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Assessment of the infectivity of malaria parasites from asymptomatic school children to *Anopheles gambiae* mosquitoes in a high transmission area in Ghana

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Asymptomatic carriage of malaria parasite is a major public health issue in Ghana and sub-Saharan Africa. These infections are mostly sub-microscopic and not detected by routine malaria screening methods. Asymptomatic malaria infection carriers serve as an infectious reservoir for malaria transmission. This study assessed the infectivity of asymptomatic children to *Anopheles gambiae* mosquitoes in a high transmission area in Ghana. Ninety-eight (98) healthy children were screened for malaria parasites by microscopy and PCR. Sub-microscopic gametocytes carriage was also determined using RT-PCR. Blood samples from asymptomatic parasite carriers were used in membrane feeding assays of laboratory colonies of *An. gambiae* mosquitoes. Infectivity was assessed by dissection of mosquito midguts and the mosquito infection rate and oocyst densities were recorded. The total participants that were asymptomatic for malaria was 73/98 (74.49%). Malaria parasite prevalence was 13.7% by microscopy and 78.08% by PCR. Sub-microscopic infections accounted for 64.38% (47/73) of the asymptomatic parasite carriers. No gametocytes were detected, however, the *Pfg*377 gene was observed in 33.33% (19/57) of the asymptomatic parasite carriers. Blood from 4 out of 19 asymptomatic carriers, associated with carriage of sub-microscopic gametocytes, were found to be infectious to the *An. gambiae* mosquitoes. The average oocyst density observed was 0.01, with an overall mosquito infection rate of 0.07. This data will be helpful in improving current malaria control efforts in Ghana.

Keywords Malaria, Membrane feeding, Oocyst, Asymptomatic infection carriers, Ghana

Transmission of malaria parasites to *An. gambiae* mosquitoes sustains the transmission cycle. Identification of all infectious reservoirs is relevant to malaria control[1,2]. Asymptomatic infection is characterized by carriage of parasites in the absence of signs and symptoms of malaria, particularly fever[3]. These infections are mostly sub-microscopic[4] and more frequent in areas of high malaria transmission[5–8]. Sub-microscopic infections with asexual malaria parasites frequently occurs in older children and adults who are asymptomatic, and this has been largely attributed to acquired immunity[9]. Individuals who are asymptomatic would normally not seek treatment, as such, they serve as carriers of gametocytes that could contribute significantly to the transmission of malaria[10–13].

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Gametocyte carriage in asymptomatic malaria infections on the other hand, is common in younger children[14]. The prevalence of gametocytes estimated by microscopy is much lower than by molecular methods such as PCR[15–17]. Across sub-Saharan Africa, gametocyte prevalence diagnosed by PCR ranging from 13% in Gambia[18]49.2% in Ghana[19] and as high as 91% in Burkina Faso[20]. Immune responses against gametocyte antigens result in the production of anti-gametocyte antibodies[21]. Some of these antibodies have been characterized as having the ability to prevent the completion of the sporogonic cycle of the parasite within the mosquito referred to as transmission blocking. Antibodies to the gametocyte antigens *Pf*s230-CO_{LI} and *Pf*s48/45-6C were among the first to be identified as transmission blocking antibodies. Antibodies to *Pf*s230-CO_{LI} and *Pf*s48/45-6C have been shown to prevent gametocyte /gamete formation (pre fertilization), whiles anti *Pf*s25 and *Pf*s28 antibodies have been shown to prevent ookinete/oocyst formation (post fertilization)[22]. In malaria endemic countries such as Burkina Faso, Ghana and Tanzania, there is the evidence of the presence of antibodies to these pre fertilization antigens[23]. In field settings, naturally acquired transmission-reducing immunity has been shown to reduce infectiousness of gametocytes to female *An. gambiae* mosquitoes[24]. Earlier studies have shown that children are more likely to have anti-gametocyte antibodies as compared to adults[25,26]hence blood samples containing gametocytes from children could be less infectious to *An. gambiae* mosquitoes as compared to blood samples from adults.

The presence of mixed malaria species or different clones of a particular strain have been reported to affect disease outcome[27–29] and response to treatment[30]. This could also affect the transmission dynamics of malaria. In human malaria infections, mixed infections with *P. falciparum* and *P. malariae* for example, have been shown to result in increased *P. falciparum* gametocytes[27,31]. Also, a comparison of the genotypes of oocysts from a mosquito, with the genotype of parasites from blood in a direct feeding assay revealed that some clones of parasites were not infectious to mosquitoes[32] whiles others were infectious.

Membrane feeding assays are used to study the infectiousness of gametocytes from natural malarial infections to *An. gambiae* mosquitoes[33]. The source of gametocytes for these assays could either be from cultured gametocytes (standard membrane feeding assay), or from blood samples from individuals with natural *Plasmodium* infections (direct membrane feeding assay). These assays are used for the study of transmission blocking interventions[34,35] as well as the evaluation of transmission reducing immunity induced by natural malaria infections[36,37]. Here, the infectiousness of gametocytes from asymptomatic children to *An. gambiae* mosquitoes was assessed by direct membrane feeding assays. This will provide information on the role of asymptomatic malaria infections to the transmission of malaria, to assist in decisions pertaining to control of infectious reservoirs of malaria in Ghana.

Results

Demographic and clinical characteristics of asymptomatic study participants

Overall, 98 participants were recruited and screened for malaria by RDT, microscopy and PCR. Twenty-five out of the 98 cases were RDT positive, but negative for microscopy and PCR, and were not characterized as negative. The rest (73) of the cases were positive for either RDT, or microscopy, and PCR, and were described as asymptomatic for malaria. The demographic and clinical characteristics of these asymptomatic individuals are represented in Table 1. Out of the 73 asymptomatic cases, 10 (13.70%) were positive for *Plasmodium* infection by microscopy. Parasite density (Geomean (95% CI)) amongst these 10 individuals who were positive for

Characteristic	Value
Median age (years) (IQR)	12 (3)
Sex ratio (Male: Female)	40:33:00
Axillary temperature (mean+/- SEM)	36.39+/- 0.05
Haemoglobin concentration (g/dL) (mean+/−SEM)	11.32+/− 0.13
Total WBC	6.15+/- 0.39
Platelet count	229.37+/- 8.87
Total positive for malaria by RDT/microscopy/PCR	73 (74.49)
No. positive by microscopy	10 (13.70)
No. positive by PCR	57 (78.08)
No. with sub microscopic infections	47 (64.38)
No with gametocyte (microscopy)	0
Gametocyte by RT-PCR (*Pf*g377)	19/57 (33.33)
Plasmodium species identification	
P. falciparum mono infection	47/57 (82.46)
P. falciparum/P. malariae	6/57 (10.53)
P. falciparum/P. ovale	4/57 (7.02)
PD (p/µl) Geomean (95%ci)	2560.75 (1383.29-6903.39)
PD (p/µl) Min–max	960–10,320

Table 1. Demographic and clinical characteristics of asymptomatic study participants. p/ul- parasite per microlitre of blood; CI- Confidence interval; IQR- Inter quartile range; g/dL – grammes per decilitre of blood; WBC-white blood cell count; SEM- Standard error of the mean.

microscopy was 2560.72 (1383.29–6903.39). PCR analysis showed that 57 out of the 73 (78.08%) asymptomatic participants had malaria infections. All cases that were microscopy positive were PCR positive. Individuals who were negative by microscopy, but positive by PCR, were described as having sub microscopic densities of parasites. This constituted 64.38% (47/73) of the total number of asymptomatic individuals. *Plasmodium* species identification indicated that 82.46% (47/57) of the PCR positive cases were mono infection with *P. falciparum* only. The rest of the infections were mixed, with 10.53% (6/57) having *P. falciparum/P. malariae* and 7.02% (4/57) having *P. falciparum/P. ovale* infections. No *P. vivax* was identified in any of the infections.

Gametocyte carriage in asymptomatic study participants

There were no gametocytes from the microscopic examination of Giemsa-stained thick blood smears. Reverse-transcriptase PCR followed by gel electrophoresis, however showed amplification of the *Pfg*377 gene in 33.33% (19/57) of the study participants. All gametocytes were clonal with observed alleles being either 300 or 350 [base pairs (bp)]. Majority (68.42%, 13/19) were of the 300 bp allele (Supplementary Fig. 1).

Mosquito infections

A total of 73 feeding experiments were carried out using blood samples from the asymptomatic study participants. In total, 2,121 female *An. gambiae* mosquitoes were fed with blood. Blood feeding rate was 56.62% (1201/2121). The total number of mosquitoes that were dissected was eight hundred and sixty-two. Infections were observed only in cases with sub microscopic gametocyte infections. Blood samples from all cases with microscopic asexual parasites did not result in mosquito infections. Out of the 19 participants who had sub microscopic gametocytes, blood samples from 21.05% (4/19) of them were infectious to the *An. gambiae* mosquitoes. Three of the participants whose blood resulted in infections were between the ages of 11–15 years, while the other participant with positive mosquito infections was greater than 15 years (Table 2). The overall prevalence of human to mosquito infectivity amongst the asymptomatic participants was 5.48% (4/73). Considering each experiment individually, the number of oocysts observed in a midgut, ranged from 1 to 13. The oocyst density for the lowest and highest oocyst counts were 0.03 and 1.86 oocyst per mid-gut, respectively. The total number of oocysts observed within infected midguts were 28, with an average oocyst density of 0.01 (Table 2).

Sero prevalence of antibodies to *Pfs*230-C0$_{LI}$ and *Pfs*48/45-6C in asymptomatic study participants

The median antibody concentration, (Inter quartile range, QR) (1,866.29, (1,186.85) for *Pfs*230-CO$_{LI}$ was significantly higher (Mann Whitney U test, 0.00), than that for *Pfs*48/45-6C (1,217.42, (IQR) 1,073.67). Seroprevalence of antibodies to *Pfs*230-C0$_{LI}$ and *Pfs*48/45-6C were 37.0% and 46.60% respectively.

Antibody responses and mosquito infections

Antibody responses to *Pfs*230C and *Pfs*48/45 varied in the 4 cases that resulted in mosquito infections. Two out of the 4 cases had antibody responses to both antigens, one case had antibody response to *Pfs*23C only; and the other case had no response to either of the 2 antigens. The median (Inter quartile range (IQR)) of antibody to *Pfs*230C amongst study participants whose blood samples resulted in mosquito infections was 1509.64 (1377.43). The median (IQR) of antibody to *Pfs*230C amongst participants whose blood samples did not result in mosquito infections was 1941.30 (1186.85). With regards to antibodies to *Pfs*48/45, the median (IQR) for cases that infected and did not infect the *An. gambiae* mosquitoes are 1364.73(2245.02), and 1217.42(940.7) respectively. Figure 1 shows a scatter plot of antibodies against *Pfs*230C and Pfs48/45 among asymptomatic study participants with and without resultant mosquito infections.

Figure 1. Antibody titers against *Pfs230C* (A) and *Pfs48/45* (B) amongst study participants whose blood samples resulted in mosquito infections (Infect), as well as those which did not result in mosquito infections (Non infect), and antibody titers for *Pfs230C* and *Pfs48/45* for asymptomatic cases (C).

Discussion

Carriers of asymptomatic malaria infections could habour gametocytes that could be infective to *Anopheles* mosquitoes[7,8,14]. Identification of such individuals for appropriate treatment is crucial for the interruption of the malaria transmission cycle[1,2]. In this study, asymptomatic school children were selected for direct membrane feeding assay to determine the infectiousness of their gametocytes to laboratory reared *An. gambiae* mosquitoes. Consistent with previous studies conducted in the same community[38]asymptomatic malaria is a persistent issue and is associated with a high prevalence of sub-microscopic infections in children. Carriage of gametocytes

Age group (years)	No. of participants blood fed to mosquitoes	No. infective to mosquitoes	No. of mosquitoes dissected	No. of infected mosquitoes with oocyst	No. of oocyst	Infection rate	Oocyst density
< 5	1	0	9	0	0	0	0
5–10	25	0	230	0	0	0	0
11–15	41	3	560	10	19	0.02	0.03
> 15	6	1	63	3	9	0.05	0.14
Total	73	0	862	13	28		

Table 2. Summary of mosquito infection studies.

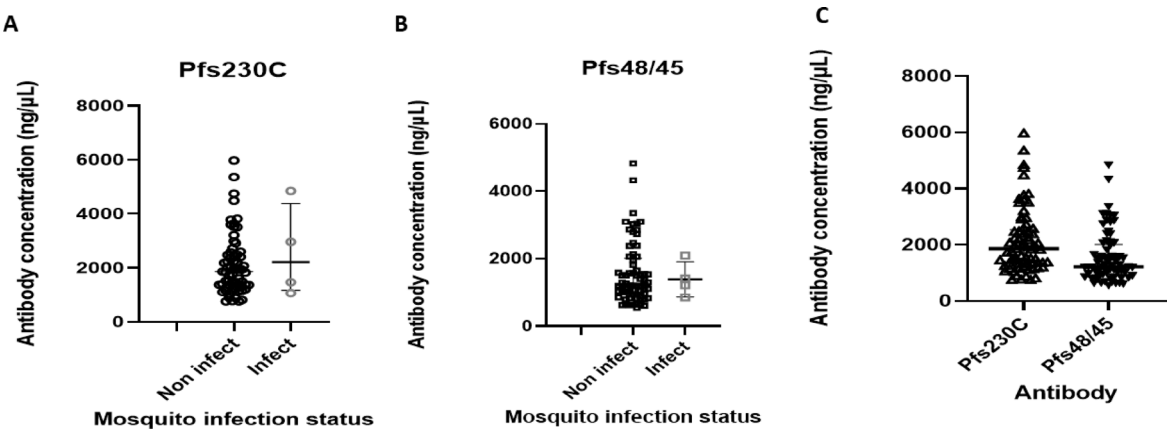

Fig. 1. Antibody titers against Pfs230C (**A**) and Pfs48/45 (**B**) amongst study participants whose blood samples resulted in mosquito infections (Infect), as well as those which did not result in mosquito infections (Non infect), and antibody titers for Pfs230C and Pfs48/45 for asymptomatic cases (**C**).

by children within the community has however been reported to be low. In previous studies, prevalence of gametocytes by microscopy was reported to be between 0 and 3.10% for off peak and peak seasons respectively[19]. There were no gametocytes recorded by microscopy in this study where sample collection was done within the peak season. Consistent with earlier studies within the Southern part of Ghana which include the site for this study, sub-microscopic gametocytes is frequently reported ranging from 20 to 49.20%[8,19].

There are varying reports on the infectivity of microscopy detected gametocytes, and sub- microscopic gametocytes to *Anopheles* mosquitoes. Presence of microscopy detecting gametocytes or sub- microscopic gametocytes alone does not guarantee the infectivity of gametocytes to *Anopheles* mosquitoes. Studies conducted in Burkina Faso reported similar mosquito infection rates for blood samples collected from study participants who were gametocyte positive by microscopy and those who had sub- microscopic gametocytes[39]. Infectivity of sub- microscopic gametocytes from symptomatic children to *Anopheles* mosquitoes have also been frequently reported in Kenya[40,41]. A similar study conducted in South East Asia (Cambodia) reported a mosquito infection of 6.25% (3/48) in 44% of individuals who carried sub-microscopic gametocyte in symptomatic cases[42]. In this same study, asymptomatic individuals on the other hand had a gametocyte prevalence of 12.60% but these gametocytes were not infectious. The implications of this is that sub-microscopic gametocytes in asymptomatic individuals were of very low densities hence could not result in mosquito infectivity. This study reports a high prevalence of sub-microscopic gametocytes with low infectivity to *Anopheles* mosquitoes.

Other factors, aside the presence of gametocytes, could influence the infectivity of gametocytes to the *An. gambiae* mosquitoes. These factors include; presence of transmission blocking antibodies[22,43–45]innate immune response to gametocytes/gametes within the mosquito[46–48]and polymorphisms in some proteins of the mosquito such as the fibrinogen-related protein – 1 (FREMP-1)[49] and the thioester binding protein-1 (TEP 1)[50]. The presence of transmission blocking antibodies (anti *Pfs*230 and *Pfs*6C) did not prevent mosquito infections in this study; two out of the 4 participants whose blood samples resulted in mosquito infections were seropositive for *Pfs*230 and *Pfs*6C. This could possibly be as a result of the presence of other factors that can affect the infectivity of gametocytes as stated earlier. Also, even though the cases were seropositive, the concentrations of these antibodies might not be high enough to prevent transmission of parasites to the *An. gambiae* mosquitoes. Unexpectedly, the highest oocyst count was observed in those cases that were seropositive for both *Pfs*230C and *Pfs*6C. Generally, the presence of these transmission blocking antibodies has been shown to greatly reduce mosquito infections in both field and laboratory assays[22,51,52]. There are instances, however, when this transmission-blocking activity has been observed either with *Pfs230* only[43] or *Pfs48/45* only[44,53]. It would be expected though, that the combined effect of these two antibodies would lead to greater transmission blocking activity.

The seroprevalence of antibodies to *Pfs230* and *Pfs48/45* reported in this study were low. This could be as a result of the presence of sub-patent gametocytes which did not elicit a strong immune response. Sub-microscopic gametocytes seemed not to elicit adequate immune responses to the gametocytes because of their very low numbers. Effective immune responses to gametocytes have been reported to be dependent on ongoing infections with high density gametocyte infections rather than low density or sub-microscopic infections[36]. The high carriage of asymptomatic malaria infections in the study participants highlights the need to expand control efforts to target asymptomatic malaria carriers to help in reducing malaria transmission.

This study had several limitations. First, Kisumu strain of *Anopheles gambiae* is very susceptible to *Plasmodium* infections due to its adaptation in the insectary. However, it was logistically challenging to breed wild-caught mosquitoes from Obom and adapt them to feed on the membrane feeder. Besides several studies have used the Kisumu strain for infection experiments. Second, a quantitative measure of the sub microscopic gametocytes will have provided additional information that could justify the low infections and also variability in oocyst count. Third, a higher number of feeds would have also improved the precision of this study.

Methods

Study site

The study site was Obom (5.6335 N″, − 1.762W″), a community in the Ga South municipality of the Greater Accra region of Ghana. Malaria transmission in Obom is perennial with high transmission[54]. The average rainfall is 790 mm along the coast and 1,270 mm in the extreme North. August is the coolest month with a temperature of 25.1 °C, whiles February and March have a temperature of 28.4 °C. Relative humidity is about 75% in February and March[8,19,38,54].

Study design and population

The study was prospective involving purposive sampling and screening of healthy school children between 6 and 17 years for asymptomatic malaria parasite infections including gametocyte carriage. Blood samples from these asymptomatic children were used in a direct membrane feeding assay, to assess the infectiousness of gametocytes from the individuals to *An.gambiae* mosquitoes. Mosquito infections were determined by microscopic examination of mercurochrome-stained mosquito midgut on day 7 after blood feeds. Oocyst density and mosquito infection rate were recorded.

Asymptomatic case definition

Participants were described as asymptomatic for malaria if they were positive for malaria by RDT, microscopy, or PCR, had an axillary temperature of ≤ 37 °C and did not present with any other signs and symptoms of malaria. The RDT was done using the One Step Malaria (HRP)-II (P.f) and (pLDH) (P.f) Antigen Rapid Test kit from SD BioLine. The kit detects the HRP II and (pLDH) from *Plasmodium falciparum* in human whole blood. Conventional PCR, employing the nested method was used for the determination of malaria parasites, all participants were screened using RDT, microscopy, and PCR.

Inclusion/exclusion criteria

Participants were included if they qualified as a case of asymptomatic malaria infection carrier and provided assent and or consent. Exclusion involved cases of symptomatic malaria or lack of consent/assent.

Sample size determination

Sample size was calculated to be a minimum of 54 feeds with the assumption of a 60% successful feeding rate and a 20% infection rate[55] with samples from asymptomatic individuals.

Participant recruitment and sample collection

Recruitment of asymptomatic study participants started in May until the end of November 2018, a period which coincided with the second rainfall season. Written informed consent was obtained from adult participants as well as parents/guardians of minors before recruitment into the study. Venous blood samples were drawn using a butterfly needle and following the appropriate protocol for venipuncture. One milliliter of venous blood was drawn each, into a heparin tube and EDTA tube. The heparin preserved samples were kept in a thermos flask at 37 °C, and immediately used for the direct membrane feeding assay. Samples preserved in EDTA were used as follows; malaria rapid diagnostic test, thick and thin blood smears for microscopy. The rest of the EDTA preserved samples were separated into plasma and packed red cells. Subsequently, 100 µl of packed red cells was immediately preserved in 500 µl of trizol, and, 100 µl of packed red cells was also preserved in 400 µl of DNA lyses buffer. These were then transported on ice from the field to the laboratory where plasma samples were preserved at -80 °C. Samples preserved in trizol, DNA lyses buffer, and the rest of the packed red cells were preserved at -20 °C for later laboratory experiments.

Rapid malaria testing

The One Step Malaria (HRP)-II (P.f) and (pLDH) (P.f) Antigen Rapid Test kit from SD BioLine, which detects the HRP II and (pLDH) from *Plasmodium falciparum* in human whole blood was used as one of the screening test for malaria. A test was recorded as positive if any of the test lines showed in addition to the control line. A test was recorded as invalid if the control line failed to show.

Giemsa staining of blood smears and malaria parasite count by microscopy

Parasite density (PD) per microlitre (µL) of blood was determined by microscopic examination of giemsa stained thick blood smears under oil immersion. Asexual parasites were counted per 200 white blood cells, and gametocytes were counted per 500 white blood cells. A white cell count of 8,000/µL was used to determine parasite density. A smear was reported as negative if there were no malaria parasites seen after 100 high power fields were observed. High discrepancies between 2 parasite counts warranted a third count by third highly experienced microscopist. The average of the 2 closest counts were then taken as the parasite count.

Membrane feeding assay and mosquito midgut dissections

Insectary colonized Kisumu strain of *An. gambiae* mosquitoes obtained from the Department of Medical Microbiology, University of Ghana Medical School and the Vestergaard Insectary of the NMIMR were used for the mosquito infectivity studies. This strain has been used for infections studies in the past (ref) For the membrane feeding assays 3–5 day old female *An. gambiae* mosquites were used. Prior to blood feeds, mosquitoes were starved for 5 h. About 60 mosquitoes per paper cup were fed with whole blood using a glass membrane feeder (Hemotek) with a 14 mm diameter. The feeder was equipped with a water jacket connected to a circulating water bath maintained at 37 °C. The *An. gambiae* mosquitoes were allowed to feed for 10 min on 200 µl of heparinized blood kept at 37 °C in a thermos flask. Unfed mosquitoes were aspirated from the cup into an empty

cup and discarded into a biohazard bin after spraying them with absolute ethanol. The number of fed mosquitoes were recorded. These were transported back to the insectary following the precautions necessary for transport of mosquitoes, and were maintained on 10% sugar solution under the following room conditions: temperature of 27 °C+/− 2 °C, humidity of 76+/− 5 g kg^{-1}, and a 12 h day/12 h night light schedule[56].

Dissection of fed *An. gambiae* mosquitoes

Seven days after the *An. gambiae* mosquitoes were fed, surviving mosquitoes were dissected on a microscope slide in phosphate buffered saline under a dissection microscope. Prior to dissection, mosquitoes were anaesthetized by chloroform and were transferred onto cotton wool soaked with normal saline in a petri dish and covered to prevent any escape. The midgut of the mosquito was carefully pulled from the posterior end of the mosquito while the thorax was held firmly by another forceps, transferred onto fresh slides and stained with 0.05% mercurochrome for 25–30 min. Stained slides were covered with a cover slip and observed under the light microscope (X10 and X40 magnification) for the presence of oocysts. The number of individuals whose blood was infective to at least 1 mosquito, the proportion of infected mosquitoes out of the total number of mosquitoes dissected, as well as the number of oocysts per infected mosquito (oocyst density) were recorded.

Plasmodium falciparum species identification

Genomic DNA was extracted from samples preserved in DNA lyses buffer using the Quick-DNA miniprep kit (cath no. D3025) from Zymo Research, California, following instructions in the manual. The concentrations and purity of the eluted DNA was checked using the nanodrop. The DNA samples were kept frozen at -20 °C until use.

Nested PCR was used for *Plasmodium* species identification using genus and species-specific primers that target the *18 S rRNA* gene[57]. In the nest 1 reaction, 5 µL of DNA template was used and 0.5 µL of nest 1 product was used for the nest 2 reaction. All reactions were carried in a volume of 15 µL. For both nest 1 and nest 2 reactions, the master mix contained 167 nM dNTP, 2.5 nM $MgCl_2$, 80 nM of each primer and 1U of One Taq polymerase. The reaction cycling conditions were: initial denaturation for 5 min at 94 °C, followed by 35 cycles of 30 s denaturation; annealing for 1 min at 55 °C (58 °C for nest 2), and extension for 1 min 68 °C; and a final extension for 5 min at 68 °C. For a positive control, a 3D7 *Plasmodium falciparum* stain was used, while a DNA no-template control was used as a negative control.

RNA extraction and conversion to cDNA

Ribonucleic acid was extracted from trizol preserved whole blood samples using the Direct zol™ RNA Miniprep plus (Zymo, USA) following manufacturer's protocol. Deoxyribonucleic acid was removed by DNase 1. Elution of RNA was done with 20 uL of DNAse/RNAse free water into DNA/RNA free tubes. Quality and concentrations of RNA was checked with a nanodrop at 260/280 absorbance. A ratio of 2 or more was accepted. In order to check for contamination with genomic DNA (gDNA). the human IL-10 gene was amplified using the extracted RNA samples. Complementary DNA (cDNA) was then synthesized using a ProtoScipt II first strand cDNA synthesis kit (New England BioLabs Inc, USA) following instructions in the manual with slight modifications. Following this, the gametocyte specific gene *Pfg*377 was amplified in a nested reverse transcriptase PCR using specific primers in a protocol earlier described by Menegon and colleagues in 2000[58]. Complementary DNA preparation from gametocytes of the *P. falciparum* NF54 was used as positive controls.

Detection and quantification of IgG antibodies to *Pfs*230-$C0_{LI}$ and *Pfs*48/45-6C

The *Pfs*230_{LI} and *Pfs*48/45-6 C proteins, which are sexual stage antigens, and produced using a *Lactococcus lactis* expression system was used in an indirect ELISA to quantify natural immune responses to these antigens as previously described[59]. These antigens, diluted in bicarbonate buffer at 1.0 µg/ml and 0.5 µg/ml respectively, were used to coat NUNC Maxisorp 96-well ELISA plates and incubated overnight at 4 °C. Plates were then blocked with 150 µL of 3% skimmed milk in phosphate buffered saline (PBS) supplemented with 0.05% Tween 20 (PBST) for an hour. Following this, patient serum (1 in 200 dilution), standard and negative and positive controls were incubated. A rabbit polyclonal anti- human immunoglobulin G (IgG) - horse raddish peroxidase (HRP) was used as a conjugate at a 1/3,000 dilution. Colour was developed with 3, 3', 5, 5' tetramethylbenzidine. The optical densities (OD) of the plasma samples were transformed into IgG concentrations (ng/µL) based on the regression curve obtained from dilutions of the PB055 (standard) using the ADAMSEL software (Ed Remarque). Positive and negative controls was used on each ELISA plate. These controls consisted of plasma from individuals who have been previously[19] described as seronegative and seropositive for the antigens. A recombinant IgG (BPO55, The Binding Site) was used as a Standard for the measurements of IgG. Cut offs for antibody responses were obtained by the formula; (Average + 2 Standard deviation) of concentrations of pooled negative control serum.

Data analysis

Data was entered and analyzed using the statistical package for social sciences (SPSS) version 24. Summaries of data were presented using tables and graphs. Age of participants was reported using median and interquartile ranges. Axillary temperature and hemoglobin results were summarized using means and standard error of means. Ratios were used to describe number of participants by gender. The median and interquartile range was reported for antibody concentrations of *Pfs*230-$C0_{LI}$ and *Pfs*48/45-6C, and the Mann Whitney U test was used to compare the differences in median antibody concentrations. A probability value of less than 0.05 was considered as statistically significant.

Data availability

The datasets generated and/or analysed during this study are available from the corresponding authors on reasonable request.

Received: 20 February 2025; Accepted: 11 June 2025
Published online: 02 July 2025

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Acknowledgements

The authors wish to acknowledge the contributions of Osafo Kantanka (Department of Microbiology, UGMS, CHS), Elijah Moses & Gloria Malibe Lagri (KMC), Elmion Korkor (LH), Degenu Proper (Department of haematology, Korle-Bu Teaching Hospital), and all volunteers as well as their guardians.

Author contributions

M.A.M., L.E.A., N.B.Q. and Y.A.A. were responsible for the study design, supervised the data collection, and contributed to the writing of the manuscript. M.A.M., I.K.S., F.K.A., H.B.A. and D.D. performed the data collection, laboratory work and analysis. M.A.M. and L.E.A. drafted the manuscript. All the authors read and approved the final manuscript.

Funding

This work was supported by funds from a World Bank African Centres of Excellence grant (WACCBIP + NCDs) and a DELTAS Africa grant *(DEL-15-007)* from the Wellcome Trust and the UK government, the National Institute of Health (NIH) grants (R01 A1123074 and D43 TW011513) to YAA. Additional funds (UGFD/11/2016–2017/002) were received from the University of Ghana through the Office of Research and Innovation and Development. The views expressed in this publication are those of the authors and not necessarily those of the funding agencies.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
The study was done in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Noguchi Memorial Institute for Medical Research (NMIMR) [protocol number: NMIMR-IRB CPD 084/16-17] as well as the Ghana Health Service (GHS) [protocol number: GHSERC-Admin/App/Ren/700/18/266].). Written informed consent was sought from adult participants, parents/guardians of minors as well as assent from minors.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-06844-7.

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