**Protocol for a systematic review and meta-analysis of the impact of first-trimester malaria infections on maternal, pregnancy and infant outcomes**

**Review Title: Impact of first-trimester malaria on pregnancy and infant outcomes: A systematic review and meta-analysis**

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**List of abbreviations used**

|  |  |
| --- | --- |
| **ANC** | Antenatal care |
| **IPD** | Individual participant data |
| **IPTp-SP** | Intermittent preventive treatment in pregnancy with Sulfadoxine-pyrimethamine |
| **IPTp-SP3+** | Three or more doses of IPTp-SP |
| **ITN** | Insecticide treated net |
| **HIV** | Human immunodeficiency virus |
| **LAMP** | Loop-mediated isothermal amplification |
| **LBW** | Low birth weight |
| **LLITN** | Long lasting insecticide treated net |
| **PCR** | Polymerase Chain Reaction |
| **PRISMA** | Preferred Reporting Items for Systematic Reviews and Meta-analysis  |
| **RDT** | Rapid diagnostic malaria test |
| **SD** | Standard deviation |
| **SGA** | Small-for-gestational-age |
| **SP** | Sulfadoxine-Pyrimethamine |
| **WHO** | World Health Organization |

# **Rationale**

In 2020, 121·9 million pregnancies occurred in malaria transmission areas, resulting in an estimated 70·9 million (58·1%) live births.1 Whilst the literature on the risks associated with malaria infections in the second and third trimester of pregnancy is well characterised, less is known about infections in early pregnancy. However, there is growing evidence about the severe health impacts of first-trimester infections on the mother and her fetus.2 *P**lasmodium falciparum* infection in early pregnancy impairs placental vasculogenesis and angiogenesis, and impacts the ability of the placenta to support foetal growth.3-7 Poor maternal and infant outcomes associated with malaria infection in the first trimester include hypertensive disorders during pregnancy, maternal anaemia, pregnancy loss, preterm birth, intrauterine growth retardation, low birth weight, and infants born small for gestational age.2,5,7-15 Furthermore, this is the period in pregnancy when women are at the highest risk of malaria infection.16-19 A modelling study suggests that over 60% of malaria infections during pregnancy occur by the end of the first trimester in unprotected women in areas of stable transmission.20 Moreover, recent evidence indicates that almost half of *P. falciparum* infections detected in the first trimester originate before pregnancy.21 A previous review by Huynh et al. on the effect of malaria in the first trimester was limited in scope and execution, restricted to high transmission areas in Africa (last search on 25 March 2014); results of six studies were presented in narrative tables without numbers, and no meta-analysis was conducted.

Many pregnancies are unintended and unplanned; in sub-Saharan Africa, East and South-East Asia and Latin America, there were an estimated 91, 58 and 69 unintended pregnancies per 1000 women aged 15-49 years, respectively (time-period 2015-2019).22 A review of 29 surveys in nine sub-Saharan African countries reported a mean unintended pregnancy rate of 33.9%.23 A mass distribution campaign in Liberia noted that only 31% women were aware of their pregnancy status during the first month.24 As a result, many women may not adapt their behaviour and use insecticide treated nets or consider implications for medication until well after the first month of pregnancy, potentially exposing the developing foetus to substances contra-indicated or not recommended in early pregnancy.

We propose a systematic review and individual participant data (IPD) meta-analysis (data permitting) on the risks and adverse pregnancy and infant outcomes associated with malaria infection in the first trimester of pregnancy. Synthesising this evidence is critical to drug developers, policymakers, and program managers to inform control strategies as this is a period of pregnancy when the current prevention policy of intermittent preventive treatment with sulfadoxine-pyrimethamine is not yet indicated and there can be provider/patient confusion on safe use of antimalarials for malaria treatment. Furthermore, this information is needed to inform the benefit-harm assessment of treatment options in this underserved group.

# **Objective of the Review**

The main objective of this review is to assess the burden and effect of first-trimester malaria infection on maternal, pregnancy and infant outcomes.

### **Primary Objective and outcomes**

To assess the effect of first-trimester malaria infection (clinical or asymptomatic) on:

* Pregnancy loss: miscarriage and stillbirth
* Maternal anaemia (haemoglobin <11 g/dl, <8 g/dl)
* Newborn low birthweight
* Preterm birth
* Small-for-gestational age

For definitions of exposures and outcomes, see Table 2.

### **Secondary objectives** **and outcomes**

To assess the effect of first-trimester malaria infection (clinical or asymptomatic) on:

2.2.1 Maternal outcomes

* Maternal haemoglobin in pregnancy
* Gestational age at maternal malaria infection
* Hypertensive disorder in pregnancy
* Maternal death

 2.2.2 Foetus during pregnancy/Newborn at delivery (for definitions, see table 2)

* Foetal growth if repeated ultrasound available during pregnancy
* Intra-uterine growth retardation
* Newborn birthweight
* Apgar score at delivery (supplement 1)
* Perinatal death (stillbirths and infant deaths in the first week of life)
* Estimated gestational age at delivery
* Infant small-for-gestational-age
* Infant haemoglobin at birth
* Infant anaemia at birth
* Congenital malaria (malaria infection of the cord blood)
* Foetal anaemia
* Infant anthropometry at birth
* If there is sufficient data/information, a composite adverse outcome of the combination of pregnancy loss, preterm birth, low birth weight and small-for-gestational-age infants will be considered

2.2.3 Infant (evaluation within 8 weeks postpartum)

This section will be evaluated in collaboration with Unger *et al*. in a review on the effect of malaria in pregnancy on the infant (Prospero CRD42020162260).

* Infant death in the first week and the first months of life (i.e., early neonatal, and neonatal mortality and infant mortality up to 8 weeks)
* Infant haemoglobin
* Infant anaemia
* All cause sick child clinic visits
* Infant clinical malaria, severe malaria, asymptomatic malaria, and submicroscopic malaria
* Infant growth: Weight for age, height for age, weight for length, mid-upper arm circumference and head circumference for age z-scores; scores of the WHO Child Growth Standards (WHO Multicentre Growth Reference Study Group)25

For definitions of exposures and outcomes please see Table 2.

**Table 1:** PICOTS Framework26

|  |  |
| --- | --- |
| **Components** | **Characteristics** |
| Population | Pregnant women in malarious regions |
| Condition | Malaria infection in the first trimester of pregnancy (any malaria species, including falciparum, vivax, ovale, malariae, and knowlesi, mixed or single infections: symptomatic or asymptomatic; uncomplicated or severe) |
| Control | No documented malaria infection in the first trimester |
| Outcomes | See specific objectives and outcomes above |
| Timing | Pregnancy to 8 weeks postpartum |
| Setting | Malaria endemic countries within sub-Saharan Africa |
| Language | The search will be conducted in English. If potentially eligible articles are identified in other languages, first an attempt will be made to identify a person who can translate the information. If this person is not available, the study will be excluded.  |

# **Methodology**

This protocol has been developed in accordance with PRISMA-P reporting guidelines and the results will be reported according to the applicable PRISMA guidelines.27-29

### **Eligibility**

### **Inclusion criteria**

* Study conducted in a malarious region: sub-Saharan Africa, Asia, Pacific, South and Central Americas.
* Primary observational and experimental studies including prospective cohort studies and trials (randomized controlled or quasi-experimental studies) with information available on first trimester malaria infection up to 13 weeks and 6 days of gestational age and one of the outcomes of interest as defined in Table 2. Because studies must have information on exposure (malaria in the first trimester) and outcome, surveys which are conducted at one time point cannot be included.
* Studies including only HIV-infected women can be included and may be used for subgroup analyses.

### **Exclusion criteria**

* No information on malaria in the first trimester.
* Studies evaluating treatment in the first trimester in the absence of a control group without malaria reporting on one of the outcomes of interest.
* Studies reporting on a history of malaria in the first trimester without a conduct of a confirmatory malaria test by the study.
* Case-control studies.
* Case Reports.
* Animal studies.
* Treatment studies where all women at enrolment have parasitemia.
* Studies outside of sub-Saharan Africa.
* Studies with a sample size of less than 30 women in the first trimester who would be eligible for inclusion in this review or with a very low number of events (e.g. < 6) which would preclude meaningful analysis
* Retrospective cohort studies.

### **Information source**

To identify eligible studies, we will use the Malaria in Pregnancy Library as the main source (<https://mip.wwarn.org>). The Malaria in Pregnancy Library combines data from over 20 sources including Medline, Web of Knowledge, Scopus, Cumulative Index to Nursing and Allied Health Literature, Bioline, the Cochrane Library databases, the WHO Global Health Library, and grey literature (i.e., reports, unpublished studies, and theses) and is updated every 4 months.30 To check completeness we will repeat the search in [PubMed](https://pubmed.ncbi.nlm.nih.gov/) (<https://pubmed.ncbi.nlm.nih.gov>), and the WorldWideScience database (<https://worldwidescience.org>). Searches will be from inception of the database to the last search date. After completion of the screening, we will repeat the search in Google Scholar (<https://scholar.google.com>) and check if any new publication is identified, until 10 consecutive result pages do not show any new results. References of included studies will be screened for additional relevant material. Experts in the field may be contacted for additional studies. It is possible that studies may be missed when malaria testing is not a prominent feature in the cohort. To be able to identify cohorts that may have been missed, available datasets about malaria in pregnancy will be screened on potential available data using repositories (e.g., WWARN) and a “malaria in pregnancy cohort registry” will be created using the Malaria in Pregnancy Library with all known cohort studies and trials, which will be additionally screened.

### **Search, screening, and selection strategies**

***Search terms***

The following search terms will be used in the Malaria in Pregnancy Library: ("Early pregnancy" OR "First Trimester" OR "First-Trimester" OR "preconception" OR "early gestation" OR "early gravidity" OR timing). No malaria term is needed because all material in this database was selected for malaria in pregnancy.

In PubMed the following search terms will be used: ("Early pregnancy" OR "First Trimester" OR "First-Trimester" OR "preconception" OR "early gestation" OR "early gravidity" OR “timing”) AND (Malaria OR falciparum OR vivax OR ovale OR knowlesi OR “mixed malaria infection”). The same terms will be used in the WorldWideScience database. The search will be conducted in English.

Because keywords may not be able to identify all relevant studies, a second search will be conducted in the “Malaria in pregnancy” library, using the keywords “Cohort” and “Trial”. The results will be additionally screened for eligible studies not identified in the first search.

***Selection process***

Two independent reviewers will screen titles, abstracts, and full-text articles if available to check if the study is eligible during the first screen. The second screen will compare the studies selected by either one of these reviewers and the two reviewers will agree on final study eligibility. The final number of studies to be obtained will be agreed upon with any disagreements on citations being resolved by consensus or by contacting a third reviewer who will serve as the tiebreaker. Studies considered eligible after full-text review by the two independent reviewers will be included in the final set of studies for inclusion. Studies with only an abstract available will not *a-priori* be excluded; an attempt to contact the authors to obtain more information will be made where the abstract does not provide enough information for inclusion. Data from reports from studies with multiple publications will be combined in one record entry, to avoid duplication of individual data. For articles retrieved in languages other than English, German, or French, the content will be verified for inclusion using a colleague who masters that language. The result of the search and selection process will be recorded in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram31 that will illustrate the number of studies rejected or retained at each step of the search and reasons for rejection.

### **Quality and bias assessment**

The papers identified by the search will be subject to quality assessment and will be graded based on the criteria from the study-design specific quality assessment tools (supplement 4 and 5). The tool for Risk of Bias In Non-randomized Studies of Exposure (ROBINS-E) (<https://www.bristol.ac.uk/population-health-sciences/centres/cresyda/barr/riskofbias/robins-e/>) will be used to assess quality in non-randomized trials and cohort studies, and the Cochrane risk-of-bias tool for randomized trials (RoB 2)32 will be used to assess randomized trials. However, for cohort studies, other quality assessment tools may be explored additionally (e.g. JBI <https://jbi.global/critical-appraisal-tools>). Quality assessment will be conducted by two reviewers (JA and AvE); a third person (SD, JH, or EO) will be used if there is disagreement. Studies labelled of very high or serious concern may be removed from the analysis.

### **Data extraction or collection process**

An attempt will be made for each eligible study to obtain the individual participant data set. Potential collaborators for the IPD analysis will be contacted by email and will be approached at least twice. If this fails (no response after two attempts in three months, or a negative response) or does not look feasible (e.g., an old study with deceased investigators), data on exposure and outcome of interest will be extracted using a standardized pretested data extraction form (supplement 2) independently by two persons (JA and AvE) and compared. Disagreement will be resolved through discussion or a third person (SD, JH, or EO) if no agreement can be reached. Where information is incomplete, an attempt may be made to contact the authors of the study involved to assess if additional information can be obtained.

Studies with authors with an interest in data-sharing will be sent a spreadsheet (supplement 3) with variables of interest. They will be requested to compile a dataset (cvs, excel or stata) with variables of interest and an anonymous identifier and upload this dataset, or an unselected dataset in WWARN (under “Malaria in pregnancy and the infant” study); alternatively, the investigators can do this for them if the WWARN agreement has been signed. For studies where a dataset has been attained for other studies/IPD meta-analyses, the investigators will be approached to find out if the dataset can be used for the purpose of this protocol. Extraction of information from studies conducted in more than one country, or different sites in one country will be split by location if there is sufficient detail available. For studies where the data cannot be shared, data will be extracted from the publication, if there is sufficient information, or authors will be asked if they can provide a table with the relevant exposures and outcomes.

### **External study-level data**

External data will be added to this file, such as GPS location from [Google Earth](https://www.google.com/earth/), and [country level HIV-prevalence data](http://aidsinfo.unaids.org) among women aged 15-49 years. Using the GPS coordinates and the midpoint of the study years, the *Plasmodium falciparum* parasite rate in 2-10 year olds as an indicator of malaria transmission intensity and ITN coverage33 will be obtained from the [Malaria Atlas Project](https://map.ox.ac.uk). For studies with no information on ITN use or use of IPTp at the time of delivery, we will obtain an estimate using national surveys ([demographic and health surveys](https://dhsprogram.com), [malaria indicator surveys](http://www.malariasurveys.org) or [multiple indicator cluster surveys](http://mics.unicef.org)) closest in time for the administrative region.

### **Exposures and outcome definitions and prioritization**

**Table 2. Definitions of exposure and outcomes**

|  |  |
| --- | --- |
| **Exposure** | Description |
| Asymptomatic malaria in the first trimester | Malaria by blood smear, rapid test, or PCR in the first trimester of pregnancy (a gestational age < 14 weeks gestation calculated from the last menstrual period for this purpose) but with no documented fever or clinical malaria as defined by the study. Any *Plasmodium* species or combination of species will be considered |
| Symptomatic malaria in the first trimester | A positive malaria test in the presence of documented fever (an axillary temperature of ≥ 37.5 °C) or clinical malaria as defined by the study. A history of malaria or malaria treatment in the first trimester will not be used as symptomatic malaria in the first trimester.  |
| Severe malaria in the first trimester  | Severe malaria: Maternal positive malaria test (RDT, blood smear, PCR or other) in the presence of one or more of the following:34* Extreme tiredness and fatigue
* Impaired consciousness
* Multiple convulsions
* Difficulty breathing or respiratory distress
* Dark or bloody urine
* Jaundice (yellowing of the eyes and skin)
* Abnormal bleeding

Laboratory* Acidosis
* Hypoglycemia
* Hyperparasitaemia
* Severe malaria anaemia
* Renal impairment

Or as defined by the source study. |
| Unexposed to malaria in the first trimester | No malaria in the first trimester is defined as the absence of documentation of a positive malaria test in this period.  |
| **Primary outcomes** |  |
| Miscarriage | Miscarriage defined as confirmed pregnancy that fails to progress beyond 27 weeks gestation or as defined by the source study. |
| Stillbirth | Stillbirth defined as a confirmed pregnancy lasting until 28 weeks or after that results in the birth of a baby showing no signs of life (death in utero), or as defined by the source study. Some studies may combine miscarriage and stillbirth; this will be noted when extracted and indicated in the analysis. |
| Maternal anaemia | Any anaemia is defined as a haemoglobin <11 g/dl. Moderate-to- severe anaemia is defined as haemoglobin <8 g/dl. If only a haematocrit is available, this will be divided by 3 to get an approximation of the estimate in g/dl.35 Maternal anaemia during pregnancy, at delivery, or within 4-8 weeks postpartum  |
| Newborn low birth weight | An infant weight < 2500 grams at birth, ideally measured within 24 hours. If weight is not available at birth, weight measured within 7 days will be used and analysis will be adjusted for time of measurement if possible.Wasting at birth will be defined as <3rd centile of corrected birthweight for gestational age using the INTERGROWTH-21st reference36 |
| Prematurity or preterm birth | Live birth before 37 complete weeks of gestational age (37 weeks + 0 days).37 Gestational age should preferably be measured by ultrasound earlier in the pregnancy. However, many methods are in use, e.g., fundal height, first day of the last menstrual period, or a scoring system such as the Ballard score at the time of delivery. All methods will be accepted and adjusted for in the analysis, if possible. |
| Small for gestational age (SGA)\* | SGA will be defined as a birthweight ≤ 10th percentile for a given gestational age and sex, using the INTERGROWTH gender specific chart.36 Additionally, the Stoppam chart will be explored as reference38  |
| **Secondary outcomes** |  |
| ***Maternal*** |  |
| Hypertensive disorder in pregnancy | Adverse event of hypertensive disorder as defined by the source study (include pre-eclampsia, eclampsia) |
| Maternal death in pregnancy or postpartum | Death from any cause related to or aggravated by pregnancy or its management (excluding accidental or incidental causes) during pregnancy and childbirth or within 42 days of termination of pregnancy, irrespective of the duration and site of the pregnancy. Although generally rare in studies, all death reports will be compiled, and classified according to first trimester malaria status. |
| ***Foetus during pregnancy/Newborn at delivery*** |  |
| Foetal growth/intra-uterine growth retardation\* | This outcome will be considered when repeated ultrasound is available during pregnancy for reliable longitudinal measurements. Intra-uterine growth retardation: foetal weight is estimated to be below the 10th percentile for its gestational age using the INTERGROWTH-21st estimated foetal weight (EFW) reference values39 |
| Apgar score at delivery | The Apgar score describes the condition of the newborn infant immediately after birth, and can be measured at 1, 5 and 10 minutes. The scoring system is available in supplement 1. Any score above 7 is considered as a good score.40  |
| Perinatal death | Stillbirths combined with infant deaths that occurred in the first week of life |
| Estimated gestational age at delivery | The outcome as presented by the study will be used. This may include by ultrasound, last menstrual period, foetal height, or Ballard score (or an equivalent score) at delivery. All methods will be accepted. In the one-stage analysis, it may be possible to adjust for the method of gestational age measurement |
| Newborn birthweight\* | Infant weight at birth in grams, ideally measured within 24 hours. If weight is not available at birth, weight measured within 7 days will be used and analysis will be adjusted for time of measurement if possible. Birthweights in kg will be reverted to grams. Using the INTERGROWTH-21st reference chart, the Z-score will be obtained for the birthweight. |
| Infant haemoglobin at birth | Haemoglobin measured by any test, recalculated to g/dl. If only a haematocrit is available, this will be divided by 3 to get an approximation of the estimate in g/dl.35 Infants are usually born with an average haemoglobin of 17 g/dl. |
| Infant anaemia at birth | A central venous haemoglobin value of below 13 g/dl or capillary haemoglobin below 14.2 g/dl or as defined by the source study. However, if IPD data is available, gestational age at delivery will be considered if prematurity is common and anaemia will be defined as < 5th percentile of a reference population.41  Moderately-to-severe anaemia will be defined as a haemoglobin value between the 1st and 5th percentile, and severe anaemia as a haemoglobin level that plots below the 1st percentile. |
| Congenital malaria | Malaria infection of the foetal cord blood or peripheral blood of the newborn at birth or within 7 days (168 hours postpartum) by any type of malaria test (any species) |
| Foetal anaemia | Haemoglobin <12.5 g/dL in umbilical cord blood or as defined by the study |
| Infant anthropometry at birth | Where available: Head circumference and length/height as continuous variable, and Z-score when adjusted by gestational age using the INTERGROWTH reference population. If there is sufficient data, abdominal and arm circumference of the newborn may be additionally considered.Stunting at birth will be defined as <3rd centile of birth length for gestational age using the INTERGROWTH-21st reference36 |
| ***Infant, within 8 weeks postpartum*** |  |
| Infant death | For this study it will be defined as death of the infant within 8 weeks postpartum. This may be categorized as follows: neonatal mortality (death in the first month postpartum), early neonatal mortality (death in the first week postpartum), and infant mortality (death from birth to 8 weeks) |
| Infant haemoglobin | Haemoglobin measured by any test within 8 weeks postpartum, recalculated to g/dl. If only a haematocrit is available, this will be divided by 3 to get an approximation of the estimate in g/dl.35 |
| Infant anaemia | Anaemia at any visit within 8 weeks postpartum, using an age-appropriate definition for anaemia (a haemoglobin concentration more than two standard deviations below the mean of similarly aged infants from a reference population not exposed to malaria in the United States)42 |
| All-cause sick child clinic visits | Any unscheduled visit to a clinic or equivalent of a sick child visit within 8 weeks postpartum, i.e., clinic visits that exclude otherwise healthy children coming for scheduled vaccination visits or scheduled follow-up visits. |
| Infant clinical malaria, and severe malaria  | Infant positive malaria test within 8 weeks postpartum (RDT, blood smear, PCR or other; any species), in the presence of documented fever (an axillary temperature or 37.5 °C or more or clinical malaria as defined by the study). Infant severe malaria: see definition for maternal severe malaria |
| Infant asymptomatic malaria | Infant positive malaria test within 8 weeks postpartum (RDT, blood smear, PCR or other; any species), in the absence of symptoms  |
| Infant submicroscopic malaria | Submicroscopic malaria (blood smear negative and PCR-positive test or a test equivalent to PCR; any species) within 8 weeks postpartum in the presence or absence of complaints |
| Weight for age (WAZ), length/height-for-age (LAZ / HAZ), weight-for length/height (WLZ / WFH), MUAC and head circumference\* | Weight and height gain defined as the age and sex standardized weight-for-age (WAZ), length- or height-for-age (LAZ/HAZ), weight-for length or height (WLZ/WHZ), arm (MUAC) and head circumference-for-age Z scores of the WHO Child Growth Standards (WHO Multicentre Growth Reference Study Group)25 |
| Underweight | Low weight for age: Moderate: Z-score <2SD from reference population; Severe: Z-score <3SD from WHO reference population |
| Stunted | Low length/height for age: Moderate: Z-score <2SD from reference population; Severe: Z-score <3SD from WHO reference population |
| Wasted | Low weight for length/height: Moderate: Z-score <2SD from reference population; Severe: Z-score <3SD from WHO reference population |
| MUAC | Low arm-circumference: Moderate: Z-score <2SD from reference population; Severe: Z-score <3SD from WHO reference population |
| Head circumference | Low head-circumference: Moderate: Z-score <2SD from reference population; Severe: Z-score <3SD from WHO reference population |
| Neurological and developmental evaluations of the infant | Neurological and developmental evaluations will be used as reported by the source studies. These may include scales and tests42  |

\*A Z-score for a measurement indicates how far the obtained measurement is from the mean, with standard deviation as the unit. A Z-score of 1.0 would indicate a value that is one standard deviation from the mean. Z-scores may be positive or negative, with a positive value indicating the score is above the mean and a negative score indicating it is below the mean. In this review, Z-scores will only be applied to outcomes where individual participant data has been obtained. Any outcome where a reference population is used can only be obtained when IPD data is available. The INTERGROWTH reference population is

derived from a multi-ethnic cohort of low-risk, well-nourished mothers with uncomplicated

pregnancies.36

Primary outcomes are based on clinical importance and common availability. Maternal anaemia is likely to be affected by chronic malaria infections and affect both the mother and the developing foetus. Infant low birth weight, and prematurity are both a reflection of intra-uterine well-being and prognostic for infant development.

### **Data synthesis**

***Analyses***

All studies will be described in a table and quality assessment presented. Analyses will be conducted in Stata and R. We will compare the outcomes of interest in the mother and infant among mothers with and without exposure. Exposure and outcomes will be used as defined in Table 2. For the analyses, different approaches will be used, and these will be pre-specified in the analysis plan before the start of the analysis. Using two-stage analyses, for each study the prevalence ratio will be calculated for outcomes available, comparing outcomes with first trimester malaria vs. outcomes without first trimester malaria. Heterogeneity between the studies in effect measures will be assessed using both the *I2* statistic and prediction intervals. Additionally, first trimester symptomatic malaria (or severe malaria) will be compared with no first trimester malaria. We anticipate that this analysis will be able to accommodate all studies with data available. Meta-regression will be conducted to assess the effect of study-level co-variates on the prevalence ratio, such as global region (Africa vs. outside of Africa), proportion of primigravidae in a study, HIV prevalence in the region, proportion of use of malaria prevention (ITNs, IPTp, other medication), and malaria transmission level. Studies will be included with malaria information up to 22 weeks gestation to allow subgroup analysis for the early months. However, a gestational age of <14 weeks will be used as the strict definition of the first trimester.

For studies which provided a dataset, additional analyses can be conducted (one stage analysis), and better adjustment for co-variates (such as maternal gravidity, age and use of malaria prevention, indicators of socio-economic status or repeated malaria infections throughout pregnancy, indicators of malaria transmission intensity, and use of malaria prevention) can be achieved. For studies with only a single time point at maternal or infant follow up, we will use prevalence ratios. For studies with multiple infant-follow up visits, time to the first event or repeated events over time will be used. For models with binary outcomes (malaria, anaemia, fever), statistical models which will be considered include Cox regression (with shared frailty) for time to event (or parametric survival models if proportional hazards assumption is not met), xtlogit, xtreg, xtgee and melogit. For incidence rate ratio we will use Poisson models (or negative binomial models when appropriate) or Hazard ratio models for repeated events. For models for continuous outcomes, xtreg, xtgee, and mixed models will be considered. When using a one-stage approach, univariate and multivariate analysis will be conducted. We will evaluate the standard deviation of the random effect as the measure of heterogeneity in mixed-level models.

***Adjusted analyses***

The co-variates examined in each analysis will differ by outcome examined and depend on availability, but will include maternal age, gravidity, season, setting (urban vs. rural), maternal HIV infection, antimalarial treatment used, use of malaria prevention (ITNs, IPTp or other regimen, IRS) by the mother and infant, use of haematinics by the infant, use of antimalarials by the infant, and type of malaria test (mother and infant). If available, other malaria test results in pregnancy can be added as confounders. Infant gender will be used for infant outcomes. For infant status at birth and growth, information on maternal anthropometry will be used if available.

***Sub-group analysis***

Subgroup analyses will be conducted by timing of infection (early or <10 weeks gestation, vs. late or 10-14 weeks of gestation), region (East vs. West Africa), maternal density of infection, uncomplicated vs. severe infections, symptomatic vs asymptomatic infections, parasite species (*P. falciparum*, *P. vivax*, mixed infections), maternal HIV status, gravidity, level of malaria transmission, and infant sex.

***Missing data***

There are different types of missing data: 1) the variable may not have been collected; 2) the variable was collected but incomplete; 3) the variable was collected but not included in the dataset. In the last case, we will contact the original authors of the study to obtain the relevant missing data. If a covariable was collected but incomplete, missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions. For a continuous variable, missing values will be imputed from random values from a normal distribution with mean and SD calculated from the available sample. For a categorical variable, missing values will be imputed from random values from a uniform distribution with probabilities *P*1, *P*2, …, and *P*k from the sample. The seed for the imputation is set as 128. Only covariates will be imputed if the variable was collected and incomplete: missing outcomes or exposure data will not be imputed.

***Sensitivity analyses***

To assess the robustness of the results, we will conduct several sensitivity analyses, including:

* Inclusion of study quality variable and study design (cohort, trial), different malaria tests where applicable, and different tests for gestational age assessment
* Imputation of partially missing data
* For binary non-repeated outcomes, we will compare the results of the two-stage approach and compare this with the results of the one-stage analysis

**Meta-bias**

Some studies or outcomes may be more likely to be reported in publications than others, e.g., because they showed specific (significant) associations in either direction or because of the perceived importance of specific finding by the authors (publication bias across studies, selective reporting within studies). We will assess which outcomes have been more frequently reported than others to assess for potential sources of meta-bias. Also, we will verify trial registries for completion of study reporting. Lastly, we will minimise reporting bias by asking authors to provide a complete set of outcomes where available.

**Confidence in cumulative evidence**

In the absence of a grading system comparable to GRADE, the system used for meta-analyses of trials, we will describe the strength of the body of evidence for the primary outcomes across the domains of risk of bias, consistency, directness, precision, and publication bias. Additional domains and outcomes may be considered where appropriate.

### **Ethics and dissemination**

All individual studies have ethical approval from relevant local ethics committees and results are or will be available in one or more publications. Results will be presented to the working group participants prior to publication and public dissemination for review. Results of the study will be published in peer-reviewed journals and presented at national and international conferences, whereby all members of the working group will be listed in slides or in the publication.

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## Supplement 1. Apgar scoring system used to evaluate newborns

|  |
| --- |
|  |
| Bpm, beats per minute (newborn heart beat). The score can be measured at 1, 5 and 10 minutes after delivery. The maximum score a newborn can obtain is a 10. A score > 7 is considered good. However, there is some controversy about this system, with some considering it unreliable in measurement and others complaining about limited predictive value for adverse infant development.40,43  |

## Supplement 2. Information to be extracted from eligible studies

Study characteristics

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Study (1st author and year) | Country | Time period | Design | Intervention (if applicable) | Sample size (by trimester if available) | Inclusion criteria | Exclusion criteria | Gestational age assessment | Outcomes available |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |

Additional variables to be extracted:

Setting (urban/rural/peri-urban)

Malaria prevention (ITN, chemoprevention, IRS)

Type of malaria test and frequency in pregnancy

HIV positivity

Age

Gravidity

Study completion rate

Antimalarial treatments for women with a positive malaria test

Outcome table

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study (1st author and year) | Outcome | With Malaria 1st trimester (n/N) | No Malaria 1st trimester (n/N) | Adjusted effect estimate if available\* |
|  |  |  |  |  |
|  |  |  |  |  |

\*Indicate the variables for which adjustment was made

See also “Teams”, excel spreadsheet “Suppl 2 Data\_extraction\_MiP first\_trimester” in the protocol folder.

## Supplement 3. Information requested for datasets from eligible studies.

See “Teams”, excel spread sheet “Suppl 3 Variable\_description first trimester” in the protocol folder.

## Supplement 4. ROBINS-E tool quality assessment

See “Teams”, file: Risk of Bias In Non-randomized Studies - of Exposures and explanation (<https://www.riskofbias.info/welcome/robins-e-tool>)

## Supplement 5. Rob 2 quality assessment

See “Teams”, file: Cochrane risk of bias tool for randomized trials (<https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2>)