# EFFECT OF ENVIRONMENTAL FACTORS ON THE PREVALENCE OF SUBMICROSCOPIC MALARIA AMONG MALAWIAN PREGNANT WOMEN

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Raskaudenaikainen malaria aiheuttaa odottaville äideille anemiaa, ja vastasyntyneelle pientä syntymäpainoa ja ennenaikaisuutta. Saharan eteläpuolisessa Afrikassa raskaana olevien naisten malaria on usein oireeton, ja parasiittien määrä perifeerisessä veressä on pieni. Perinteisellä mikroskopialla raskaana olevien malariainfektioita jää huomaamatta. PCR-tekniikalla on mahdollista havaita pienen parasiittitiheyden submikroskooppiset infektiot. Tässä tutkimuksessa selvitettiin ympäristötekijöiden vaikutusta submikroskooppisen malarian esiintyvyyteen raskaana olevilla naisilla Malawissa.

Tutkimuksessa käytetty aineisto on kerätty vuosien 2003 ja 2006 välillä Lungwenan kylässä Malawissa. Terveyskeskuksen äitiysklinikalta rekrytoiduilta raskaana olevilta naisilta otettiin tutkimuksen alussa verinäyte, josta tunnistettiin *P. falciparumin* laktaattidehydrogenaasi-geeniä real-time PCR -menetelmällä. Malarian esiintyvyyttä verrattiin ympäristötekijöihin, joita selvitettiin tutkimuksen alussa täytetyn kyselykaavakkeen avulla.

Submikroskooppisen malarian esiintyvyys oli 40,6 %. Hyttysverkon omisti 73,5 % raskaana olevista naisista ja heistä 82,4 % oli edellisenä yönä nukkunut sen alla. Hyttysverkon omistaminen alensi merkitsevästi malariainfektion riskiä (AOR = 0.6, 95 % CI 0.5–0.8, $p < 0.001$), sen sijaan hyttysverkon käytöllä ei ollut merkitsevää yhteyttä malariainfektioon. Muista ympäristötekijöistä merkitsevästi malariainfektion riskiä vähensi hyttysverkon käsitteleminen hyttysmyrkyllä, asuintalon ikkunat, suurempi henkilömäärä taloudessa ja kuiva vuodenaika. Lisäksi malarian riskiä suurensi, jos juomaveden lähteenä oli suojaamaton kaivo, järvi, lampi tai joki.

Tärkeimpänä malarialta suojaavana tekijänä raskaana olevilla naisilla nousi esiin hyttysverkot ja niiden jatkuva käyttö. Yleisesti asuinolojen parantaminen vähentäisi riskiä sairastua malariaan. Köyhyyden vastaiset keinot vaikuttanevat suotuisasti myös raskaudenaikaisen malarian estämiseen.

# ABBREVIATIONS USED

| | |
|---|---|
| AOR | Adjusted odds ratio |
| CI | Confidence interval |
| DBS | Dried blood spot |
| DNA | Deoxyribonucleic acid |
| gDNA | genomic DNA |
| Hb | Haemoglobin |
| HIV | Human immunodeficiency virus |
| IPTp | Intermittent preventive treatment in pregnancy |
| IRS | Indoor residual spraying |
| ITN | Insecticide treated net |
| IUGR | Intrauterine growth retardation |
| LAIS | Lungwena Antenatal Intervention Study |
| LBW | Low birth weight |
| LDH | Lactate dehydrogenase |
| LLIN | Long-lasting insecticidal net |
| MIS | Malaria Indicator Survey |
| OR | Odds ratio |
| PBS | Phosphate-buffered saline |
| PCR | Polymerase chain reaction |
| RDT | Rapid diagnostic test |
| SD | Standard deviation |
| SP | Sulfadoxine-pyrimethamine |
| WHO | World Health Organization |

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# 1 INTRODUCTION

Malaria is caused by a parasite of *Plasmodium*-species, of which most common is *Plasmodium falciparum*. According to World Health Organization (WHO), approximately 212 million cases of malaria occurred worldwide in 2015 and the incidence has decreased by 21 % between 2010 and 2015. Malaria related deaths have declined by 29 % between 2010 and 2015. Majority of malaria cases and deaths related to malaria occur in Africa. (1) However, malaria still creates a remarkable worldwide burden, even though morbidity and mortality caused by malaria has reduced within past decade, especially among pregnant women and children under five years old.

## 1.1 Etiology and pathogenesis of malaria

*Plasmodium*-species that infect humans are *P. falciparum*, *P. vivax, P. malariae* and *P. ovale*. *P. falciparum* is the most dangerous, and can cause serious complications and even lead to death. Malaria is transmitted to humans by *Anopheles*-mosquito. (2,3)

When the mosquito stings human, it releases *Plasmodium* sporozoites to the blood within saliva of the mosquito. The sporozoites transfer to liver, where each sporozoite mature to few thousands of merozoites. For *P.falciparum* this stage last about 5–6 days. The merozoites break the liver cells and are released to the bloodstream where they invade red blood cells and continue maturing. After 1–3 days divided merozoites break the red blood cell, enter the blood stream again and infect more red blood cells. The infected red blood cells become more prone to stick to each other and the endothelium of small blood vessels, creating thrombosis and haemorrhage. This leads to end organ damage, especially in brain, kidneys, gastrointestinal tract, lungs and liver. (2,3)

The incubation period is 7–30 days. Symptoms caused by malaria are fever, headache, nausea, vomiting and myalgias. Fever is typically cyclic but especially in *P. falciparum* infection, fever can be anything between cyclic and continuous. The length of cycle depends on the species infecting and is between 36–72 hours. (2,3)

## 1.2 Malaria in pregnancy

Ten years ago, it was estimated that 25 million pregnant women every year is at risk of *P. falciparum* infection in sub-Saharan Africa in a review by Desai et al. (4). In areas of stable transmission, the prevalence of malaria in pregnancy at the time of delivery was estimated as 25 % for peripheral or placental infection. This was evaluated as underestimate, since the data it's based on, are prevalence data at one time-point and determined by light microscopy. Thus, infections outside the particular time-point haven't been considered, and low-grade parasitaemia, which are not detectable by light microscopy, are not included. For detection of those submicroscopic infections, polymerase chain reaction (PCR) or placental histology would be needed. (4)

Recently, studies from high transmission areas have shown prevalence of malaria among pregnant women with microscopy from 2.3 % in Malawi (5) to as high as 99 % in South-Eastern region of Nigeria (6). Ten articles between years 2009 and 2015 with microscopic prevalence of malaria among pregnant women in high transmission areas was reviewed in this study (5-14). The median prevalence of malaria was 13.5 % (range from 2.3 % to 99 %). Four studies reviewed had prevalence of submicroscopic malaria in high transmission areas in pregnant women, determined by PCR (5,11,14,15). The median prevalence with PCR was 19.1 % (range from 10.7 % to 29.5 %).

It seems that the prevalence of malaria in pregnancy has declined along with the overall incidence of malaria. Even prevalence determined by PCR and thus including submicroscopic infections, is not as high as before. These suggest that the utilization of malaria control measures in both general population and among pregnant women has been successful.

In high transmission areas malaria in adults is often asymptomatic. That is due to acquired immunity as a result of constant exposure and prior infections in childhood (3). This immunity does not cover from infection, but reduces the risk of severe disease. The acquired immunity protects pregnant women too and the malaria infections are often asymptomatic. But pregnant women are still predisposed to malaria and the risk for severe symptomatic disease is higher than among non-pregnant women. Additional immunocompromising state besides pregnancy, e.g. HIV, further increase the risk of severe disease. Also in areas of seasonal or low transmission, the probability of symptomatic malaria is higher. (3,4) In a study reported by Mayor et al., the clinical complaints suggestive to malaria were not associated with prevalence

of malaria, indicating that traditional signs and symptoms of malaria doesn't predict malaria infection in pregnant women (14).

In pregnant women, the infected red blood cells sequester to placenta causing placental infection (3). As a consequence, the rate of parasitaemia in peripheral blood is low, and will most likely be undetected by light microscopy. Immunity also results as low-grade parasitaemia in peripheral blood. To detect these submicroscopic infections, more sensitive methods like PCR and placental histology are needed. (3,4)

Although malaria in pregnancy is mostly asymptomatic and severe disease is rare, malaria infection during pregnancy has remarkable negative consequences which not only affect maternal health but also birth outcomes. Most significant maternal outcome is anaemia. Of all severe anaemia among pregnant women in Africa, it is estimated that 25 % is caused by malaria (3,4). Maternal anaemia was significantly more common in pregnant women with asymptomatic malaria in studies by Douamba et al. (33 % vs. 10 %, $p = 0.0002$) (10) and Matangila et al. (adjusted OR = 5, 95 % CI 2.3–10.1, $p < 0.001$) (11) . Moreover, a study by Mayor et al. found that submicroscopic infection was associated with increased risk of maternal anaemia (OR = 2.80, 95 % CI 1.46–5.38, $p = 0.002$) (14). Thus, asymptomatic and submicroscopic malaria also cause anaemia.

The maternal mortality is also one of the negative outcomes caused by malaria and it remains to be poorly estimated. The mortality rates caused directly or indirectly by malaria range from 0.5 % to 23 % in hospital studies and from 2.9 % to 17.6 % in community-based studies (4).

For birth outcome, the most commonly reported adverse effect is an increased risk of low birthweight (LBW, defined as birthweight < 2500 g) (4). LBW is highly associated with increase in infant mortality. The cause for LBW is thought to be intrauterine growth retardation (IUGR) or preterm birth which are partly caused by malaria in pregnancy. Also, association between placental malaria and stillbirth has been shown. (4)

Mohammed et al. carried out a case-control study to show association between submicroscopic *P. falciparum* infection and LBW in women having LBW babies or not (16). The prevalence of malaria at delivery was determined by microscopy, placental histology and PCR. There were no microscopy positive samples. The prevalence of submicroscopic malaria was 27.5 % in case-group and 7 % in control group with PCR. Histologically positive placental malaria was

not significantly higher in case group. The overall malaria prevalence with histology and PCR combined was significant too, 53.0 % and 30 % in cases and controls, respectively. Only submicroscopic infections were significantly associated with LBW (OR = 6.89, 95 % CI 2.2–20.8, p = 0.001), whereas placental malaria diagnosed by placental histology had no significant association with LBW. (16)

On the contrary, the other study conducted by Cohee et al. found that submicroscopic infections were not associated with LBW, preterm delivery or maternal anaemia (17). Rantala et al. also found no association between submicroscopic infections and LBW (5). In South Africa, Tsoka-Gwegweni and Kleinschmidt found low prevalence of malaria (0.07 %) among pregnant women and no association to anaemia or LBW (18). The diagnosis was made by rapid diagnostic tests (RDT). There was great loss in study population during the study period, and less than half of the enrolled was in follow up at the time of delivery. Malaria tests were planned to be taken at least three times during the study period until delivery, but there is no information on how this was fulfilled. Authors recognize, that the numbers are too low to show evidence of association. (18)

In conclusion, the evidence of the association between submicroscopic malaria infections and LBW is somewhat contradictory. On the other hand, microscopic malaria, either peripheral of placental, increases the risk of LBW unequivocally (4).

In addition, the risk of all-cause anaemia is higher among infants born to mothers with placental malaria. Placental infections reduce the transfer of maternal antibodies to the fetus causing increasing risk to many other infectious diseases besides malaria. Earlier studies suggest that malaria during pregnancy creates increasing overall morbidity and developmental problems for the child later in life. These conclusions are still uncertain and require more research. (4)

## 1.3 Laboratory diagnosis of malaria

Diagnosis of malaria is based on light microscopy of thick and thin blood smears of peripheral blood. However, microscopy is unable to detect low-grade parasitaemia which are common among pregnant women, thus more sensitive methods like PCR are needed. For field use immunochromatografic rapid diagnostic tests (RDT) has been developed. In pregnant women, placental infection is usual, hence placental histology is also possible at delivery. (2-4)

Rantala et al. compared real-time PCR and microscopy in malaria diagnosis in pregnant women at delivery (5) and determined sensitivity of 90.9 % and specificity of 91.2 % for real time PCR compared to microscopy. The sensitivity of microscopy compared to PCR was 20%. Matangila et al. compared microscopy, RDTs and nested PCR in *P. falciparum* diagnosis in pregnant women (11). Compared to PCR, the sensitivity was 67.3 % (95 % CI 52.5–80.1) and specificity 97.4 % (95 % CI 92.7–99.5) for microscopy. RDTs had sensitivity of 81.6 % (95 % CI 68.0–91.2) and specificity of 94.9 % (95 % CI 89.2–98.1) compared to PCR. (11) Mayor et al. compared microscopy to real time PCR and had sensitivity of 22.7 % for microscopy (14).

Above results show that the ability of microscopy to find infected pregnant women is inadequate. The higher sensitivity for microscopy (67.3 %) reported by Matangila et al. compared to the two others (20 % and 22.7 %) might be explained by higher density of parasites in the study population and thus a greater amount of infections detected by microscopy.

Cohee et al. used quantitative PCR for diagnosis of submicroscopic malaria infections and compared it to placental histology at delivery (17). Submicroscopic infections were significantly associated with placental malaria infection, in fact all who had submicroscopic infection at delivery had placental infection. Unexpectedly, there was also histologically diagnosed placental infections with no submicroscopic infection at any point during pregnancy (39/110). That was explained by possible submicroscopic infections occurring and clearing before the enrolment or between the sampling. (17)

These results suggest that submicroscopic infections most likely are evidence of placental infection. But even with PCR some placental malaria can be left undetected. Even though, of current diagnostic methods, PCR has shown the greatest ability to recognize malaria infections among pregnant women. Also, it’s important to detect malaria infection early in pregnancy to prevent placental infections. As PCR is not conveniently available in developing countries for daily use, RDTs are a better option for field diagnosis. In fact, RDTs were shown to detect some of the submicroscopic infections also (11). For prevalence studies, PCR or placental histology should be used as a diagnostic method to avoid too low prevalence data among pregnant women.

## 1.4 Risk factors and prevention of malaria in pregnancy

The known risk factors for microscopic malaria in pregnancy on high transmission areas are primigravidity, younger maternal age and second trimester (4). On low transmission and seasonal malaria areas the gravidity hasn't been so strongly associated to risk of malaria. These suggest that in high and stable transmission areas immunity acquired is associated to both age and parity. (4) For the prevention of malaria in pregnancy WHO recommends the use of long-lasting insecticidal nets (LLINs), intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) in areas of moderate to high transmission and prompt diagnosis and effective treatment of malaria (19).

Maternal characteristics as risk factors are quite commonly investigated (4,6,8,12,15,20,21). But environmental factors affecting malaria prevalence hasn't been much studied, with the exception of bed net availability and usage (10-12,15,22,23). Obliviously, a lot of studies about the benefits of IPTp has been done, but they are not referred here.

In many countries, Malaria Indicator Survey (MIS) has been conducted regularly to determine the availability and usage of malaria control measures including insecticide treated bed nets (ITN) and determining the prevalence of malaria in risk groups and/or general population. In Eritrea at MIS 2012 the coverage of bed nets was high, at least one bed net was owned by 90 % of households and 87 % had at least one ITN. Of pregnant women, 60 % had slept under an ITN the previous night. The malaria prevalence in general population was 1.1 % (95 % CI 0.9–1.3). (24)

In South Sudan, MIS 2009 showed that the prevalence of malaria was 24.5 % (95 % CI 23.0–26.1) among general population and among pregnant women 9.9 % (95 % CI 7.4–13.1) (7). The proportion of households with at least one bed net was 59.3 % (95 % CI 57.5–61.6), and 35.9 % (95 % CI 31.9–40 .2) of pregnant women had slept under the net the previous night. The malaria prevalence was high and the reasons suggested were low coverage and utilization of interventions, and low knowledge levels. (7) In these reports no analyses of the association between owning or using ITNs and the prevalence of malaria were made. It seems that in Eritrea where ITN coverage is high, the prevalence of malaria is essentially lower than in South Sudan where ITN coverage was lower, at least in 2009.

For environmental factors affecting malaria prevalence among pregnant women, not many studies were available. In six studies the association between malaria prevalence in pregnancy

and ITN coverage or usage was analyzed. Four of them did not find significant association between ITN use and prevalence of malaria (10,12,15,22), two did (11,23). The effect of season in the prevalence of malaria among pregnant women was analyzed in three studies (13-15) with inconsistent findings. High-risk season was associated with higher malaria prevalence in one study (13), but two studies found no association between dry or rainy season and prevalence of malaria (14,15). The effect of housing conditions and materials of walls, roofs, floors and windows on the prevalence of malaria was investigated in general population (9). The poor wall materials were found to be associated with prevalence of malaria. Another study found association between household size and malaria prevalence in pregnant women (6). Only two of the studies above included submicroscopic infections to the analysis (14,15).

The need for malaria control strategies specifically targeted to pregnant women was evaluated in two studies. In the South African study of Tsoka-Gwegweni and Kleinschmidt the prevalence of malaria was low (0.07 %) (18). They suggest that malaria control measures for entire population benefit also during pregnancy, and there is no need for measures specifically aimed for pregnant women. In Umkhanayakude health district, where the study was conducted and malaria transmission is high, yearly indoor residual spraying (IRS) of insecticide for every house is conducted. (18) That is an extensive strategy which does not apply most African countries.

Another study by Boudová et al. investigated if a universal bed net campaign would reduce the burden of malaria among pregnant women in Malawi (15). Following the bed net campaign, the use of bed nets increased from 50.3 % to 66.2 %. At the same time the prevalence of malaria decreased from 28.4 % to 15.0 %. However, there was no association between malaria infection and bed net use in individual level. Contradictory to the study by Tsoka-Gwegweni and Kleinschmidt (18), this study suggests that besides universal anti-malarial measures, specific strategies targeting pregnant women are still needed. All women had their first or second pregnancy, being in higher risk of malaria, which may explain the high prevalence. (15) And in the end, the bed net coverage in Malawi is still quite low, and as a universal method it cannot be compared with the yearly IRS of every household.

# 2 AIMS

This study aims to evaluate the effect of environmental factors on the prevalence of malaria among pregnant women in a malaria endemic area. The focus is on detailed environmental factors which might affect prevalence of malaria caused by *P. falciparum*. As described above, environmental factors in malaria among pregnant women have not been investigated thoroughly. Furthermore, none of the studies found were investigating environmental factors widely but rather were focusing on some specific aspect. In this study, the following environmental factors were analyzed: season, household size, earning cash money, material of the walls and roof, windows, the source of the drinking water, sanitary facilities, bed net household availability, the impregnation of bed net and the usage of the bed.

# 3 MATERIALS AND METHODS

## 3.1 Study site and study population

The data used in this study is from Lungwena Antenatal Intervention Study (LAIS) which was conducted in rural Malawi between 2003 and 2007. In the area malaria transmission is high and stable. The details of the original study were reported elsewhere (25). In brief, the study population was drawn from the pregnant women who presented at the health centre in Lungwena, Mangochi District, Southern Malawi, between 14$^{th}$ and 26$^{th}$ gestation week. In addition to gestational age the enrolment criteria were felt movements of the fetus, availability for follow up and informed consent. The sample size has been determined for the primary study, which was an intervention study with three intervention groups and preterm delivery as the primary outcome. The three intervention groups were control group who received standard IPTp-SP with two doses during pregnancy, first intervention group who received SP monthly, and second intervention group who received SP monthly and azithromycin twice. A follow up until delivery was at four week intervals until 36$^{th}$ gestation week and weekly thereafter. (25)

1320 women were enrolled and questionnaires of background and malaria measures were filled. The forms included information of participant and her partner, household, economics, housing, malaria measures and medical and obstetric histories. In this study, only the enrolment data were used.

## 3.2 Laboratory analysis

At enrolment, maternal peripheral venous blood samples were collected as dried blood spot (DBS) and blood films. Giemsa-stained thick and thin blood films were prepared and subsequently interpreted by an experienced microscopist in a local research laboratory. For PCR diagnosis of *P. falciparum,* 1309 DBS samples were collected. DNA were extracted from DBS and real-time PCR was performed to detect *Plasmodium falciparum* as follows.

For extraction of genomic DNA (gDNA) from DBS, each DBS sample was punched 6 times (3x3 mm). The punch was sterilized between each sample. Punched DBS were put into 96-well plate and 1 ml of 1xPBS buffer and 5 µl of 10 % saponin were added. The plate was stored overnight at 4°C. Next day, after centrifuging, the supernatant was aspirated and discarded. Again 1 ml of 1xPBS was added and the plate was incubated at 4°C for 30 minutes. Previous

procedure for supernatant was repeated. After discarding fluid, 100 µl of sterile water and 50 µl of 20 % Chelex were added. Plate was covered with foil and incubated in 95–99°C water bath for 12 minutes with vortex every 2–3 minutes. The plate was then centrifuged at 1 500 rpm for 5 minutes. The fluid was transferred to new plate and then centrifuged the new plate at 1 500 rpm for 10 minutes. The final fluid containing Chelex extracted DNA was once more transferred to new plate and stored at -20°C.

Real time PCR detected lactate dehydrogenase (LDH) gene in *Plasmodium falciparum*. Primers specific to the gene were used for amplification of the LDH gene of *P. falciparum* by PCR. The amplification is detected in real time when the oligonucleotide probe anneals to targeted sequence and fluoresces. The procedure of real time PCR was as follows. PCR master mix (MM) was made mixing 12.5 µl of TaqMan Mastermix, 1.25 µl LDH forward primer (20 uM), 1.25 µl LDH reverse primer (20 uM), 0.25 µl LDH probe (20 uM) and 7.75 µl of sterile water for each sample. Thus, with reaction volume of 25 µl including 2µl of gDNA, MM had primers at 1000 nM and probe at 200 nM. In 96-well reaction plate, 23 µl of MM was dispensed to every well and 2 µl of DNA was transferred from sample plate. Each plate included four serial 10-fold dilutions (1, 0.1, 0.01, 0.001 µg/ml) of 3D7 DNA of *P. falciparum* as positive controls and two negative controls of nuclease free water. Each plate was made in duplicate.

Before real time PCR, the plate was centrifuged for 1 minute at 300 rpm at 4°C. The plates were run on ABI 7900HT real time system. The cycling conditions were: 50°C for 2 min, 95°C for 10 min and 45 cycles of 95°C for 15 s and 60°C for 1 min. Samples were considered *P. falciparum* positive if the amplification curves in both reaction plates reached the threshold line. If only other reached the threshold line, the sample was re-run.

## 3.3 Statistical analysis

The chosen environmental variables were: the season at the enrolment (November-April – rainy season), the number of persons in household, does the participant or her partner earn cash money of their work, the building material of the walls and roof of the house, windows of the house, the source of the drinking water, sanitary facilities, bed net household availability, the impregnation of bed net and the usage of the bed net determined by if the participant had slept under the net previous night or not.

The variables were categorized as follows: household size ( ≤ 3, 4–6, ≥ 7), earning cash (never or irregularly, the participant or her partner regularly, both the participant and her partner regularly), material of the walls (grass or mud [including poles and grass, poles and mud and only mud], mud brick, burnt brick), material of the roof (grass, iron sheets, tiles), windows (no, yes [including open windows and windows with screen or glass]), drinking water (piped water or borehole, protected well, and unprotected well, lake, river or pond), sanitary facilities (none, pit latrine [including ventilated improved pit latrine and regular pit latrine]), bed net ownership (no, yes), impregnation of the net (never, more than 6 months ago, less than 6 months ago), sleeping under the net the previous night (no, yes) and season (rainy, dry).

The analysis was performed using IBM SPSS Statistics version 23. Only participants with PCR result were included in the analysis (n = 1309). The frequencies of the variables were determined as absolute numbers and percentages, and 95 % confidence intervals were calculated. To measure the correlation between environmental factors and the prevalence of malaria, binary logistic regression was used. Univariate logistic regression was performed to each variable separately. The prevalence of malaria determined by real time PCR was the dependent variable. Multiple logistic regression was performed including the variables with p-value < 0.1 in primary analysis, to determine adjusted odds ratios and 95 % confidence intervals. Multivariable analysis had to be done in two sets, since part of the questions only considered those who owned bed net. First set included all variables filling the significance criteria, except the ownership of the bed net, and the second set included all but impregnation of the bed net. The difference between these two analyses were not significant, and thus the results are from the first analysis to all but bed net ownership. For final analysis, $p < 0.05$ was considered significant.

## 3.4 Ethical considerations

The original LAIS was performed according to Good Clinical Practice guidelines (ICH-GCP) and its protocol was approved by the College of Medicine, Research and Ethics Committee, University of Malawi and the Ethical Committee of Pirkanmaa Hospital District, Finland (25). Informed consent was collected from each participant.

# 4 RESULTS

## 4.1 General characteristics and the prevalence of malaria

The characteristics of the 1309 women in this study is summarized in Table 1. The mean age of the participants was 24.9 (SD 6.4) years. Twenty-three percent had their first pregnancy. Most had not completed standard schooling (7 years), the mean completed years being 2.2 (SD 2.7). Most were married (97.4 %) and approximately two thirds were employed (62.7 %). At the point of enrolment, almost half (48.1 %) had anaemia (Hb < 110 g/l).

The overall prevalence of malaria was 8.9 % (95 % CI 7.4–10.4) and 40.6 % (95 % CI 37.9–43.3) by light microscopy and real time PCR, respectively (Table 2). The type of malaria in light microscopy positive samples was determined as *P. falciparum* in 95 (81.9 %) cases and *P. malariae* in 21 (18.1 %) cases.

## 4.2 Environmental factors potentially affecting the malaria prevalence

The distribution of the environmental factors potentially affecting malaria prevalence are categorized in Table 3. Approximately half of the households had 3 or less persons (51.3 %), while 42.5 % had 4 to 6 persons and 6.2 % had seven or more. The participant or her partner never or irregularly earned cash money of their work in 35.8 % of the families, either the participant or her partner earned cash money of their work regularly in 56.8 % of the families and only in 7.4 % both the participant and her partner earned cash money regularly.

Material of the walls of the main house was poles and grass, poles and mud or mud in only 24 (1.8 %) participants. The material was mostly either mud brick (71.2 %) or burnt brick (27.0 %). Material of the roof of the main house was mostly grass (94.6 %) and only 5.4 % had iron sheets. None had tiles as the material of the roof. Most of the participants had no windows in their houses (83.5 %). Of those who had, 75 (34.7 %) had screen or glass on their windows otherwise the windows were open. Drinking water from borehole or pipes was used by 85.2 % of the participants. Protected well was used by 4.4 % and unprotected well, lake, river or pond by 10.4 %. Any kind of pit latrine was available for 94.2 % of the pregnant women, and 5.8 % had no sanitary facilities.

## Table 1 Characteristics of the pregnant women

| Characteristic (N) | |
|---|---|
| **Age (years) (1308)** | |
| Mean (SD) | 24.9 (6.4) |
| **BMI (kg/m$^2$) (1307)** | |
| Mean (SD) | 21.8 (2.2) |
| **Parity (1309)** | |
| Primigravidae, n (%) | 299 (22.9) |
| Multigravidae, n (%) | 1010 (77.2) |
| **Education (years) (1307)** | |
| Mean (SD) | 2.2 (2.7) |
| **Marital status (1308)** | |
| Married, n (%) | 1274 (97.4) |
| Unmarried, n (%) | 34 (2.6) |
| **Employment (1302)** | |
| Employed, n (%) | 817 (62.7) |
| Unemployed, n (%) | 485 (37.3) |
| **Anaemia (Hb < 110 g/l) (1309), n (%)** | 630 (48.1) |

N = total number, SD = standard deviation, n = number, % = percentage, Hb = Haemoglobin

## Table 2 Prevalence of malaria with microscopy and real time PCR

| | n (%) | 95 % CI |
|---|---|---|
| **Microscopic parasitaemia (1307)** | | |
| Negative | 1191 (91.1) | 89.6–92.6 |
| Positive | 116 (8.9) | 7.4–10.4 |
| **Malaria type (116)** | | |
| *P. falciparum* | 95 (81.9) | 74.9–88.9 |
| *P. malariae* | 21 (18.1) | 11.1–25.1 |
| **PCR result (1309)** | | |
| Negative | 777 (59.4) | 56.7–62.1 |
| Positive | 532 (40.6) | 37.9–43.3 |

n = number, % = percentage, CI = confidence interval, PCR = polymerase chain reaction

At least one bed net was owned by 73.5 % of the pregnant women. Of those who had the bed net 82.4 % informed having slept under the net the previous night. The impregnation of the bed net had been done less than 6 months ago in 46.3 %, more than 6 months ago in 47.8 % and never in 5.8 % of the bed nets.

The enrolment season was equally divided, of the pregnant women 52.9 % enrolled during the rainy season, and 47.1 % enrolled during the dry season.

## Table 3 Environmental factors potentially affecting the malaria prevalence

| Environmental factor (N) | n | % [95 % CI] |
|---|---|---|
| **Household size (1307)** | | |
| ≤ 3 | 670 | 51.3 [48.6–54.0] |
| 4-6 | 556 | 42.5 [39.8–45.2] |
| ≥ 7 | 81 | 6.2 [4.9–7.5] |
| **Earning cash (1309)** | | |
| Never or irregularly | 469 | 35.8 [33.2–38.4] |
| 1 person regularly | 743 | 56.8 [54.1–59.5] |
| 2 persons regularly | 97 | 7.4 [6.0–8.8] |
| **Material of the walls (1307)** | | |
| Grass or mud | 24 | 1.8 [1.1–2.5] |
| Mud brick | 930 | 71.2 [68.7–73.7] |
| Burnt brick | 353 | 27.0 [24.6–29.4] |
| **Material of the roof (1303)** | | |
| Grass | 1232 | 94.6 [93.4–95.8] |
| Iron sheets | 71 | 5.4 [4.2–6.6] |
| **Windows (1307)** | | |
| No | 1091 | 83.5 [81.5–85.5] |
| Yes | 216 | 16.5 [14.5–18.5] |
| **Drinking water (1308)** | | |
| Piped water or borehole | 1115 | 85.2 [83.3–87.1] |
| Protected well | 57 | 4.4 [3.3–5.5] |
| Unprotected well, lake, river or pond | 136 | 10.4 [8.7–12.1] |
| **Sanitary facilities (1304)** | | |
| None | 76 | 5.8 [4.5–7.1] |
| Pit latrine | 1228 | 94.2 [92.9–95.5] |
| **Bed net (1308)** | | |
| No | 346 | 26.5 [24.1–28.9] |
| Yes | 962 | 73.5 [71.1–75.9] |
| **Impregnation of the net (958)** | | |
| Never | 56 | 5.85 [4.3–7.3] |
| More than 6 months ago | 458 | 47.8 [44.6–51.0] |
| Less than 6 months ago | 444 | 46.35 [43.1–49.5] |
| **Sleeping under the net (961)** | | |
| No | 169 | 17.6 [15.2–20.0] |
| Yes | 792 | 82.4 [80.0–84.8] |
| **Season (1309)** | | |
| Rainy | 692 | 52.9 [50.2–55.6] |
| Dry | 617 | 47.1 [44.4–49.8] |

N = total number, n = number, % = percentage, CI = confidence interval

## 4.3 The association between environmental factors and submicroscopic malaria

Household size, having windows or not, drinking water source, bed net ownership, impregnation of the bed net and the season of enrolment were significantly associated with risk of malaria (Table 4). The participants who had four or more persons in their household were less likely to have *P. falciparum* infection compared to households with 1–3 persons (AOR = 0.5, 95 % CI 0.4–0.7, $p < 0.001$ and AOR = 0.4, 95 % CI 0.2–0.9, $p = 0.01$ for 4–6 persons and ≥ 7 persons, respectively).

Having windows, with or without screen, in the main house decreased the risk of malaria compared to those who had no windows in their main house (AOR = 0.6, 95 % CI 0.4–0.9, $p = 0.01$). Those whose source of drinking water was unprotected well, lake, river or pond had significantly higher risk for *P. falciparum* infection compared to those whose source was piped water or borehole (AOR = 1.9, 95 % CI 1.2–3.0, $p = 0.01$). Protected well as a water source had no significant association with malaria compared to piped water or borehole.

The ownership of at least one bed net significantly lowered the risk of malaria (AOR = 0.6, 95 % CI 0.5–0.8, $p < 0.001$). Unexpectedly the impregnation of the bed net significantly reduced the risk of malaria in the group with impregnation more than 6 months ago compared to those who had never impregnated the net (AOR = 0.4, 95 % CI 0.2–0.7, $p = 0.001$), while impregnation less than 6 months ago had no significant association with malaria prevalence.

Those who enrolled during the dry season had lower risk for malaria compared to those who enrolled during the rainy season (AOR = 0.6, 95 % CI 0.5–0.8, $p < 0.001$).

## Table 4 Association of environmental factors to malaria

| Environmental factor (N) | Prevalence of *P. falciparum* % (n/N) | OR [95% CI] | AOR [95% CI] | p |
|---|---|---|---|---|
| **Household size (1307)** | | | | |
| ≤ 3 | 48.7 (326/670) | 1 | 1 | |
| 4-6 | 31.8 (117/556) | 0.5 [0.4–0.6] | 0.5 [0.4–0.7] | <0.001 |
| ≥ 7 | 33.3 (27/81) | 0.5 [0.3–0.9] | 0.4 [0.2–0.9] | 0.01 |
| **Earning cash (1309)** | | | | |
| Never or irregularly | 41.2 (193/469) | 1 | | |
| 1 person regularly | 41.0 (305/743) | 1.0 [0.8–1.3] | | |
| 2 persons regularly | 35.1 (34/97) | 0.8 [0.5–1.2] | | |
| **Material of the walls (1307)** | | | | |
| Grass or mud | 37.5 (9/24) | 1 | | |
| Mud brick | 42.4 (394/930) | 1.2 [0.5–2.8] | | |
| Burnt brick | 36.3 (128/353) | 0.9 [0.4–2.2] | | |
| **Material of the roof (1303)** | | | | |
| Grass | 41.1 (506/1232) | 1 | | |
| Iron sheets | 31.0 (22/71) | 0.6 [0.4–1.1] | | |
| **Windows (1307)** | | | | |
| No | 42.2 (460/1091) | 1 | 1 | |
| Yes | 32.4 (70/216) | 0.7 [0.5–0.9] | 0.6 [0.4–0.9] | 0.01 |
| **Drinking water (1308)** | | | | |
| Piped water or borehole | 39.7 (443/1115) | 1 | 1 | |
| Protected well | 31.6 (18/57) | 0.7 [0.4–1.2] | 0.9 [0.4–1.8] | 0.7 |
| Unprotected well, lake, river or pond | 51.5 (70/136) | 1.6 [1.1–2.3] | 1.9 [1.2–3.0] | 0.01 |
| **Sanitary facilities (1304)** | | | | |
| None | 48.7 (37/76) | 1 | | |
| Pit latrine | 40.1 (493/1228) | 0.7 [0.4–1.1] | | |
| **Bed net (1308)** | | | | |
| No | 49.4 (171/346) | 1 | 1 | |
| Yes | 37.4 (360/962) | 0.6 [0.5–0.8] | 0.6 [0.5–0.8] | <0.001 |
| **Impregnation of the net (958)** | | | | |
| Never | 53.6 (30/56) | 1 | 1 | |
| More than 6 months ago | 30.6 (140/458) | 0.4 [0.2–0.7] | 0.4 [0.2–0.7] | 0.001 |
| Less than 6 months ago | 42.3 (188/444) | 0.6 [0.4–1.1] | 0.6 [0.4–1.1] | 0.1 |
| **Sleeping under the net (961)** | | | | |
| No | 36.7 (62/169) | 1 | | |
| Yes | 37.6 (298/792) | 1.0 [0.7–1.5] | | |
| **Season (1309)** | | | | |
| Rainy | 46.1 (319/692) | 1 | 1 | |
| Dry | 34.5 (213/617) | 0.6 [0.5–0.8] | 0.6 [0.5–0.8] | <0.001 |

N = total number, % = percentage, n = number, OR = odds ratio, AOR = adjusted odds ratio, CI = confidence interval, p-value is considered significant when <0.05

# 5 DISCUSSION

The prevalence of submicroscopic *P. falciparum* infection among pregnant Malawian women at enrolment was 40.6 %. The type of malaria in microscopy positive samples was determined to be *P. falciparum* in 81.9 % of the samples and *P. malariae* in 18.1 % of the samples. Thus, the prevalence of malaria caused by all *Plasmodium* species might be a little higher, but not considerably. The prevalence of malaria is determined by real time PCR to take into account submicroscopic infections as they remarkably increase the burden of malaria as well. The results can show us new potential ways to reduce the risk of malaria.

At the time of enrolment, the duration of pregnancy was 14$^{th}$ to 26$^{th}$ week i.e. second trimester and the women had not yet received any intermittent preventive malaria medication. The prevalence is high compared to other studies that have reported submicroscopic prevalence of malaria among pregnant women (11,14,15). The study by Matangila et al. was conducted in 2012 in Democratic Republic of the Congo (11). The women were enrolled on their first antenatal visit, so they had not received any IPTp, and women of all trimesters were recruited. The prevalence of submicroscopic malaria was 29.5 % (95 % CI 22.7–37.1 %). Mayor et al. reported prevalence of 23.2 % among Mozambican pregnant women (14). The women were recruited between 2003 and 2005 in Maternity Clinic where they attended with clinical complaints suggestive to malaria. Women of all trimesters were included. No information of received IPTp was provided. In the study by Boudová et al., the prevalence was determined at enrolment with women on 28$^{th}$ or less gestation weeks, on their first or second pregnancy in Blantyre, Malawi in 2014 (15). The prevalence had declined from 28.4 % in 2009 to 18.5 % in 2014. In all these studies, the study sites are high and stable transmission areas.

In this study, the DBS were collected between 2003 and 2006. Based on results by Boudová et al. (15), we could assume that the prevalence of submicroscopic malaria among pregnant women in Lungwena, Mangochi District, Malawi, has also declined within past decade. The data of overall incidence of malaria according to WHO are consistent with this assumption (1). The prevalence determined in the study by Mayor et al. (14) is from selected group with suitable symptoms, thus the prevalence among all pregnant women might have been higher. Another explanation could be that possible IPTp have reduced the prevalence. However, this is not very likely explanation since, according to WHO (1), IPTp was adopted in Mozambique only in 2006.

The coverage of bed nets was high, at least one bed net was owned by 73.5 % of pregnant women and 82.4 % of those who had bed net reported having slept under the net the previous night. The type of net or the insecticide used is not known, but 46.3 % of the nets were treated with insecticide within 6 months and at least those can be regarded as ITNs. If the determination of an ITN is that the net has been impregnated at least once at any time, then the amount of ITNs would be 94.1 % of the bed nets. Owning a bed net significantly lowered the risk of having submicroscopic *P. falciparum* infection (AOR = 0.6). Impregnation of the bed net more than 6 months ago had protective effect against malaria (AOR = 0.4). However, impregnation less than 6 months ago had no significant association with the prevalence of malaria. Also, usage of the bed net had no effect on the risk of malaria.

In most of the studies, where the association between the prevalence and bed net use or ownership was analyzed, the prevalence of malaria was determined by microscopy (10-12,22,23). Douamba et al. (10) investigated the prevalence of asymptomatic malaria in Ouagadougou, Burkina Faso, where malaria transmission is high but seasonal. The study was conducted during the malaria transmission season in 2010. The prevalence of malaria was 30 % and 24 % with RDT and microscopy, respectively. Only 42 % of pregnant women used ITN. The proportion of pregnant women who owned but not used ITN was not informed. There was no significant difference in the microscopic prevalence of *P. falciparum* malaria between those who used ITN and those who did not use. (10) There were some inconsistencies in the article within the text and tables, and the results regarding ITN use were not provided in the table.

Simultaneously, Cisse et al. (12) conducted a similar study in Bobo-Dioulasso, Burkina Faso. The prevalence of *P.falciparum* among pregnant women was 18.1 % (95 % CI: 15.1–21.2) by microscopy. The ownership of at least one ITN was low, 46.9 % of the pregnant women had ITN. There was no data of the use of ITNs. The ownership of an ITN had no significant association with malaria (AOR = 0.8, 95 % CI 0.5–1.5, p = 0.4). The difference in prevalence reported by Douamba et al. (10), was suspected to be due to lower use of IPTp-SP in Ouagadougou. (12)

The prevalence of malaria at delivery in an area of high coverage of ITNs among women who did not receive IPT in pregnancy was studied by Kabanywanyi et al. in Tanzania (22). Malaria transmission is moderate to high and perennial. Malaria prevalence was determined with microscopy from placental blood sample at delivery. The ITN coverage was indeed high with

91 % reporting having slept under a bed net the previous night at home. Of these, 23 % had had the net treated with insecticide within one year. The prevalence of malaria was 8 %. The association between ITN use and prevalence of malaria was not significant. On the other hand, those who used bed net had significantly lower risk of delivering LBW baby (OR = 0.22, 95 % CI 0.08–0.59 for women who used treated bed net and OR = 0.34, 95 % CI 0.16–0.74 for women who used untreated bed net). (22)

Abeku et al. (23) evaluated the effectiveness of malaria interventions in Ethiopia and Uganda. They found that those who had slept under an ITN the previous night, had significantly lower risk of malaria (2.3 % vs. 5.7 %, p = 0.020). However, the association was in general population and no analysis including only pregnant women were made. Furthermore, this association was seen in only one of four study sites. (23) On the other hand, Matangila et al. (11) found that the utilization of bed net was significantly associated with lower risk of malaria among pregnant women (AOR = 0.4, 95 % CI 0.2–0.7, p < 0.01). The analyses were made using microscopic prevalence of malaria as a dependent factor. The bed net availability was similar to the studies that did not find any associations. Only 43.1 % of the pregnant women had bed net and 69.8 % of them was ITN. The bed net was owned and used the previous night by 35.2 % of the participants. The microscopic prevalence of malaria was also similar (21.6 %). (11)

The association between bed net use and submicroscopic malaria infection was analyzed by Boudová et al. (15). As described before the prevalence of malaria decreased significantly while the coverage of ITNs increased due to universal bed net campaign. In individual level, however, the association between submicroscopic malaria and bed net use was not significant (OR = 0.8, 95 % CI 0.6–1.1, p = 0.23).

It seems that there is no association between bet net use and microscopically detectable malaria infection among pregnant women. As described before, the sensitivity of microscopy is not good enough to find low parasitaemia which is common among pregnant women. With more sensitive diagnostic method the association might have been found. In fact, the results by Kabanywanyi et al. (22) suggest that submicroscopic infections not detected were present and increased the risk of having LBW baby among those who did not use bed net. On the other hand, Boudová et al. (15) found no association even though the submicroscopic infections were included in the analysis. They speculated that due to high coverage of bed nets in the area, the transmission is lower and thus pregnant women are less exposed to malaria.

In this study, the bed net ownership lowered the risk of submicroscopic malaria. The use of the net the previous night prior the enrolment, however, had no effect on the risk of submicroscopic malaria. The women might have started to use the bed net only after they have found out being pregnant. The susceptibility to malaria may increase as early as 8 weeks' gestation, at least in primigravidae (4). Early infection may have led to placental malaria with submicroscopic peripheral parasitaemia. This could also explain the negative results of other studies concentrating on the bed net use the previous night. Also, the explanation of Boudová et al. about lower transmission in high bed net coverage area (15), may explain results in this study too.

The result of impregnation of the bed net more than 6 months ago being more protective against malaria than impregnation less than 6 months ago, is unexpected. The cross tabulation of impregnation of the bed net and utilization of the bed net showed that the result cannot be explained by lower utilization rate of those who had impregnated the net recently. On the contrary, a greater proportion of those who had impregnated the net less than 6 months ago had used the net the previous night. Most likely some other factors explain this result, for example the group that had longer time of the impregnation can be generally wealthier. Also, maternal characteristic such as age and parity can be unevenly distributed among the groups. These subsequent analyses were not done in this connection. The prevalence of malaria was significantly lower among those who had ever impregnated the bed net compared to those who had never impregnated the net.

In this study, those who had more than three persons in their household were in lower risk of having submicroscopic malaria compared to those who had three or less. Those who had 4–6 persons or ≥7 persons had equally decreased risk of *P. falciparum* infection. Our results are similar as previous study by Gunn et al. (6). They found that those who had more people in the household had lower risk of high parasitaemia (OR = 0.94, 95 % CI 0.89–0.99, $p < 0.05$ for every one person increase in household size). The study was conducted in Nigeria and the prevalence of microscopic malaria was very high, 99 %. Of malaria positive, the amount of high parasitaemia was 38 %. The effect was explained by assumed multiparity of those who had more persons in their household and the increased immunity to malaria among multiparous women. (6) The increased immunity can explain at least the lower risk for high parasitaemia, but for submicroscopic low-grade parasitaemia other explanations should be considered. Parity

hasn’t shown protective effect on submicroscopic malaria, at least in the studies reviewed here (14,15).

Of housing conditions, having windows compared to no windows at all, lowered the risk of submicroscopic malaria. A vast majority (83.5 %) of the pregnant women had no windows at all in their houses. Having a window, with or without screen, was associated with more regular income suggesting that those who had windows were generally wealthier.

The material of walls or roof had no association to the prevalence of submicroscopic malaria among pregnant women. Sonko et al. (9) investigated if socio-economic status is associated to prevalence of malaria in Gambia. The data of the study was from MIS from November 2010 to January 2011. The survey covered 4 500 households and the questionnaires included questions about housing conditions and materials of walls, roofs, floors and windows. A blood sample was collected from women 15–49 years old and children 6 months to 14 years old, to determine malaria infection. In addition, blood samples were collected from every 4$^{th}$ household for general population prevalence. RDT and microscopy was used for diagnosis. The overall prevalence of malaria in general population was 8.7 % (95 % CI 7.4–10.2). The method of diagnosis used to determine prevalence was not clearly stated, but it is supposedly RDT. For general population having poor wall materials was associated with higher prevalence of malaria (OR = 1.6, 95 % CI 1.1–2.3, p = 0.01). Poor wall materials were defined as cane/palm/trunks, mud/dirt, stone with mud and mud/krinting (woven bamboo) in comparison to good wall materials defined as bricks, cement block, covered adobe and other. For children aged 6 to 59 months and children aged 5 to 14 years, in addition to poor wall materials, also poor floors, roofs and windows were significantly associated with the prevalence of malaria. In general population, however, the association was not significant. (9) Associations among pregnant women were not analyzed, but the results suggest that the risk of malaria in risk groups is higher in houses with poor materials. Explanation for this association could be that poor house materials allow the mosquitos to enter the house more easily and thus predispose the inhabitants to infection.

Having unprotected well, lake, pond or river as the source of drinking water increased the risk of submicroscopic malaria infection compared to piped water or borehole as source of drinking water. The sites that increase risk of malaria are the ones which are potential for mosquito

breeding (26). The probability of being stung by a mosquito carrying *P. falciparum* and infected is higher.

Mayor et al. (14) and Boudová et al. (15) both analyzed the association between the prevalence of submicroscopic malaria and season divided in dry and rainy season. The details of the studies have been explained above. Neither found significant association between submicroscopic malaria and season. In this study the prevalence of submicroscopic malaria was more common among those who enrolled at the rainy season compared to those who enrolled at the dry season. The rainy season was determined between November and April (27). It could be argued that division to high-risk and low-risk season would have been more convenient. That would have required calculation of the prevalence of malaria in each month during the study period and assessment of annual trends to determine the high- and low-risk seasons (13). Also, yearly weather conditions were not known and they may affect the risk of malaria.

High- and low-risk seasons were determined by Jäckle et al. (13) who evaluated the impact of seasonality in the risk of malaria in pregnancy. The study site was rural Gabon, where there is little seasonal variation in malaria transmission. The study period was from January 2008 to December 2011. The prevalence of malaria among pregnant women was 16 % by microscopy. The season was significantly associated with the risk of malaria, being higher in the high-risk season (OR = 1.91, 95 % CI 1.39–2.63, $p < 0.001$). The high- and low-risk seasons were determined based on annual trends during the study period. This seasonal variation was even more pronounced in high-risk group of young and pauciparous women. (13) The definition of high- and low-risk seasons differed a lot from the dry and rainy seasons in the area. Thus, these results are not straight comparable to those in this study. The usefulness of these results is questionable, since the high- and low-risk seasons vary depending on weather conditions and mosquito breeding. The same definition for high- and low-risk season is not applicable every year. Also, because of the method used in defining the high- and low-risk seasons, the positive result is somewhat expectable.

The analyses done in this study indicated that the chosen environmental factors cover a minor part of all the affecting factors. Malaria control measures specifically targeted to pregnant women, such as IPTp-SP, have a remarkable role in preventing malaria. The prevalence of submicroscopic malaria in this same study population was only 10.7 % at delivery (5) and all of

the women had received at least two doses of IPTp-SP during pregnancy. This suggest that IPTp given has not only prevented but also cleared some of the infections.

As a conclusion, for prevention of submicroscopic infections among pregnant women, high coverage of bed nets is essential. All women in fertile age should use bed net regardless of their known state of pregnancy. High coverage of bed nets in the community apparently protect pregnant women from malaria infection (15). The results considering the seasonal effect on the risk of malaria are inconsistent. Even in stable transmission areas seasonal variation in the prevalence of malaria most likely exists (13), but the pattern will be hard to predict. Thus, bed nets should be used all around the year, regardless of the season. General measures against poverty would improve the housing conditions related to malaria infection. The strategies targeted to prevent poverty would most likely also prevent malaria among pregnant women. Prevention of malaria in pregnancy has to be a sum of methods affecting transmission from different directions.

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