Fetal growth and birth weight are independently reduced by malaria infection and curable sexually transmitted and reproductive tract infections in Kenya, Tanzania, and Malawi: A pregnancy cohort study

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Highlights

- Largest study to date on the effect of malaria on fetal growth using ultrasound
- First to asses fetal growth after sexually transmitted/reproductive tract infection
- Dual burden of the infections negatively impact fetal growth
- First and second times mothers are especially vulnerable to the infections
- Integrated antenatal care is needed to reduce the burden

Fetal growth and birth weight are independently reduced by malaria infection and curable sexually transmitted and reproductive tract infections in Kenya, Tanzania, and Malawi: A pregnancy cohort study

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ABSTRACT

Objective

Malaria and sexually transmitted and reproductive tract infections (STIs/RTIs) are highly prevalent in sub-Saharan Africa and associated with poor pregnancy outcomes. We investigated the individual and combined effects of malaria and curable STIs/RTIs on fetal growth in Kenya, Tanzania, and Malawi.

Methods

This study was nested within a randomized trial comparing monthly intermittent preventive treatment for malaria in pregnancy with sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine, alone or combined with azithromycin. Fetal weight gain was assessed by serial prenatal ultrasound. Malaria was assessed monthly, and *Treponema pallidum*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Chlamydia trachomatis* and bacterial vaginosis at enrolment and in the third trimester. The effect of malaria and STIs/RTIs on fetal weight/birthweight Z-scores was evaluated using mixed-effects linear regression.

Results

1,435 pregnant women had fetal/birth weight assessed 3,950 times. Compared to women without malaria or STIs/RTIs (n=399), malaria-only (n=267), STIs/RTIs-only (n=410) or both (n=353) were associated with reduced fetal growth (adjusted mean difference in fetal/birth weight Z-score [95% CI]: malaria=-0.18 [-0.31,-0.04], p=0.01]; STIs/RTIs=-0.14 [-0.26,-0.03], p=0.01]; both=-0.20 [-0.33,-0.07], p=0.003). Paucigravidae experienced the greatest impact.

Conclusion

Malaria and STIs/RTIs are associated with poor fetal growth especially among paucigravidae women with dual infections. Integrated antenatal interventions are needed to reduce the burden of both malaria and STIs/RTIs.

Keywords

Malaria in pregnancy, sexually transmitted infection, reproductive tract infection, bacterial vaginosis, fetal growth, birthweight.

INTRODUCTION

Despite efforts to reduce its burden [1], an estimated 46 to 52 million pregnancies were at risk of malaria infection in sub-Saharan Africa in 2020 [2]. Most malaria infections (>80%) during pregnancy remain asymptomatic [3] yet are associated with maternal anemia and impaired fetal growth [4, 5], leading to small-for-gestational-age (SGA), low birthweight (LBW) newborns, and preterm delivery [6].

Curable sexually transmitted and other reproductive tract infections (STIs/RTIs) such as syphilis (*Treponema pallidum*), chlamydia (*Chlamydia trachomatis*), gonorrhoea (*Neisseria gonorrhoeae*), trichomoniasis (*Trichomonas vaginalis*) and bacterial vaginosis are also common in sub-Saharan Africa [7]. Syphilis screening and treatment is part of standard antenatal care throughout sub-Saharan Africa, but other STIs/RTIs are managed via syndromic algorithms [8]. Like malaria, most STIs/RTIs are asymptomatic and often remain undetected and untreated [9]. Exposure to STIs/RTIs during pregnancy is associated with poor birth outcomes such as preterm birth and LBW [7].

Infants born preterm, SGA, or with LBW are at increased risk of neonatal morbidity and mortality [6] and possibly cardio-metabolic diseases in adult life [10].

Despite malaria and STIs/RTIs being highly prevalent in sub-Saharan Africa, few studies have investigated their dual-impact on fetal growth and pregnancy outcomes [11]. Fetal growth evaluation requires accurate gestational age estimation and serial ultrasound to assess fetal weight. Most studies in sub-Saharan Africa relied on LBW and SGA at birth as proxy indicators of intrauterine growth restriction. However, both have limitations in identifying intrauterine growth restriction. Firstly, LBW may result

from either intrauterine growth restriction, preterm delivery, or both [12]. Secondly, SGA newborns may be growth-retarded or constitutionally small but healthy [13]. Finally, newborns may have failed to achieve their biological growth potential but still be above the cut-off for LBW or SGA [13].

Only a few and small studies have used ultrasound to assess the effect of malaria on fetal growth [4, 5, 14, 15]. To our knowledge, no study has investigated the effects of STIs/RTIs on fetal growth trajectories or the consequences of both malaria and STIs/RTIs using ultrasound.

METHODS

Study design and population

This cohort study was nested in a randomized partially placebo-controlled trial conducted from March 2018 to August 2019 involving 4,680 pregnant women comparing monthly intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine versus dihydroartemisinin-piperaquine, alone or combined with a single course of azithromycin at enrolment conducted in Kenya, Tanzania and Malawi [16]. Of these women, one-third were randomly selected into a nested cohort for fetal growth monitoring by serial ultrasound. In order to have a power of 80% to detect an expected proportion of women with STIs/RTIs was 40% in sulfadoxine-pyrimethamine arm compared to 30% in dihydroartemisinin-piperaquine / dihydroartemisinin-piperaquine + azithromycin, with alpa=0.025, 432 women per treatment arm were needed. To allow for 13% loss to follow-up, 500 women were recruited per arm. Women attending antenatal care were enrolled if HIV-negative, had a

viable singleton pregnancy between 16 and 28 weeks gestation, no known heart disease, had not received sulfadoxine-pyrimethamine during the current pregnancy, and had no known allergy to the study drugs.

Data collection procedures

Details of data collection have been described elsewhere [16]. In brief, demographic data and medical history were collected at enrolment. Women were screened for urinary tract infection (using urine dipsticks) and hypertensive disorders (blood pressure >140/90 mmHg ± proteinuria), prior medication usage, and maternal anthropometrics were recorded at each antenatal visit. Hemoglobin level was assessed (Hemocue 301 or 201) at enrolment, in the third trimester, and at delivery.

Estimation of gestational and fetal weight

Using ultrasound and standard methodology, gestational age was estimated based on crown-rump length until 13^{+6} weeks [17], and from 14^{+0} weeks by using an algorithm of head circumference and femur length [18], head circumference only [18] or femur length only [19], depending on availability of fetal biometrics. Serial ultrasound was performed at enrolment if gestational age was ≥ 22 weeks, at approximately 25-28 weeks gestation, and at approximately 32-35 weeks, and fetal weights were estimated based on head circumference, abdominal circumference and femur length [20].

Detection of malaria

Women were screened for malaria at enrolment using malaria rapid diagnostic tests (mRDTs) (CareStart™ Malaria Pf/PAN (HRP2/pLDH) Ag Combo) as per national policy

in Kenya and Tanzania. In all three countries, women with fever (≥ 37.5^{0}C) or recent history of fever were also screened with mRDTs.

In Kenya and Malawi, regardless of treatment arm, women with positive mRDTs were treated with artemether-lumefantrine, and IPTp dosing was deferred for four weeks. In Tanzania, women with positive mRDTs in the sulfadoxine-pyrimethamine arm were treated with artemether-lumefantrine, and IPTp was deferred for four weeks. However, women in the dihydroartemisinin-piperaquine and dihydroartemisinin-piperaquine/azithromycin groups who had positive mRDTs at enrolment were given their first course of IPTp but at later visits artemether-lumefantrine was administrated if mRDTs were positive, and IPTp was deferred for four weeks.

Peripheral maternal venous blood was collected at all visits and at delivery, along with cord and placental blood. Thick and thin blood smears were prepared, Giemsa stained, and independently double-read by experienced microscopists; where results were discordant, a third reading was performed to determine the final result [16]. Dried blood spots were also prepared for quantitative real-time polymerase chain reaction (qRT-PCR) [16]. Finally, placental biopsies were taken at delivery for malaria histology [16].

Detection of STIs/RTIs

As part of standard care, pregnant women were pre-screened for HIV. Women living with HIV were provided treatment per national guidelines and excluded from the study. All women were subsequently screened for syphilis with SD-Bioline point of care tests and, if positive, they were treated with 2.4 million units intramascular benzathine penicillin G. Additionally, clinic staff routinely asked women if they had experienced any symptoms associated with STIs/ RTIs. At any visit, if a woman responded in the

affirmative, she was treated by the clinic staff according to national syndromic management guidelines recommended by the WHO [8]. Apart from routine care, clinic staff collected vaginal swabs and stored them on site until the end of the trial, at which time the samples were shipped to a regional reference laboratory in East Africa for retrospective batch analysis. Serum and vaginal swab samples were collected at enrolment and between 32-36 weeks. Serum samples were tested for rapid plasma reagin and confirmatory syphilis testing with *Treponema pallidum* Hemagglutination assays. Vaginal samples were tested for chlamydia and gonorrhoea DNA by RT-PCR (*Artus*® CT/NG QS-RGQ Kit), trichomoniasis with SACASE™ Real-TM Kit, and bacterial vaginosis using the Nugent scoring.

Pregnancy outcome

At delivery, birthweight was measured using digital scales (Seca GmbH & Co. KG., precision 10g or ADE M112600, precision 5g) and head and abdominal circumferences using flexible tape. Birthweights recorded >1 hour post-delivery were adjusted for the physiological weight loss [21].

Statistical analysis

Analyses were conducted using Stata software, v16 (Stata Corp, Texas, USA). Malaria exposure was defined as testing positive at any time-point by any assay: mRDT, microscopy, qRT-PCR, and/or placental histology. STIs/RTIs exposure was defined for individual STIs/RTIs and as a composite variable with positive test for any STIs/RTIs at any time-point. For the longitudinal analyses, women were considered negative until their first malaria and/or STIs/RTIs episode and thereafter considered positive. Four

unique exposure groups were generated to assess if malaria and STIs/RTIs co-infection affected growth trajectories; a control group with neither malaria nor STIs/RTIs; malaria-only; STIs/RTIs-only; and malaria plus STIs/RTIs.

The primary outcome was Z-scores for fetal weights and birthweight using a sex-specific Tanzanian reference chart [22] based on previous evidence indicating that a local growth curve is more representative than the international growth curve [23]. Our approach aligns with recent recommendations by the International Federation of Gynecology and Obstetrics (FIGO) on the accuracy of growth curves [24]. Secondary outcomes were birthweight Z-scores alone, growth trajectories based only on fetal weights Z-score, SGA (birthweight <10th percentile) [22], LBW (birthweight <2.5Kg), preterm delivery (gestational age <37 weeks), and newborn abdominal circumference in millimeters and head circumference in millimeters or Z-scores based on INTERGROWTH-21st reference [25].

Women with a non-viable pregnancy outcome (miscarriage, stillbirths), twin pregnancy, severe congenital malformations, or missing data on malaria and STIs/RTIs were excluded. Furthermore, observations with weights measured <14 days apart, gestational age <18 weeks or ≥45 weeks, birthweights <250g or ≥6,500g, or fetal/birthweight Z-score >±5, were excluded.

Linear regression models and linear mixed-effects models were used to assess the effect of malaria and/or STIs/RTIs on birth size and growth trajectories respectively. All crude models were adjusted for study design factors (study arm, site, and gravidity [paucigravidae, i.e. primi- and secundigravidae, and multigravidae]). In mixed models, these same design factors were included as fixed effects, gestational age at visit was

included as a time factor, and individual participant as a random effect to account for within-subject clustering. In addition, other potential confounders, selected based on the statistical analysis plan for the main trial, including rainfall patterns, malaria transmission intensity, patterns of parasite resistance to sulfadoxine-pyrimethamine, maternal age, gestational age at enrolment or delivery, socioeconomic status, maternal body-mass index, bednet use, number of IPTp doses received, hemoglobin levels, and sex of the fetus/newborns, were considered if associated with the outcome variable with a $p<0.2$ in the univariate models and retained in final models if p-values were <0.1.

Malaria infection is more detrimental in paucigravidae and undernourished women than in multigravidae and well-nourished counterparts. Thus, we fitted models with interaction terms to investigate possible effect-modification between malaria and gravidity or malaria and maternal body-mass index. The interaction between malaria and STIs/RTIs was also assessed.

To assess if the effect on growth trajectories was due to poor growth close to delivery, models only including Z-scores for fetal weights but not birthweight, were also generated. Finally, as fetal weight gain is mainly in the third trimester, a linear regression model was generated with a single fetal weight Z-score in the third trimester as the outcome, and malaria infections or STIs/RTIs occurring before the fetal weight estimation as exposure.

Additionally, a dose-response relationship was assessed by comparing the impact of number of malaria episodes on birth weight Z-score using the group with one malaria episode as the reference group. Furthermore, the model on STIs/RTIs was repeated after categorizing STIs/RTIs exposure by: 1) composite STIs/RTIs only at enrolment,

between weeks 32 and 36, or both at enrolment and between weeks 32 to 36; 2) only one type of STIs/RTIs, or multiple STIs/RTIs. Finally, we assessed the effect of malaria and STIs/RTIs on SGA, LBW and preterm delivery using Poisson regression with robust error variance.

RESULTS

Study population

Of the 1,586 women randomly selected for fetal growth monitoring, 1,435 were eligible for analyses. Of the 1,435 participants, 573 (39.9%) were >22 weeks at enrolment and had fetal weight assessed,1,007 (70.2%) had fetal weight assessed between approximately 25-28 weeks and 1,045 (72.8%) between approximately 32-35 weeks. Birthweights were available for 1,325 (92.3%) participants. Thus, 3,950 observations of fetal weight/birthweights were included in the longitudinal analysis (Figure S1).

The distribution of the 1,435 women was similar across study arms and countries. The mean age was 24.9 (SD 5.8) years. Only 2.8% were underweight (body-mass index <18.5 Kg/m^2) at enrolment, whereas 33.2% were overweight (25-29.9 Kg/m^2) or obese (≥30 Kg/m^2). Among the newborns, 13.4% were SGA, 4.3% were preterm, and 8.1% were LBW (Table 1). Baseline maternal characteristics were similar between included and excluded mother-newborn dyads, except that; a higher proportion of excluded women were from Malawi and paucigravidae, the proportion of bed net use at enrolment also differed significantly between the two groups and this proportion was lower among excluded women (Table S1).

Malaria infection was common: 43.4% (623/1,435) of women had at least one episode during pregnancy, and 46.3% (364/787) of paucigravidae had malaria (Table 2.a). Malaria prevalence varied across study sites and arms, being highest in Malawi and in the sulfadoxine-pyrimethamine arm (Table S2). Women with malaria had lower socioeconomic status, were younger, had lower body-mass index and hemoglobin levels at enrolment, and more often came from rural areas (Table S2).
Similarly, a high prevalence of STIs/RTIs was observed, with over half of the women having STIs/RTIs detected either at enrolment, in the third trimester, or at both timepoints (Table 2.b). Bacterial vaginosis was the most common, with 34.6% (449/1,297) of the women testing positive for bacterial vaginosis at least once during pregnancy. Only 1.9% (27/1,407) and 4.2% (54/1,298) of the women had syphilis and gonorrhoea, respectively. Among women with STIs/RTIs a higher proportion were from Tanzania. Women with and without STIs/RTIs had similar demographic characteristics across study arms (Table S3). Fetal biometry in second and third trimesters by gestational age and gravidity is described in Table S4.

Effect of malaria and STIs/RTIs on growth trajectories

There was a trend towards lower mean birthweight Z-scores among women with malaria infection and STIs/RTIs compared to women without (adjusted mean difference [aMD] [95% CI] malaria:-0.10 [-0.22,0.02], p=0.09; STIs/RTIs:aMD=-0.09 [-0.21,0.02], p=0.12) (Table 3.a+b). Malaria exposure was also associated with a higher proportion of newborns being SGA (aRR:1.50 [1.14-1.97], p=0.004) (Table 3.a). The effect was more

evident among paucigravidae women with malaria or STIs/RTIs (Malaria: aMD for birthweight Z-score= -0.19 [-0.35,-0.03], p=0.02 and SGA aRR=1.84 [1.26-2.69], p=0.002); STIs/RTIs:aMD for birthweight Z-score =-0.17 [-0.33,-0.01], p=0.04) (Table 3.a+b). There was a tendency towards a dose-response relationship between the number of malaria episodes and impact on birthweight Z-score, although this was not statistically significant (1 vs 2 malaria episodes aMD -0.12 [-0.41,0.16], p=0.39; 1 vs 3+ malaria episodes aMD -0.32 [-0.72, 0.09], p=0.13). Infection with both malaria and STIs/RTIs in paucigravid women had an even more pronounced effect on birthweight Z-scores (aMD=-0.34 [-0.57, -0.11], p=0.003) (Table 4a) and SGA (aRR=2.53 [1.37-4.67], p=0.003) (Table 4.b). The same effect on birthweight and risk of SGA was not observed among multigravidae (Tables 3 and 4).

Neither head circumference nor abdominal circumference differed significantly among malaria or STIs/RTIs exposed compared to non-exposed newborns (Tables 3 and 4).

No statistically significant effect of the individual STIs/RTIs on birthweight was observed, albeit there was a trend towards lower birthweight Z-score among newborns whose mothers had bacterial vaginosis (crude MD=-0.13 [-0.21, 0.08], p=0.06) (Table S5).

The effects of malaria and STIs/RTIs on growth trajectories were investigated using mixed-effect regression models on fetal weights and birthweight Z-scores (Table 5). Malaria infection was associated with a lower weight Z-score over time (aMD=-0.12 [-0.22, -0.03], p=0.01) (Table 5.a). The effects differed significantly by gravidity strata ($P_{interaction}$=0.01) and were more pronounced among paucigravide (weight Z-score [95% CI] over time aMD=-0.17(-0.31, -0.04), p=0.01) than multigravidae (aMD=-0.07 [-0.21, 0.07], p=0.34) (Table 5.b+c). There were no significant interaction between BMI and

malaria ($P_{interaction}$=0.48). STIs/RTIs also reduced weight Z-score over time (aMD=-0.11, -0.20, -0.01, p=0.03), again with paucigravidae being most affected (Table 5.d+e).

The magnitude of the effect on growth trajectories was similar after exposure to malaria-alone (aMD=-0.18 (-0.31, -0.04), p=0.01), STIs/RTIs-alone (aMD=-0.14, -0.26, -0.03, p=0.01) or to malaria plus STIs/RTIs (aMD=-0.20, -0.33, -0.07, p=0.003) (Tables 5.g), and there was a non-significant interaction between malaria and STIs/RTIs ($P_{interaction}$=0.18). Again, infection with both malaria and STIs/RTIs impacted growth trajectories more in paucigravidae than multigravidae (aMD=-0.30, -0.48, -0.11, p=0.001 vs -0.11, -0.30, 0.09, p=0.28) (Table 5.h+i).

Models containing only fetal weight Z-scores but not birthweight yielded similar results (Tables 3 and 4.a).

Fetal weight in the 3rd trimester, assessed by a single measure, was also lower among paucigravidae after malaria (aMD=-0.25, -0.47, -0.03, p=0.02), but not after STIs/RTIs (Table S6). Fetal weight gain over time was lower among women with STIs/RTIs at enrolment than women with STIs/RTIs both at enrolment and in the third trimester (Table S7). The individual STIs/RTIs were not significantly associated with impaired fetal growth, although there was a trend towards lower fetal/birthweight Z-score for trichomoniasis (aMD=-0.11, -0.23, -0.02, p=0.09) (Table S7). Finally, having multiple STIs/RTIs did not further reduce fetal weight gain compared to having a single STI/RTI (Table S7).

DISCUSSION

There was a high burden of malaria and STIs/RTIs; almost 25% of the women had both conditions during pregnancy. This is consistent with previous studies demonstrating a high prevalence of either malaria [26], STIs/RTIs [27], or both [11].

In the current study, fetal growth trajectories were negatively affected by infection with malaria and STIs/RTIs alone or combined. Malaria in pregnancy is characterized by placental sequestration of malaria-infected erythrocytes resulting in placental inflammation [12], poor vascular development [28] and altered flow in the umbilical and uterine arteries [29]. This may explain the association between malaria and fetal growth restriction. Previous smaller longitudinal studies found reduced fetal biometry and weights in the second [15] and third trimester [4] and an increased risk of fetal SGA [14]. We observed a negative impact on fetal growth trajectories based both on fetal weights and birthweights as well as solely on ultrasound-estimated fetal weights. This suggests that the negative effect occurs continuously *in utero* and not only close to birth. Paucigravidae experienced the greatest negative impact on fetal growth trajectories, a finding consistent with gravidity-associated epidemiology of malaria in pregnancy [6].

The mechanism by which STIs/RTIs affect fetal growth is not well elucidated. One mechanism may be that ascending genital infections lead to intrauterine infection and inflammation, damaging the trophoblast cells and resulting in placental dysfunction [30]. Previous studies on STIs/RTIs used birthweight as a proxy for intrauterine growth restriction [31]. Our study is the first to conduct serial prenatal ultrasound measurements, demonstrating a significant negative association between STIs/RTIs and fetal growth trajectories. Having infection with both malaria and STIs/RTIs was

particularly deleterious to pregnancies of paucigravidae, perhaps due to the dual placental insult occurring in this group. However, the interaction between the dual infection was insignificant. This suggests a non-synergistic effect, although this could also be due to the small sample size and the limited power to detect interactions.

Fetal weight gain was reduced over time among women who tested positive for STIs/RTIs at enrolment but not when considering STIs/RTIs occurring only at week 32-36. This suggests that the negative effect of STIs/RTIs on fetal growth alterations is set early in pregnancy, well before fetal growth peaks in the third trimester. Thus, intervention later in pregnancy may not interrupt the causal pathway to reduced fetal growth. Previous studies found a significant association between bacterial vaginosis and SGA at birth, while others have reported a non-significant association [31].

The effect of STIs/RTIs may also depend on the type and number of infections. Our study indicated that the negative effect of STIs/RTIs on fetal growth might mainly be due to bacterial vaginosis or trichomoniasis. Bacterial vaginosis was the most common cause of STIs/RTIs, especially among women with only one type of STIs/RTIs, and the high prevalence of bacterial vaginosis provided more statistical power to detect an impact on fetal growth. This might explain why having only one type compared to multiple types of STIs/RTIs appeared to be strongly associated with impaired fetal growth.

Our findings have implications for antenatal care and public health in areas where both malaria and STIs/RTIs are prevalent. The dual burden of malaria and STIs/RTIs is under-appreciated in the antenatal care setting and in the research community. This

may partly be explained by both malaria infections and STIs/RTIs being largely asymptomatic among pregnant women [9]. Thus, etiological assays to quantify the true dual burden of infections are needed. A systematic review of malaria and STIs/RTIs among pregnant women attending antenatal care facilities in sub-Saharan Africa identified 171 studies with relevant data points for pooling; none reported the prevalence of dual infection [7].

Current antenatal care includes screening strategies for malaria, HIV, and syphilis. Our study suggests the importance of antenatally targeting other STIs/RTIs as well. Women in this study received IPTp to prevent malaria at each antenatal visit and high-quality care in the clinical trial context with treatment of all detected malaria, syphilis, and symptomatic STIs/RTIs. Nonetheless, a consequential and deleterious effect was still observed – even after adjusting for the type and number of IPTp doses. This emphasizes the need to strengthen community sensitisation and public health awareness about the prevalence, consequences and prevention strategies of these infections. As both malaria and STIs/RTIs are often asymptomatic [27], universal early screening and treatment of both conditions may be warranted [26, 32], especially as point-of-care tests for STIs/RTIs are available, in addition to syphilis and HIV [33]. The importance of early syphilis screening and treatment on pregnancy outcomes has been well demonstrated [32]. A similar emphasis on early intervention is needed for other STIs/RTIs, particularly in low and middle income countries with high disease burdens.

Strength and limitation

This is the largest study to date utilising ultrasound for fetal weight estimation concurrently with in-depth testing for malaria and STIs/RTIs. High-quality obstetric ultrasound was ensured by thorough training of sonographers, review of all ultrasound images at the beginning of the study and thereafter 10% randomly selected scans – all performed by a medical doctor with extensive experience in obstetric ultrasound (CS). All anthropometric measurements were performed twice, with a third reading for discrepancies and the average of the two closest readings was considered definitive. Birthweight measured >1 hour after delivery were also adjusted for physiological weight loss [21].

However, this study also has some limitations. First, fetal weight and birthweight were converted into Z-score using the STOPPAM reference chart, as we have previously demonstrated this reference chart to be more appropriate for the setting [23]. However, a similar reference for head circumference and abdominal circumference is not available, and the INTERGROWTH-21st was therefore used for head circumference [25]. Second, previous studies indicated that malaria in either the first or second trimester might be the most detrimental [4, 5]. However, women were enrolled from the second trimester onward. Thus, malaria infections occurring in the first trimester were not accounted for, and some women may wrongly have been classified as malaria-negative, resulting in an underestimation of the true burden. Third, miscarriage and stillbirth may be due to malaria and/or STIs/RTIs but were excluded in the analyses. Fourth, the prevalence of STIs/RTIs at enrolment were lower among the excluded women, and may represent some selection bias. Finally, some residual confounders could not be ruled out, including genetic factors. However, these are unlikely to have influenced the results

as they would be expected to be relatively infrequent and balanced between study exposure groups.

CONCLUSION

Both malaria and STIs/RTIs were common and associated with poor fetal growth, especially among paucigravidae women with dual infections. Early antenatal intervention is key to reducing the dual burden of malaria and STIs/RTIs. Public health awareness campaigns against these infections are urgently needed, alongside screening for all STIs/RTIs and promoting early antenatal care-seeking, to optimise pregnancy outcomes in low and middle income countries.

Author Contributions

GM, RMC, MM, MA, DTRM, JPAL, FOtK and CS conceived and designed the study. GM, RMC, MM, HB, DTRM, QS, GRG, CM, SG, OAM, VM, KSP, HH, PM, RK, JPAL, SK, FM, JRG, MA, FOtK, and CS contributed to the data acquisition. QS, CM, HH, RK, SK, and MA coordinated the laboratory component. GM conducted the statistical analysis and wrote the first draft of the manuscript. All authors contributed to data interpretation and critical revision for important intellectual content. All authors approved the final version submitted.

Conflict of interest

All authors declare no competing interests.

Disclaimer: The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

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The funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Ethical approval statement

This study was approved by independent ethics committees in Tanzania (NIMR/HQ/R.8a/Vol.1X/2533), Malawi (P.02/17/2110) and Kenya (SERU 75-3421). Individual, written informed consent was obtained prior to enrolment or any study procedure. CDC Human Research Protections Office reviewed and approved CDC participation as non-engaged.

Data sharing

Individual participant data is available from the Worldwide Antimalarial Resistance Network (WWARN) data repository.

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TABLES AND FIGURE LEGENDS

Tables

Supplementary figure and tables

Table S5. Association between birthweight and each STIs/RTIs

Table S6. The association between malaria and STIs/RTIs with fetal weight Z-score in the third trimester among paucigravide women

Table S7. Effect of STIs/RTIs during pregnancy on fetal growth trajectories as Z-score of fetal weight and birth weight

Table 1. Characteristics of mother-newborn pairs

Characteristic		N	n (%) / mean (SD)
	Baseline data		
Study site:		1,435	
	Tanzania		557 (38.8)
	Malawi		416 (29.0)
	Kenya		462 (32.2)
Study settings:		1,435	
	Rural		1,065 (74.2)
	Semi-urban		309 (21.5)
	Urban		61 (4.3)
Study interventions arms:		1,435	
	SP		481 (33.5)
	DP		472 (32.9)
	DP+AZ		482 (33.6)
Education level:		1,433	
	None		126 (8.8)
	Primary		787 (54.9)
	Secondary		440 (30.7)
	Higher		80 (5.6)
Marital status:		1,435	
	Married		1,235 (86.1)
	Single		156 (10.9)
	Others		44 (3.0)
Socio-economic status		1,435	
	Low		506 (35.3)
	Middle		466 (32.5)
	High		462 (32.2)
History of smoking		1,435	3 (0.2)
Alcohol use		1,435	11 (0.8)
Age at enrolment, years [a]		1,431	24.9 (5.8)
Height at enrolment, Cm [a]		1,432	158.5 (7.1)
Weight at enrolment, Kg [a]		1,431	60.6 (10.7)
BMI at enrolment, Kg/m^2		1,428	
	<18.5 (Undernutrition)		40 (2.8)
	18.5-24.9 (Normal)		914 (64.0)
	25-29.9 (Overweight)		347 (24.3)
	>=30 (Obese)		127 (8.9)
MUAC at enrolment, cm [a]		1,428	26.9 (3.4)
GA at enrolment, days [a]		1,435	146.8 (24.2)
Bed net use at enrolment		1,435	1,138 (79.3)
	Bed net use last night	1,138	1,116 (98.0)
	Treated bed net	1,137	891 (78.3)
IRS sprayed in household		1,435	78 (5.4)
	Morbidity during pregnancy		

Hypertensive disorders:	1,435	
Essential hypertension [b]		4 (0.3)
Pregnancy induced hypertension [c]		8 (0.6)
Pre-eclampsia [d]		2 (0.1)
Urinary tract infection [e]	1,435	221 (15.4)
Hemoglobin (g/dL) at enrolment [a]	1,433	11.0 (1.5)
Pregnancy outcome		
Place of delivery:	1,338	
Hospital		1,039 (77.7)
Health centre		202 (15.1)
Home		67 (5.0)
On the way		30 (2.2)
Mode of delivery:	1,330	
Spontaneous vaginal		1,196 (89.9)
Cesarean Section		132 (9.9)
Forcep/Vacuum		2 (0.2)
Live birth (GA>28)	1,342	1,342 (100.0)
Male newborn	1,342	663 (49.4)
GA at delivery, days	1,339	278 (13.0)
Preterm delivery	1,339	58 (4.3)
Birthweight, grams [a, f]	1,325	3087 (470)
Low birthweight (<2500g)	1,325	107 (8.1)
Small for gestational age [g]	1,299	174 (13.4)
Large for gestational age [h]	1,299	96 (7.4)

SP: sulfadoxine-pyrimethamine, DP: dihydroartemisinin-piperaquine, AZ: azithromycin, IRS: insecticide residue spray, BMI: body mass index, MUAC: mid upper arm circumference, GA: gestational age, [a] mean (standard deviation), [b] systolic blood pressure (sBP)>=140 or diastolic blood pressure (dBP)>=90 mmhg before GA 20 weeks measured twice at least 4 hours part, [c] sBP>=140 or dBP>=90 mmhg measured twice at least 4 hours apart after GA 20 weeks without proteinuria, [d] hypertension with proteinuria after GA 20 weeks, [e] positive urine leucocytes and nitrites, [f] The majority (78%) of the newborns were measured within 12 hours of birth, and weight measured >1 hour after birth were adjusted for the physiological weight loss [21], [g] <10th percentile based on a Tanzanian reference chart [22], [h] >90th percentile using Tanzanian reference chart [22].

Table 2: Prevalence of Malaria and STIs/RTIs

		Paucigravidae [a]		Multigravidae	*P*-value
A. Malaria exposure: [b]	N	n (%) / mean (SD)	N	n (%) / mean (SD)	
At Enrolment	787	233 (29.6)	648	144 (22.2)	**0.002**
During pregnancy	787	160 (20.3)	648	105 (16.2)	**0.045**
At Delivery	787	84 (10.7)	648	67 (10.3)	0.84
Cumulative malaria prevalence [c]	787	364 (46.3)	648	259 (40.0)	**0.017**
Number of malaria episodes	787		648		
0		423 (53.7)		389 (60.0)	**0.02**
1		249 (31.6)		198 (30.6)	
2		79 (10.0)		42 (6.5)	
3+		36 (4.6)		19 (2.9)	
Gestational age at first malaria detection, days [d]		177 (50)		187 (58)	**0.02**
Malaria by trimester [e]					
Never malaria		423 (53.7)		389 (60.0)	**0.006**
2nd trimester only		190 (24.1)		120 (18.5)	
3rd trimester only		94 (11.9)		92 (14.2)	
Both 2nd and 3rd trim.		80 (10.2)		47 (7.3)	
Symptomatic malaria	787	119 (15.1)	648	55 (8.5)	**<0.001**

B. STIs/RTIs exposure

		Negative	Positive at enrolment only	Positive at weeks 32-36 only	Positive at both enrolment and weeks 32-36
	N	n (%)	n (%)	n (%)	n (%)
Composite STIs/RTIs [f]	1,429	666 (46.6)	304 (21.3)	134 (9.4)	325 (22.7)
Specific STIs/RTIs [g]:					
Bacterial vaginosis	1,297	848 (65.4)	214 (16.5)	79 (6.1)	156 (12.0)
Trichomoniasis	1,301	999 (76.8)	104 (8.0)	106 (8.2)	92 (7.1)
Chlamydia	1,297	1,080 (83.3)	137 (10.6)	38 (2.9)	42 (3.2)
Gonorrhoea	1,298	1,244 (95.8)	32 (2.5)	17 (1.3)	5 (0.4)
Syphilis	1,407	1,380 (98.1)	26 (1.9)	1 (0.1)	0 (0)
	No STIs/RTIs		STIs/RTIs		
Symptomatic STIs/RTIs	666	4 (0.6)	763	11 (1.4)	**0.19**

[a] 1st and 2nd pregnancy, [b] Malaria was confirmed by any positive test: real time polymerase chain reaction, malaria rapid diagnostic test, microscopy, and at delivery also included placental histology, 599/623 (96%) of the women got the first malaria attack before the 3rd trimester, [c] the total number for enrolment, during and delivery visits is not equal to the cumulative number as some women had >1 episodes which are not mutually exclusive, GA: gestational age, [d] Mean (standard deviation), P by chi^2 for the difference in proportions or t-test for the mean difference. [e] The malaria prevalence in the 2nd and in the 3rd trimester was significantly different for paucigravidae (McNemar, p<0.001), and close to significant for multigravidae (McNemar, p=0.06). STIs/RTIs: Sexually transmitted/reproductive tract infections [f] women who were never tested for STIs/RTIs were excluded in all analyses where STIs/RTIs were considered as an exposure. The STIs/RTIs prevalence at enrolment and in the 3rd trimester was significantly different (McNemar, p<0.001),. [g] Women who were never tested for the specific STI/RTI were excluded in all analyses where the specific STIs/RTIs was considered as an exposure.

Table 3: Fetal weight and newborn anthropometrics at delivery by malaria infection and composite STIs/RTIs status

A. Effect of malaria infection		No malaria		Malaria		unadjusted		adjusted	
	Outcome	n	Mean (SD)	n	Mean (SD)	mean difference (95% CI)	p-value	mean difference (95% CI)	p-value
All gravidae	BW, grams	738	3115 (471)	587	3053 (466)	-45.7 (-97.1, 5.8)	0.08	-36.5 (-80.6, 7.62)	0.11
	BW Z-score [a]	722	-0.09 (1.1)	569	-0.24 (1.1)	**-0.12 (-0.24, -0.01)**	**0.04**	-0.10 (-0.22, 0.02)	0.09
	HC, mm	688	343 (18.6)	566	342 (20.0)	-0.62 (-2.7, 1.5)	0.56	-0.12 (-2.0, 1.8)	0.91
	HC Z-score [b]	675	0.38 (1.3)	551	0.30 (1.3)	-0.03 (-0.17, 0.12)	0.72	-0.01 (-0.15, 0.13)	0.89
	AC, mm	665	322 (21.8)	553	320 (26.6)	-1.14 (-3.9, 1.6)	0.42	-0.88 (-3.5, 1.8)	0.51
	FW-Z-score [a]	834	0.11 (1.3)	284	-0.20 (1.3)	**-0.18 (-0.30, -0.06)**	**0.004**	**-0.15 (-0.27, -0.03)**	**0.016**
Paucigravidae	BW, grams	371	3110 (472)	342	3008 (443)	**-100.2 (-168.4, -32.1)**	**0.004**	**-71.7 (-131.8, -11.6)**	**0.02**
	BW Z-score [a]	362	-0.13 (1.1)	328	-0.34 (1.1)	**-0.22 (-0.38, -0.05)**	**0.01**	**-0.19 (-0.35, -0.03)**	**0.02**
	HC, mm	337	342 (19.6)	331	341 (19.7)	-0.74 (-3.7, 2.2)	0.62	-0.76 (-1.98, 3.5)	0.58
	HC Z-score [b]	328	0.26 (1.3)	319	0.24 (1.2)	-0.01 (-0.18, 0.20)	0.93	-0.02 (-0.17, -0.22)	0.81
	AC, mm	323	320 (22.2)	325	319 (24.0)	-1.8 (-5.4, 1.81)	0.34	-0.13 (-3.6, 3.4)	0.94
	FW-Z-score [a]	421	0.02 (1.3)	176	-0.30 (1.3)	**-0.21 (-0.37, -0.04)**	**0.014**	**-0.20 (-0.36, -0.03)**	**0.018**
Multigravidae	BW, grams	367	3120 (471)	245	3115 (491)	20.6 (-58.0, 99.2)	0.61	12.2 (-53.7, 78.0)	0.72
	BW Z-score [a]	360	-0.05 (1.1)	241	-0.10 (1.03)	-0.01 (-0.19, 0.16)	0.87	-0.02 (-0.19, 0.16)	0.86
	HC, mm	351	344 (17.5)	235	343 (20.4)	-0.61 (-3.7, 2.5)	0.7	-1.1 (-3.8, 1.8)	0.49
	HC Z-score [b]	347	0.39 (1.1)	232	0.38 (1.4)	-0.05 (-0.27, 0.17)	0.66	-0.02 (-0.23, 0.20)	0.88
	AC, mm	342	323 (21.3)	228	322 (29.7)	-0.61 (-4.9, 3.7)	0.78	-1.6 (-5.7, 2.5)	0.45
	FW-Z-score [a]	413	0.19 (1.3)	108	-0.03 (1.3)	-0.15 (-0.34, 0.04)	0.11	-0.13 (-0.32, 0.05)	0.17
	Outcome	n	n (%)	n	n (%)	RR (95% CI)	p-value	RR (95% CI)	p-value
All gravidae	SGA [a]	725	77 (10.6)	574	97 (16.9)	**1.50 (1.14, 1.98)**	**0.004**	**1.50 (1.14, 1.97)**	**0.004**
	LBW	738	52 (7.1)	587	55 (9.4	1.24 (0.86, 1.79)	0.25	1.28 (0.89,1.85)	0.19
	PT	746	27 (3.6)	593	31 (5.2)	1.50 (0.90, 2.48)	0.18	1.43 (0.86, 2.36)	0.17
Paucigravidae	SGA [a]	363	36 (9.9)	330	63 (19.1)	**1.86 (1.27,2.71)**	**0.001**	**1.84 (1.26, 2.69)**	**0.002**
	LBW	371	27 (7.3)	342	36 (10.5)	1.40 (0.87, 2.25)	0.17	1.33 (0.81, 2.18)	0.25
	PT	373	8 (2.1)	346	21 (6.1)	**3.03 (1.37, 6.71)**	**0.01**	**2.84 (1.29, 6.27)**	**0.01**
Multigravidae	SGA [a]	362	41 (11.3)	244	34 (13.9)	1.12 (0.75, 1.69)	0.58	1.11 (0.73, 1.67)	0.63

	LBW	367	25 (6.8)	245	19 (7.8)	1.00 (0.55, 1.80)	0.99	1.23 (0.70, 2.17)	0.47
	PT	373	19 (5.1)	247	10 (4.1)	0.83 (0.38, 1.80)	0.63	0.81 (0.38, 1.71)	0.58

B. Effect of STIs/RTIs		**No STIs/RTIs**		**STIs/RTIs**		**unadjusted**		**adjusted**	
	Outcome	n	Mean (SD)	n	Mean (SD)	mean difference (95% CI)	p-value	mean difference (95% CI)	p-value
All gravidae	BW, grams	608	3107 (476)	711	3071 (465)	-31.5 (-82.0, 19.0)	0.22	-38.7 (-81.8, 4.3)	0.08
	BW Z-score [a]	589	-0.09 (1.1)	697	-0.21 (1.04)	-0.10 (-0.21, -0.02)	0.1	-0.09 (-0.21, 0.02)	0.12
	HC, mm	579	342 (21.1)	669	344 (17.4)	0.60 (-1.5, 2.7)	0.57	0.21 (-1.7, 2.1)	0.83
	HC Z-score [b]	561	0.32 (1.4)	659	0.37 (1.2)	0.003 (-0.14, 0.14)	0.96	0.01 (-0.13, 0.15)	0.93
	AC, mm	554	321 (26.9)	658	321 (21.5)	-0.05 (-2.8, 2.7)	0.97	-0.75 (-3.3, 1.8)	0.57
	FW-Z-score [a]	602	0.06 (1.3)	514	-0.01(1.3)	-0.08 (-0.20, 0.03)	0.14	-0.10 (-0.21, 0.01)	0.08
Paucigravidae	BW, grams	738	3115 (471)	587	3053 (466)	-38.6 (-106.7, 29.5)	0.27	-55.5 (-114.8, 3.7)	0.07
	BW Z-score [a]	722	-0.09 (1.1)	569	-0.24 (1.1)	**-0.17 (-0.33, -0.01)**	**0.04**	**-0.17 (-0.33, -0.01)**	**0.04**
	HC, mm	688	343 (18.6)	566	342 (20.0)	0.01 (-2.9, 2.9)	0.99	0.52 (-3.2, 2.1)	0.7
	HC Z-score [b]	675	0.38 (1.3)	551	0.30 (1.3)	-0.02 (-0.21, 0.17)	0.85	-0.02 (-0.21, 0.17)	0.82
	AC, mm	665	322 (21.7)	553	320 (26.6)	-0.90 (-4.5, 2.7)	0.62	-2.0 (-5.5, 1.4)	0.25
	FW-Z-score [a]	307	-0.07 (1.3)	288	-0.08 (1.3)	-0.03 (-0.19, 0.02)	0.67	-0.08 (-0.23, 0.08)	0.33
Multigravidae	BW, grams	738	3115 (471)	587	3053 (466)	-19.8 (-94.8, 56.4)	0.62	-17.2 (-79.8, 45.4)	0.59
	BW Z-score [a]	722	-0.09 (1.1)	569	-0.24 (1.1)	-0.02 (-0.18, 0.15)	0.85	-0.01 (-0.16, 0.17)	0.96
	HC, mm	688	343 (18.6)	566	342 (20.0)	1.3 (-1.7, 4.3)	0.4	1.10 (-1.6, 3.8)	0.43
	HC Z-score [b]	675	0.38 (1.3)	551	0.30 (1.3)	0.03 (-0.18, 0.24)	0.79	0.04 (-0.16, 0.25)	0.68
	AC, mm	665	322 (21.7)	553	320 (26.6)	0.78 (-3.4, 4.8)	0.73	0.26 (-3.7, 4.1)	0.92
	FW-Z-score [a]	295	0.19 (1.3)	226	0.08 (1.3)	-0.14 (-0.30, 0.02)	0.1	-0.13 (-0.29, 0.03)	0.12
	Outcome	n	n (%)	n	n (%)	RR (95% CI)	p-value	RR (95% CI)	p-value
All gravidae	SGA [a]	593	72 (12.1)	700	101 (14.4)	1.14 (0.86,1.50)	0.38	1.17 (0.88,1.55)	0.28
	LBW	608	50 (8.2)	711	56 (7.9)	0.93 (0.64, 1.34)	0.69	1.04 (0.72, 1.49)	0.85
	PT	614	35 (5.7)	719	22 (3.1)	**0.58 (0.35, 0.98)**	**0.041**	**0.58 (0.35, 0.97)**	**0.04**
Paucigravidae	SGA [a]	297	36 (12.1)	391	62 (15.9)	1.30 (0.89, 1.91)	0.18	1.37 (0.93,2.02)	0.11
	LBW	307	26 (8.5)	401	36 (9.0)	1.10 (0.67, 1.80)	0.7	1.26 (0.79, 2.00)	0.34
	PT	311	20 (6.4)	403	9 (2.3)	**0.37 (0.17, 0.82)**	**0.01**	**0.38 (0.17, 0.82)**	**0.01**
Multigravidae	SGA [a]	296	36 (12.2)	309	39 (12.6)	0.96 (0.63, 1.45)	0.84	0.94 (0.62, 1.42)	0.75
	LBW	30	24 (8.0)	310	20 (6.5)	0.74 (0.42, 1.28)	0.28	0.81 (0.44,	0.5

	1						1.50)	
PT	30	15 (5.0)	316	13 (4.1)	0.86 (0.43,1.71)	0.67	0.89 (0.43,	0.74
	3						1.81)	

Malaria infection was defined as any positive test: real time polymerase chain reaction, malaria rapid diagnostic test, microscopy, and at delivery also included placental histology, AC: abdominal circumference at delivery, BW: birthweight adjusted for time since delivery [21], [a] based on Tanzanian reference chart [22], FW: fetal weight (the mean is for the last FW but the models included all longitudinal FW measurements), HC: head circumference at delivery, [b] based on intergrowth-21 reference chart [25] but it does not include AC, LBW: low BW (<2.5Kg), PT: preterm delivery, SGA: small for gestational age (BW <10th percentiles), STIs/RTIs: Sexually transmitted/reproductive tract infections defined as composite of any STIs/RTIs, SD: standard deviation, P: P-value from linear regression model for newborn anthropometrics or mixed effect linear model for the FW. The unadjusted models included the *a priori* selected co-variables gravidity (paucigravidae defined as primi- or secundigravidae vs multigravidae defined as 3 or more pregnancies), study arm and site, and the adjusted models included gravidity, study arm and site, maternal body mass index, maternal age, and gestational age at enrolment and/or delivery. Furthermore, newborn sex if the outcome was not z-scores.

Table 4: Fetal weight and newborn anthropometrics at delivery by malaria infection, composite STIs/RTIs, or both

A. Continuous Outcome		Analysis	None		Malaria only		STIs/RTIs only		STIs/RTIs + malaria		Malaria-only vs None		STIs/RTIs-only vs None		dual infection vs None		Malaria-only vs STI only	
			n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	mean difference (95% CI)	p-value	mean difference (95% CI)	p-value	mean difference (95% CI)	p-value	mean difference (95% CI)	p-value
All gravidae	BW, grams	Unadjusted	354	3140 (473)	254	3061 (477)	381	3090 (470)	330	3050 (459)	-64.8 (-140.0, 10.4)	0.09	-47.9 (-114.8, 19.1)	0.16	-70.3 (-140.8, 0.16)	0.05	-16.9 (-91.8, 57.9)	0.66
		Adjusted									-47.8 (-112.3, 16.7)	0.15	-50.8 (-107.8, 6.3)	0.08	**-67.3 (-127.5, -7.2)**	**0.03**	3.0 (-61.0, 67.0)	0.93
	BW Z-score [a]	Unadjusted	345	0.001 (1.1)	244	-0.22 (1.1)	375	-0.17 (1.04)	322	-0.25 (1.1)	**-0.19 (-0.36, -0.02)**	**0.03**	-0.15 (-0.31, 0.001)	0.05	**-0.20 (-0.36, -0.04)**	**0.02**	-0.04 (-0.21, 0.13)	0.66
		Adjusted									-0.16 (-0.33, -0.01)	0.07	-0.14 (-0.29, 0.01)	0.07	**-0.17 (-0.34, -0.01)**	**0.03**	-0.02 (-0.19, 0.15)	0.83
	HC, mm	Unadjusted	329	342 (18.0)	250	341 (24.5)	356	344 (19.0)	313	343 (15.4)	-0.18 (-3.2, 2.9)	0.91	0.94 (-1.8, 3.7)	0.5	0.04 (-2.9, 2.9)	0.98	-1.12 (-4.16, 1.92)	0.47
		Adjusted									0.49 (-2.3, 3.3)	0.73	0.72 (-1.8, 3.2)	0.58	0.06 (-2.6, 2.7)	0.96	-0.23 (-3.00, 2.54)	0.87
	HC Z-score [b]	Unadjusted	322	0.33 (1.3)	239	0.30 (1.4)	350	0.42 (1.3)	309	0.32 (1.2)	0.07 (-0.13, 0.28)	0.51	0.07 (-0.11, 0.26)	0.45	-0.01 (-0.21, 0.18)	0.9	-0.002 (-0.21, 0.20)	0.98
		Adjusted									0.09 (-0.11, 0.30)	0.38	0.08 (-0.11-0.26)	0.4	-0.01 (-0.18, 0.20)	0.96	0.01 (-0.19, 0.22)	0.9
	AC, mm	Unadjusted	313	322 (20.8)	341	321 (33.3)	349	321 (22.7)	309	320 (20.0)	-0.61 (-4.6, 3.4)	0.77	0.34 (-3.3, 4.0)	0.85	-1.1 (-4.9, 2.7)	0.58	-0.96 (-4.94, 3.02)	0.64
		Adjusted									-0.38 (-4.4, 3.7)	0.86	0.47 (-3.2, 4.1)	0.8	-0.71 (-4.5, 3.1)	0.71	-0.85 (-4.84, 3.14)	0.68
	FW-Z-score [a]	Unadjusted	458	0.18 (1.3)	144	-0.32 (1.3)	375	0.02 (1.3)	139	-0.07 (1.3)	**-0.31 (-0.48,-0.13)**	**<0.001**	**-0.16 (-0.30, -0.02)**	**0.02**	**-0.22 (-0.38,-0.05)**	**0.01**	-0.15 (-0.33, 0.03)	0.1
		Adjusted									**-0.23 (-0.41, -0.06)**	**0.01**	**-0.15 (-0.28, -0.01)**	**0.03**	**-0.21 (-0.38, -0.05)**	**0.01**	-0.09 (-0.26, 0.09)	0.33
Paucigravidae	BW, grams	Unadjusted	161	3134 (456)	146	3032 (476)	208	3088 (485)	193	2995 (418)	-100.1 (-202.4, 2.3)	0.06	-40.8 (-133.9, 52.4)	0.39	**-132.7 (-228.3,-37.0)**	**0.007**	-59.3 (-157.4, 38.7)	0.24
		Adjusted									-58.5 (-148.0, 31.1)	0.2	-47.2 (-128.3, 34.0)	0.25	**-121.0 (-205.0,-37.2)**	**0.005**	-11.3 (-97.0, 74.5)	0.79
	BW Z-score [a]	Unadjusted	157	-0.04 (1.1)	139	-0.23 (1.1)	203	-0.21 (1.1)	186	-0.42 (1.1)	-0.19 (-0.43, 0.08)	0.13	-0.15 (-0.37, 0.07)	0.16	**-0.36 (-0.59, -0.14)**	**0.002**	-0.04 (-0.26, 0.19)	0.76
		Adjusted									-0.17 (-0.41, 0.09)	0.18	-0.16 (-0.38, 0.06)	0.15	**-0.34 (-0.57, -0.11)**	**0.003**	-0.01 (-0.24, 0.23)	0.94
	HC, mm	Unadjusted	147	341 (17.	145	341 (24.	188	343 (20.	183	342	1.02 (-3.3, 5.3)	0.64	1.4 (-2.6,	0.49	-0.42 (-4.5,	0.84	1.6 (-2.2,	0.4

			7)		5)		9)		(14.7)			5.5)		3.7)		5.5)	
		Adjusted								3.2 (-0.81, 7.2)	0.12	1.5 (-2.2, 5.2)	0.42	-0.40 (-3.4, 4.2)	0.83	-0.40 (-4.6, 8.8)	0.85
	HC Z-score [b]	Unadjusted	143	0.16 (1.3)	137	0.31 (1.3)	183	0.34 (1.3)	179	0.22 (1.1)	0.22 (-0.06, -0.51)	0.12	0.17 (-0.10, 0.43)	0.21	0.01 (-0.26, 0.28)	0.95	0.06 (-0.22 0.33)	0.68
		Adjusted									0.24 (-0.04, 0.53)	0.09	0.17 (-0.10, 0.42)	0.22	0.02 (-0.25, 0.29)	0.89	0.08 (-0.19, 0.35)	0.57
	AC, mm	Unadjusted	138	320 (20.3)	141	320 (27.4)	183	320 (23.6)	181	317 (21.0)	-0.24 (-5.7, 5.2)	0.93	0.27 (-4.8, 5.3)	0.92	-2.3 (-7.5, 2.8)	0.38	-0.52 (-5.7, 4.7)	0.85
		Adjusted									0.33 (-5.1-5.8)	0.9	-0.01 (-5.1, 5.1)	0.99	-1.4 (-6.6, 3.7)	0.59	0.34 (-4.8, 5.6)	0.9
	FW-Z-score [a]	Unadjusted	215	0.10 (1.3)	92	-0.45 (1.2)	205	-0.06 (1.3)	83	-0.12 (1.3)	**-0.32 (-0.56, -0.09)**	**0.008**	-0.11 (-0.30, 0.09)	0.27	-0.21 (-0.44, 0.01)	0.07	-0.22 (-0.45, 0.02)	0.08
		Adjusted									**-0.27 (-0.50, -0.03)**	**0.03**	-0.11 (-0.31, 0.08)	0.25	**-0.24 (-0.47, -0.02)**	**0.03**	-0.14 (-0.73, 0.10)	0.27
Multigravidae	BW, grams	Unadjusted	193	3145 (488)	108	3099 (477)	173	3093 (452)	137	3127 (503)	-29.7 (-142.3, 82.5)	0.6	-60.0 (-157.0, 37.1)	0.23	9.3 (-96.2, 114.7)	0.86	31.0 (-85.7, 146.2)	0.61
		Adjusted									-9.8 (-122.1, 102.4)	0.86	-44.5 (-141.2, 52.2)	0.37	27.4 (-77.7, 132.5)	0.61	34.7 (-80.3, 149.7)	0.55
	BW Z-score [a]	Unadjusted	188	0.03 (1.1)	105	-0.21 (1.1)	172	-0.13 (1.02)	136	-0.02 (1.0)	-0.20 (-0.45, 0.05)	0.11	-0.16 (-0.37, 0.06)	0.15	0.0001 (-0.023, 0.23)	0.99	-0.05 (-0.30, 0.21)	0.72
		Adjusted									-0.15 (-0.41, 0.09)	0.21	-0.12 (-0.34, 0.09)	0.25	0.04 (-0.19, 0.27)	0.73	-0.03 (-0.29, 0.22)	0.8
	HC, mm	Unadjusted	182	344 (18.3)	105	341 (24.5)	168	345 (16.6)	130	345 (16.2)	-1.9 (-6.1, 2.6)	0.41	0.47 (-3.4, 4.3)	0.81	0.80 (-3.4, 5.0)	0.71	-2.3 (-6.9, 2.2)	0.31
		Adjusted									-1.6 (-6.1, 2.8)	0.46	0.71 (-3.1, 4.5)	0.72	1.4 (-2.8, 5.6)	0.51	-2.4 (-6.9, 2.1)	0.3
	HC Z-score [b]	Unadjusted	179	0.46 (1.3)	102	0.28 (1.5)	167	0.50 (1.2)	130	0.46 (1.2)	-0.11 (-0.42, 0.20)	0.5	-0.02 (-0.28, 0.25)	0.90	-0.001 (-0.29, 0.29)	1	-0.09 (-0.41, 0.23)	0.58
		Adjusted									-0.10 (-0.41, 0.21)	0.53	-0.04 (-0.31, 0.23)	0.77	-0.004 (-0.29-0.29)	0.98	-0.06 (-0.38, 0.26)	0.71
	AC, mm	Unadjusted	175	323 (21.1)	100	322 (40.3)	166	323 (21.6)	128	323 (18.1)	-1.3 (-7.4, 4.9)	0.68	0.31 (-5.0, 5.6)	0.91	0.16 (-5.6, 5.9)	0.96	-1.6 (-7.9, 4.7)	0.62
		Adjusted									1.0 (-7.2, 5.2)	0.75	0.50 (-4.8, 5.8)	0.85	0.38 (-5.4, 6.2)	0.9	-1.5 (-7.8, 4.8)	0.64
	FW-Z-score [a]	Unadjusted	243	0.25 (1.3)	52	-0.08 (1.2)	170	0.11 ª1.3)	56	0.01 (1.4)	**-0.28 (-0.55, -0.02)**	**0.03**	**-0.21 (-0.40, -0.02)**	**0.03**	-0.23 (-0.48, 0.02)	0.08	-0.08 (-0.35, 0.19)	0.58
		Adjusted									-0.24 (-0.50, 0.03)	0.08	-0.16 (-0.35, 0.03)	0.1	-0.21 (-0.46, 0.04)	0.1	-0.06 (-0.33, 0.21)	0.68

B. Binary outcomes		analysis	None		Malaria only		STI/RTI only		STIs/RTIs + malaria		Malaria -only vs none		STI/RTI-only vs none		dual infection vs None		Malaria -only vs STI/RTI only	
			n	n (%)	n	n (%)	n	n (%)	n	n (%)	RR (95%CI)	p-value	RR (95%CI)	p-value	RR (95%CI)	p-value	RR (95%CI)	p-value
All gravidae	SGA [a]	Unadjusted	346	33 (9.5)	247	39 (15.8)	376	44 (11.7)	324	57 (17.6)	**1.60 (1.05,2.45)**	**0.03**	1.19 (0.78,1.82)	0.41	**1.67 (1.12, 2.50)**	**0.01**	1.34 (0.91,1.99)	0.14
		Adjusted									**1.58 (1.03, 2.42)**	**0.04**	1.21 (0.80, 1.85)	0.37	**1.71 (1.14, 2.55)**	**0.01**	1.30 (0.87,1.94)	0.2
	LBW	Unadjusted	354	26 (7.3)	254	24 (9.5)	381	26 (6.8)	330	30 (9.1)	1.25 (0.74,2.10)	0.4	0.92 (0.56,1.56)	0.78	1.13 (0.68, 1.86)	0.65	1.34 (0.78,2.30)	0.28
		Adjusted									1.12 (0.68,1.86)	0.66	0.92 (0.55,1.56)	0.76	1.29 (0.79,2.09)	0.31	1.21 (0.70,2.11)	0.49
	PT	Unadjusted	359	17 (4.7)	255	18 (7.1)	384	9 (2.3)	335	13 (3.9)	1.47 (0.78,2.80)	0.23	0.51 (0.24, 1.12)	0.09	0.92 (0.46,1.85)	0.81	**2.87 (1.31,6.26)**	**0.01**
		Adjusted									1.14 (0.61,2.14)	0.68	**0.35 (0.14,0.89)**	**0.03**	1.19 (0.58,2.38)	0.65	**3.28 (1.27,8.48)**	**0.01**
Paucigravidae	SGA [a]	Unadjusted	157	12 (7.6)	140	24 (17.4)	204	24 (11.8)	187	38 (20.3)	**2.20 (1.14,4.23)**	**0.02**	1.54 (0.80,2.95)	0.2	**2.53 (1.37, 4.68)**	**0.003**	1.43 (0.85,2.43)	0.18
		Adjusted									**2.11 (1.08, 4.10)**	**0.03**	1.53 (0.80, 2.92)	0.2	**2.53 (1.37, 4.67)**	**0.003**	1.38 (0.81,2.36)	0.24
	LBW	Unadjusted	161	11 (6.8)	146	15 (10.3)	208	16 (7.8)	193	20 (10.4)	1.47 (0.71,3.05)	0.3	1.17 (0.56,2.44)	0.68	1.53 (0.75, 3.10)	0.24	1.26 (0.64,2.49)	0.51
		Adjusted									1.07 (0.54,2.12)	0.85	0.99 (0.48, 2.05)	0.99	1.57 (0.81,3.07)	0.19	1.07 (0.54,2.13)	0.84
	PT	Unadjusted	163	6 (3.7)	148	14 (9.5)	208	2 (0.96)	195	7 (3.6)	**2.54 (1.06,6.07)**	**0.04**	0.26 (0.05,1.26)	0.10	1.11 (0.39,3.14)	0.83	**9.93 (2.14,46.01)**	**0.003**
		Adjusted									**2.44 (1.02,5.84)**	**0.05**	0.25 (0.05,1.23)	0.09	1.07 (0.39,2.92)	0.9	**9.55 (2.03,44.81)**	**0.004**
Multigravidae	SGA [a]	Unadjusted	189	21 (11.1)	107	15 (14.0)	172	20 (11.6)	137	19 (13.9)	1.17 (0.64,1.13)	0.6	0.98 (0.56, 1.71)	0.95	1.19 (0.65,2.18)	0.95	0.52 (0.25,1.10)	0.09
		Adjusted									1.13 (0.62, 2.08)	0.68	0.95 (0.54, 1.66)	0.85	1.20 (0.68. 2.18)	0.56	0.69 (0.31,1.56)	0.37
	LBW	Unadjusted	193	15 (7.8)	108	9 (8.3)	173	10 (5.8)	137	10 (7.3)	1.01 (0.45,2.28)	0.97	0.72 (0.34,1.50)	0.38	1.41 (0.59,3.40)	0.44	0.38 (0.14,0.99	0.05
		Adjusted									1.53 (0.68,3.42)	0.3	0.93 (0.38,2.27)	0.88	1.64 (0.72,3.71)	0.24	0.35 (0.19,1.62)	0.28
	PT	Unadjusted	196	11 (5.6)	107	4 (3.7)	176	7 (4.0)	140	6 (4.3)	0.68 (0.21,2.23)	0.52	0.73 (0.30, 1.71)	0.46	0.93 (0.27,3.21)	0.91	1.04 (0.18,5.9)	0.96
		Adjusted									0.54 (0.28,1.06)	0.07	0.86 (0.33, 2.27)	0.77	0.63 (0.26, 1.53)	0.30	1.02 (0.18,3.9)	0.98

Malaria infection was defined as any positive test: real time polymerase chain reaction, malaria rapid diagnostic test, microscopy, and at delivery also included placental histology, AC: abdominal circumference at delivery, BW: birthweight adjusted for time since delivery [21], [a] based on Tanzanian reference chart [22], FW: fetal weight (mean is for the last FW but the models included all longitudinal FW), HC: head circumference at delivery, [b] based on intergrowth-21 reference chart [25] but it does not include AC, LBW: low BW (<2.5Kg), PT: preterm delivery, SGA: small for gestational age (BW <10th percentiles), STIs/RTIs: Sexually transmitted/reproductive tract infections defined as composite of any STIs/RTIs, SD: standard deviation, P: P-value from linear regression model for newborn anthropometrics or mixed effect linear model for FW. Mixed effects linear model was used for continuous longitudinal outcomes while ordinary linear regression was used for continuous delivery outcomes and GLM commands (Poisson regression with robust error variance) was used for binary outcomes, The unadjusted models included the *a priori* selected co-variables gravidity, study arm and site, and the adjusted models included gravidity, study arm and site, maternal body mass index, maternal age, and gestational age at enrolment and/or delivery. Furthermore, newborn sex if the outcome was not z-scores.

Table 5: Effect of malaria infection and composite STIs/RTIs during pregnancy on fetal growth trajectories as Z-scores of fetal weights and birth weights

Exposure groups		Crude model [a]			Adjusted model [b]		
	n	mean difference	95% CI	*p*-value	mean difference	95% CI	*p*-value
A. Effect of malaria infection all gravidae pairs [c]							
No malaria	812	Ref			Ref		
Malaria	623	**-0.14**	**-0.24, -0.05**	**0.003**	**-0.12**	**-0.22, -0.03**	**0.01**
B. Effect of malaria infection among paucigravidae [c]							
No malaria	423	Ref			Ref		
Malaria	364	**-0.19**	**-0.32 - 0.06**	**0.004**	**-0.17**	**-0.31, -0.04**	**0.01**
C. Effect of malaria infection among multigravidae [c]							
No malaria	389	Ref			Ref		
Malaria	259	-0.09	-0.23 -0.06	0.24	-0.07	-0.21, 0.07	0.34
D. Effect of composite STIs/RTIs among all women-newborn pairs [d]							
No STIs/RTIs	666	Ref			Ref		
STIs/RTIs	763	**-0.10**	**-0.20, -0.01**	**0.03**	**-0.11**	**-0.20, -0.01**	**0.03**
E. Effect of composite STIs/RTIs among paucigravidae women-newborn pairs [d]							
No STIs/RTIs	348	Ref			Ref		
STIs/RTIs	434	-0.10	-0.24, 0.03	0.11	-0.13	-0.26, 0.001	0.05
F. Effect of composite STIs/RTIs among multigravidae women-newborn pairs [d]							
No STIs/RTIs	318	Ref			Ref		
STIs/RTIs	329	-0.10	-0.24, 0.04	0.15	-0.08	-0.21, 0.06	0.25
G. Effect of malaria infection and/or composite STI/RTI among all women-newborn pairs [e]							
No malaria no STIs/RTIs	399	Ref			Ref		
Malaria only	267	**-0.22**	**-0.35, -0.08**	**0.002**	**-0.18**	**-0.31, -0.04**	**0.01**
STIs/RTIs only	410	**-0.15**	**-0.27, -0.04**	**0.01**	**-0.14**	**-0.26, -0.03**	**0.01**
Malaria and STIs/RTIs	353	**-0.21**	**-0.35, -0.08**	**0.001**	**-0.20**	**-0.33, -0.07**	**0.003**
H. Effect of malaria infection and/or composite STIs/RTIs among paucigravidae women-newborn pairs [e]							
No malaria no STIs/RTIs	193	Ref			Ref		
Malaria only	155	**-21**	**-0.41,-0.02**	**0.027**	-0.17	-0.36, 0.02	0.08

STIs/RTIs only	228	-12	-0.29, 0.04	0.15	-0.13	-0.30, 0.03	0.11
Malaria and STIs/RTIs	206	**-0.28**	**-0.46, -0.10**	**0.002**	**-0.30**	**-0.48, -0.11**	**0.001**
I. Effect of malaria infection and/or composite STIs/RTIs among multigravidae women-newborn pairs [e]							
No malaria no STIs/RTIs	206	Ref			Ref		
Malaria only	112	**-0.23**	**-0.43, -0.03**	**0.02**	-0.18	-0.38, 0.02	0.08
STIs/RTIs only	182	**-0.19**	**-0.35, 0.03**	**0.02**	-0.16	-0.32, 0.001	0.05
Malaria and STIs/RTIs	147	-0.13	-0.33, 0.06	0.18	-0.11	-0.30, 0.09	0.28

The growth trajectories was based on Z-scores for both fetal weights by ultrasound and birthweight and was assessed using mixed effects regression model, [a] adjusted for gravidity, study arm and site (n=1,329 for all and 717 for paucigravidae), [b] adjusted for gravidity, study arm, site and other covariates including maternal age, maternal body mass index, gestational age at enrolment and delivery (n=1,319 for all and 708 for paucigravidae women-newborn pairs). [c] Considered malaria positive from when the first malaria attack occurred, [d] Considered STIs/RTIs positive from when STIs/RTIs was first diagnosed [e] Consider positive for both malaria and STIs/RTIs from when both diseases had occurred. Malaria was defined as any positive test: real time polymerase chain reaction, malaria rapid diagnostic test, microscopy, and at delivery also included placental histology (5 participants with malaria but no STIs/RTIs data were excluded), STIs/RTIs: Sexually transmitted/reproductive tract infections defined as composite of any STIs/RTIs, NA: not applicable as crude coefficients were not significant.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: