or stillbirth), birthweight, newborn sex. Where available: HIV status, rural residence, IRS, smoker, iron and folate supplementation, maternal anthropometry and placental histology. These last four variables were not used in the current analyses. Each individual dataset was checked for ranges and consistency; information was compared with publications where possible, and variables were created or reformatted for merging. No irregularities were identified when checking the IPD data. For aggregated data, the study information was extracted independently by AMvE and AM using excel sheets and compared, and only used if both persons agreed on the extracted information.

### <span id="page-7-1"></span>Supplement 4: Supplemental information on quality assessment

In this individual patient data analysis, participating studies were surveys, trials and cohorts. Although some included studies were trials, these were not selected because of their research question but because of the availability of the exposure and outcomes of interest (submicroscopic malaria). For this reason, we used an adaptation of the Newcastle Ottawa Scale. We assessed the availability of primary exposures (malaria by test and compartment) and outcomes of interest (submicroscopic malaria prevalence, birthweight, gestational age, haemoglobin), and if the assessment of the outcomes was blind for the exposure, as far as could be assessed. Confounders were defined as factors that were associated with the exposure and the outcome but were not on the causal pathway. We assessed whether important known confounders were available such as age, gravidity, setting (rural or urban place of living) and HIV status. We recorded the availability of co-variates such as recent malaria treatment and malaria prevention (net use, ITN, IRS, IPTp). In the current study, submicroscopic malaria prevalence was our outcome of interest, and we used the criteria as described in Table S0. Studies could attain one point for each question, with a maximum of six points. We considered scores of 5 or 6 as indicators of high quality of the contributing data. Studies were not excluded from the analyses based on their quality score, but a sensitivity analysis was conducted to assess the impact of low-quality studies. The quality score information was included in the sensitivity analysis as a dichotomy (score of 5 or 6 vs. <5) or continuous score, to assess if results would differ by quality of the study (Table S23). We used the same criteria for studies where data was extracted from the articles when we were not able to retrieve the data set.

### *Table S0: Criteria for quality assessment*



### <span id="page-8-0"></span>Supplement 5: Supplemental information on variables and multivariable models

We added information on geospatial coordinates of the study sites (longitude and latitude obtained from Google Earth: [https://www.google.com/earth/\)](https://www.google.com/earth/), and the prevalence of sulfadoxinepyrimethamine (SP) resistance markers for studies in Africa only (Ala437Gly and Lys540Glu substitutions in the *dhps* gene), obtained from the publication, study authors, or existing prevalence maps of *P. falciparum dhps* mutations).<sup>1,2</sup>

The following covariates were included in the analyses as appropriate and available:

- Maternal covariates: Age, gravidity, HIV infection, use of malaria prevention (ITNs, IPTp, IRS) or antimalarials, gestational age or trimester in pregnancy or at delivery
- Location or study site co-variates: malaria season, location of living (rural vs. urban), indicator of malaria transmission intensity for study site at the time of study, prevalence of molecular markers of SP resistance in the study area around the time of the study (*Pfdhps*-A437G and *Pfdhps*-K540E), species, region

• Study level covariates: study design, risk of bias assessment, details of blood smear reading. Random effects models were used to allow for heterogeneity in risk across studies. The  $I^2$  was used as indicator of heterogeneity.

Studies in Asia and the Pacific were combined as "Asia". An indicator of malaria transmission intensity was obtained from the Malaria Atlas Project (2020-layer, [https://malariaatlas.org\)](https://malariaatlas.org/) for the mid-year of the study for meta-analyses and for the study year for the one-stage IPD analyses. The Malaria Atlas Project estimates reflect the average prevalence of *P. falciparum* infection among children 2-10 years of age to within five kilometres of any location. We defined low malaria transmission as a *Pf*PR<sub>2-10</sub> <10%, moderate transmission as PfPR<sub>2-10</sub> 10-34%, and high transmission as *PfPR*<sub>2-10</sub> ≥35%. Low transmission can be considered similar to hypo-endemic malaria.<sup>3</sup> Hyperendemic and holo-endemic malaria are officially defined as a *PfPR*<sub>2-10</sub> of 50-75%, and >75%, respectively; however, malaria transmission has declined and in our participating studies only five were in high and one in a holo-endemic transmission area.<sup>4</sup> To obtain meaningful sample sizes, we reduced the cut-off for high transmission to *Pf*PR<sub>2-10</sub> ≥35%.

Age was used in three groups, defined as <20 years, 20-29 years, and 30+ years, which was used as baseline. Gravidity was used in three groups (primigravidae, secundigravidae and gravidae 3+). A few studies had age data and gravidity only as categories available and not as a continuous variable, and for this reason, the total number available in categories is higher than for age or gravidity as continuous variables.

We checked collinearity between age and gravidity before including these variables in multivariate analysis. We evaluated collinearity using the variance inflation factor (VIF) and tolerance, defined as 1/VIF. A tolerance value lower than 0.1 is comparable to a VIF of 10 and may merit further investigation.<sup>5</sup> Results were as follows:



To indicate net use, we defined "any net" to indicate a net of any type that was used as defined by the study, e.g. in the previous night, during pregnancy, or available in the household, and ITN use as reported by the participant. Several trials and cohorts provided ITNs at the time of enrolment in pregnancy; if this was reported, we considered at the time of delivery that these participants used ITNs. This information was not included in the variable "any net use" at the time of delivery. There was wide variety in report of antimalarials for time period, number of times and type of antimalarial. Additionally, trials used several different treatments as IPTp or IST. We combined reported antimalarial use and IPTp in one variable, indicating a history of antimalarial use in pregnancy. For malaria prevalence during pregnancy, gestational age was included in multivariable models as a continuous variable, whereas at the time of delivery we included the variable "preterm", to indicate if the gestational age was before 37 weeks of gestation at the time of delivery. Rainy season was defined according to the dataset where this was available or using the date variable and information from the source article or author where available, or from the internet for the location of the study when first-mentioned sources were not available. For studies where no visit date was available, we assigned the midyear of the study as study year. To assess the effect of quality of blood smear reading, variables were created for each study indicating the number of high power fields examined before declaring a slide negative (100 high power fields versus 200 or more), and a variable

indicating number of persons reading the slides (1 person and 10% quality control versus 2 persons and a third person in case of disagreements).

*Prevalence.* Estimates for prevalence by study were generated within the merged dataset for the IPD analyses. A dataset was created with prevalence by study, and this was merged with the dataset from individual studies from the extracted data for the two-stage analysis. The Freeman-Tukey double arcsine transformation was used for metaprop.6 The variance stabilizing transformation of the proportions as proposed by Freeman and Tukey normalizing the outcomes of proportions before pooling, $6$  is defined as;

$$
\sin \sqrt{\frac{r_i}{n_i+1}} + \sin \sqrt{\frac{r_i+1}{n_i+1}}
$$

The asymptotic variance of the transformed variable is defined as  $\frac{1}{n_1+0.5}$ .

This transformation is intended to achieve approximate normality. This method has the advantage that the variances of the arcsine-based transformations depend only on the sample sizes, which are typically fixed, known values.<sup>8</sup> A continuity correction of 0.5 was used for cells with 0-values in the meta-analysis of odds ratios.9

Because of the high heterogeneity, the pooled summary estimate of the prevalence of submicroscopic and microscopic malaria was reported in combination with the median and range, allowing the readers their own judgment, and providing a range that may cover the likely result. Individual participant data analysis was conducted to try to address some of the heterogeneity through the covariate adjustment for dataset where the individual participant data was available.

*Fever.* For the random-effect multivariable logit models of fever (xtlogit), the main interest was the relationship between malaria infection and fever, and the modifying effect of transmission level or gravidity (as potential indicators of background immunity) on this relationship. Adjustment was made for base model covariates and gestational age. Although we explored the effects of history of antimalarial use, ITN or any type of net use, HIV infection, and IRS, we did not use these variables in the models because of the drop in sample size; however, the most important results are described in supplement 8. We only presented the relationship between malaria and fever in pregnancy because at delivery, multiple additional causes of fever can be present due to the delivery process.

*Species.* Malaria species was not examined for placental blood because of the differences in ability to sequester in the placenta by species.

*Factors associated with microscopic and submicroscopic malaria.* We explored factors which are known to affect exposure to infected mosquito bites (incidence of infection: ITN use and net use, IRS, rainy season, level of malaria transmission, continent, rural vs. urban setting), factors associated with clearance of parasites in the blood (antimalarial treatment) and factors known to affect immunological response to malaria (age, gravidity, gestational age and HIV-status). Multiple imputations could not be conducted for missing information, as these variables were not missing at random but, in many cases, reflected the study design/protocol. The last group of factors may affect chronicity of the infection, once an infection has been acquired. Multivariable models were made for Africa and America/Asia separately. In areas of moderate and high malaria transmission, a gravidity specific pattern of malaria is well known, with primigravidae having the highest prevalence of malaria, and a decrease in prevalence with increasing pregnancy number which may be due to the development of gravidity specific immunity to malaria.<sup>10,11</sup> In most high transmission regions the prevalence does not differ much by gravidity among gravida 3+. However, in areas of low

transmission, such a pattern is not seen, with primi- and multigravidae having a similar prevalence of malaria. Because of these patterns, an interaction term between gravidity and transmission level was explored in models for Africa. The model improved with the interaction term, and for this reason in Africa analyses were stratified by by high (*Pf*PR2-10 ≥35%) and moderate-to-low transmission areas (*Pf*PR2-10 <35%); Gravidity was divided into primigravidae [G1], secundigravidae [G2], and multigravidae [G3+], except for analyses with non-convergence, when categories could be combined. . There were no known HIV-infected participants in America/Asia, so this covariate was not evaluated in this region. In some models at delivery, study design could not be included because of collinearity.

*Outcome of submicroscopic malaria at enrolment in the consecutive scheduled visit.* Nine studies had information on submicroscopic malaria at follow up visits. All studies had scheduled study visits every 4-6 weeks. We explored the outcome of submicroscopic malaria at enrolment at the subsequent scheduled study visit. Note that submicroscopic malaria at enrolment is generally detected too late to have clinical consequences in the form of treatment; however, in trials women generally will receive treatment at enrolment, and in cohort studies in Africa, IPTp with SP may have started. To avoid labelling inadvertent unscheduled as scheduled study visits, a minimum interval of 14 days was used between enrolment and consecutive scheduled study follow up visit. No maximum limit was set to allow the largest sample size available. We calculated prevalence of submicroscopic, microscopic and no malaria infection at the 2<sup>nd</sup> scheduled study visit when submicroscopic malaria was present at enrolment and calculated the time interval in days. We explored several definitions for time interval between visits, with or without upper or lower limits and these models gave similar results. Co-variates explored were base model variables, interval between study visits in days, markers of SP resistance and antimalarial treatment at enrolment, and all were initially included in the multivariable model. All locations in moderate-to-low transmission areas were in high transmission areas, and for this reason, a combination variable of SP resistance and transmission level was created, indicating low-moderate transmission-high SP resistance, high transmission-low SP resistance, and high transmission-high SP resistance. Factors with a p-value >=0.1 (Wald test) in both parts of the model (for submicroscopic and microscopic malaria) in the multivariate model were removed. Additionally, removed factors were one by one tested (and added) if they improved the subsequent multivariable model with the p-value <0.1 (in at least one part of the model).

### <span id="page-11-0"></span>Supplement 6: Differences with protocol

The protocol is available at https://www.wwarn.org/tools-resources/subpatent-malaria-studygroup-protocol. In the main multivariable analyses, variables were not removed based on their pvalues, because this would not allow comparison of the effect of variables across time points, level of malaria transmission or region. Because of the variability in availability of co-factors, the choice was made for a base model and additional factors with limited sample size. This study was restricted to microscopic and submicroscopic malaria; RDTs, placental histology and NAAT-test characteristics were not evaluated in the current article. To allow assessment of the different categories at the same time (microscopic, and submicroscopic malaria versus no infection), a multinomial model was used instead of xtlogit. Because of the high observed heterogeneity, prevalence results were also summarised as study median and range.

# <span id="page-12-0"></span>Supplemental Results

# <span id="page-12-1"></span>Supplement 7: Molecular methods used, proportion of microscopy positive/NAAT-negative results and sensitivity of microscopy Of the 68 participating studies, quantititative real time PCR was used in 30 studies (44.1%), nested PCR in 31 studies (45.6%) and 4 studies used LAMP (5.9%). For the three remaining studies, the molecular method was unavailable for one study; one used both methods, and one used LDRFMA ("polymerase chain reaction /ligase detection reaction-fluorescent microsphere assay", Stanisic et al. 2015).<sup>12</sup> The 18S rRNA gene was most commonly (67·6%) targeted in studies using PCR.

The proportion of microscopy positive/PCR negative test results ranged from 0-5.9% in pregnancy (n=54, mean 1.0%, sd 1.3, median 0.6, IQR 0-1.6%; median of 0 in 11 subgroups in the Americas, 1.3%, IQR 0.1-2.0% in 11 subgroups in Asia, and 1.0%, IQR 0.2-2.0% in 32 subgroups in Africa; [Table](#page-30-0)  [S9\)](#page-30-0). The pooled sensitivity of microscopy to detect NAAT-positive infections was 36.7% (30.8-42.9, *I 2* =97%) during pregnancy, 27.4% (22.6-32.5, *I 2* =91%) for maternal and 26.7% (21.4-32.4, *I 2* =90%) for placental blood at delivery.

### <span id="page-12-2"></span>Supplement 8: Additional information on fever and malaria

Gravidity was not associated with fever in the overall model and in America/Asia, but the odds of fever were higher among primigravidae in the model for Africa overall (aOR=1.58, 1.12-2.23, p=0.0095 compared to gravidae 3+), and in high malaria transmission areas (aOR=2.29, 1.08-2.62, p=0.0222), but not in moderate-to-low transmission areas (aOR 1.42, 0.81-2.50, p=0.22). Only in the model for Africa overall was an interaction noted between transmission level and gravidity. An age <20 years was associated with fever in moderate-to-low transmission areas in Africa as a protective factor (aOR=0.38, 0.18-0.78, p=0.0092). Of the variables available for a limited sample size (HIV, antimalarial use for treatment or prevention, ITN or bednet use), antimalarial use was associated with fever in the overall model (aOR=2.02, 1.48-2.77, p<0.0001, N=8094), and ITN use was a protective factor (aOR 0.69, 0.51-0.94, p=0.0166, N=7967). Results for malaria tests at delivery were similar to pregnancy (data not shown). The probability of being febrile decreased with the progression of pregnancy but this trend was not malaria specific as it was evident among women with microscopic infections, submicroscopic infections and uninfected women [\(Figure S3\)](#page-59-0); gestational age was associated with fever in the overall model (aOR 0.98, 0.97-0.99, p<0.0001), but the interaction terms between gestational age and malaria were not significant (p=0.29 and p=0.43 for interaction term of gestational age with submicroscopic and microscopic malaria, respectively).

# <span id="page-12-3"></span>Supplement 9: Additional analyses

Evaluation of predictors of infection that were restricted to data collected at the first ANC visit only, reduced the sample size to about one third (Africa) and a quarter (Americas/Asia) of the full sample. There was no data from  $1<sup>st</sup>$  ANC visits among multigravidae in high transmission areas or the Americas (Table S15). Except for the model in moderate transmission areas, it was not possible to include *PfPR*<sub>2-10</sub>. Compared to the full model, less factors were associated with malaria infections, but that may have been due to the difference in sample size. In both transmission areas in Africa, young women (<20 years compared to 30+ years) and primigravidae (compared to gravidae 3+) were at risk for microscopic malaria, and young age remained a risk factor for submicroscropic malaria. In Asia, microscopic infections were more prevalent among women < 20 years (aOR 2.60, 95% CI 1.37- 4.93) compared to women aged 30+ years.

Submicroscopic malaria was present throughout pregnancy and this was consistent by region, transmission intensity and gravidity [\(Figure S6\)](#page-67-0). Only in primi- and secundigravidae in high

transmission areas in Africa was the proportion of submicroscopic malaria less than microscopic malaria; for multigravidae and all gravidities in other transmission levels, submicroscopic infections were more common than microscopic among NAAT-positive infections [\(Figure S6\)](#page-67-0). Among studies with follow-up visits, the longest documented period of submicroscopic malaria was 161 days over five consecutive visits for a woman in Benin.

In Africa, compared to using 200+ high power fields before declaring a negative smear, using 100 power fields was associated with a decreased detection of malaria, whereas the use of 1 reader and 10% quality control compared to 2+ readers was associated with an increase in the detection of microscopic but not submicroscopic malaria (no malaria as reference group, Table S25). In this African model, rainy season changed from a significant into a non-significant factor for microscopic malaria; the association between other significant factors (age, gravidity and transmission level) strengthened or stayed the same. In the Americas and Asia, no effect of quality of slide reading was detected on the detection of malaria, and the association between malaria and other factors (age, gravidity, transmission) remained similar (Table S25).

In sensitivity analyses evaluating the quality of blood smear reading, only in moderate-to-low transmission areas in Africa an effect was seen, with definition of a negative slide as "no parasites in 100 high power fields" resulting in lower detection of microscopic malaria (aOR 0.32, 95%CI 0.19- 0.55) compared to a definition of "no parasites in 200+ high power fields" (Table S25). Having 1 reader and 10% quality control of slides resulted in a higher prevalence of microscopic malaria (aOR of 4.31, 2.21-8.41) compared to 2+ readers. Indicators of the quality of blood smear reading were not predictive in high transmission areas in Africa or in the Americas/Asia.

Using a different indicator of transmission level (malaria infection by PCR in the first trimester among all gravidae) resulted in different sample size by transmission region in Africa. However, overall the same variables remained important as predictors of submicroscopic and microscopic malaria (Table S25). In Africa in moderate-to-low transmission areas, the aOR of PfPR<sub>2-10</sub> for submicroscopic malaria was 0.97, 0.94-0.99 (indicating a 3% decrease in submicroscopic malaria with 1% increase in transmission level); the corresponding aOR for the alternative transmission indicator was 1.12, 1.04-1.21 (indicating a 12% increase in submicroscopic malaria with a 1% increasing transmission level, which seemed high). In the Americas/Asia, the aOR of *PfPR*<sub>2-10</sub> for microscopic malaria was 1.00, 0.96-1.04 (p=0.98); the corresponding aOR for the alternative transmission level indicator for microscopic malaria was 1.04, 1.01-1.07 (p=0.0130) (Table S25).

A funnel plot for included studies for submicroscopic malaria infection in pregnancy can be seen in Figure S7; The graph looks reasonably symmetrical, suggesting no clear publication bias. The p-value for the Egger test was 0.43, suggesting there was no indication of a small study effect.

Information on existing versus newly diagnosed HIV infection as part of antenatal screening was not available to differentiate between women with known HIV infection on antiretroviral therapy and prophylaxis with daily cotrimoxazole (which has antimalarial properties)13 with known HIV infection versus women with newly diagnosed HIV infections not yet on antiretrovirals and cotrimoxazole. It was encouraging to see that treatment with dihydroartemisinin-piperaquine was associated with protection from submicroscopic infections at the subsequent visit among the limited sample of women from whom information was available at enrolment and follow-up. This indicates that effective malaria treatment reduces subsequent risks for submicroscopic malaria.

### **References**

1. Malaria Atlas Project. Maps. 2023.

[https://data.malariaatlas.org/maps?layers=Malaria:202206\\_Global\\_Pf\\_Parasite\\_Rate](https://data.malariaatlas.org/maps?layers=Malaria:202206_Global_Pf_Parasite_Rate) (accessed April 05, 2023).

2. World Wide Antimalarial Resistance Network (WWARN). Molecular Surveyor. 2023. <http://www.wwarn.org/dhfr-dhps-surveyor/#0> (accessed January 23, 2023).

3. Hay SI, Smith DL, Snow RW. Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infect Dis* 2008; **8**(6): 369-78.

4. Weiss DJ, Lucas TCD, Nguyen M, et al. Mapping the global prevalence, incidence, and mortality of Plasmodium falciparum, 2000-17: a spatial and temporal modelling study. *Lancet* 2019; **394**(10195): 322-31.

5. O'Brien MO. A caution regarding rules of thumb for variance inflation factors. *Quality & Quantity* 2007; **41**: 673-90.

6. Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. *Arch Public Health* 2014; **72**(1): 39.

7. Freeman MF, Tukey JW. Transformations related to the angular and the square root. *Ann Math Stat* 1950; **21**(4): 607-11.

8. Lin L, Xu C. Arcsine-based transformations for meta-analysis of proportions: Pros, cons, and alternatives. *Health Sci Rep* 2020; **3**(3): e178.

9. Ren Y, Lin L, Lian Q, Zou H, Chu H. Real-world performance of meta-analysis methods for double-zero-event studies with dichotomous outcomes using the cochrane database of systematic reviews. *J Gen Intern Med* 2019; **34**(6): 960-8.

10. Mayor A, Bardaji A, Macete E, et al. Changing trends in *P. falciparum* burden, immunity, and disease in pregnancy. *N Engl J Med* 2015; **373**(17): 1607-17.

11. Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 2007; **7**(2): 93-104.

12. Stanisic DI, Moore KA, Baiwog F, et al. Risk factors for malaria and adverse birth outcomes in a prospective cohort of pregnant women resident in a high malaria transmission area of Papua New Guinea. *Trans R Soc Trop Med Hyg* 2015; **109**(5): 313-24.

13. Klement E, Pitche P, Kendjo E, et al. Effectiveness of co-trimoxazole to prevent *Plasmodium falciparum* malaria in HIV-positive pregnant women in sub-Saharan Africa: an open-label, randomized controlled trial. *Clin Infect Dis* 2014; **58**(5): 651-9.

# Supplemental Tables

# Table S1: Characteristics of included studies, in order of continent and time period of study

<span id="page-15-1"></span><span id="page-15-0"></span>



16





NA: Not available. NR, not reported.

Only for Pf: NAAT only for Pf detection. Only Pf: NAAT species detection not specified, but only Pf infections reported

\*ITN use reported at enrolment in pregnancy for surveys or cohorts in pregnancy, or at enrolment for surveys at delivery; use during pregnancy or use in the last night

† Any net, not clear if ITN or not

*Van Eijk et al Supplement sMIP IPD-meta (03apr23)\_suppl\_no\_tc*

# Table S2: Description of malaria treatments, IPTp and study arms of trials in IPD studies (by continent and year of study)

<span id="page-19-0"></span>





AZ, azithromycin. ACT, artemisinin-based combination therapy. AQ, amodiaquine. AS, artesunate. CQ, chloroquine. CTX, cotrimoxazole. DP, dihydroartemisinin–piperaquine. EFV, efavirenz-based antiretroviral therapy. LPV-r, lopinavir/ritonavir-based antiretroviral therapy. NA, not applicable or available. IPT, intermittent preventive treatment. IPTp, intermittent preventive treatment in pregnancy. IST, intermittent screening and treatment. MQ pyrimethamine. PQ, primaquine. SP, sulfadoxine-pyrimethamine. ST: screen and treat.

<span id="page-22-0"></span>

## Table S3: Risk of bias assessment of included IPD studies



# Table S4: Risk of bias assessment of included aggregated data studies

<span id="page-24-0"></span>

# Table S5: Characteristics of participants by timing of test and source of blood, IPD and aggregated data



\*Substudies: different locations within study, or groups if stratified enrolment

<span id="page-25-0"></span>†One study with locations both in Americas and Asia

‡Studies could be conducted in different regions or areas with different malaria endemicity



<span id="page-26-0"></span>Abbreviations: HIV, human immunodeficiency virus. IPD, individual participant data. NAATs, nucleic acid amplification tests (PCR or LAMP). ITN, insecticide treated net. IRS, indoor residual spraying.

\*Participants with microscopy positive/PCR negative test results excluded

† In brackets (number of studies where variable is missing because data was not collected or available | number of participants involved; number of studies with some participants missing this information | number of partic this variable is missing)

‡A history of fever in the past week or documented fever as defined by the study

§Either reported ITN use at enrolment or delivery or received ITN at the start of a cohort or trial in pregnancy (delivery only)

At delivery, 43% of women with a maternal blood result and 49% of women with a placental blood result had received 2 or more doses of an antimalarial

# Table S7: Pregnancy: Prevalence of malaria by microscopy compared to NAATs by study (IPD, order by region and country)

<span id="page-27-0"></span>



Abbreviations: BS: microscopy. LAMP: loop-mediated isothermal amplification. NAAT: nucleic acid amplification test. PCR: polymerase chain reaction. PNG: Papua New Guinea. G12: women in their first and second pregnancy. G35: women in their third to fifth pregnancy. For analyses in the main paper, the groups "microscopy" positive/NAAT-negative" has been excluded.

\**Pf*PR2-10: Estimated prevalence in the study area for the mid-year of the study for *P. falciparum* among children 2-10 years of age (2020-layer, Malaria Atlas Project: https://malariaatlas.org). † LAMP was used in the following studies: Ahmed 2019,<sup>13</sup> Kakuru 2016,<sup>47</sup> Briggs,<sup>50</sup> Vasquez 2018<sup>4</sup> and Vasquez 2020<sup>5</sup>. ‡ Enrolment in this study was stratified by gravidity.

Several studies had enrolment criteria that may have affected parasite prevalence, these included (where known, in alphabetical order of first author): Ahmed 2019<sup>13</sup>: HIVnegative women, no severe malaria, no antimalarial treatment in previous month. Chico  $2017^{89}$ : No antimalarials or antibiotics in the previous 4 weeks. Daud  $2014^{38}$ : Hb  $>= 7.5$ g/dl. Denoeud-Ndam 201334: HIV-infected women, last SP dose one month before enrolment, 2 weeks after any other antimalarial intake. Desai 201543: HIV-negative women, no IPTp received yet, no severe anaemia (not further defined). Gavina 2018<sup>3</sup>: No antimalarial treatment in the past 2 weeks, no signs of severe malaria. Kakuru 2016<sup>47</sup>: HIVnegative women. Kapito-Tembo 2010:<sup>55</sup> HIV-infected women. Madanitsa 2016<sup>39</sup>: HIV-negative women, 1<sup>st</sup> ANC visit, Hb >7 g/dl, no IPTp received yet. Matangila 2014<sup>61</sup>: No fever or other symptoms of malaria. Ntoumi 2016<sup>42</sup>: No history of clinical malaria in the past 2 weeks, no fever for the past 48 hours, axillary temperature ≤37.5° at enrolment. Quakyi 2019<sup>66</sup>: HIV-negative women. Stanisic 2015<sup>9</sup>: 1<sup>st</sup> ANC visit, Hb ≥5 g/dl. Tadesse 2020<sup>68</sup>: No severe malaria symptoms, no antimalarials in the past 4 weeks. Unger 2015<sup>90</sup>: 1<sup>st</sup> ANC visit, Hb ≥6 g/dl. Vasquez 2018<sup>4</sup>: No antimalarials in the past three days. Vasquez 2020<sup>5</sup>: No history of malaria or antimalarial use in the past 3 months, no positive malaria test at previous ANC, no severe malaria. Williams 201635: 1st ANC visit.

*Van Eijk et al Supplement sMIP IPD-meta (03apr23)\_suppl\_no\_tc*

# Table S8: Delivery: Prevalence of malaria by microscopy compared to NAATs by study (IPD, order by region and country)

<span id="page-29-0"></span>



Abbreviations: BS: microscopy. LAMP: loop-mediated isothermal amplification. NAAT: nucleic acid amplification test. PCR: polymerase chain reaction. PNG: Papua New Guinea. G12: women in their first and second pregnancy. G35: women in their third to fifth pregnancy. For analyses in the main paper, the group "microscopy positive/NAAT-negative" has been excluded. \*  $PfPR_{2-10}$ : Estimated prevalence in the study area for the mid-year of the  $\mu$  and the manner and the contract of the c this study was stratified by gravidity.

Table S9: Median and interquartile range of microscopy positive/NAAT negative test results overall and by region



<span id="page-30-0"></span>IQR, interquartile range. N, number of substudies. NAAT, nucleic acid amplification test.

# Table S10: Pregnancy: Pooled prevalence and odds ratio of fever by malaria test results



<span id="page-31-0"></span>Abbreviations: aOR, adjusted odds ratio. CI, confidence interval. OR, Odds ratio.

Factors with a p-value  $\leq 0.05$  printed in bold

Notes: The following stata-procedures were used: pooled prevalence: metaprop, two-stage analyses OR: metan, one-stage analyses OR: xtlogit. Note that for the procedures meta-prop and metan, studies with 0-values in sample the control group (metan) were not included in the meta-analyses which may explain some of the differences between median and pooled prevalence and pooled OR and aOR.

\*Adjusted for region (Africa vs. Americas/Asia in overall model), gestational age, transmission level as continuous variable, season, study year, gravidity, age and first antenatal visit. The inclusion of gestational age l size (254 in overall model) but results with and without gestational age were similar (data not shown). An interaction was noted between malaria status and region (Africa vs. Americas/Asia combined, see table insert) for t values for interaction terms between malaria status and gestational age were  $\geq 0.05$  for each model (p=0.0661 for interaction term of model among parasitaemic women). Malaria species were not associated with fever in mo parasitaemic women by region (data not shown).





# Table S11: Pooled prevalence of species as assessed by PCR among microscopic and submicroscopic infections by region, meta-analysis

<span id="page-32-0"></span>NA, not available. Notes: Pooled estimates using metaprop procedure, Stata. Note that because of the weighting of the studies in the random-effects models, proportion of P. falciparum and P. vivax are not adding up to 100. \*All regions in Africa combined because of limited sample in moderate-tolow transmission areas

### Table S12: Malaria species as assessed by PCR as risk factor for submicroscopic malaria among PCR-positive infections, IPD\*



<span id="page-33-0"></span>aOR, adjusted Odds ratio. CI, confidence interval. IPD, individual participant data.

\*Submicroscopic malaria as outcome of interest, with microscopic malaria as reference group, and species as exposure as a variable with 4 categories with *P. falciparum* as the reference category. Models were adjusted for variables in the baseline model (age, gravidity, season, level of malaria transmission and 1<sup>st</sup> antenatal visit for enrolment and study design at delivery). +Mixed infections: combinations of P. falciparum, P. vivax, P. malariae or P. ovale. ‡Other mono-infections: mono-infections with P. ovale or P. malariae § Comparison: P. vivax versus any other infection, univariate analyses, because of small sample size 1 Pregnancy: univariate analyses because of small sample size

# Table S13: Multivariable analyses of factors associated with submicroscopic and microscopic infections by gravidity, base model

<span id="page-34-0"></span>




ANC, antenatal clinic. aOR, adjusted Odds ratio. CI, confidence interval. G1, primigravidae. G2, secundigravidae. G3+, multigravidae (excluding secundigravidae). PfPR<sub>2-10</sub>, Plasmodium falciparum prevalence among children aged 2-10 years at the location and year of study visit, as estimated by the Malaria Atlas Project. Factors where the p-value is <0·05 are printed in bold. Note that studies in Asia and the Pacific were included under "Asia"; studies in central or south America were included under "Americas".

Notes: Results for first ANC visits may have been affected by the data available, with only one study including first ANC visits only in high transmission areas for which only women in their first and second pregnancy were eligible; they had a lower prevalence of malaria compared to the other studies in this group,. In Asia/Americas, two studies included first ANC visits only and these were conducted in areas with higher malaria transmission than the other studies in this group.

Table S14: Multivariable analyses of factors associated with submicroscopic and microscopic *Plasmodium falciparum* and *Plasmodium vivax* infection in Asia and America, IPD base model



OR, adjusted Odds ratio. CI, confidence interval. G1, primigravidae. G2, secundigravidae. G3+, multigravidae (excluding secundigravidae). PfPR2-10, Plasmodium falciparum prevalence among children aged 2-10 years at the location and year of study visit, as estimated by the Malaria Atlas Project. Factors where the p-value is <0·05 are printed in bold. Note that studies in Asia and the Pacific were included under "Asia"; studies in central or south America were included under "Americas". \*The line for combination of G1 and G2 was added to allow the assessment of the effect size of the combination of these groups, using the same reference group (G3+) and the same base model. Note that this estimate is from a separate model.

Table S15: Multivariable analyses of factors associated with submicroscopic and microscopic malaria infections in pregnancy by region, first ANC visits only, IPD base model



Age (years)



ANC, antenatal clinic. aOR, adjusted Odds ratio. CI, confidence interval. G1, primigravidae. G2, secundigravidae. G3+, multigravidae (excluding secundigravidae). Available data: number of participants with an outcome on submicroscopic, microscopic and no malaria. Factors where the p-value is <0.05 are printed in bold. Note that studies in Asia and the Pacific were included under "Asia". \*Transmission level: Africa, high transmission: PfPR<sub>2-10</sub> ≥35%; Africa, moderate-to-low transmission: PfPR<sub>2-10</sub> <35%: only 1 study (two sublocations) PfPR<sub>2-10</sub> <10%. In the model for America/Asia, PfPR<sub>2-10</sub> was included as a continuous variable in the models. In the high transmission area in Africa, *Pf*PR<sub>2-10</sub> was not included because of non-convergence of the models; there were 3 sublocations with *Pf*PR<sub>2-10</sub> range of 47-63%. In Asia, PfPR<sub>2-10</sub> was not included because of the limited range of PfPR<sub>2-10</sub> (0-4%) with only 174 participants when PfPR<sub>2-10</sub> in the range of 2-4%, leading to distortion of results (e.g., for submicroscopic malaria vs. no malaria the aOR was 0.59, 0.45-0.78, suggesting a jump of 41% in odds for every percentage increase of *Pf*PR<sub>2-10</sub> which is improbable). Removal of *Pf*PR<sub>2-10</sub> did not lead to meaningful differences for the estimates of the other co-variates. †Available data (data with information on microscopic, submicroscopic and no malaria): Africa, high transmission areas N=6746, 9 sublocations (microscopic 2455, submicroscopic 1406, no malaria infections 2885); Africa, moderate-to-low transmission areas N=8350, 25 sublocations (microscopic 949, submicroscopic 1858, no malaria infection 5543); Americas/Asia N=10,305, 23 sublocations (microscopic 373, submicroscopic 919, no malaria infection 9013).

Table S16: Multivariable models by region to assess the effect of inclusion of the variable "Gestational age" into the analyses of submicroscopic and microscopic malaria infections, IPD





Table S17: Multivariable models in Africa to assess the effect of inclusion of the variable "HIV infection" into the analyses of submicroscopic and microscopic malaria infections, IPD





Table S18: Multivariable models in Africa to assess the effect of inclusion of the variable "Antimalarial use" into the analyses of submicroscopic and microscopic malaria infections, IPD



Notes: Because of non-convergence of the model, age was collapsed from 5 into 3 categories. The variable "First ANC visit" was not included; all involved studies included women at any ANC visit, or it was unknown.



Notes: Because of non-convergence of the models, age was collapsed from 5 into 3 categories and gravidity into two (primigravidae versus multigravidae). The variable "First ANC visit" was not included; all involved studies included women at any ANC visit, or it was unknown.





Table S19: Delivery: Multivariable analyses of factors associated with submicroscopic and microscopic malaria infections peripheral blood, IPD: Base model and variables of interest with limited sample size





aOR, adjusted Odds ratio. G1, primigravidae. G2, secundigravidae. G3+, multigravidae (excluding secundigravidae). ITN, insecticide treated net. IRS, indoor residual spraying. Available data: number of participants with an outcome on submicroscopic, microscopic and no malaria. Note: there was insufficient information about IRS to allow useful models. There was insufficient information on HIV in Asia/Americas. \*High transmission: *Pf*PR2-10 ≥35%. Moderate and low transmission: *Pf*PR2-10 <35%. †Available data: Africa, high transmission N=4976, 10 sublocations (microscopic 511, submicroscopic 826, and no malaria infections 3639 observations). Africa, moderate-to-low transmission N=10,737 (microscopic 505, submicroscopic 1232, no malaria infection 9000), 36 sublocations. Asia/Americas 6,102 (microscopic 111, submicroscopic 446 and no malaria infection 5545), 16 sublocations. **‡** Africa high transmission, subgroup analysis: HIV infection: model with HIV infection only, non-convergence of adjusted models, very low numbers of HIV-infected women with malaria. Setting, subgroup analysis: survey and PfPR<sub>2-10</sub> removed because of non-convergence. Only 1 cohort study in rural area. Any net use, subgroup analysis: only univariate analysis because of non-convergence.

Table S20: Delivery: Multivariable analyses of factors associated with placental submicroscopic and microscopic malaria infections, IPD: Base model and variables of interest with limited sample size





aOR, adjusted Odds ratio. G1, primigravidae. G2, secundigravidae. G3+, multigravidae (excluding secundigravidae). ITN, insecticide treated net. IRS, indoor residual spraying. Available data: number of participants with an outcome on submicroscopic, microscopic and no malaria. Note: there was insufficient information about IRS to allow useful models. There was Insufficient information on HIV in Asia/Americas. \*High transmission: PfPR<sub>2-10</sub> ≥35%, moderate-to-low transmission PfPR<sub>2-10</sub> <35%. †Available data: Africa, high transmission N=5391, 11 sublocations (microscopic 750, submicroscopic 738 and no malaria infection 3903 observations). Africa, moderate-to-low transmission N=8048 (microscopic 440, submicroscopic 877 and no malaria infection 6731), 33 sublocations. Asia/Americas N=5001 (microscopic 97, submicroscopic 235, and no malaria infection 4669), 18 sublocations.  $\ddagger$  Africa high transmission; HIV subgroup analyses: only age and gravidity included because of non-convergence. Setting subgroup analysis: *Pf*PR2-10 removed because of non-convergence.

### *Van Eijk et al Supplement sMIP IPD-meta (03apr23)\_suppl\_no\_tc* Table S21: Microscopy and fever results at second scheduled study visit in pregnancy after submicroscopic malaria at enrolment



Fever was defined as documented fever (<37.5 °C) or a history of fever in the past 1-7 days as per the definition used in the source studies.

\*Meta-prop procedure Stata

†Comparing the difference in days using multinomial model (GSEM) with no malaria as reference: p=0.6352 comparing submicroscopic at 2<sup>nd</sup> visit vs. none, and p=0.3492 comparing microscopic vs. none for overall model. Among with fever at 2<sup>nd</sup> study visit:  $p=0.1826$  and  $p=0.2943$  comparing submicroscopic at 2<sup>nd</sup> visit vs. none, and microscopic vs. none for overall model, respectively. Comparing difference in days for submicroscopic vs. mi

## Table S22: Sensitivity analysis: Comparing IPD and aggregated data for study outcomes



\*Pooled estimate from Stata procedure metaprop

†Wilcoxon rank-sum test (or Mann-Whitney test): this tests the hypothesis that two independent samples are from populations with the same distribution

## Table S23: Sensitivity analysis: Effect of quality assessment factor in multivariable models





aOR, adjusted Odds ratio. Note: adjusted for baseline model as reported in Table 2, S14, and S15

\*Interaction noted between continent and submicroscopic malaria: for this reason, data are additionally presented by continent

Table S24: Sensitivity analysis: Effect of quality of blood smear reading on risk factors for submicroscopic and microscopic malaria, multivariable models in pregnancy, IPD







aOR, adjusted Odds ratio. CI, confidence interval. G1, primigravidae. G2, secundigravidae. G3+, multigravidae (excluding secundigravidae). Available data: number of participants with an outcome on submicroscopic, microscopic and no malaria. Factors where the p-value is <0.05 are printed in bold. Note that studies in Asia and the Pacific were included under "Asia"; studies in middle or south America were included under "Americas". \* High transmission: *PfPR*<sub>2-10</sub> ≥35%, moderate-to-low transmission *PfPR*<sub>2-10</sub> <35%. † Available data (data with information on microscopic, submicroscopic and no malaria): Africa, high transmission areas N=6790, 9 sublocations; Africa, moderate-to-low transmission areas N=8306, 24 sublocations; Americas/Asia N=10,305, 23 sublocations. †In Africa: High transmission: *PfPR*<sub>2-10</sub> ≥35%, moderate and low transmission combined: PfPR<sub>2-10</sub> <35%: only 1 study <10% in Africa. In the model for America/Asia, one study in Indonesia, 9% of data, had PfPR<sub>2-10</sub> of 25%, all other studies PfPR<sub>2-10</sub> 0-5%, with 81% PfPR<sub>2-10</sub> of <2%. ‡Comparison group for first ANC visit: not first ANC visit or unknown if first visit or not. First ANC visit was not included in high transmission area because of non-convergence for subgroups (cells with 0 values).

Table S25: Sensitivity analysis: Multivariable analyses of factors associated with submicroscopic and microscopic malaria infections in pregnancy by region, using an alternative measure of transmission level\*, IPD







aOR, adjusted Odds ratio. CI, confidence interval. G1, primigravidae. G2, secundigravidae. G3+, multigravidae (excluding secundigravidae). ITN, insecticide treated net. IRS, indoor residual spraying. Available data: number of participants with an outcome on submicroscopic, microscopic and no malaria. Factors where the p-value is <0.05 are printed in bold. Note that studies in Asia and the Pacific were included under "Asia"; studies in central or south America were included under "Americas".

\*Alternative measure of transmission level: Parasite prevalence among all gravidae, measured in the first trimester by NAAT (PCR or LAMP). Africa, high transmission: alternative measure ≥35%; Africa, moderateto-low transmission: alternative measure <35%: only 1 study (two sublocations). †Available data (data with information on microscopic, submicroscopic or no malaria): Africa, high transmission areas: N=9479, 19 sublocations; Africa, moderate-to-low transmission areas in Africa; N=5222, 13 sublocations; Americas/Asia: N=10,261, 22 sublocations. ‡Comparison group for first ANC visit: Not first ANC visit or unknown if first visit or not. § Africa, high transmission: The alternative indicator was not included in the model of antimalarial use because of non-convergence. Africa, moderate-to-low transmission: In model for rural setting, first ANC visit not included because of non-convergence. In model for antimalarial use only age and rainy season included in models with no malaria as reference, and only rainy season included in submicroscopic vs. microscopic model because of non-convergence. Model for IRS conducted without indicator of malaria transmission and gravidity collapsed into primigravidae versus multigravidae because of nonconvergence. ¶ There was insufficient information on HIV infection in studies in the Americas and Asia.

# Supplemental Figures

## Figure S1: Map of included studies



Legend: Green: *PfPR*<sub>2-10</sub> < 10%. Blue: *PfPR*<sub>2-10</sub> 10-34%. Red: *PfPR*<sub>2-10</sub> ≥ 35%.

Figure S2A: Proportion of submicroscopic malaria among NAAT-positive test results by malaria transmission level among first ANC attendees



Figure S2B: Proportion of submicroscopic malaria among NAAT-positive test results in pregnancy by malaria transmission level by gravidity



NAAT, Nucleic acid amplification test (PCR or LAMP). *Pf*PR2-10, *Plasmodium falciparum* prevalence among children aged 2-10 years at the year of study visit, as estimated by the Malaria Atlas Project. Note that studies in Asia and the Pacific were combined under "Asia"; studies in middle or south America were combined under "Americas".

Markers: Orange: studies in Africa; Green: studies in Asia; Red: studies in the Americas. The relationship and 95% CI were estimated for Africa only, using fractional polynomials logistic regression with robust variance (primigravidae p=0.004 and p<0.0001 for comparison with linear and noncovariate model, respectively; secundigravidae p=0.008 and p<0.0001, respectively; multigravidae p=0.011 and p<0.0001, respectively)

## *Van Eijk et al Supplement sMIP IPD-meta (03apr23)\_suppl\_no\_tc* Figure S1: Probability of fever by malaria status, gestational age and region

 $\overline{4}$  $\overline{4}$  $\overline{4}$ Africa, high transmission America / Asia Africa, low to moderate (≥35% PfPR<sub>2-10</sub>) transmission (< $35\%$  PfPR<sub>2-10</sub>) က္ က္ <u>က္</u>  $\overline{N}$  $\overline{N}$  $\mathbf{\Omega}$  $\nabla_{\mathbf{r}}$  $\overline{\mathcal{L}}$  $\overline{\mathbf{r}}$  $\circ$  $\circ$  $\circ$  $\overline{36}$  $\overline{36}$  $\overline{36}$  $2<sup>8</sup>$  $20$ 28  $20$  $28$  $\overline{20}$  $12$  $12$  $12$ Gestational age in weeks Gestational age in weeks Gestational age in weeks - Submicroscopic malaria - Microscopic malaria **←** No malaria Notes: Individual participant data, using one-stage model for fever during pregnancy. The adjusted odds ratios (aOR) and 95% confidence intervals for gestational age were overall 0.96, 0.94-0.98 (p=0.0007), America/Asia aOR=0.98, 0.97- 0.99s16 (p=0.4816), Africa, moderate-low transmission, aOR=0.98, 0.92-1.04 (p=0.1400), and Africa high transmission, aOR=0.95, 0.93-0.97 (p=0.0007). For additional information, see [Table S10.](#page-31-0)

Figure S2: Prevalence of submicroscopic and microscopic malaria in studies with regular follow up visit in pregnancy by gestational age









Figure S3: Proportion of submicroscopic malaria (forest plot), microscopic malaria (column) and no malaria (column) at a subsequent scheduled follow up visit in pregnancy among women with submicroscopic malaria at enrolment, by region



CI, confidence interval. PNG, Papua New Guinea. PfPR<sub>2-10</sub>, Plasmodium falciparum prevalence in children 2-10 years of age as an indicator of level of malaria transmission at the midyear of study, Malaria Atlas Project.

## Figure S4: Probability and 95% confidence interval of microscopic and submicroscopic malaria by gestational age



• Microscopic malaria. • Submicroscopic malaria. • Proportion submicroscopic among NAAT-positives

*Pf*PR2-10, *Plasmodium falciparum* prevalence in children 2-10 years of age as an indicator of level of malaria transmission at the midyear of study, Malaria Atlas Project. Estimates were from models adjusted for age, type of gestational age assessment, and year of study.

Notes: In high malaria transmission areas there was not sufficient information before 10 weeks of gestational age. For G3+ in moderate-to-low transmission areas, there was insufficient information after 30 weeks of gestational age.

*Van Eijk et al Supplement sMIP IPD-meta (03apr23)\_suppl\_no\_tc* Figure S7: Funnel plot for studies with information on submicroscopic malaria infection during pregnancy



## References

1. Bardaji A, Martinez-Espinosa FE, Arevalo-Herrera M, et al. Burden and impact of *Plasmodium vivax* in pregnancy: A multi-centre prospective observational study. *PLoS Negl Trop Dis* 2017; **11**(6): e0005606.

2. Elbadry MA, Tagliamonte MS, Raccurt CP, et al. Submicroscopic malaria infections in pregnant women from six departments in Haiti. *Trop Med Int Health* 2017; **22**(8): 1030-6.

3. Gavina K, Gnidehou S, Arango E, et al. Clinical outcomes of submicroscopic infections and correlates of protection of VAR2CSA antibodies in a longitudinal study of pregnant women in Colombia. *Infect Immun* 2018; **86**(4).

4. Vasquez AM, Medina AC, Tobon-Castano A, et al. Performance of a highly sensitive rapid diagnostic test (HS-RDT) for detecting malaria in peripheral and placental blood samples from pregnant women in Colombia. *PLoS One* 2018; **13**(8): e0201769.

5. Vásquez AM, Vélez G, Medina A, et al. Evaluation of highly sensitive diagnostic tools for the detection of *P. falciparum* in pregnant women attending antenatal care visits in Colombia. *BMC Pregnancy Childbirth* 2020; **20**(1): 440.

6. Parekh FK, Davison BB, Gamboa D, Hernandez J, Branch OH. Placental histopathologic changes associated with subclinical malaria infection and its impact on the fetal environment. *Am J Trop Med Hyg* 2010; **83**(5): 973-80.

7. Arango EM, Samuel R, Agudelo OM, Carmona-Fonseca J, Maestre A, Yanow SK. Molecular detection of malaria at delivery reveals a high frequency of submicroscopic infections and associated placental damage in pregnant women from northwest Colombia. *Am J Trop Med Hyg* 2013; **89**(1): 178-83.

8. Agudelo O, Arango E, Maestre A, Carmona-Fonseca J. Prevalence of gestational, placental and congenital malaria in north-west Colombia. *Malar J* 2013; **12**(1): 341.

9. Stanisic DI, Moore KA, Baiwog F, et al. Risk factors for malaria and adverse birth outcomes in a prospective cohort of pregnant women resident in a high malaria transmission area of Papua New Guinea. *Trans R Soc Trop Med Hyg* 2015; **109**(5): 313-24.

10. Singh N, Bharti PK, Singh MP, et al. What is the burden of submicroscopic malaria in pregnancy in central India? *Pathog Glob Health* 2015; **109**(1): 30-8.

11. Unger HW, Ome-Kaius M, Wangnapi RA, et al. Sulphadoxine-pyrimethamine plus azithromycin for the prevention of low birthweight in Papua New Guinea: a randomised controlled trial. *BMC Med* 2015; **13**(1): 9.

12. Pava Z, Burdam FH, Handayuni I, et al. Submicroscopic and asymptomatic *Plasmodium* parasitaemia associated with significant risk of anaemia in Papua, Indonesia. *PLoS One* 2016; **11**(10): e0165340.

13. Ahmed R, Poespoprodjo JR, Syafruddin D, et al. Efficacy and safety of intermittent preventive treatment and intermittent screening and treatment versus single screening and treatment with dihydroartemisinin-piperaquine for the control of malaria in pregnancy in Indonesia: a cluster-randomised, open-label, superiority trial. *Lancet Infect Dis* 2019; **19**(9): 973-87.

14. Ahmed R, Asih PPB, Noviyanti R, et al. The clinical burden of microscopically patent and sub-microscopic *P. falciparum* and *P. vivax* malaria in pregnancy in Indonesia. Challenges in malaria research: Core science and innovation, Oxford, UK 22-24 September 2014. p. P2.

15. Ahmed R, Levy EI, Maratina SS, et al. Performance of four HRP-2/pLDH combination rapid diagnostic tests and field microscopy as screening tests for malaria in pregnancy in Indonesia: a cross-sectional study. *Malar J* 2015; **14**(1): 420.

16. Walker-Abbey A, Djokam RR, Eno A, et al. Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. *Am J Trop Med Hyg* 2005; **72**(3): 229-35.

17. Mockenhaupt FP, Rong B, Till H, et al. Submicroscopic *Plasmodium falciparum* infections in pregnancy in Ghana. *Trop Med Int Health* 2000; **5**(3): 167-73.

18. Mockenhaupt FP, Ulmen U, von Gaertner C, Bedu-Addo G, Bienzle U. Diagnosis of placental malaria. *J Clin Microbiol* 2002; **40**(1): 306-8.

19. Malhotra I, Dent A, Mungai P, et al. Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya. *PLoS Med* 2009; **6**(7): e1000116.

20. Leke RF, Bioga JD, Zhou J, et al. Longitudinal studies of *Plasmodium falciparum* malaria in pregnant women living in a rural Cameroonian village with high perennial transmission. *Am J Trop Med Hyg* 2010; **83**(5): 996-1004.

21. Adegnika AA, Verweij JJ, Agnandji ST, et al. Microscopic and sub-microscopic *Plasmodium falciparum* infection, but not inflammation caused by infection, is associated with low birth weight. *Am J Trop Med Hyg* 2006; **75**(5): 798-803.

22. Menendez C, Bardaji A, Sigauque B, et al. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. *PLoS ONE* 2008; **3**(4): e1934.

23. Luntamo M, Rantala AM, Meshnick SR, et al. The effect of monthly sulfadoxine-pyrimethamine, alone or with azithromycin, on PCR-diagnosed malaria at delivery: a randomized controlled trial. *PLoS ONE* 2012; **7**(7): e41123.

24. Landis SH, Lokomba V, Ananth CV, et al. Impact of maternal malaria and under-nutrition on intrauterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo. *Epidemiol Infect* 2009; **137**(2): 294-304.

25. Dobano C, Berthoud T, Manaca MN, et al. High production of pro-inflammatory cytokines by maternal blood mononuclear cells is associated with reduced maternal malaria but increased cord blood infection. *Malar J* 2018; **17**(1): 177.

26. Cottrell G, Moussiliou A, Luty AJ, et al. Submicroscopic *Plasmodium falciparum* infections are associated with maternal anemia, premature births, and low birth weight. *Clin Infect Dis* 2015; **60**(10): 1481-8.

27. Oduwole OA, Ejezie GC, Odey FA, et al. Congenital malaria in calabar, Nigeria: the molecular perspective. *Am J Trop Med Hyg* 2011; **84**(3): 386-9.

28. Cohee LM, Kalilani-Phiri L, Boudova S, et al. Submicroscopic malaria infection during pregnancy and the impact of intermittent preventive treatment. *Malar J* 2014; **13**(1): 274.

29. Natureeba P, Ades V, Luwedde F, et al. Lopinavir/Ritonavir-based antiretroviral treatment (ART) versus Efavirenz-based ART for the prevention of malaria among HIV-infected pregnant women. *J Infect Dis* 2014; **210**(12): 1938-45.

30. Patel JC, Mwapasa V, Kalilani L, et al. Absence of association between sickle trait hemoglobin and placental malaria outcomes. *Am J Trop Med Hyg* 2016; **94**(5): 1002-7.

31. Kattenberg JH, Tahita CM, Versteeg IA, et al. Evaluation of antigen detection tests, microscopy, and polymerase chain reaction for diagnosis of malaria in peripheral blood in asymptomatic pregnant women in Nanoro, Burkina Faso. *Am J Trop Med Hyg* 2012; **87**(2): 251-6.

32. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, et al. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: A multicentre randomized controlled trial. *PLoS Med* 2014; **11**(9): e1001733.

33. Gonzalez R, Desai M, Macete E, et al. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-infected women receiving cotrimoxazole prophylaxis: A multicenter randomized placebo-controlled trial. *PLoS Med* 2014; **11**(9): e1001735.

34. Denoeud-Ndam L, Zannou DM, Fourcade C, et al. Cotrimoxazole prophylaxis versus mefloquine intermittent preventive treatment to prevent malaria in HIVinfected pregnant women: two randomized controlled trials. *J Acquir Immune Defic Syndr* 2013; **65**(2): 198-206.

35. Williams J, Njie F, Cairns M, et al. Non-falciparum malaria infections in pregnant women in West Africa. *Malar J* 2016; **15**(1): 53.

36. Mahamar A, Andemel N, Swihart B, et al. Malaria infection is common and associated with perinatal mortality and preterm delivery despite widespread use of chemoprevention in Mali: An observational study 2010 to 2014. *Clin Infect Dis* 2021; **73**(8): 1355-61.

37. Arinaitwe E, Ades V, Walakira A, et al. Intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy: a cross-sectional study from Tororo, Uganda. *PLoS ONE* 2013; **8**(9): e73073.

38. Daud II, Opinya FO, Midem D, et al. Improved pregnancy outcomes in a prospective study of pregnant women enrolling in an antenatal clinic in Western Kenya. *Health* 2015; **6**: 2651-6.

39. Madanitsa M, Kalilani L, Mwapasa V, et al. Scheduled intermittent screening with rapid diagnostic tests and treatment with dihydroartemisinin-piperaquine versus intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy in Malawi: An open-label randomized controlled trial. *PLoS Med* 2016; **13**(9): e1002124.

40. Nkhoma M, Ashorn P, Ashorn U, et al. Providing lipid-based nutrient supplement during pregnancy does not reduce the risk of maternal *P falciparum* parasitaemia and reproductive tract infections: a randomised controlled trial. *BMC Pregnancy Childbirth* 2017; **17**(1): 35.

41. Mosha D, Chilongola J, Ndeserua R, Mwingira F, Genton B. Effectiveness of intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy on placental malaria, maternal anaemia and birthweight in areas with high and low malaria transmission intensity in Tanzania. *Trop Med Int Health* 2014; **19**(9): 1048-56.

42. Francine N, Damien B, Anna F, Michael K, Christevy VJ, Felix KK. Characterization of asymptomatic *Plasmodium falciparum* infection and its risk factors in pregnant women from the Republic of Congo. *Acta Trop* 2016; **153**: 111-5.

43. Desai M, Gutman J, L'lanziva A, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. *Lancet* 2015; **386**(10012): 2507-19.

44. Chaponda EB, Chandramohan D, Michelo C, Mharakurwa S, Chipeta J, Chico RM. High burden of malaria infection in pregnant women in a rural district of Zambia: a cross-sectional study. *Malar J* 2015; **14**: 380.

45. Braun V, Rempis E, Schnack A, et al. Lack of effect of intermittent preventive treatment for malaria in pregnancy and intense drug resistance in western Uganda. *Malar J* 2015; **14**: 372.

46. Arnaldo P, Rovira-Vallbona E, Langa JS, et al. Uptake of intermittent preventive treatment and pregnancy outcomes: health facilities and community surveys in Chokwe district, southern Mozambique. *Malar J* 2018; **17**(1): 109.

47. Kakuru A, Jagannathan P, Muhindo MK, et al. Dihydroartemisinin-piperaquine for the prevention of malaria in pregnancy. *N Engl J Med* 2016; **374**(10): 928-39.

48. Mbouamboua Y, Koukouikila-Koussounda F, Ntoumi F, et al. Sub-microscopic *Plasmodium falciparum* infections in matched peripheral, placental and umbilical cord blood samples from asymptomatic Congolese women at delivery. *Acta Trop* 2019; **193**: 142-7.

49. Accrombessi M, Fievet N, Yovo E, et al. Prevalence and associated risk factors of malaria in the first trimester of pregnancy: A preconceptional cohort study in Benin. *J Infect Dis* 2018; **217**(8): 1309-17.

50. Briggs J, Ategeka J, Kajubi R, et al. Impact of microscopic and submicroscopic parasitemia during pregnancy on placental malaria in a high-transmission setting in Uganda. *J Infect Dis* 2019; **220**(3): 457-66.

51. Singer LM, Newman RD, Diarra A, et al. Evaluation of a malaria rapid diagnostic test for assessing the burden of malaria during pregnancy. *Am J Trop Med Hyg* 2004; **70**(5): 481-5.

52. Perrault SD, Hajek J, Zhong K, et al. Human immunodeficiency virus co-infection increases placental parasite density and transplacental malaria transmission in Western Kenya. *Am J Trop Med Hyg* 2009; **80**(1): 119-25.

53. Adam I, Elbasit IE, Salih I, Elbashir MI. Submicroscopic *Plasmodium falciparum* infections during pregnancy, in an area of Sudan with a low intensity of malaria transmission. *Ann Trop Med Parasitol* 2005; **99**(4): 339-44.

54. Bouyou-Akotet MK, Nzenze-Afene S, Ngoungou EB, et al. Burden of malaria during pregnancy at the time of IPTp/SP implementation in Gabon. *Am J Trop Med Hyg* 2010; **82**(2): 202-9.
55. Kapito-Tembo AP. Malaria and anemia in HIV-infected pregnant women in Malawi: associations with cotrimoxazole prophylaxis, submicroscopic malaria, iron supplementation and iron deficiency: The University of North Carolina at Chapel Hill; 2010.

56. Newman PM, Wanzira H, Tumwine G, et al. Placental malaria among HIV-infected and uninfected women receiving anti-folates in a high transmission area of Uganda. *Malar J* 2009; **8**(1): 254.

57. Nwaefuna EK, Afoakwah R, Orish VN, Egyir-Yawson A, Boampong JN. Effectiveness of intermittent preventive treatment in pregnancy with sulphadoxinepyrimethamine against submicroscopic falciparum malaria in Central Region, Ghana. *J Parasitol Res* 2015; **2015**: 959427.

58. Elbashir HM, Salih MM, Elhassan EM, Mohmmed AM, Elbashir MI, Adam I. Polymerase chain reaction and histology in diagnosis of placental malaria in an area of unstable malaria transmission in Central Sudan. *Diagn Pathol* 2011; **6**(1): 128.

59. Kyabayinze DJ, Zongo I, Cunningham J, et al. HRP2 and pLDH-based rapid diagnostic tests, expert microscopy, and PCR for detection of malaria infection during pregnancy and at delivery in areas of varied transmission: A prospective cohort study in Burkina Faso and Uganda. *PLoS ONE* 2016; **11**(7): e0156954.

60. Kashif AH, Adam GK, Mohmmed AA, Elzaki SE, Abdelhalim AM, Adam I. Reliability of rapid diagnostic test for diagnosing peripheral and placental malaria in an area of unstable malaria transmission in eastern Sudan. *Diagn Pathol* 2013; **8**(1): 59.

61. Matangila JR, Lufuluabo J, Ibalanky AL, Inocencio da Luz RA, Lutumba P, van Geertruyden JP. Asymptomatic *Plasmodium falciparum* infection is associated with anaemia in pregnancy and can be more cost-effectively detected by rapid diagnostic test than by microscopy in Kinshasa, Democratic Republic of the Congo. *Malar J* 2014; **13**: 132.

62. Lamptey H, Ofori MF, Kusi KA, et al. The prevalence of submicroscopic *Plasmodium falciparum* gametocyte carriage and multiplicity of infection in children, pregnant women and adults in a low malaria transmission area in Southern Ghana. *Malar J* 2018; **17**(1): 331.

63. Fadlelseed OE, Osman ME, Shamseldin NM, Elhussein AB, Adam I. *Plasmodium falciparum* genotypes in matched peripheral, placental and umbilical cord blood in an area characterised by unstable malaria transmission in eastern Sudan. *Heliyon* 2017; **3**(6): e00326.

64. Ruh E, Bateko JP, Imir T, Taylan-Ozkan A. Investigation of pregnancy-associated malaria by microscopy, rapid diagnostic test and PCR in Bandundu, the Democratic Republic of Congo. *Trans R Soc Trop Med Hyg* 2018; **112**(1): 8-13.

65. Natureeba P, Kakuru A, Muhindo M, et al. Intermittent preventive treatment with dihydroartemisinin-piperaquine for the prevention of malaria among HIVinfected pregnant women. *J Infect Dis* 2017; **216**(1): 29-35.

66. Quakyi I, Tornyigah B, Houze P, et al. High uptake of intermittent preventive treatment of malaria in pregnancy is associated with improved birth weight among pregnant women in Ghana. *Sci Rep* 2019; **9**(1): 19034.

67. Samuels AM, Towett O, Seda B, et al. Diagnostic performance of ultra-sensitive rapid diagnostic tests for malaria in pregnant women attending antenatal clinics in western Kenya. 68th annual meeting of the American Society of Tropical Medicine and Hygiene, November 20-24, National Harbor, Maryland, USA; 2019: Am J Trop Med Hyg; 2019. p. 276.

68. Tadesse G, Kamaliddin C, Doolan C, et al. Active case detection of malaria in pregnancy using loop-mediated amplification (LAMP): a pilot outcomes study in South West Ethiopia. *Malar J* 2020; **19**(1): 305.

69. Chauvin P, Menard S, Iriart X, et al. Prevalence of *Plasmodium falciparum* parasites resistant to sulfadoxine/pyrimethamine in pregnant women in Yaounde, Cameroon: emergence of highly resistant pfdhfr/pfdhps alleles. *J Antimicrob Chemother* 2015; **70**(9): 2566–71.

70. Enosse S, Magnussen P, Abacassamo F, et al. Rapid increase of *Plasmodium falciparum* dhfr/dhps resistant haplotypes, after the adoption of sulphadoxinepyrimethamine as first line treatment in 2002, in southern Mozambique. *Malar J* 2008; **7**: 115.

71. Gutman J, Mwandama D, Wiegand RE, et al. In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitaemia in pregnant women in Machinga District, Malawi. *Malar J* 2015; **14**: 197.

72. Mita T, Venkatesan M, Ohashi J, et al. Limited geographical origin and global spread of sulfadoxine-resistant dhps alleles in *Plasmodium falciparum* populations. *J Infect Dis* 2011; **204**(12): 1980-8.

73. Moussiliou A, De Tove YS, Doritchamou J, et al. High rates of parasite recrudescence following intermittent preventive treatment with sulphadoxine-pyrimethamine during pregnancy in Benin. *Malar J* 2013; **12**: 195.

74. Esu E, Tacoli C, Gai P, et al. Prevalence of the Pfdhfr and Pfdhps mutations among asymptomatic pregnant women in Southeast Nigeria. *Parasitol Res* 2018; **117**(3): 801-7.

75. Artimovich E, Schneider K, Taylor TE, et al. Persistence of sulfadoxine-pyrimethamine resistance despite reduction of drug pressure in Malawi. *J Infect Dis* 2015; **212**(5): 694-701.

76. Tumwebaze P, Tukwasibwe S, Taylor A, et al. Changing antimalarial drug resistance patterns identified by surveillance at three sites in Uganda. *J Infect Dis* 2017; **215**(4): 631-5.

77. Tahita MC, Tinto H, Erhart A, et al. Prevalence of the dhfr and dhps mutations among pregnant women in rural Burkina Faso five years after the introduction of intermittent preventive treatment with sulfadoxine-pyrimethamine. *PLoS One* 2015; **10**(9): e0137440.

78. Guerra M, Neres R, Salgueiro P, et al. *Plasmodium falciparum* genetic diversity in continental Equatorial Guinea before and after introduction of artemisinin-based combination therapy. *Antimicrob Agents Chemother* 2017; **61**(1).

79. Gupta H, Macete E, Bulo H, et al. Drug-resistant polymorphisms and copy numbers in *Plasmodium falciparum*, Mozambique, 2015. *Emerg Infect Dis* 2018; **24**(1): 40- 8.

80. Lucchi NW, Okoth SA, Komino F, et al. Increasing prevalence of a novel triple-mutant dihydropteroate synthase genotype in *Plasmodium falciparum* in western Kenya. *Antimicrob Agents Chemother* 2015; **59**(7): 3995-4002.

81. Ndiaye D, Dieye B, Ndiaye YD, et al. Polymorphism in dhfr/dhps genes, parasite density and ex vivo response to pyrimethamine in *Plasmodium falciparum* malaria parasites in Thies, Senegal. *Int J Parasitol Drugs Drug Resist* 2013; **3**: 135-42.

82. Desai M, Gutman J, Taylor SM, et al. Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy for malaria in pregnancy at clearing infections and preventing low birth weight. *Clin Infect Dis* 2016; **62**(3): 323–33.

83. Diawara F, Steinhardt LC, Mahamar A, et al. Measuring the impact of seasonal malaria chemoprevention as part of routine malaria control in Kita, Mali. *Malar J* 2017; **16**(1): 325.

84. Baraka V, Ishengoma DS, Fransis F, et al. High-level *Plasmodium falciparum* sulfadoxine-pyrimethamine resistance with the concomitant occurrence of septuple haplotype in Tanzania. *Malar J* 2015; **14**: 439.

85. Nkoli Mandoko P, Rouvier F, Matendo Kakina L, et al. Prevalence of *Plasmodium falciparum* parasites resistant to sulfadoxine/pyrimethamine in the Democratic Republic of the Congo: emergence of highly resistant pfdhfr/pfdhps alleles. *J Antimicrob Chemother* 2018; **73**(10): 2704-15.

86. Baraka V, Delgado-Ratto C, Nag S, et al. Different origin and dispersal of sulfadoxine-resistant *Plasmodium falciparum* haplotypes between Eastern Africa and Democratic Republic of Congo. *Int J Antimicrob Agents* 2017; **49**(4): 456-64.

87. Conrad MD, Mota D, Foster M, et al. Impact of intermittent preventive treatment during pregnancy on *Plasmodium falciparum* drug resistance-mediating polymorphisms in Uganda. *J Infect Dis* 2017; **216**(8): 1008-17.

88. Huijben S, Macete E, Mombo-Ngoma G, et al. Counter-selection of antimalarial resistance polymorphisms by intermittent preventive treatment in pregnancy. *J Infect Dis* 2020; **221**(2): 293-303.

89. Chico RM, Chaponda EB, Ariti C, Chandramohan D. Sulfadoxine-Pyrimethamine exhibits dose-response protection against adverse birth outcomes related to sexually transmitted and reproductive tract infections. *Clin Infect Dis* 2017; **64**(8): 1043-51.

90. Unger HW, Aho C, Ome-Kaius M, et al. Impact of intermittent preventive treatment in pregnancy with azithromycin-containing regimens on maternal nasopharyngeal carriage and antibiotic sensitivity of Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus: a cross-sectional survey at delivery. *J Clin Microbiol* 2015; **53**(4): 1317-23.