Estimating Annual Fluctuations in Malaria Transmission Intensity and in the Use of Malaria Control Interventions in Five Sub-Saharan African Countries

Article *in* The American journal of tropical medicine and hygiene · September 2020

DOI: 10.4269/ajtmh.19-0795

CITATION
1

READS
178

29 authors, including:

Elisha Adeniji
Kintampo Health Research Centre, Ghana, Kintampo
19 PUBLICATIONS **742** CITATIONS
SEE PROFILE

Kwaku Poku Asante
Kintampo Health Research Centre
326 PUBLICATIONS **6,502** CITATIONS
SEE PROFILE

Owusu Boahen
Kintampo Health Research Centre, Kintampo, Ghana
25 PUBLICATIONS **2,425** CITATIONS
SEE PROFILE

Guillaume Compaoré
Centre de Recherche en Santé de Nouna
21 PUBLICATIONS **185** CITATIONS
SEE PROFILE

Am. J. Trop. Med. Hyg., 00(0), 2020, pp. 1–10
doi:10.4269/ajtmh.19-0795

Estimating Annual Fluctuations in Malaria Transmission Intensity and in the Use of Malaria Control Interventions in Five Sub-Saharan African Countries

RTS,S Epidemiology EPI-MAL-005 Study Group
Elisha Adeniji,[1] Kwaku Poku Asante,[1] Owusu Boahen,[1] Guillaume Compaoré,[2] Boubacar Coulibaly,[2] Seyram Kaali,[1] Youssouf Kabore,[3] Mathieu Lamy,[4]* John Lusingu,[5,6] Anangisye Malabeja,[5] Petra Mens,[7] Mattéa Orsini,[8] Lucas Otieno,[9] Walter Otieno,[9] Seth Owusu-Agyei,[1] Janet Oyieko,[9] Jean-Yves Pirçon,[10] Nicolas Praet,[10] François Roman,[10] Ali Sie,[2] Valentine Sing'oei,[9] Sodiomon B. Sirima,[3] Khadime Sylla,[11] Roger Tine,[11] Alfred B. Tiono,[3] Mathilda Tivura,[1] Effua Usuf,[12] and Stéphanie Wéry[10]†

[1]*Kintampo Health Research Centre, Ghana Health Service, Kintampo, Ghana;* [2]*Centre de Recherche en Santé de Nouna, Nouna, Burkina Faso;* [3]*Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso;* [4]*Aixial c/o GSK, Wavre, Belgium;* [5]*National Institute for Medical Research (NIMR), Korogwe, Tanzania;* [6]*University of Copenhagen, Copenhagen, Denmark;* [7]*Parasitology Unit, Department of Medical Microbiology, Academic Medical Center, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands;* [8]*4Clinics c/o GSK, Wavre, Belgium;* [9]*KEMRI - Walter Reed Project, US Army Medical Research Directorate-Kenya, Kombewa, Kenya;* [10]*GSK, Wavre, Belgium;* [11]*Département de Parasitologie, Centre de Recherche de Keur Socé, Faculté de Médecine, Université Cheikh Anta Diop, Dakar, Senegal;* [12]*Medical Research Council Unit, The Gambia at London School of Hygiene and Tropical Medicine, Fajara, The Gambia*

Abstract. RTS,S/AS01$_E$ malaria vaccine safety, effectiveness, and impact will be assessed in pre- and post-vaccine introduction studies, comparing the occurrence of malaria cases and adverse events in vaccinated versus unvaccinated children. Because those comparisons may be confounded by potential year-to-year fluctuations in malaria transmission intensity and malaria control intervention usage, the latter should be carefully monitored to adequately adjust the analyses. This observational cross-sectional study is assessing *Plasmodium falciparum* parasite prevalence (*Pf*PR) and malaria control intervention usage over nine annual surveys performed at peak parasite transmission. *Plasmodium falciparum* parasite prevalence was measured by microscopy and nucleic acid amplification test (quantitative PCR) in parallel in all participants, and defined as the proportion of infected participants among participants tested. Results of surveys 1 (S1) and 2 (S2), conducted in five sub-Saharan African countries, including some participating in the Malaria Vaccine Implementation Programme (MVIP), are reported herein; 4,208 and 4,199 children were, respectively, included in the analyses. *Plasmodium falciparum* parasite prevalence estimated using microscopy varied between study sites in both surveys, with the lowest prevalence in Senegalese sites and the highest in Burkina Faso. In sites located in the MVIP areas (Kintampo and Kombewa), *Pf*PR in children aged 6 months to 4 years ranged from 24.8% to 27.3%, depending on the study site and the survey. Overall, 89.5% and 86.4% of children used a bednet in S1 and S2, of whom 68.7% and 77.9% used impregnated bednets. No major difference was observed between the two surveys in terms of *Pf*PR or use of malaria control interventions.

INTRODUCTION

Substantial investment to expand existing malaria interventions has resulted in a reduction in the global incidence rate of malaria between 2010 and 2017.[1] However, between 2015 and 2017, after stagnation, a slight upward trend in malaria incidence was observed. Malaria remains a major cause of death worldwide, with approximately 93% of all malaria deaths in 2017 occurring in Africa.[1] To reach the Global Technical Strategy for Malaria 2016–2030 target of reducing global malaria incidence and mortality rates by at least 90% by 2030,[2] the need for safe and effective malaria vaccines that prevent disease and death and decrease transmission to enable malaria eradication was endorsed and documented in the WHO Malaria Vaccine Technology Roadmap.[3]

GlaxoSmithKline (GSK) has developed, in partnership with the PATH Malaria Vaccine Initiative, a pre-erythrocytic *Plasmodium falciparum* malaria vaccine, RTS,S/AS01$_E$ (GSK™, Wavre, Belgium), for routine immunization of infants and children living in sub-Saharan African (SSA) malaria-endemic countries. In 2015, the European Medicines Agency adopted a positive opinion for the use of the vaccine in children aged 6 weeks to 17 months at the first dose.[4] In January 2016, the WHO recommended a pilot implementation of RTS,S/AS01$_E$ in children as of 5 months of age in three to five epidemiological SSA settings with moderate to high malaria transmission.[5] In April 2017, the WHO announced the vaccine introduction based on a cluster-randomized design in pilot areas of Ghana (GH), Kenya (KE), and Malawi through the national expanded programs on immunization, in the framework of the Malaria Vaccine Implementation Programme (MVIP).[6] Today, RTS,S/AS01$_E$ is the first vaccine implemented for the prevention of malaria.

To assess vaccine safety, effectiveness, and impact, GSK designed a pre- and a post-vaccine introduction observational study (Clinical Trials.gov identifiers: NCT02374450 and NCT03855995, respectively), allowing comparison of the occurrence of malaria cases and adverse events in vaccinated versus unvaccinated children. In parallel with those studies, the present observational cross-sectional annual study (NCT02251704) is estimating *P. falciparum* parasite prevalence (*Pf*PR) and the use of malaria control interventions during up to nine consecutive years, applying standardized methodologies and multiple diagnostic testing. More specifically, considering the WHO recommendation to operate the MVIP in moderate to high transmission areas of SSA, this study will allow 1) characterizing the malaria transmission intensity (MTI) before RTS,S/AS01$_E$ vaccine introduction in different countries/areas, including the ones participating in the MVIP; 2) monitoring overtime fluctuations of MTI and of the use of malaria control interventions before and after vaccine

* Address correspondence to Mathieu Lamy, GSK Biologicals, Ave. de Fleming, 20, Wavre 1300, Belgium. E-mail: mathieu.x.lamy@gsk.com
† At the time of Survey 1, SW was an employee of Keyrus Biopharma c/o GSK.

introduction in those areas to adjust the pre- and post-vaccine introduction comparison analyses for potential year-to-year and/or geographical variations.

We present here the results of the first two annual surveys that were conducted before vaccine introduction. On completion of all surveys, the data collected in this study involving approximately 50,000 participants representing multiple sites in various SSA countries will provide a unique perspective on malaria prevalence variations across Africa.

MATERIALS AND METHODS

Ethics. The study was approved by national independent Ethics Committees and local institutional review boards in Burkina Faso (BF), GH, KE, Senegal (SN), and Tanzania (TZ), and conducted in accordance with the provisions of the International Conference on Harmonisation and Good Clinical Practice guidelines.

Study population. Individuals aged 6 months to < 10 years, whose parents or legally acceptable representative had provided informed consent for study participation, were randomly selected each year in each of the study sites (see Study design section) using population listings generated from local Health and Demographic Surveillance Systems (HDSS) and following a stratification by age-group (see the Supplemental Appendix Section 1). Children in care, or actively participating in any trial involving the administration of an investigational malaria vaccine and/or drug, were excluded.

Study design. Malaria transmission intensity levels are consensus indicators developed by the Global Malaria Eradication Programme to measure malaria endemicity[7] using a standardized methodology. There are several methods for estimating MTI, including entomological inoculation rates (EIRs), serological conversion rates (SCRs), and blood parasite prevalence. Although EIR is a standard method, the measure is challenging, and the interpretation and comparability of the setting may be difficult because of vector heterogeneity.[8] The methodology and interpretation of SCRs to classify the intensity of malaria is still not commonly used. *Plasmodium falciparum* prevalence, despite requiring trained staff for slide reading, provides a standardized and relatively easy to implement method to assess MTI in study sites of varied transmission intensity, and has often been used in previous epidemiological studies.[7,9,10] Therefore, *Pf*PR was the selected method to assess potential variations in MTI in the present study.

The study is multicentric with study sites corresponding to geographically limited catchment areas located in low, moderate, or high MTI regions of SSA, and having an HDSS in place.

Up to nine annual cross-sectional surveys will be conducted during the malaria peak transmission period in each study site (from mid-September to mid-December in Western African sites and from mid-April to mid-August in Eastern African sites). To optimize the detection of the peak transmission, each study site has been equipped with a weather station to record meteorological data such as rainfall, temperature, and humidity. Surveys were conducted during the course of the rainy season preferably when rains decrease, which should correspond to the period of highest malaria transmission. In this article, results of the first two annual surveys are presented. Survey 1 (S1) and survey 2 (S2) were conducted in seven study sites in five SSA countries: BF (Nouna, Saponé), GH (Kintampo), KE (Kombewa), SN (Keur Socé, Niakhar), and TZ (Korogwe). Sites in GH (Kintampo) and KE (Kombewa) are part of the areas where the RTS,S/AS01$_E$ vaccination will be implemented through the national Expanded Programs on Immunization in the framework of the MVIP. It is important to note that this study is also conducted in SSA areas where the RTS,S/AS01$_E$ vaccine will not be introduced in the framework of the MVIP because GSK initiated the study before the WHO recommendation.[5,11]

Data collection. Demographic details (age and gender), medical history, and information on care-seeking behaviors (hospitalization for malaria within the last 3 months, visits to healthcare provider for fever or malaria treatment in the previous 14 days, anti-malaria therapy received within the last 14 days); malaria control intervention usage (bednets [new {not older than a year}, pierced/torn, impregnated], indoor residual spraying [IRS]); the usage of coils, repellents, and local herbs; and malaria potential risk factors (rural/urban area, house construction materials, use of electricity, and open/closed water source) were recorded for all participants at the time of the survey. Axillary body temperature was measured and recorded during the survey visit.

To assess within-site heterogeneity of *Pf*PR, study areas were mapped by villages using grid referencing and divided into 3–14 segments with a minimum of 10 enrolled individuals per segment. Segments will remain unchanged for the duration of the study.

Assessment of parasitemia. Both microscopy and Nucleic Acid Amplification Tests (NAATs) were used in parallel on all participants to assess parasitemia. The latter are expected to be more sensitive and specific, particularly in cases of low parasite density.[12,13] In brief, a blood sample was collected by finger prick for thin and thick blood films for the microscopy assessment and filter paper blood spots for NAATs. Blood smears were examined by two local independent microscopists, and any discrepancies were settled by a third reader. Parasitemia was measured by the examination of 100 high-powered fields on thick smear to determine the presence of parasites; in the case of a positive result, additional 100 fields were examined to assess the presence of multiple species. *Plasmodium* species and sexual forms were identified on thin blood film. Parasite density was computed as the geometric mean of two readings, counting parasites against 200 white blood cells on thin blood film, assuming 8,000 white blood cells/μL. Parasite density was categorized as low (< 2,500 parasites/μL), medium (2,500–9,999), high (10,000–19,999), or very high (> 20,000). In the case of low density (< 10 parasites against 200 leukocytes), parasite count was conducted against 500 white blood cells. The parasite count technique was replicated to count gametocytes. In parallel with microscopy, asexual and sexual parasitemia was assessed by NAATs using both real-time quantitative PCR (QT-PCR) assay and real-time nucleic acid sequence-based amplification (QT-NASBA) assay. Quantitative PCR allowed detection of asexual and sexual parasites combined, the final output being qualitative (positive or negative) and semi-quantitative (high, medium, low, and negative). Real-time nucleic acid sequence-based amplification allowed detection of gametocytes, the final output being qualitative (positive or negative). Details for blood slide and NAAT assessment of parasitemia are available in the Supplemental Appendix (Sections 2 and 3, respectively).

If fever (i.e., axillary temperature ≥ 37.5°C) was recorded at the time of the visit or reported to have occurred within 24 hours before the visit, a malaria rapid diagnostic test (RDT) was conducted using blood from the finger prick sampling. If the RDT was positive, then treatment was given according to national guidelines. Moreover, any participant identified as being parasite positive following microscopy was traced to receive treatment according to national guidelines.

Statistical methods. The planned sample size was 600 participants per study site and per survey distributed as 400 participants between the ages of 6 months to 4 years and 200 participants between the ages of 5 and 9 years. The sample size was calculated to ensure sufficiently narrow CIs around study site–specific *Pf*PR estimates (with a maximum residual standard error of 0.25).

Plasmodium falciparum parasite prevalence and prevalence of gametocytes were estimated as the proportion of participants infected, or carrying gametocytes, respectively, among participants tested. Prevalences were estimated by age and by site. The agreement between the two diagnostic methods used in the framework of this study (parasitemia measured by microscopy versus NAATs) was described using the Cohen's kappa coefficient and assessed using the Landis and Koch scale.[14] The prevalence of *Plasmodium* species other than *P. falciparum* was estimated as the proportion of infected participants among participants tested. The within-site heterogeneity between segments was tested using Cochran's *Q*-test based on inverse variance weights.

Malaria control intervention coverage was estimated as the proportion of users among participants for which this information was available. The care-seeking behaviors (treatment sought for malaria or fever in the 14 days before the visit and hospitalization for malaria in the last 3 months before the visit) were described as the proportion of participants having sought for health care among all participants. In addition, a risk factor analysis for malaria infection (dependent variable: *P. falciparum* parasitemia as measured by microscopy) was conducted using a multivariable logistic regression with study site as cluster and using a backward strategy for the selection of significant explanatory variables, that is, predefined potential risk factors and/or the use of malaria control interventions (Supplemental Appendix Section 4). Age was computed as a continuous variable.

RESULTS

Survey 1 and S2 data were collected between October 2014 and August 2015, and between September 2015 and July 2016, respectively. During S1 and S2, 4,215 and 4,204 children were enrolled and 4,208 and 4,199 were included in the analyses (Figure 1), with a balanced distribution across the seven study sites (Supplemental Table 1). Across all sites, 51.3% of participants in S1 and 50.3% in S2 were males (Supplemental Table 2).

Year-to-year variation in *P. falciparum* prevalence. *Plasmodium falciparum* parasite prevalence estimated using microscopy varied between study sites in both surveys, with the lowest prevalence figures in Senegalese sites and the highest in BF (Table 1, Figure 2). In Kintampo and Kombewa sites that are located in the MVIP areas, *Pf*PR in children aged 6 months to 4 years ranged from 24.8% to 27.3% depending on the study site and the survey. In both surveys and across all sites except in SN, *Pf*PR was lower in the 6-month to < 5-year than in the 5-year to < 10-year age-group. *Plasmodium falciparum* parasite prevalence was similar in S1 and S2, except for a higher prevalence in S2 in participants from the 6-month

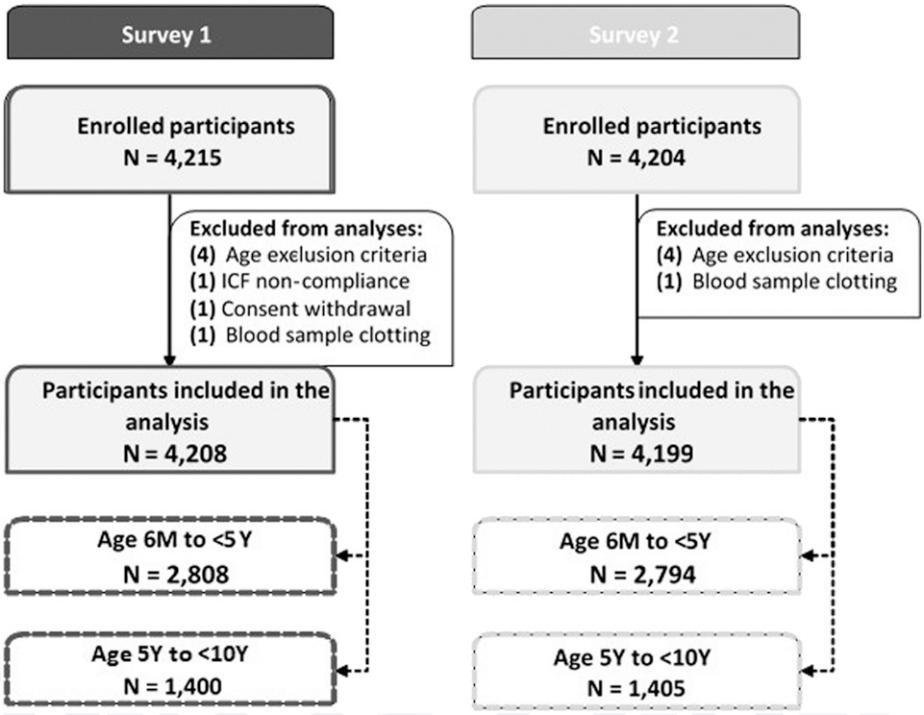


FIGURE 1. Study design overview and study population included in the analysis. ICF = inform consent form; *N* = number of participants in each group; 6 M to < 5 Y = 6 months to younger than 5 years; 5 Y to < 10 Y = 5 years to younger than 10 years.

TABLE 1

Plasmodium falciparum parasitemia prevalence measured by microscopy by study site and survey according to age-group

		P. falciparum parasitemia prevalence measured by microscopy slide reading							
		Survey 1				Survey 2			
Age-group	Study site	*n*	*N*	Proportion (%)	95% CI	*n*	*N*	Proportion (%)	95% CI
6 M to < 5 Y	Nouna, BF	249	404	61.6	56.7; 66.4	321	400	80.3	76.0; 84.0
	Saponé, BF	162	403	40.2	35.4; 45.2	182	399	45.6	40.7; 50.6
	Kintampo, GH	99	400	24.8	20.6; 29.3	109	400	27.3	22.9; 31.9
	Kombewa, KE	100	402	24.9	20.7; 29.4	108	401	26.9	22.7; 31.6
	Keur Socé, SN	2	400	0.5	0.1; 1.8	4	397	1.0	0.3; 2.6
	Niakhar, SN	6	398	1.5	0.6; 3.3	1	397	0.3	0.01; 1.4
	Korogwe, TZ	29	401	7.2	4.9; 10.2	6	400	1.5	0.6; 3.2
5 to < 10 Y	Nouna, BF	154	202	76.2	69.8; 81.9	167	200	83.5	77.6; 88.4
	Saponé, BF	137	201	68.2	61.2; 74.5	134	200	67.0	60.0; 73.5
	Kintampo, GH	115	200	57.5	50.3; 64.4	103	200	51.5	44.4; 58.6
	Kombewa, KE	96	197	48.7	41.6; 55.9	81	198	40.9	34.0; 48.1
	Keur Socé, SN	1	200	0.5	0.01; 2.8	2	203	1.0	0.1; 3.5
	Niakhar, SN	3	200	1.5	0.3; 4.3	2	204	1.0	0.1; 3.5
	Korogwe, TZ	34	200	17.0	12.1; 22.9	12	200	6.0	3.1; 10.3

BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania; *n* = number of participants positive for *P. falciparum* parasitemia measured by microscopy; *N* = number of participants with known result for *P. falciparum* microscopy; *P. falciparum* = *Plasmodium falciparum*; 6 M to < 5 Y = 6 months to younger than 5 years; 5 Y to < 10 Y = 5 years to younger than 10 years; 95% CI = exact 95% CI.

to < 5-year age-group in BF Nouna and lower prevalence in S2 in both the 6-month to < 5-year and 5-year to < 10-year age-groups in participants from TZ Korogwe. Significant within-site heterogeneity was detected in all sites, except in SN: BF Nouna (S1 $P < 0.0001$, S2 $P = 0.0184$), BF Saponé (S1 $P = 0.0321$, S2 $P < 0.0001$), TZ Korogwe (S1 $P = 0.0037$, S2 $P = 0.0275$), KE Kombewa (S1 $P = 0.0220$, S2 $P < 0.0001$), and GH Kintampo (S2 only $P = 0.0065$).

Similar trends were observed when measured by QT-PCR, with *Pf*PR varying from 2.7% in SN Niakhar to 69.9% in BF Nouna (not measured in SN Keur Socé) in S1 and from 1.3% in SN Keur Socé to 76.3% in BF Nouna in S2 (Supplemental Table 3).

The prevalence of gametocytes measured by microscopy ranged from 0.5% to 4.0% in S1 and from 0.0% to 5.3% in S2 in all sites, except in BF (15.8% and 22.7% in S1 and 12.8% and 18.2% in S2 in Nouna and Sapone, respectively). Among participants tested positive for asexual parasites, 7.5–66.7% carried gametocytes in S1 (Kintampo and Keur Socé, respectively) and 0.0–83.3% in S2 (Niakhar and Keur Socé, respectively) (Table 2). The proportion of infected participants carrying gametocytes as estimated by QT-NASBA ranged from 13.3% in SN Niakhar to 59.2% in BF Sapone in S1 (not measured in Keur Socé) and from 7.1% in SN Niakhar to 66.7% in SN Keur Socé in S2 (Supplemental Table 3).

Agreement between diagnostic tests. Across surveys, approximately 21.6% of participants with a positive QT-PCR result had a negative microscopy reading (285 of 1,268 positive participants per QT-PCR in S1 and 296 of 1,417 in S2) and around 8.8% of participants positive for microscopy had a negative result with QT-PCR (92 of 1,075 positive participants per microscopy in S1 and 110 of 1,231 in S2) (Table 3). The proportion of participants with a negative result by microscopy among participants with a positive QT-PCR ranged

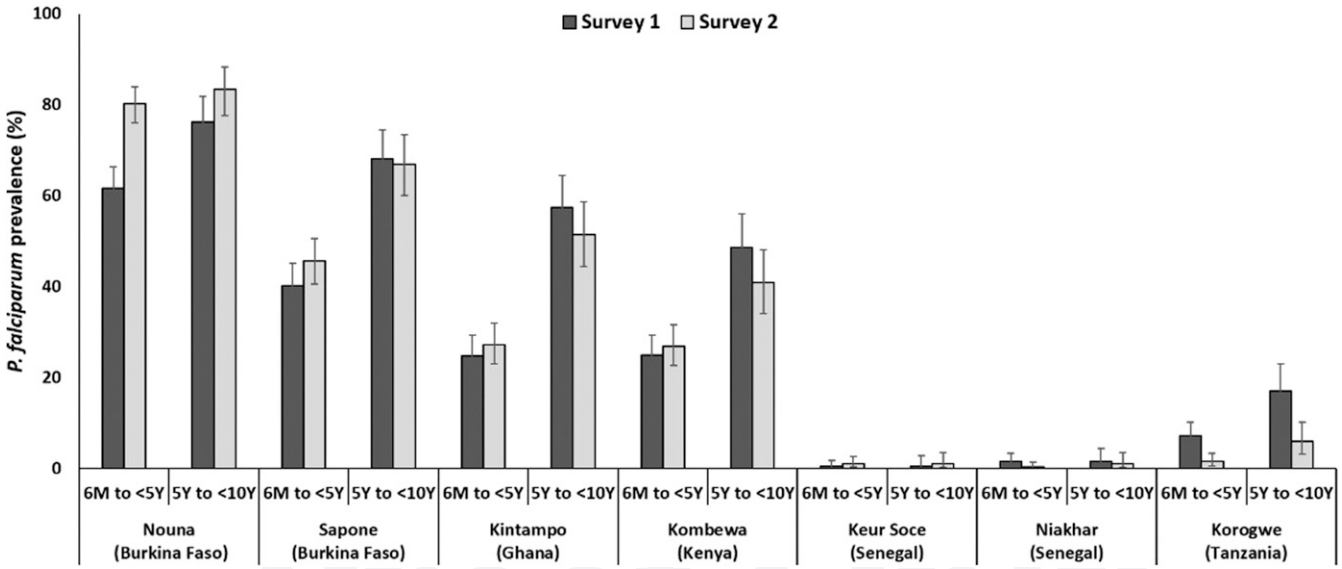


FIGURE 2. *Plasmodium falciparum* parasitemia prevalence measured by microscopy, by age category, and by site. 6 M to < 5 Y = 6 months to younger than 5 years; 5 Y to < 10 Y = 5 years to younger than 10 years. Error bars depict 95% CI.

TABLE 2

Gametocyte results measured by microscopy according to *P. falciparum* infection status per study site and survey

	Gametocytes measured by microscopy			
	Participants positive for *P. falciparum* by microscopy		Participants tested for *P. falciparum* by microscopy	
Study site, *n* (%)	Survey 1	Survey 2	Survey 1	Survey 2
Nouna, BF	*N* = 403	*N* = 488	*N* = 606	*N* = 600
Positive	89 (22.1)	76 (15.6)	96 (15.8)	77 (12.8)
Saponé, BF	*N* = 299	*N* = 316	*N* = 604	*N* = 599
Positive	111 (37.1)	84 (26.6)	137 (22.7)	109 (18.2)
Kintampo, GH	*N* = 214	*N* = 212	*N* = 600	*N* = 600
Positive	16 (7.5)	23 (10.9)	18 (3.0)	32 (5.3)
Kombewa, KE	*N* = 196	*N* = 189	*N* = 599	*N* = 599
Positive	19 (9.7)	8 (4.2)	24 (4.0)	9 (1.5)
Keur Socé, SN	*N* = 3	*N* = 6	*N* = 600	*N* = 600
Positive	2 (66.7)	5 (83.3)	5 (0.8)	5 (0.8)
Niakhar, SN	*N* = 9	*N* = 3	*N* = 598	*N* = 601
Positive	1 (11.1)	0.0 (0)	3 (0.5)	0.0 (0)
Korogwe, TZ	*N* = 63	*N* = 18	*N* = 601	*N* = 600
Positive	7 (11.1)	1 (5.6)	8 (1.3)	1 (0.2)

BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania; *n* (%) = number (percentage) of participants in a given category; *N* = total number of participants; *P. falciparum* = *Plasmodium falciparum*.

between 42.9% and 78.6% in sites with low MTI (Niakhar, Keur Socé, and Korogwe).

Across all sites, Cohen's kappa coefficient between qualitative results of the two methods of measurement of parasitemia (microscopy versus QT-PCR) using the Landis and Koch scale showed a substantial agreement in both surveys (S1: kappa = 0.75 [95% CI: 0.73; 0.78]; S2: kappa = 0.78 [95% CI: 0.75; 0.80]). Cohen's kappa coefficient between semi-quantitative results for parasitemia measured by microscopy versus QT-PCR also showed a substantial agreement in both S1 (kappa = 0.65 [95% CI: 0.63; 0.68]) and S2 (kappa = 0.66 [95% CI: 0.64; 0.68]).

Between 25.0% (192 of 767 in S2) and 33.8% (185 of 548 in S1) of participants tested positive for gametocytes by QT-NASBA were also detected positive by microscopy (Supplemental Table 4).

Prevalence of *Plasmodium* species other than *P. falciparum*. Infection with *Plasmodium malariae* was observed in 1.5% of participants in S1 and 3.0% of participants in S2 (Supplemental Table 5). Coinfection with both *P. malariae* and *P. falciparum* was more frequent than single infection with *P. malariae* alone in both surveys (S1: 4.6% versus 0.2%; S2: 7.4% versus 1.2%). Of the 62 participants in S1 and 128 participants in S2 infected with *P. malariae*, 55 (88.7%) and 91 (71.1%) were also infected with *P. falciparum*, respectively. Infection with *Plasmodium ovale* was low in both S1 and S2 (0.5% and 0.2% of participants). *Plasmodium vivax* was not observed in S1 and in only one participant in S2. Infections with species other than *P. falciparum* were mostly observed in sites of medium-to-high *P. falciparum* prevalence.

Year-to-year variation in the use of malaria control interventions. Overall, 89.5% and 86.4% of children used a bednet the night before the survey in S1 and S2, respectively (Table 4, Supplemental Table 6). The highest use of bednets was in KE Kombewa (97.5%) in S1 and TZ Korogwe (99.2%) in S2, and the lowest in GH Kintampo (70.2%) in S1 and SN Niakhar (69.6%) in S2 (Table 4). A decrease in usage between the two surveys was observed in Kombewa, Keur Socé, and Niakhar, whereas an increase was observed in Kintampo and Korogwe.

Overall, 70–80% of the bednets were impregnated, 60–70% were new, and approximately 25% were torn (Supplemental Table 6). Details for the characterization of bednet usage (new, impregnated, and pierced/torn) by study site are shown in Figure 3.

Participant's recall of IRS in the past 12 months was recorded for a very low number of participants (across surveys, 4.1% overall), mainly in SN (Supplemental Table 7).

Overall, usage of coils and repellents was limited, around 10% of the population in both surveys with variations per site between 1% and 40% according to the survey (Supplemental Table 7).

Year-to-year variation in reported fever and care-seeking behaviors. Fever in the last 24 hours was reported for approximately a quarter of the participants in both surveys, with differences across study sites ranging from 3.6% in BF Saponé to 63.8% in KE Kombewa in S1 and from 3.0% in SN Keur Socé to 71.6% in KE Kombewa in S2 (Supplemental Table 8). Across all sites, occurrence of fever was higher in *P. falciparum*–infected versus non-infected participants (35.6% versus 21.1% in S1 and 33.5% versus 22.5% in S2, respectively). In both surveys, fever was more frequently reported by participants with higher parasite densities (Supplemental Table 9).

In S1, 15.7% of participants had sought treatment against malaria or fever in the 14 days before the survey compared with 12.8% in S2, ranging from 0.0% to up to 33.3% depending on the study site and on the survey (Table 5, Supplemental Table 10). *Plasmodium falciparum*–infected children sought treatment against malaria or fever more often than non-infected children (20.1% versus 14.0% in S1 and 20.0% versus 9.8% in S2, respectively).

The proportion of participants hospitalized for malaria was 2.6% in S1 and 2.8% in S2, with no marked difference between *P. falciparum*–infected and non-infected participants 2.8% versus 2.5% in S1 and 3.4% versus 2.6% in S2, respectively; (Table 5, Supplemental Table 10**)**.

Association between potential risk factors and *P. falciparum* infection. An exploratory multivariable model was used to assess the association between potential risk factors or malaria control interventions and *P. falciparum* infection. Across both surveys, houses equipped with electricity (odds ratio [OR]: S1 0.75 [95% CI: 0.61; 0.93]; S2 0.89 [95% CI: 0.80; 0.98]), cement/plaster walls versus mud (OR: S1 0.81 [95% CI: 0.69; 0.96]; S2 0.87 [95% CI: 0.76; 0.99]), and nets on all windows (OR: S1 0.73 [95% CI: 0.63; 0.84]; S2 0.79 [95% CI: 0.66; 0.94]) were associated with a lower risk of infection with *P. falciparum*. In addition, older age was associated with a higher risk of infection (OR: 1.14 [95% CI: 1.05; 1.24] in S1 and 1.09 [95% CI: 1.02; 1.16] in S2; Supplemental Tables 11 and 12).

Figure 4 represents a plain language summary, which elaborates on the epidemiologic study relevance that could be shared with patients by healthcare professionals.

DISCUSSION

Characterizing MTI in different SSA settings. Using *Pf*PR as a proxy, this study aims at characterizing MTI[15] in different SSA settings, including areas in GH and KE where the RTS,S/AS01$_E$ malaria vaccine is currently introduced in the framework of the MVIP. More specifically, considering the WHO

TABLE 3
Agreement between microscopy and QT-PCR test results by survey

Study site	*P. falciparum* measured by microscopy		Survey 1 QT-PCR Positive (*N* = 1,268) *n*	%	Survey 1 QT-PCR Negative (*N* = 2,069) *n*	%	Survey 1 Total *N* = 3,337	Survey 2 QT-PCR Positive (*N* = 1,417) *n*	%	Survey 2 QT-PCR Negative (*N* = 2,737) *n*	%	Survey 2 Total (*N* = 4,154)
Nouna, BF	Positive	*n*	347	90.1	38	9.9	385	400	82.1	87	17.9	487
		%	85.5	–	21.7	–		87.5	–	61.3	–	
	Negative	*n*	59	30.1	137	69.9	196	57	50.9	55	49.1	112
		%	14.5	–	78.3	–		12.5	–	38.7	–	
	Total	*n*	406	–	175	–		457	–	142	–	–
Saponé, BF	Positive	*n*	212	99.1	2	0.9	214	312	98.7	4	1.3	316
		%	86.2	–	1.1	–		80.0	–	1.9	–	
	Negative	*n*	34	16.0	178	84.0	212	78	27.6	205	72.4	283
		%	13.8	–	98.9	–		20.0	–	98.1	–	–
	Total	*n*	246	–	180	–	–	390	–	209	–	–
Kintampo, GH	Positive	*n*	188	90.0	21	10	209	201	94.8	11	5.2	212
		%	81.4	–	6.3	–		71.5		3.4	–	
	Negative	*n*	43	12.1	313	87.9	356	80	20.6	308	79.4	388
		%	18.6	–	93.7	–		28.5		96.6	–	
	Total	*n*	231	–	334	–	–	281	–	319	–	–
Kombewa, KE	Positive	*n*	174	88.8	22	11.2	196	185	97.9	4	2.1	189
		%	66.4	–	6.5	–		78.4	–	1.1	–	
	Negative	*n*	88	21.8	315	78.2	403	51	12.4	359	87.6	410
		%	33.6	–	93.5	–		21.6		98.9	–	
	Total	*n*	262	–	337	–	–	236		363	–	–
Keur Socé, SN	Positive	*n*	–	–	–	–	–	4	66.7	2	33.3	6
		%	–	–	–	–		57.1		0.4	–	
	Negative	*n*	–	–	–	–	–	3	0.5	547	99.5	550
		%	–	–	–	–		42.9	–	99.6	–	
	Total	*n*	–		–	–	–	7	–	549	–	–
Niakhar, SN	Positive	*n*	7	87.5	1	12.5	8	3	100.0	0	0.0	3
		%	46.7	–	0.2			21.4	–	0.0	–	–
	Negative	*n*	8	1.4	549	98.6	557	11	1.8	587	98.2	598
		%	53.3	–	99.8	–		78.6	–	100.0	–	–
	Total	*n*	15	–	550	–	–	14	–	587	–	–
Korogwe, TZ	Positive	*n*	55	87.3	8	12.7	63	16	88.9	2	11.1	18
		%	50.9	–	1.6	–		50.0	–	0.4	–	–
	Negative	*n*	53	9.9	485	90.1	538	16	2.7	566	97.3	582
		%	49.1	–	98.4	–		50.0	–	99.6	–	–
	Total	*n*	108	–	493	–	–	32	–	568	–	–
Overall total	Positive	*n*	983	91.4	92	8.6	1,075	1,121	91.1	110	8.9	1,231
		%	77.5		4.4	–		79.1		4.0	–	
	Negative	*n*	285	12.6	1,977	87.4	2,262	296	10.1	2,627	89.9	2,923
		%	22.5	–	95.6	–		20.9	–	96.0	–	
	Total	*n*	1,268	–	2,069	–	–	1,417	–	2,737	–	–

BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania; *n* = number of participants in a given category; *P. falciparum* = *Plasmodium falciparum*; QT-PCR = quantitative PCR; % = percentage of participants with available results.

recommendation to operate the MVIP in moderate to high transmission areas of SSA, *Pf*PR in children aged 6 months to 4 years in Kintampo and Kombewa sites was high, ranging from 24.8% to 27.3% depending on the study site and the survey. Similar *Pf*PR was estimated by Drakeley et al.,[9] indicating a stable mesoendemic MTI level (*Pf*PR = 10–50%) in those areas.[10,16] *Plasmodium falciparum* parasite prevalence varied largely between sites as it was expected from various preselected transmission intensity areas. In both surveys, the two sites in BF had the highest *Pf*PR and the two sites in SN, the lowest. *Plasmodium falciparum* parasite prevalence rates recorded in this study are in line with previous findings.[9,16–18]

Across most sites, the *Pf*PR was lower in the 6-month to < 5-year than in the 5-year to < 10-year age-group. In addition, a risk factor analysis highlighted an association between older age and higher risk of infection. Those results corroborate the findings of other studies, identifying increasing age as a risk factor for carrying malaria blood stage parasites.[9,16,19] This may potentially be explained by the fact that younger children benefit from a more focused usage of control interventions (bednets).[20] Another explanation would be increased immunity in older children due to repeated exposure to the parasite,

TABLE 4
Bednet usage the night before the survey by study site and survey

Site, country	Bednet usage the night before the survey: Survey 1 (*N* = 4,208) *N*	% (95% CI)	Survey 2 (*N* = 4,199) *n*	% (95% CI)
Nouna, BF	547	90.3 (87.6; 92.5)	530	88.3 (85.5; 90.8)
Saponé, BF	557	92.2 (89.8; 94.2)	534	89.1 (86.4; 91.5)
Kintampo, GH	421	70.2 (66.3; 73.8)	497	82.8 (79.6; 85.8)
Kombewa, KE	584	97.5 (95.9; 98.6)	534	89.1 (86.4; 91.5)
Keur Socé, SN	568	94.7 (92.6; 96.3)	520	86.7 (83.7; 89.3)
Niakhar, SN	547	91.5 (88.9; 93.6)	418	69.6 (65.7; 73.2)
Korogwe, TZ	544	90.5 (87.9; 92.7)	595	99.2 (98.1; 99.7)
Overall	3,768	89.5 (88.6; 90.5)	3,628	86.4 (85.3; 87.4)

BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania; *n* (%) = number (percentage) of children using a bednet the night before the visit in each site; *N* = total number of participants; 95% CI = exact 95% CI.

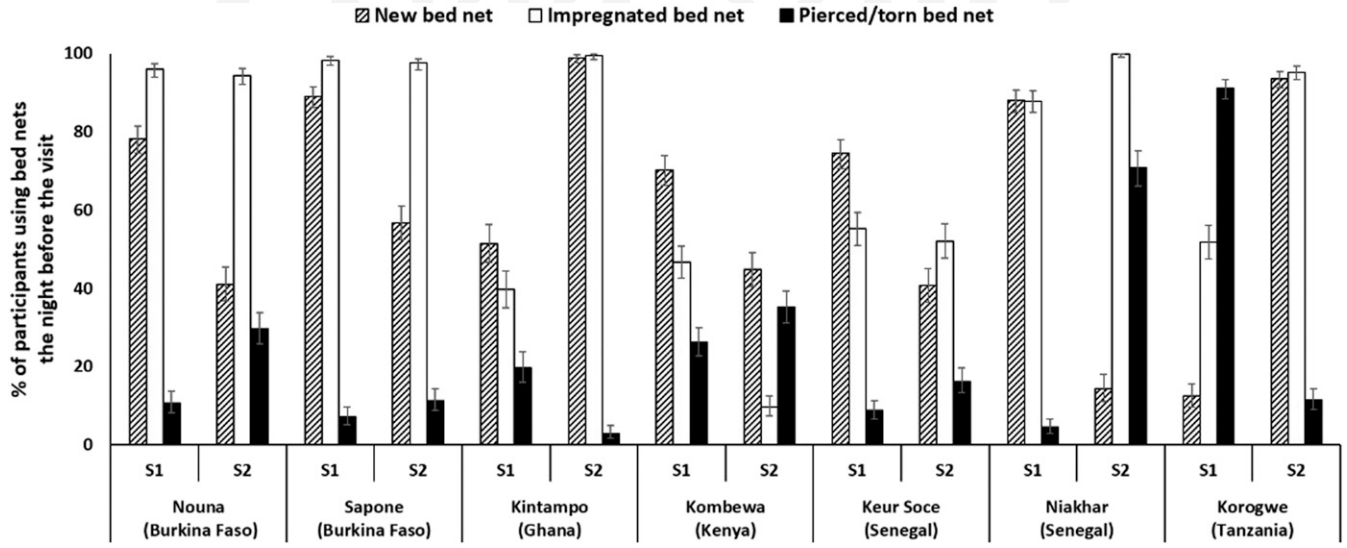


FIGURE 3. Bednet characterization according to obsolescence, impregnation, and condition, by survey and by site. impregnated bednet = bednet dipped in an insecticide liquid before or after purchase; new bednet = bednet not older than 1 year; S1 = survey 1; S2 = survey 2. Error bars depict 95% CI.

leading to asymptomatic carriage and a lower probability to be treated than in symptomatic children.[21,22]

Infection with *P. malariae* was observed in a few participants, and infection with *P. ovale* was rare, which supports observations from other studies conducted across SSA.[23–26] Across all sites and surveys, only one *P. vivax* infection was identified by microscopy in TZ, which differs from Twohig et al.'s[27] recent findings of growing evidence of this species in SSA. A high proportion of coinfections with *P. falciparum* was observed in *P. malariae*–infected participants (between 70% and 90%) and *P. ovale*–infected participants (around 60%). Similarly, high percentages of *P. falciparum* coinfection in *P. malariae*–infected participants was previously reported in Guinea (97%),[23] Uganda (91%),[24] and in the Democratic Republic of the Congo (90%),[25] with lower percentages reported in rural BF (67%)[26] and Benin (34%).[23]

Approximately 20% of participants positive for *P. falciparum* by QT-PCR were undetected by microscopy. Classifying the study sites by MTI, this proportion was higher in low MTI sites. This might be explained by a relatively lower sensitivity of microscopy readings than nucleic acid related techniques, particularly in low parasite density infections, which are more frequently observed in low MTI areas.[12,13] Nevertheless, the kappa statistic estimated a substantial agreement between microscopy and NAAT techniques using either qualitative or semi-quantitative real-time PCR.

Monitoring year-to-year variations in MTI and in the use of malaria control interventions. In addition, the present results are establishing a standardized baseline for further estimation of the year-to-year variations in *Pf*PR and in the use of malaria control interventions, which are key variables to be monitored before and after vaccine introduction in the MVIP areas. Little variation in *P. falciparum* prevalence between the first two annual surveys was observed among most sites. Usage of bednets as a malaria control intervention was high in all sites and in both surveys (ranging 70–99%). Some variation in the usage of bednets was observed in all sites, except in BF.

In S2, participants not using mosquito coils, insecticide sprays, or repellents against malaria vectors were significantly less likely to be infected with *P. falciparum* (OR: S2 0.88 [95% CI: 0.782; 0.988]); however, no such association was observed in S1. Various entomological studies have questioned the efficacy of repellents and coils as effective malaria prevention measures and highlighted the false sense of protection perceived by the user.[28–30] Our data do not allow to draw robust conclusions at this stage, and the trend observed in S2 should be closely monitored in the subsequent cross-sectional surveys.

Study limitations. As with all interview–questionnaire-based studies, this study could have been subject to information or recall bias as data related to bednet usage, control interventions usage, and care-seeking behaviors were collected from parents' recollection as opposed to objective observation. The impact of these potential information biases on the study results is estimated to be limited because of the proximity in time between the occurrence of the event for

TABLE 5
Care-seeking behaviors (treatment sought for fever and hospitalization for malaria) according to *P. falciparum* infection status by microscopy and survey

		P. falciparum infected			*P. falciparum* not infected			Total		
Care-seeking behavior	Survey	*N*	*N*	% (95% CI)	*n*	*N*	% (95% CI)	*n*	*N*	% (95% CI)
Treatment sought for malaria or fever in the previous 14 days	S1	238	1,187	20.1 (17.8; 22.4)	424	3,021	14.0 (12.8; 15.3)	662	4,208	15.7 (14.6; 16.9)
	S2	247	1,232	20.0 (17.8; 22.4)	290	2,967	9.8 (8.7; 10.9)	537	4,199	12.8 (11.8; 13.8)
Hospitalization for malaria in the past 3 months	S1	33	1,187	2.8 (1.9; 3.9)	76	3,021	2.5 (2.0; 3.1)	109	4,208	2.6 (2.1; 3.1)
	S2	42	1,232	3.4 (2.5; 4.6)	77	2,967	2.6 (2.1; 3.2)	119	4,199	2.8 (2.4; 3.4)

n (%) = number (percentage) of children in each group; *N* = total number of participants; *P. falciparum* = *Plasmodium falciparum*; S1 = survey 1; S2 = survey 2; 95% CI = exact 95% CI.

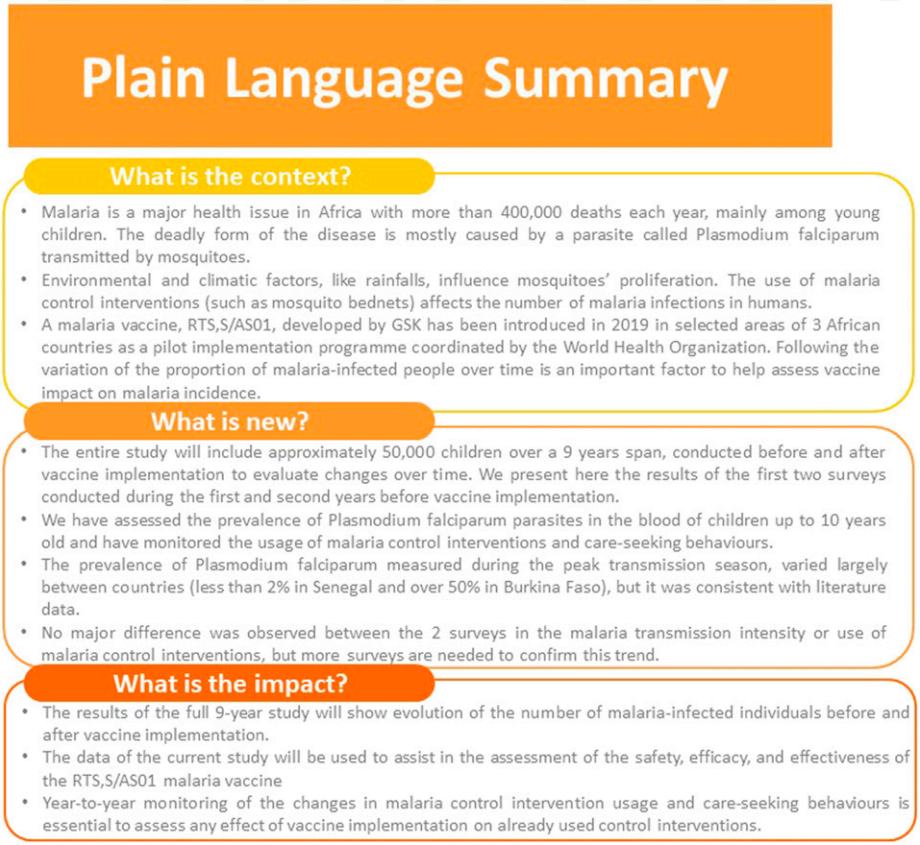


FIGURE 4. Plain language summary. This figure appears in color at www.ajtmh.org.

which information is collected and the interview itself. Another study limitation may be related to both sensitivity and specificity of microscopy slide readings. Slide reading performances may indeed vary depending on the parasite density and species identification. However, the kappa statistic estimated a substantial agreement between microscopy and QT-PCR, the latter being able to detect a higher number of low density infections than microscopy and RDT.[12,31] Moreover, this should mainly impact low transmission settings due to the high proportion of low-density infections.

CONCLUSION

The present article summarizes the results of the first two annual surveys of a larger malaria prevalence study aiming at characterizing MTI in light of the use of malaria control interventions and other environmental factors. Our results confirm that the high *Pf*PR observed in study sites that are part of the MVIP is in line with the WHO recommendation to operate the program in moderate to high transmission areas. In addition, our results are key to inform on the potential occurrence of annual fluctuations in MTI that may influence the assessment of the RTS,S/AS01$_E$ vaccine safety, effectiveness, and impact. The observations based on the first two surveys of this study do not indicate major temporal changes in terms of *Plasmodium* prevalence or use of malaria control interventions, but more surveys are needed to confirm this trend. The data generated in this study will be used to create variables to adjust the temporal and concurrent comparison analyses of the RTS,S/AS01$_E$ vaccine safety, impact, and effectiveness study. More specifically, the year- and site-specific *Pf*PR computed on unvaccinated study participants will be included as covariates in the regression models of the safety, impact, and effectiveness study to assess annual fluctuations and/or changes due to other malaria control interventions.

To a broader extent, the large sample size of this study (approximately 50,000 participants during the course of 9 years) and the use of a standardized methodology across multiple sites in various countries in SSA using multiple testing will provide a unique perspective on malaria prevalence variations across Africa.

Received October 25, 2019. Accepted for publication August 7, 2020.

Published online September 21, 2020.

Note: Supplemental Appendix and tables appear at www.ajtmh.org.

Acknowledgments: We thank the children, their families, and their communities who participated in this study; the investigators, and their clinical and laboratory teams for their contribution to the study and their support and care of participants; and also thank Shyam Prasad TR (TCS c/o GSK) for data management, Aparna Yarraguntla for statistical analysis (ICON, c/o GSK), Roland Vaudry (GSK) and William Zonta (Modis, Belgium c/o GSK) for publication management, and Sarah Benns (Independent medical writer on behalf of GSK) for drafting the manuscript.

Financial support: This trial was sponsored and funded by GlaxoSmithKline Biologicals SA (GSK). GSK also funded all costs associated with the development and the publishing of the present manuscript.

Disclosure: F. R., J.-Y. P., and S. W. are employees of the GSK group of companies. At the time of the study, N. P. was an employee of the

GSK group of companies. F. R., J.-Y. P., and N. P. hold shares in the GSK group of companies. A. M., A. S., A. B. T., B. C., E. A., G. C., J. L., J. O., K. P. A., K. S., L. O., M. T., O. B., P. M., R. T., S. K., S. O.-A., S. B. S., V. S., W. O., and Y. K., via their institutions, received grants from the GSK group of companies for the conduct of this study/work. A. S., A. B. T., B. C., E. A., G. C., J. O., K. P. A., L. O., M. T., O. B., S. K., S. O.-A., S. B. S., V. S., W. O., and Y. K., via their institutions, received grants from the GSK group of companies for the conduct of studies outside the submitted work. L. O. report grants from GSK Trust in Science outside the submitted work. M. L. is an employee of Aixial on behalf of the GSK group of companies. M. O. is an employee of 4Clinics on behalf of the GSK group of companies. At the time of the study, E. U. was a consultant for the GSK group of companies. GSK makes available anonymized individual participant data and associated documents from interventional clinical studies which evaluate medicines, upon approval of proposals, submitted to www.clinicalstudydatarequest.com. To access data for other types of GSK sponsored research, for study documents without patient-level data, and for clinical studies not listed, please submit an enquiry via the website. All authors reviewed and commented on a draft version of the manuscript and gave their final approval for it to be submitted for publication.

Authors' addresses: Elisha Adeniji, Kwaku Poku Asante, Owusu Boahen, Seyram Kaali, Seth Owusu-Agyei, and Mathilda Tivura, Kintampo Health Research Centre, Ghana Health Service, Accra, Ghana, E-mails: elisha.adeniji@kintampo-hrc.org, kwakupoku.asante@kintampo-hrc.org, owusu.boahen@kintampo-hrc.org, kaali.seyram@kintampo-hrc.org, sowusuagyei@uhas.edu.gh, and mathilda.tivura@kintampo-hrc.org. Guillaume Compaoré, Boubacar Coulibaly, and Ali Sie, Centre de Recherche en Santé de Nouna, Nouna, Burkina Faso, E-mails: guidedeo@yahoo.fr, boubacar@fasonet.bf, and alisie.crsn@fasonet.bf. Youssouf Kabore, Sodiomon B. Sirima, and Alfred B. Tiono, Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso, E-mails: kabyouf@yahoo.fr, s.sirima.cnlp@fasonet.bf, and t.alfred@fasonet.bf. Mathieu Lamy, Aixial c/o GSK, Wavre, Belgium, E-mail: mathieu.x.lamy@gsk.com. John Lusingu, National Institute for Medical Research (NIMR), Korogwe, Tanzania, and University of Copenhagen, Copenhagen, Denmark, E-mail: jpalusingu@yahoo.co.uk. Anangisye Malabeja, National Institute for Medical Research (NIMR), Korogwe, Tanzania, E-mail: malabeja1@yahoo.com. Petra Mens, Parasitology Unit, Department of Medical Microbiology, Academic Medical Center, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands, E-mail: p.f.mens@amc.uva.nl. Mattéa Orsini, 4Clinics c/o GSK, Wavre, Belgium, E-mail: mattea.x.orsini@gsk.com. Lucas Otieno, Walter Otieno, Janet Oyieko, and Valentine Sing'oei, KEMRI - Walter Reed Project, US Army Medical Research Directorate-Kenya, Kombewa, Kenya, E-mails: lucas.tina@usamru-k.org, walter.otieno@usamru-k.org, janet.oyieko@usamru-k.org, and valentine.singoei@usamru-k.org. Jean-Yves Pirçon, François Roman, and Stéphanie Wéry, GSK, Wavre, Belgium, E-mails: jean-yves.x.pircon@gsk.com, francois.p.roman@gsk.com, and stephanie.x.wery@gsk.com. Nicolas Praet, GSK, Wavre, Belgium, and Janssen Pharmaceutica NV, Beerse, Belgium, E-mail: nicolas.x.praet@gsk.com. Khadime Sylla and Roger Tine, Faculté de Médecine, Département de Parasitologie, Centre de Recherche de Keur Socé, Université Cheikh Anta Diop, Dakar, Senegal, E-mails: khasylla@gmail.com and rogertine@hotmail.com. Effua Usuf, Medical Research Council Unit, The Gambia at London School of Hygiene and Tropical Medicine, Fajara, The Gambia, E-mail: effuau@gmail.com.

REFERENCES

1. World Health Organization, 2018. *World Malaria Report 2018*. Available at: https://www.who.int/malaria/publications/world-malaria-report-2018/report/en/. Accessed March 28, 2019.
2. World Health Organization, 2015. *Global Technical Strategy for Malaria 2016–2030*. London, United Kingdom: WHO.
3. World Health Organization, Malaria Vaccine Funders Group, 2013. *Malaria Vaccine Technology Roadmap 2013*. Available at: https://www.malariavaccine.org/sites/www.malariavaccine.org/files/content/page/files/TRM_update_nov13.pdf. Accessed February 18, 2019.
4. European Medicines Agency SMH, 2015. *First Malaria Vaccine Receives Positive Scientific Opinion from EMA 2015*. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2015/07/news_detail_002376.jsp&mid=WC0b01ac058004d5c1. Accessed March 15, 2018.
5. World Health Organization, 2018. Malaria vaccine, 2016. WHO position paper–January 2016. *Wkly Epidemiol Rec 91:* 33–52.
6. World Health Organization, 2017. *Ghana, Kenya and Malawi to Take Part in WHO Malaria Vaccine Pilot Programme*. Available at: https://www.afro.who.int/news/ghana-kenya-and-malawi-take-part-who-malaria-vaccine-pilot-programme. Accessed March 20, 2019.
7. Hay SI, Smith DL, Snow RW, 2008. Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infect Dis 8:* 369–378.
8. Hay SI, Rogers DJ, Toomer JF, Snow RW, 2000. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, internet access and review. *Trans R Soc Trop Med Hyg 94:* 113–127.
9. Drakeley C et al., 2017. Longitudinal estimation of *Plasmodium falciparum* prevalence in relation to malaria prevention measures in six sub-Saharan African countries. *Malar J 16:* 433.
10. Smith DL, Guerra CA, Snow RW, Hay SI, 2007. Standardizing estimates of the *Plasmodium falciparum* parasite rate. *Malar J 6:* 131.
11. World Health Organization, 2017. *Malaria Vaccine Implementation Programme (MVIP)*. Available at: https://www.who.int/immunization/diseases/malaria/malaria_vaccine_implementation_programme/about/en/. Accessed March 22, 2019.
12. World Health Organization, 2019. *Malaria: Areas of Work – Diagnostic Testing*. Available at: https://www.who.int/malaria/areas/diagnosis/nucleic-acid-amplification-tests/en/. Accessed February 18, 2019.
13. Okell LC, Ghani AC, Lyons E, Drakeley CJ, 2009. Submicroscopic infection in *Plasmodium falciparum*-endemic populations: a systematic review and meta-analysis. *J Infect Dis 200:* 1509–1517.
14. Landis JR, Koch GG, 1977. The measurement of observer agreement for categorical data. *Biometrics 33:* 159–174.
15. The malERA Refresh Consultative Panel on Combination Interventions and Modelling, 2017. malERA: an updated research agenda for combination interventions and modelling in malaria elimination and eradication. *PLoS Med 14:* e1002453.
16. Diallo A et al., 2017. An epidemiological study to assess *Plasmodium falciparum* parasite prevalence and malaria control measures in Burkina Faso and Senegal. *Malar J 16:* 63.
17. Sylla K et al., 2015. Sero-epidemiological evaluation of *Plasmodium falciparum* malaria in Senegal. *Malar J 14:* 275.
18. Tine RCK et al., 2013. Parasitic infections among children under five years in Senegal: prevalence and effect on anaemia and nutritional status. *ISRN Parasitol 2013:* 272701.
19. Ceesay SJ et al., 2008. Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet 372:* 1545–1554.
20. Nankabirwa J, Brooker SJ, Clarke SE, Fernando D, Gitonga CW, Schellenberg D, Greenwood B, 2014. Malaria in school-age children in Africa: an increasingly important challenge. *Trop Med Int Health 19:* 1294–1309.
21. Felger I, Maire M, Bretscher MT, Falk N, Tiaden A, Sama W, Beck H-P, Owusu-Agyei S, Smith TA, 2012. The dynamics of natural *Plasmodium falciparum* infections. *PLoS One 7:* e45542.
22. Aron JL, 1983. Dynamics of acquired immunity boosted by exposure to infection. *Math Biosci 64:* 249–259.
23. Ceesay SJ et al., 2015. Malaria prevalence among young infants in different transmission settings, Africa. *Emerg Infect Dis 21:* 1114–1121.
24. Asua V, Tukwasibwe S, Conrad M, Walakira A, Nankabirwa JI, Mugenyi L, Kamya MR, Nsobya SL, Rosenthal PJ, 2017. *Plasmodium* species infecting children presenting with malaria in Uganda. *Am J Trop Med Hyg 97:* 753–757.

25. Doctor SM et al., 2016. Low prevalence of *Plasmodium malariae* and *Plasmodium ovale* mono-infections among children in the Democratic Republic of the Congo: a population-based, cross-sectional study. *Malar J 15:* 350.
26. Gneme A, Guelbeogo WM, Riehle MM, Tiono AB, Diarra A, Kabre GB, Sagnon N, Vernick KD, 2013. *Plasmodium* species occurrence, temporal distribution and interaction in a child-aged population in rural Burkina Faso. *Malar J 12:* 67.
27. Twohig KA, Pfeffer DA, Baird JK, Price RN, Zimmerman PA, Hay SI, Gething PW, Battle KE, Howes RE, 2019. Growing evidence of *Plasmodium vivax* across malaria-endemic Africa. *PLoS Negl Trop Dis 13:* e0007140.
28. Avicor SW, Wajidi MFF, Owusu EO, 2017. To coil or not to coil: application practices, perception and efficacy of mosquito coils in a malaria-endemic community in Ghana. *Environ Sci Pollut Res Int 24:* 21138–21145.
29. Hogarh JN, Agyekum TP, Bempah CK, Owusu-Ansah EDJ, Avicor SW, Awandare GA, Fobil JN, Obiri-Danso K, 2018. Environmental health risks and benefits of the use of mosquito coils as malaria prevention and control strategy. *Malar J 17:* 265.
30. Lukwa N, Chiwade T, 2008. Lack of insecticidal effect of mosquito coils containing either metofluthrin or esbiothrin on *Anopheles gambiae* sensu lato mosquitoes. *Trop Biomed 25:* 191–195.
31. World Health Organization, 2017. *Malaria Policy Advisory Committee (MPAC) Meeting Report 2017*. Geneva, Switzerland: WHO. Contract No.: WHO/HTM/GMP/MPAC/2017.21.

The following are supplemental files and will be published online only

Supplemental Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Appendix“Estimating annual fluctuations in malaria transmission intensity and in the use of malaria control interventions in 5 sub-Saharan African countries”

TABLE OF CONTENTS

PAGE

LIST OF SUPPLEMENTALTABLES

PAGE

1. SELECTION OF CHILDREN PARTICIPATING IN THE SURVEY

The participant selection process was repeated each year independently meaning that the individuals were different in each cross-sectional survey except if they were re-selected in a subsequent survey by chance. The population listings generated from the demographic surveillance allowed for sampling of the required individuals according to stratification by age group as follows (the number of individuals was approximately plus or minus 5 children):

- 60 children aged 6 months to <1 year
- 120 children aged 1 year
- 120 children aged 2 years
- 50 children aged 3 years
- 50 children aged 4 years
- 40 children aged 5 years
- 40 children aged 6 years
- 40 children aged 7 years
- 40 children aged 8 years
- 40 children aged 9 years.

2. DETERMINATION OF PARASITEMIA BY MICROSCOPY

Assessing parasite presence

A 100-field examination of the thick film was conducted to assess presence of parasites and species.[1]

Negative result: 100 fields free of parasites were to be read before a slide was declared negative.

Positive result: If parasites were present within reading of 100 fields, the slide was positive. Positive slides were examined for a further 100 fields to ensure all species present were detected.

Identification of *Plasmodium* species

Positive parasitemia identified on any thick blood film was always identified to species. This was done on thin blood film except in cases of low parasitemia.

Parasite density counting against assumed 8000 leukocytes per microliter

In this method of estimating parasite density, it was assumed that there were 8000 leukocytes per microliter of blood.

- If upon counting 200 leukocytes, 10 or more parasites had been counted, the results were to be recorded as the parasites per 200 leukocytes.
- If upon counting 200 leukocytes, 9 or fewer parasites had been recorded, the reader was to continue counting until 500 leukocytes had been counted and the number of parasites per 500 leukocytes were to be recorded.
- It should be noted that the count was to be by species, and counts for *P. falciparum*were to be made for both gametocytes and asexual parasites.

Criteria for concordance for double reading of slides

All slides were read twice, by two independent readers to quantify the *P. falciparum* parasite presence and density. If slides were judged to be discordant, a third independent read was to be organized in the following cases:

A. The result from one reader was negative and the one of the other was positive.

B. For high and medium positive parasitemia results (blood parasitemia >400/μL), the higher count divided by the lower count was >2.

C. For low parasitemia (blood parasitemia ≤400/μL), the highest reading density was more than one $\log_{10}$ higher than the lowest reading.

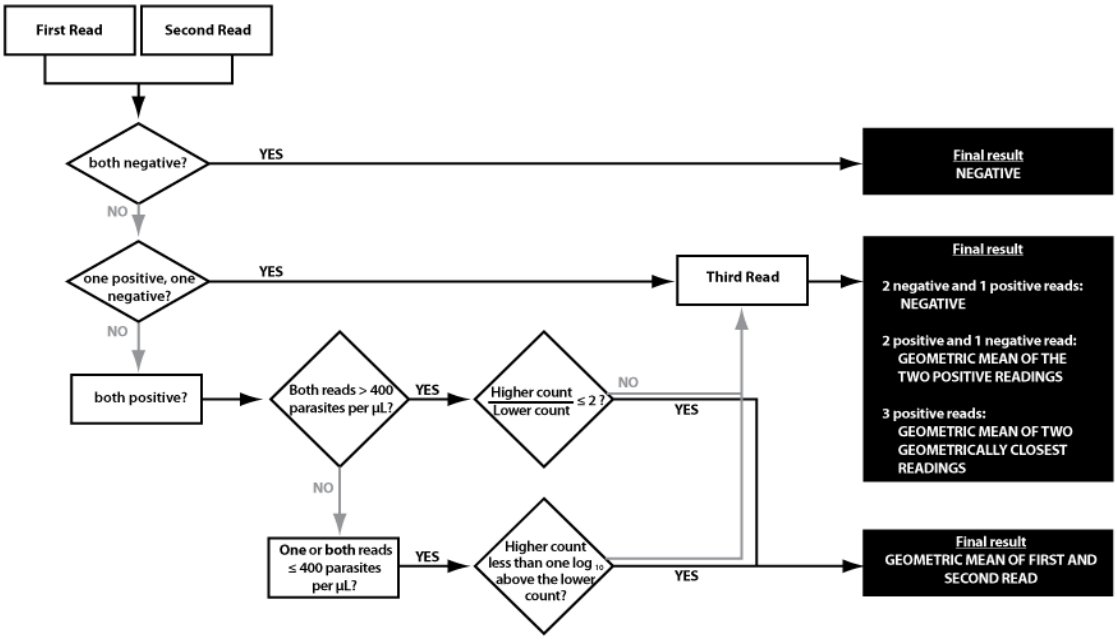
If the parasitemia result was high or medium in one slide and the result from the other slide reading was low, i.e. one was >400/μL and the other was ≤400/μL, criterion (C) was to be applied.

Determination of final result

If there were two concordant results, the final result was the geometric mean of the two readings.

If the first two readings were discordant then the final result was to follow the following principles:

- For cases of positive/negative discrepancy (A), the majority decision was to be adopted. If the decision was positive, the final result was the geometrical mean of the two positives.
- For cases of three positive reads (B and C), the final result was to be the geometric mean of the two geometrically closest readings.



3. DETERMINATION OF PARASITEMIA BY NUCLEIC ACID AMPLIFICATION TEST (NAAT)

Determination of parasitemia by nucleic acid amplification test (NAAT) in the study used the following tests:

- quantitative polymerase chain reaction(QT-PCR) for the detection of both DNA (asexual parasites) and RNA (gametocytes);
- quantitative nucleic acid sequence-based amplification (QT-NASBA) for the specific detection of RNA, identifying sexual stages parasites (gametocytes).

Both were performed at AMC (Academic Medical Center, Amsterdam, The Netherlands). Details for QT-PCR are presented in Error: Reference source not found et al, 2001[2] and for QT-NASBAin Error: Reference source not found et al, 2004, 2004.[3]

4. LIST OF PRE-DEFINED MALARIA CONTROL INTERVENTIONS AND POTENTIAL RISK FACTORS

List of malaria control intervention explanatory variables tested in themultivariable logistical regression model:

Malaria or fever treatment sought for in the past 14 days	Yes vs. No
Malaria hospitalization in the last 3 months	Yes vs. No
Antimalarial or any other medication consumed within 14 days prior to study visit	Yes vs. No
Antimalarial drug consumed in the past 14 days	Yes vs. No
Other medication consumed over 14 days prior to study visit	Yes vs. No
Subject sleep under a bednet last night	Yes vs. No
New net (less than 1 year)	No Bednet vs. No
	Yes vs. No
Impregnated bednet	Yes vs. No
	No Bednet vs. No
Pierced/torn bednet	Yes vs. No
	No Bednet vs. No
Number of holes	Less than 5 vs. More or equal to 5
	No Pierced Bednet vs. More or equal to 5
	No Bednet vs. More or equal to 5
Use of mosquito coils over 7 days	Yes vs. Missing/No
Use of insecticide sprays over 7 days	Yes vs. Missing/No
Use of commercial repellents over 7 days	Yes vs. Missing/No
Use of traditional repellents over 7 days	Yes vs. Missing/No
Use of none of above over 7 days	Yes vs. Missing/No
Use of indoor residual spraying (IRS) in the past 12 months to spray interior	Yes vs. No
Use of indoor residual spraying - number of months ago	>4 vs. 1-2
	No Residual Spray vs. 1-2
	3-4 vs. 1-2

IRS = Indoor Residual Spraying, application of a residual insecticide to internal walls and ceilings of housing structures.[4]

List of potential risk factor explanatory variables tested in the multivariable logistical regression model:

Age (in years)	Continuous
Gender	Male vs. Female
Number of persons living in the same part of the house	4-5 vs. ≤3
	>5 vs. ≤3
Number of persons enrolled into the study	1 vs. 2
	3 vs. 2
	>3 vs. 2
Localization	Urban area vs. Rural area
	Semi-Rural Area vs. Rural area
Type of location	Town (>10,000 and < 50,000 habitants) vs. Countryside
	Small city (>50,000 and < 1mil. habitants) vs. Countryside
	Large city (>1 mil. habitants) vs. Countryside (<10,000
Main house construction material: Walls	Clay vs. Mud
	Cement/Plaster vs. Mud
	Brick vs. Mud
	Other vs. Mud
	Cement /Paint vs. Mud
Main house construction material: Floor	Carpet vs. Natural floor*
	Ceramic tiles vs. Natural floor*
	Parquet/ polished wood vs. Natural floor*
	Clay vs. Natural floor*
	Cement vs. Natural floor*
	Rudimentary floor** vs. Natural floor*
Main house construction material: Roof	Tiles vs. Grass/Palm
	Other vs. Grass/Palm
	Iron sheet vs. Grass/Palm
	Clay vs. Grass/Palm
Main house construction material: Windows/eaves	Other vs. Open
	No Windows vs. Open
	Closed vs. Open
	Partially open vs. Open
Main house construction material: Nets	Nets present on some windows vs. Nets not present
	Nets present on all windows vs. Nets not present
	Other vs. Nets not present
Main source of drinking water	Closed water source† vs. Open water source††
Is the open source in the compound	No open water vs. No
	Yes vs. No
Presence of electricity	Yes vs. No

* Natural floor = earth, sand, dung.

** Rudimentary floor = wood, palm, bamboo.

† Closed water source (piped water, tube well, dug well, protected well).

†† Open water source (unprotected well, spring water, rainwater, tanker truck, surface water).

5. SUPPLEMENTAL TABLES

5.1. Study Population and Demographic Characteristics

Supplemental Table 1 Number of individuals included in the analysis by study site, age group and survey

Study site	6M–<5Y		5–<10Y		All ages	
	Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2
	n	n	n	n	n	n
Nouna, BF	404	400	202	200	606	600
Saponé, BF	403	399	201	200	604	599
Kintampo, GH	400	400	200	200	600	600
Kombewa, KE	402	401	197	198	599	599
KeurSocé, SN	400	397	200	203	600	600
NiakharSN	398	397	200	204	598	601
Korogwe, TZ	401	400	200	200	601	600
All	2.808	2.794	1.400	1.405	4.208	4.199

6M–<5Y = Individuals aged 6 months to less than 5 years at informed consent.
5–<10Y = Individuals aged 5 years to less than 10 years at informed consent.
n = number of individuals in a given category.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

Supplemental Table 2 Summary of demographic characteristics of individuals by study siteand survey

Study site	Characteristics		Survey 1	Survey 2
Nouna, BF			**N=606**	**N=600**
	Age at informed consent (years)	Mean±SD	3.99±2.69	4.00±2.74
		Range	0.53–9.90	0.56–9.97
	Age group (years)	6M–<5Y; n (%)	404 (66.7)	400 (66.7)
		5–<10Y; n (%)	202 (33.3)	200 (33.3)
	Gender	Female; n (%)	304 (50.2)	300 (50.0)
		Male; n (%)	302 (49.8)	300 (50.0)
Saponé, BF			**N=604**	**N=599**
	Age at informed consent (years)	Mean±SD	4.05±2.77	4.06±2.75
		Range	0.51–9.93	0.50–9.89
	Age group (years)	6M–<5Y; n (%)	403 (66.7)	399 (66.6)
		5–<10Y; n (%)	201 (33.3)	200 (33.4)
	Gender	Female; n (%)	295 (48.8)	283 (47.3)
		Male; n (%)	309 (51.2)	316 (52.8)
Kintampo, GH			**N=600**	**N=600**
	Age at informed consent (years)	Mean±SD	3.99±2.74	4.02±2.74
		Range	0.55–9.81	0.58–9.94
	Age group (years)	6M–<5Y; n (%)	400 (66.7)	400 (66.7)
		5–<10Y; n (%)	200 (33.3)	200 (33.3)
	Gender	Female; n (%)	278 (46.3)	297 (49.5)
		Male; n (%)	322 (53.7)	303 (50.5)
Kombewa, KE			**N=599**	**N=599**
	Age at informed consent (years)	Mean±SD	4.05±2.78	4.08±2.75
		Range	0.57–10.00	0.58–9.97
	Age group (years)	6M–<5Y; n (%)	402 (67.1)	401 (66.9)
		5–<10Y; n (%)	197 (32.9)	198 (33.1)
	Gender	Female; n (%)	279 (46.6)	315 (52.6)
		Male; n (%)	320 (53.4)	284 (47.4)
KeurSocé, SN			**N=600**	**N=600**
	Age at informed consent (years)	Mean±SD	4.04±2.74	4.07±2.76
		Range	0.55–9.96	0.60–9.92
	Age group (years)	6M–<5Y; n (%)	400 (66.7)	397 (66.2)
		5–<10Y; n (%)	200 (33.3)	203 (33.8)
	Gender	Female; n (%)	303 (50.5)	307 (51.2)
		Male; n (%)	297 (49.5)	293 (48.8)
Niakhar, SN			**N=598**	**N=601**
	Age at informed consent (years)	Mean±SD	4.02±2.77	4.08±2.77
		Range	0.54–9.99	0.70–9.83
	Age group (years)	6M–<5Y; n (%)	398 (66.6)	397 (66.1)
		5–<10Y; n (%)	200 (33.4)	204 (33.9)
	Gender	Female; n (%)	309 (51.7)	280 (46.6)
		Male; n (%)	289 (48.3)	321 (53.4)
Korogwe, TZ			**N=601**	**N=600**
	Age at informed consent (years)	Mean±SD	4.01±2.75	4.03±2.73
		Range	0.50–9.95	0.54–9.97
	Age group (years)	6M–<5Y; n (%)	401 (66.7)	400 (66.7)
		5–<10Y; n (%)	200 (33.3)	200 (33.3)

Study site	Characteristics		Survey 1	Survey 2
	Gender	Female; n (%)	282 (46.9)	306 (51.0)
		Male; n (%)	319 (53.1)	294 (49.0)
Overall			**N=4,208**	**N=4,199**
	Age at informed consent (years)	Mean±SD	4.02±2.75	4.05±2.75
		Range	0.50–10.00	0.50–9.97
	Age group (years)	6M–<5Y; n (%)	2,808 (66.7)	2,794 (66.5)
		5–<10Y; n (%)	1,400 (33.3)	1,405 (33.5)
	Gender	Female; n (%)	2,050 (48.7)	2,088 (49.7)
		Male; n (%)	2,158 (51.3)	2,111 (50.3)

N = total number of individuals overall or per site.
n = number of individuals in a given category.
SD = standard deviation.
Range = minimum and maximum values.
6M-<5Y = Individuals aged 6 months to less than 5 years at informed consent.
5-<10Y = Individuals aged 5 yearsto less than 10 years at informed consent.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

5.2. *Plasmodium* parasite prevalence

Supplemental Table 3 ***P. falciparum*** **results (asexual parasites and gametocytes) by study site andsurvey measured by NAAT (QT-PCR and QT-NASBA)**

Study site	Survey 1		Survey 2	
	P. falciparum **measured by QT-PCR**	**Presence of gametocytes measured by QT-NASBA***	***P. falciparum*** **measured by QT-PCR**	**Presence of gametocytes measured by QT-NASBA***
Nouna, BF; n (%)	**N=581**	**N'=406**	**N=599**	**N'=456**[†]
Positive	406 (69.9)	205 (50.5)	457 (76.3)	268 (58.8)
Negative	175 (30.1)	201 (49.5)	142 (23.7)	188 (41.2)
Saponé, BF; n (%)	**N=426**	**N'=245**[†]	**N=599**	**N'=390**
Positive	246 (57.7)	145 (59.2)	390 (65.1)	229 (58.7)
Negative	180 (42.3)	100 (40.8)	209 (34.9)	161 (41.3)
Kintampo, GH; n	**N=565**	**N'=229**[†]	**N=600**	**N'=281**
Positive	231 (40.9)	94 (41.0)	281 (46.8)	134 (47.7)
Negative	334 (59.1)	135 (59.0)	319 (53.2)	147 (52.3)
Kombewa, KE, n	**N=599**	**N'=262**	**N=599**	**N'=236**
Positive	262 (43.7)	81 (30.9)	236 (39.4)	120 (50.8)
Negative	337 (56.3)	181 (69.1)	363 (60.6)	116 (49.2)
KeurSocé, SN;** n	**-**	**-**	**N=556**	**N'=6**[†]
Positive	-	-	7 (1.3)	4 (66.7)
Negative	-	-	549 (98.7)	2 (33.3)
Niakhar, SN; n (%)	**N=565**	**N'=15**	**N=601**	**N'=14**
Positive	15 (2.7)	2 (13.3)	14 (2.3)	1 (7.1)
Negative	550 (97.3)	13 (86.7)	587 (97.7)	13 (92.9)
Korogwe, TZ; n (%)	**N=601**	**N'=108**	**N=600**	**N'=32**
Positive	108 (18.0)	21 (19.4)	32 (5.3)	11 (34.4)
Negative	493 (82.0)	87 (80.6)	568 (94.7)	21 (65.6)

NAAT = Nucleic Acid Amplification Test.
QT-PCR = Quantitative Polymerase Chain Reaction.
QT-NASBA = Quantitative Nucleic Acid Sequence-Based Amplification.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.
N = number of individuals tested by QT-PCR for detection of*P. falciparum DNA and/or RNA* per site.
N' = number of individuals tested positive by QT-PCR being tested by QT-NASBA for detection of *P. falciparum* gametocytes RNA per site.
n = number of individuals in a given category.
*Test for detection of gametocytes, QT-NASBA (RNA specific), was only performed on samples positive by QT-PCR (detecting both DNA and RNA).
**KeurSocé, SN had no valid NAAT results in Survey 1.
[†] Only individuals with available results are included in N or N'.

Supplemental Table 4 Number of individuals carrying gametocytes measured by microscopy compared to gametocytes results measured by QT-NASBA by study site and by survey

Presence of gametocytes measured by microscopy, n (%)	Presence of Gametocytes (measured by QT-NASBA)				Total	
	Positive		Negative			
Study site	**Survey 1**	**Survey 2**	**Survey 1**	**Survey 2**	**Survey 1**	**Survey 2**
Nouna, BF	**N=205**	**N=268**	**N=201**	**N=188**	**N=406**	**N=456**
	66 (32.2)	69 (25.7)	20 (10.0)	7 (3.7)	86 (21.2)	76 (16.7)
Saponé, BF	**N=145**	**N=229**	**N=100**	**N=161**	**N=245**	**N=390**
	81 (55.9)	89 (38.9)	15 (15.0)	16 (9.9)	96 (39.2)	105 (26.9)
Kintampo, GH	**N=94**	**N=134**	**N=135**	**N=147**	**N=229**	**N=281**
	12 (12.8)	24 (17.9)	1 (0.7)	5 (3.4)	13 (5.7)	29 (10.3)
Kombewa, KE	**N=81**	**N=120**	**N=181**	**N=116**	**N=262**	**N=236**
	18 (22.2)	7 (5.8)	4 (2.2)	2 (1.7)	22 (8.4)	9 (3.8)
KeurSocé, SN*	**-**	**N=4**	**-**	**N=2**	**-**	**N=6**
	-	3 (75.0)	-	0	-	3 (50.0)
Niakhar, SN	**N=2**	**N=1**	**N=13**	**N=13**	**N=15**	**N=14**
	1 (50.0)	0	1 (7.7)	0	2 (13.3)	0
Korogwe, TZ	**N=21**	**N=11**	**N=87**	**N=21**	**N=108**	**N=32**
	7 (33.3)	0	1 (1.1)	1 (4.8)	8 (7.4)	1 (3.1)
Overall	**N=548**	**N=767**	**N=717**	**N=648**	**N=1,265**	**N=1,415**
	185 (33.8)	192 (25.0)	42 (5.9)	31 (4.8)	227 (17.9)	223 (15.8)

QT-PCR = Quantitative Polymerase Chain Reaction.
QT-NASBA = Quantitative Nucleic Acid Sequence-Based Amplification.
N = number of individuals with positive parasitemia as measured by QT-PCR in the given category.
n = number of gametocyte positive individuals measured by microscopy in the given category.
*KeurSocé, SN had no valid QT-PCR results in Survey 1.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

Supplemental Table 5 Prevalence of *Plasmodium species*other than *P. falciparum* measured by microscopy by study site, *P. falciparum* infection status and survey

Study site	*Plasmodium*parasitemia; n (%)	*Pf* infected		*Pf* not infected		Total	
		Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2
Nouna, BF		**N=403**	**N=488**	**N=203**	**N=112**	**N=606**	**N=600**
	P. malariae	2 (0.5)	30 (6.1)	2 (1.0)	27 (24.1)	4 (0.7)	57 (9.5)
	P. vivax	0	0	0	0	0	0
	P. ovale	1 (0.2)	0	2 (1.0)	0	3 (0.5)	0
Saponé, BF		**N=299**	**N=316**	**N=305**	**N=283**	**N=604**	**N=599**
	P. malariae	28 (9.4)	29 (9.2)	3 (1.0)	6 (2.1)	31 (5.1)	35 (5.8)
	P. vivax	0	0	0	0	0	0
	P. ovale	1 (0.3)	0	0	0	1 (0.2)	0
Kintampo, GH		**N=214**	**N=212**	**N=386**	**N=388**	**N=600**	**N=600**
	P. malariae	9 (4.2)	15 (7.1)	2 (0.5)	3 (0.8)	11 (1.8)	18 (3.0)
	P. vivax	0	0	0	0	0	0
	P. ovale	3 (1.4)	1 (0.5)	5 (1.3)	1 (0.3)	8 (1.3)	2 (0.3)
Kombewa, KE		**N=196**	**N=189**	**N=403**	**N=410**	**N=599**	**N=599**
	P. malariae	16 (8.2)	16 (8.5)	0	1 (0.2)	16 (2.7)	17 (2.8)
	P. vivax	0	0	0	0	0	0
	P. ovale	8 (4.1)	4 (2.1)	0	3 (0.7)	8 (1.3)	7 (1.2)
KeurSocé, SN		**N=3**	**N=6**	**N=597**	**N=594**	**N=600**	**N=600**
	P. malariae	0	0	0	0	0	0
	P. vivax	0	0	0	0	0	0
	P. ovale	0	0	0	0	0	0
Niakhar, SN		**N=9**	**N=3**	**N=589**	**N=598**	**N=598**	**N=601**
	P. malariae	0	0	0	0	0	0
	P. vivax	0	0	0	0	0	0
	P. ovale	0	0	0	0	0	0
Korogwe, TZ		**N=63**	**N=18**	**N=538**	**N=582**	**N=601**	**N=600**
	P. malariae	0	1 (5.6)	0	0	0	1 (0.2)
	P. vivax	0	1 (5.6)	0	0	0	1 (0.2)
	P. ovale	0	1 (5.6)	3 (0.6)	0	3 (0.5)	1 (0.2)
Overall		**N=1,187**	**N=1,232**	**N=3,021**	**N=2,967**	**N=4,208**	**N=4,199**
	P. malariae	55 (4.6)	91 (7.4)	7 (0.2)	37 (1.2)	62 (1.5)	128 (3.0)
	P. vivax	0	1 (0.1)	0	0	0	1 (0.02)
	P. ovale	13 (1.1)	6 (0.5)	10 (0.3)	4 (0.1)	23 (0.5)	10 (0.2)

Pf infected = Individualsinfected with *P. falciparum*parasitemia measured by microscopy.
Pf not infected = Individualsnot infected with *P. falciparum*parasitemia measured by microscopy.
N = total number of individuals overall or per site.
n = number of individuals in a given category.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

5.3. Malaria control interventions

Supplemental Table 6 Number of individuals having slept under a bednet the night before the visit and characterization of bednets by study site and survey

Study site		Survey 1	Survey 2
Nouna, BF		**N=606**	**N=600**
Participant slept under a bednet the night before the visit	n	547	530
	% (95% CI)	90.3 (87.6;92.5)	88.3 (85.5;90.8)
New bednet (less than 1 year)*	n	428	218
	% (95% CI)	78.2 (74.5;81.6)	41.1 (36.9;45.5)
Impregnated†bednet*	n	525	501
	% (95% CI)	96.0 (94.0;97.5)	94.5 (92.2;96.3)
Pierced/torn bednet*	n	59	158
	% (95% CI)	10.8 (8.3;13.7)	29.8 (25.9;33.9)
Saponé, BF		**N=604**	**N=599**
Participant slept under a bednet the night before the visit	n	557	534
	% (95% CI)	92.2 (89.8;94.2)	89.1 (86.4;91.5)
New bednet (less than 1 year)*	n	496	303
	% (95% CI)	89.0 (86.2;91.5)	56.7 (52.4;61.0)
Impregnated†bednet*	n	548	521
	% (95% CI)	98.4 (97.0;99.3)	97.6 (95.9;98.7)
Pierced/torn bednet*	n	40	61
	% (95% CI)	7.2 (5.2;9.7)	11.4 (8.9;14.4)
Kintampo, GH		**N=600**	**N=600**
Participant slept under a bednet the night before the visit	n	421	497
	% (95% CI)	70.2 (66.3;73.8)	82.8 (79.6;85.8)
New bednet (less than 1 year)*	n	217	492
	% (95% CI)	51.5 (46.7;56.4)	99.0 (97.7;99.7)
Impregnated†bednet*	n	167	495
	% (95% CI)	39.7 (35.0;44.5)	99.6 (98.6;100)
Pierced/torn bednet*	n	83	15
	% (95% CI)	19.7 (16.0;23.8)	3.0 (1.7;4.9)
Kombewa, KE		**N=599**	**N=599**
Participant slept under a bednet the night before the visit	n	584	534
	% (95% CI)	97.5 (95.9;98.6)	89.1 (86.4;91.5)
New bednet (less than 1 year)*	n	410	239
	% (95% CI)	70.2 (66.3;73.9)	44.8 (40.5;49.1)
Impregnated†bednet*	n	273	52
	% (95% CI)	46.7 (42.6;50.9)	9.7 (7.4;12.6)
Pierced/torn bednet*	n	153	188
	% (95% CI)	26.2 (22.7;30.0)	35.2 (31.2;39.4)
KeurSocé, SN		**N=600**	**N=600**
Participant slept under a bednet the night before the visit	n	568	520
	% (95% CI)	94.7 (92.6;96.3)	86.7 (83.7;89.3)
New bednet (less than 1 year)*	n	423	212
	% (95% CI)	74.5 (70.7;78.0)	40.8 (36.5;45.1)
Impregnated†bednet*	n	314	271
	% (95% CI)	55.3 (51.1;59.4)	52.1 (47.7;56.5)
Pierced/torn bednet*	n	50	85
	% (95% CI)	8.8 (6.6;11.4)	16.3 (13.3;19.8)

Study site		Survey 1	Survey 2
Niakhar, SN		**N=598**	**N=601**
Participant slept under a bednet the night before the visit	n	547	418
	% (95% CI)	91.5 (88.9;93.6)	69.6 (65.7;73.2)
New bednet (less than 1 year)*	n	482	60
	% (95% CI)	88.1 (85.1;90.7)	14.4 (11.1;18.1)
Impregnated[†]bednet*	n	481	418
	% (95% CI)	87.9 (84.9;90.5)	100 (99.1;100)
Pierced/torn bednet*	n	25	296
	% (95% CI)	4.6 (3.0;6.7)	70.8 (66.2;75.1)
Korogwe, TZ		**N=601**	**N=600**
Participant slept under a bednet the night before the visit	n	544	595
	% (95% CI)	90.5 (87.9;92.7)	99.2 (98.1;99.7)
New bednet (less than 1 year)*	n	68	557
	% (95% CI)	12.5 (9.8;15.6)	93.6 (91.3;95.4)
Impregnated[†]bednet*	n	282	567
	% (95% CI)	51.8 (47.5;56.1)	95.3 (93.3;96.9)
Pierced/torn bednet*	n	496	69
	% (95% CI)	91.2 (88.5;93.4)	11.6 (9.1;14.4)
Overall		**N=4,208**	**N=4,199**
Participant slept under a bednet the night before the visit	n	3,768	3,628
	% (95% CI)	89.5 (88.6;90.5)	86.4 (85.3;87.4)
New bednet (less than 1 year)*	n	2,524	2,081
	% (95% CI)	67.0 (65.5;68.5)	57.4 (55.7;59.0)
Impregnated[†]bednet*	n	2,590	2,825
	% (95% CI)	68.7 (67.2;70.2)	77.9 (76.5;79.2)
Pierced/torn bednet*	n	906	872
	% (95% CI)	24.0 (22.7;25.4)	24.0 (22.7;25.5)

95% CI = Exact 95% confidence interval.
N = total number of individuals overall or per site.
n = number of individuals in a given category.
*Denominator = number of individuals who slept under a bednet the night before the visit.
[†]Dipped in liquid insecticidebefore or after purchase.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

Supplemental Table 7 Number of individuals having used insect repellents or insecticides by study site and survey

Study site		Total	
		Survey 1	Survey 2
Nouna, BF		**N=606**	**N=600**
Use of mosquito coils over 7 days	n	109	211
	% (95% CI)	18.0 (15.0:21.3)	35.2 (31.3:39.1)
Use of insecticide sprays over 7 days	n	14	14
	% (95% CI)	2.3 (1.3:3.8)	2.3 (1.3:3.9)
Use of commercial repellents over 7 days	n	9	14
	% (95% CI)	1.5 (0.7:2.8)	2.3 (1.3:3.9)
Use of traditional repellents over 7 days	n	1	2
	% (95% CI)	0.2 (0.0:0.9)	0.3 (0.0:1.2)
Use of none of the above over 7 days	n	473	364
	% (95% CI)	78.1 (74.5:81.3)	60.7 (56.6:64.6)
Use of IRS in past 12 months	n	0	14
	% (95% CI)	0 (0.0:0.6)	2.3 (1.3:3.9)
Saponé, BF		**N=604**	**N=599**
Use of mosquito coils over 7 days	n	1	16
	% (95% CI)	0.2 (0.0:0.9)	2.7 (1.5:4.3)
Use of insecticide sprays over 7 days	n	0	3
	% (95% CI)	0 (0.0:0.6)	0.5 (0.1:1.5)
Use of commercial repellents over 7 days	n	1	3
	% (95% CI)	0.2 (0.0:0.9)	0.5 (0.1:1.5)
Use of traditional repellents over 7 days	n	4	0
	% (95% CI)	0.7 (0.2:1.7)	0 (0.0:0.6)
Use of none of the above over 7 days	n	598	577
	% (95% CI)	99.0 (97.9:99.6)	96.3 (94.5:97.7)
Use of IRS in past 12 months	n	0	0
	% (95% CI)	0 (0.0:0.6)	0 (0.0:0.6)
Kintampo, GH		**N=600**	**N=600**
Use of mosquito coils over 7 days	n	84	30
	% (95% CI)	14.0 (11.3:17.0)	5.0 (3.4:7.1)
Use of insecticide sprays over 7 days	n	25	24
	% (95% CI)	4.2 (2.7:6.1)	4.0 (2.6:5.9)
Use of commercial repellents over 7 days	n	0	0
	% (95% CI)	0 (0.0:0.6)	0 (0.0:0.6)
Use of traditional repellents over 7 days	n	1	0
	% (95% CI)	0.2 (0.0:0.9)	0 (0.0:0.6)
Use of none of the above over 7 days	n	490	560
	% (95% CI)	81.7 (78.3:84.7)	93.3 (91.0:95.2)
Use of IRS in past 12 months	n	2	0
	% (95% CI)	0.3 (0.0:1.2)	0 (0.0:0.6)
Kombewa, KE		**N=599**	**N=599**
Use of mosquito coils over 7 days	n	23	7
	% (95% CI)	3.8 (2.4:5.7)	1.2 (0.5:2.4)
Use of insecticide sprays over 7 days	n	5	0
	% (95% CI)	0.8 (0.3:1.9)	0 (0.0:0.6)
Use of commercial repellents over 7 days	n	1	2
	% (95% CI)	0.2 (0.0:0.9)	0.3 (0.0:1.2)
Use of traditional repellents over 7 days	n	2	0
	% (95% CI)	0.3 (0.0:1.2)	0 (0.0:0.6)
Use of none of the above over 7 days	n	568	590
	% (95% CI)	94.8 (92.7:96.5)	98.5 (97.2:99.3)
Use of IRS in past 12 months	n	0	0
	% (95% CI)	0 (0.0:0.6)	0 (0.0:0.6)
KeurSocé, SN		**N=600**	**N=600**
	n	8	4

Study site		**Total**	
Use of mosquito coils over 7 days	% (95% CI)	1.3 (0.6:2.6)	0.7 (0.2:1.7)
Use of insecticide sprays over 7 days	n	5	0
	% (95% CI)	0.8 (0.3:1.9)	0 (0.0:0.6)
Use of commercial repellents over 7 days	n	1	1
	% (95% CI)	0.2 (0.0:0.9)	0.2 (0.0:0.9)
Use of traditional repellents over 7 days	n	11	81
	% (95% CI)	1.8 (0.9:3.3)	13.5 (10.9:16.5)
Use of none of the above over 7 days	n	581	514
	% (95% CI)	96.8 (95.1:98.1)	85.7 (82.6:88.4)
Use of IRS in past 12 months	n	286	0
	% (95% CI)	47.7 (43.6:51.7)	0 (0.0:0.6)
Niakhar, SN		**N=598**	**N=601**
Use of mosquito coils over 7 days	n	13	11
	% (95% CI)	2.2 (1.2:3.7)	1.8 (0.9:3.3)
Use of insecticide sprays over 7 days	n	3	4
	% (95% CI)	0.5 (0.1:1.5)	0.7 (0.2:1.7)
Use of commercial repellents over 7 days	n	44	0
	% (95% CI)	7.4 (5.4:9.8)	0 (0.0:0.6)
Use of traditional repellents over 7 days	n	102	22
	% (95% CI)	17.1 (14.1:20.3)	3.7 (2.3:5.5)
Use of none of the above over 7 days	n	477	565
	% (95% CI)	79.8 (76.3:82.9)	94.0 (91.8:95.8)
Use of IRS in past 12 months	n	12	28
	% (95% CI)	2.0 (1.0:3.5)	4.7 (3.1:6.7)
Korogwe, TZ		**N=601**	**N=600**
Use of mosquito coils over 7 days	n	8	26
	% (95% CI)	1.3 (0.6:2.6)	4.3 (2.8:6.3)
Use of insecticide sprays over 7 days	n	0	20
	% (95% CI)	0 (0.0:0.6)	3.3 (2.0:5.1)
Use of commercial repellents over 7 days	n	0	2
	% (95% CI)	0 (0.0:0.6)	0.3 (0.0:1.2)
Use of traditional repellents over 7 days	n	1	1
	% (95% CI)	0.2 (0.0:0.9)	0.2 (0.0:0.9)
Use of none of the above over 7 days	n	592	552
	% (95% CI)	98.5 (97.2:99.3)	92.0 (89.5:94.0)
Use of IRS in past 12 months	n	0	0
	% (95% CI)	0 (0.0:0.6)	0 (0.0:0.6)
Overall		**N=4 208**	**N=4 199**
Use of mosquito coils over 7 days	n	246	305
	% (95% CI)	5.8 (5.2:6.6)	7.3 (6.5:8.1)
Use of insecticide sprays over 7 days	n	52	65
	% (95% CI)	1.2 (0.9:1.6)	1.5 (1.2:2.0)
Use of commercial repellents over 7 days	n	56	22
	% (95% CI)	1.3 (1.0:1.7)	0.5 (0.3:0.8)
Use of traditional repellents over 7 days	n	122	106
	% (95% CI)	2.9 (2.4:3.5)	2.5 (2.1:3.0)
Use of none of the above over 7 days	n	3.779	3.722
	% (95% CI)	89.8 (88.9:90.7)	88.6 (87.6:89.6)
Use of IRS in past 12 months	n	300	42
	% (95% CI)	7.1 (6.4:7.9)	1.0 (0.7:1.3)

N = total number of individuals overall or per site.
n = number of individuals in a given category.
95% CI = Exact 95% confidence limits.
IRS = Indoor Residual Spraying, application of a residual insecticide to internal walls and ceilings of housing structures.[4]
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

5.4. Care-seeking behaviours

SupplementalTable 8 Number of individualspresenting with fever reported in the last 24 hours and measured at visit by study site,*P. falciparum* infection status and survey

Study site		*Pf* infected		*Pf* not infected		Total	
		Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2
Nouna, BF		**N=403**	**N=488**	**N=203**	**N=112**	**N=606**	**N=600**
Fever in the last 24 hours*	n	138	120	38	29	176	149
	% (95% CI)	34.2 (29.6;39.1)	24.6 (20.8;28.7)	18.7 (13.6;24.8)	25.9 (18.1;35.0)	29.0 (25.5;32.8)	24.8 (21.4;28.5)
Fever at visit**	n	29	32	3	5	32	37
	% (95% CI)	7.2 (4.9;10.2)	6.6 (4.5;9.1)	1.5 (0.3;4.3)	4.5 (1.5;10.1)	5.3 (3.6;7.4)	6.2 (4.4;8.4)
Saponé, BF		**N=299**	**N=316**	**N=305**	**N=283**	**N=604**	**N=599**
Fever in the last 24 hours*	n	15	32	7	15	22	47
	% (95% CI)	5.0 (2.8;8.1)	10.1 (7.0;14.0)	2.3 (0.9;4.7)	5.3 (3.0;8.6)	3.6 (2.3;5.5)	7.8 (5.8;10.3)
Fever at visit**	n	13	25	8	8	21	33
	% (95% CI)	4.4 (2.3;7.3)	7.9 (5.2;11.5)	2.6 (1.1;5.1)	2.8 (1.2;5.5)	3.5 (2.2;5.3)	5.5 (3.8;7.7)
Kintampo, GH		**N=214**	**N=212**	**N=386**	**N=388**	**N=600**	**N=600**
Fever in the last 24 hours*	n	102	105	83	142	185	247
	% (95% CI)	47.7 (40.8;54.6)	49.5 (42.6;56.5)	21.5 (17.5;25.9)	36.6 (31.8;41.6)	30.8 (27.2;34.7)	41.2 (37.2;45.2)
Fever at visit**	n	22	22	11	8	33	30
	% (95% CI)	10.3 (6.6;15.2)	10.4 (6.6;15.3)	2.8 (1.4;5.0)	2.1 (0.9;4.0)	5.5 (3.8;7.6)	5.0 (3.4;7.1)
Kombewa, KE		**N=196**	**N=189**	**N=403**	**N=410**	**N=599**	**N=599**
Fever in the last 24 hours*	n	127	146	255	283	382	429
	% (95% CI)	64.8 (57.7;71.5)	77.2 (70.6;83.0)	63.3 (58.4;68.0)	69.0 (64.3;73.5)	63.8 (59.8;67.6)	71.6 (67.8;75.2)
Fever at visit**	n	18	15	14	4	32	19
	% (95% CI)	9.2 (5.5;14.1)	7.9 (4.5;12.8)	3.5 (1.9;5.8)	1.0 (0.3;2.5)	5.3 (3.7;7.5)	3.2 (1.9;4.9)
KeurSocé, SN		**N=3**	**N=6**	**N=597**	**N=594**	**N=600**	**N=600**
Fever in the last 24 hours*	n	0	3	37	15	37	18
	% (95% CI)	0 (0.0;70.8)	50.0 (11.8;88.2)	6.2 (4.4;8.4)	2.5 (1.4;4.1)	6.2 (4.4;8.4)	3.0 (1.8;4.7)
Fever at visit**	n	1	2	42	62	43	64

Study site		*Pf* infected		*Pf* not infected		Total	
		Survey 1	**Survey 2**	**Survey 1**	**Survey 2**	**Survey 1**	**Survey 2**
	% (95% CI)	33.3 (0.8;90.6)	33.3 (4.3;77.7)	7.0 (5.1;9.4)	10.4 (8.1;13.2)	7.2 (5.2;9.5)	10.7 (8.3;13.4)
Niakhar, SN		**N=9**	**N=3**	**N=589**	**N=598**	**N=598**	**N=601**
Fever in the last 24 hours*	n	4	1	131	141	135	142
	% (95% CI)	44.4 (13.7;78.8)	33.3 (0.8;90.6)	22.2 (18.9;25.8)	23.6 (20.2;27.2)	22.6 (19.3;26.1)	23.6 (20.3;27.2)
Fever at visit**	n	1	1	14	10	15	11
	% (95% CI)	11.1 (0.3;48.2)	33.3 (0.8;90.6)	2.4 (1.3;4.0)	1.7 (0.8;3.1)	2.5 (1.4;4.1)	1.8 (0.9;3.3)
Korogwe, TZ		**N=63**	**N=18**	**N=538**	**N=582**	**N=601**	**N=600**
Fever in the last 24 hours*	n	36	6	87	44	123	50
	% (95% CI)	57.1 (44.0;69.5)	33.3 (13.3;59.0)	16.2 (13.2;19.6)	7.6 (5.5;10.0)	20.5 (17.3;23.9)	8.3 (6.2;10.8)
Fever at visit**	n	24	1	15	5	39	6
	% (95% CI)	38.1 (26.1;51.2)	5.6 (0.1;27.3)	2.8 (1.6;4.6)	0.9 (0.3;2.0)	6.5 (4.7;8.8)	1.0 (0.4;2.2)
Overall		**N=1,187**	**N=1,232**	**N=3,021**	**N=2,967**	**N=4,208**	**N=4,199**
Fever in the last 24 hours*	n	422	413	638	669	1,060	1,082
	% (95% CI)	35.6 (32.8;38.4)	33.5 (30.9;36.2)	21.1 (19.7;22.6)	22.5 (21.1;24.1)	25.2 (23.9;26.5)	25.8 (24.5;27.1)
Fever at visit**	n	108	98	107	102	215	200
	% (95% CI)	9.1 (7.5;10.9)	8.0 (6.5;9.6)	3.5 (2.9;4.3)	3.4 (2.8;4.2)	5.1 (4.5;5.8)	4.8 (4.1;5.5)

*Pf*infected = Individuals infected with *P. falciparum*parasitemia measured by microscopy.
Pf not infected = Individuals not infected with *P. falciparum*parasitemia measured by microscopy.
N = total number of individualsoverall or per site.
n = number of individuals in a given category.
95%CI = exact 95% confidence limits.
*Fever in the 24h prior to the visit reported during the visit.
**Temperature recorded at visit after axillary conversion ≥ 37.5°C.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN= Senegal; TZ = Tanzania.

Supplemental Table 9 Number of individuals presenting with feverreported in the last 24 hours and measured at visit by study site,parasite density measured by microscopyand survey

Study site	Parasitemia	Negative		Low		Medium		High		Very high	
		Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2
Nouna, BF		**N=203**	**N=112**	**N=198**	**N=307**	**N=105**	**N=100**	**N=48**	**N=39**	**N=52**	**N=42**
Fever in the last 24 hours*	n	38	29	44	67	39	18	24	13	31	22
	% (95% CI)	18.7 (13.6;24.8)	25.9 (18.1;35.0)	22.2 (16.6;28.7)	21.8 (17.3;26.9)	37.1 (27.9;47.1)	18.0 (11.0;26.9)	50.0 (35.2;64.8)	33.3 (19.1;50.2)	59.6 (45.1;73.0)	52.4 (36.4;68.0)
Fever at visit**	n	3	5	5	12	3	3	7	3	14	14
	% (95% CI)	1.5 (0.3;4.3)	4.5 (1.5;10.1)	2.5 (0.8;5.8)	3.9 (2.0;6.7)	2.9 (0.6;8.1)	3.0 (0.6;8.5)	14.6 (6.1;27.8)	7.7 (1.6;20.9)	26.9 (15.6;41.0)	33.3 (19.6;49.5)
Saponé, BF		**N=305**	**N=283**	**N=167**	**N=151**	**N=81**	**N=93**	**N=32**	**N=31**	**N=19**	**N=41**
Fever in the last 24 hours*	n	7	15	6	6	5	11	0	4	4	11
	% (95% CI)	2.3 (0.9;4.7)	5.3 (3.0;8.6)	3.6 (1.3;7.7)	4.0 (1.5;8.4)	6.2 (2.0;13.8)	11.8 (6.1;20.2)	0 (0.0;10.9)	12.9 (3.6;29.8)	21.1 (6.1;45.6)	26.8 (14.2;42.9)
Fever at visit**	n	8	8	4	4	3	7	2	4	4	10
	% (95% CI)	2.6 (1.1;5.1)	2.8 (1.2;5.5)	2.4 (0.7;6.1)	2.6 (0.7;6.6)	3.7 (0.8;10.4)	7.5 (3.1;14.9)	6.3 (0.8;20.8)	12.9 (3.6;29.8)	21.1 (6.1;45.6)	24.4 (12.4;40.3)
Kintampo, GH		**N=386**	**N=388**	**N=123**	**N=118**	**N=39**	**N=38**	**N=16**	**N=23**	**N=36**	**N=33**
Fever in the last 24 hours*	n	83	142	49	43	19	23	8	14	26	25
	% (95% CI)	21.5 (17.5;25.9)	36.6 (31.8;41.6)	39.8 (31.1;49.1)	36.4 (27.8;45.8)	48.7 (32.4; 65.2)	60.5 (43.4;76.0)	50.0 (24.7;75.3)	60.9 (38.5;80.3)	72.2 (54.8;85.8)	75.8 (57.7;88.9)
Fever at visit**	n	11	8	9	3	1	3	1	4	11	12
	% (95% CI)	2.8 (1.4;5.0)	2.1 (0.9;4.0)	7.3 (3.4;13.4)	2.5 (0.5;7.3)	2.6 (0.1;13.5)	7.9 (1.7;21.4)	6.3 (0.2;30.2)	17.4 (5.0;38.8)	30.6 (16.3;48.1)	36.4 (20.4;54.9)
Kombewa, KE		**N=403**	**N=410**	**N=100**	**N=84**	**N=42**	**N=44**	**N=25**	**N=15**	**N=29**	**N=46**
Fever in the last 24 hours*	n	255	283	64	61	21	33	16	13	26	39
	% (95% CI)	63.3 (58.4;68.0)	69.0 (64.3;73.5)	64.0 (53.8;73.4)	72.6 (61.8;81.8)	50.0 (34.2;65.8)	75.0 (59.7;86.8)	64.0 (42.5;82.0)	86.7 (59.5;98.3)	89.7 (72.6;97.8)	84.8 (71.1;93.7)
Fever at visit**	n	14	4	2	1	4	3	2	2	10	9
	% (95% CI)	3.5 (1.9;5.8)	1.0 (0.3;2.5)	2.0 (0.2;7.0)	1.2 (0.0;6.5)	9.5 (2.7;22.6)	6.8 (1.4;18.7)	8.0 (1.0;26.0)	13.3 (1.7;40.5)	34.5 (17.9;54.3)	19.6 (9.4;33.9)

Study site	Parasitemia	Negative		Low		Medium		High		Very high	
		Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2
KeurSocé, SN		**N=597**	**N=594**	**N=2**	**N=2**	**-**	**N=3**	**N=1**	**-**	**-**	**N=1**
Fever in the last 24 hours*	n	37	15	0	0	-	2	0	-	-	1
	% (95% CI)	6.2 (4.4;8.4)	2.5 (1.4;4.1)	0 (0.0;84.2)	0 (0.0;84.2)	-	66.7 (9.4;99.2)	0 (0.0;97.5)	-	-	100 (2.5;100)
Fever at visit**	n	42	62	0	1	-	0	1	-	-	1
	% (95% CI)	7.0 (5.1;9.4)	10.4 (8.1;13.2)	0 (0.0;84.2)	50.0 (1.3;98.7)	-	0 (0.0;70.8)	100 (2.5;100)	-	-	100 (2.5;100)
Niakhar, SN		**N=589**	**N=598**	**N=6**	**N=1**	**N=1**	**-**	**N=1**	**N=1**	**N=1**	**N=1**
Fever in the last 24 hours*	n	131	141	2	0	0	-	1	0	1	1
	% (95% CI)	22.2 (18.9;25.8)	23.6 (20.2;27.2)	33.3 (4.3;77.7)	0 (0.0;97.5)	0 (0.0;97.5)	-	100 (2.5;100)	0 (0.0;97.5)	100 (2.5;100)	100 (2.5;100)
Fever at visit**	n	14	10	0	0	0	-	0	0	1	1
	% (95% CI)	2.4 (1.3;4.0)	1.7 (0.8;3.1)	0 (0.0;45.9)	0 (0.0;97.5)	0 (0.0;97.5)	-	0 (0.0;97.5)	0 (0.0;97.5)	100 (2.5;100	100 (2.5;100)
Korogwe, TZ		**N=538**	**N=582**	**N=24**	**N=10**	**N=18**	**N=2**	**N=3**	**N=2**	**N=18**	**N=4**
Fever in the last 24 hours*	n	87	44	9	3	10	1	2	1	15	1
	% (95% CI)	16.2 (13.2;19.6)	7.6 (5.5;10.0)	37.5 (18.8;59.4)	30.0 (6.7;65.2)	55.6 (30.8;78.5)	50.0 (1.3;98.7)	66.7 (9.4;99.2)	50.0 (1.3;98.7)	83.3 (58.6;96.4)	25.0 (0.6;80.6)
Fever at visit**	n	15	5	3	1	6	0	1	0	14	0
	% (95% CI)	2.8 (1.6;4.6)	0.9 (0.3;2.0)	12.5 (2.7;32.4)	10.0 (0.3;44.5)	33.3 (13.3;59.0)	0 (0.0;84.2)	33.3 (0.8;90.6)	0 (0.0;84.2)	77.8 (52.4;93.6)	0 (0.0;60.2)
Overall		**N=3,021**	**N=2,967**	**N=620**	**N=673**	**N=286**	**N=280**	**N=126**	**N=111**	**N=155**	**N=168**
Fever in the last 24 hours*	n	638	669	174	180	94	88	51	45	103	100
	% (95% CI)	21.1 (19.7;22.6)	22.5 (21.1;24.1)	28.1 (24.6;31.8)	26.7 (23.4;30.3)	32.9 (27.5;38.6)	31.4 (26.0;37.2)	40.5 (31.8;49.6)	40.5 (31.3;50.3)	66.5 (58.4;73.8)	59.5 (51.7;67.0)
Fever at visit**	n	107	102	23	22	17	16	14	13	54	47
	% (95% CI)	3.5 (2.9;4.3)	3.4 (2.8;4.2)	3.7 (2.4;5.5)	3.3 (2.1;4.9)	5.9 (3.5;9.3)	5.7 (3.3;9.1)	11.1 (6.2;17.9)	11.7 (6.4;19.2)	34.8 (27.4;42.9)	28.0 (21.3;35.4)

Low = <2,500 parasites/ µL; Medium = 2,500 – 9,999 parasites/ µL; High 10,000 – 19,999 parasites/ µL; Very high ≥ 20,000 parasites/ µL.
N= total number of individualsoverall or per site.
n= number of individuals in a given category.

95%CI = Exact 95% confidence limits.
*Fever in the 24h prior to the visit reported during the visit.
**Temperature recorded at visit after axillary conversion ≥ 37.5°C.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

Supplemental Table 10 Number of individualshaving sought treatment for malaria or fever in thepast 14 days and individuals hospitalized for malaria in the last 3 months by study site, *P. falciparum* infection status and survey

Study site		*Pf* infected		*Pf* not infected		Total	
		Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2
Nouna, BF		**N=403**	**N=488**	**N=203**	**N=112**	**N=606**	**N=600**
Seeking malaria or fever treatment in past 14 days	n	132	125	70	33	202	158
	% (95% CI)	32.8 (28.2;37.6)	25.6 (21.8;29.7)	34.5 (28.0;41.5)	29.5 (21.2;38.8)	33.3 (29.6;37.2)	26.3 (22.8;30.1)
Malaria hospitalization in the last 3 months	n	7	12	5	3	12	15
	% (95% CI)	1.7 (0.7;3.5)	2.5 (1.3;4.3)	2.5 (0.8;5.7)	2.7 (0.6;7.6)	2.0 (1.0;3.4)	2.5 (1.4;4.1)
Saponé, BF		**N=299**	**N=316**	**N=305**	**N=283**	**N=604**	**N=599**
Seeking malaria or fever treatment in past 14 days	n	12	21	25	25	37	46
	% (95% CI)	4.0 (2.1;6.9)	6.6 (4.2;10.0)	8.2 (5.4;11.9)	8.8 (5.8;12.8)	6.1 (4.3;8.3)	7.7 (5.7;10.1)
Malaria hospitalization in the last 3 months	n	2	4	4	5	6	9
	% (95% CI)	0.7 (0.1;2.4)	1.3 (0.3;3.2)	1.3 (0.4;3.3)	1.8 (0.6;4.1)	1.0 (0.4;2.1)	1.5 (0.7;2.8)
Kintampo, GH		**N=214**	**N=212**	**N=386**	**N=388**	**N=600**	**N=600**
Seeking malaria or fever treatment in past 14 days	n	32	67	118	131	150	198
	% (95% CI)	15.0 (10.5;20.4)	31.6 (25.4;38.3)	30.6 (26.0;35.4)	33.8 (29.1;38.7)	25.0 (21.6;28.7)	33.0 (29.2;36.9)
Malaria hospitalization in the last 3 months	n	9	17	33	38	42	55
	% (95% CI)	4.2 (1.9;7.8)	8.0 (4.7;12.5)	8.5 (6.0;11.8)	9.8 (7.0;13.2)	7.0 (5.1;9.3)	9.2 (7.0;11.8)
Kombewa, KE		**N=196**	**N=189**	**N=403**	**N=410**	**N=599**	**N=599**
Seeking malaria or fever treatment in past 14 days	n	42	30	126	76	168	106
	% (95% CI)	21.4 (15.9;27.8)	15.9 (11.0;21.9)	31.3 (26.8;36.0)	18.5 (14.9;22.6)	28.0 (24.5;31.8)	17.7 (14.7;21.0)
Malaria hospitalization in the last 3 months	n	11	9	21	24	32	33
	% (95% CI)	5.6 (2.8;9.8)	4.8 (2.2;8.8)	5.2 (3.3;7.9)	5.9 (3.8;8.6)	5.3 (3.7;7.5)	5.5 (3.8;7.7)
KeurSocé, SN		**N=3**	**N=6**	**N=597**	**N=594**	**N=600**	**N=600**
Seeking malaria or fever treatment in past 14 days	n	0	0	0	0	0	0
	% (95% CI)	0 (0.0;70.8)	0 (0.0;45.9)	0 (0.0;0.6)	0 (0.0;0.6)	0 (0.0;0.6)	0 (0.0;0.6)
Malaria hospitalization in the last 3 months	n	0	0	1	1	1	1
	% (95% CI)	0 (0.0;70.8)	0 (0.0;45.9)	0.2 (0.0;0.9)	0.2 (0.0;0.9)	0.2 (0.0;0.9)	0.2 (0.0;0.9)

Study site		*Pf* infected		*Pf* not infected		Total	
		Survey 1	Survey 2	Survey 1	Survey 2	Survey 1	Survey 2
Niakhar, SN		**N=9**	**N=3**	**N=589**	**N=598**	**N=598**	**N=601**
Seeking malaria or fever treatment in past 14 days	n	0	0	6	0	6	0
	% (95% CI)	0 (0.0;33.6)	0 (0.0;70.8)	1.0 (0.4;2.2)	0 (0.0;0.6)	1.0 (0.4;2.2)	0 (0.0;0.6)
Malaria hospitalization in the last 3 months	n	0	0	0	0	0	0
	% (95% CI)	0 (0.0;33.6)	0 (0.0;70.8)	0 (0.0;0.6)	0 (0.0;0.6)	0 (0.0;0.6)	0 (0.0;0.6)
Korogwe, TZ		**N=63**	**N=18**	**N=538**	**N=582**	**N=601**	**N=600**
Seeking malaria or fever treatment in past 14 days	n	20	4	79	25	99	29
	% (95% CI)	31.7 (20.6;44.7)	22.2 (6.4;47.6)	14.7 (11.8;18.0)	4.3 (2.8;6.3)	16.5 (13.6;19.7)	4.8 (3.3;6.9)
Malaria hospitalization in the last 3 months	n	4	0	12	6	16	6
	% (95% CI)	6.3 (1.8;15.5)	0 (0.0;18.5)	2.2 (1.2;3.9)	1.0 (0.4;2.2)	2.7 (1.5;4.3)	1.0 (0.4;2.2)
Overall		**N=1,187**	**N=1,232**	**N=3,021**	**N=2,967**	**N=4,208**	**N=4,199**
Seeking malaria or fever treatment in past 14 days	n	238	247	424	290	662	537
	% (95% CI)	20.1 (17.8;22.4)	20.0 (17.8;22.4)	14.0 (12.8;15.3)	9.8 (8.7;10.9)	15.7 (14.6;16.9)	12.8 (11.8;13.8)
Malaria hospitalization in the last 3 months	n	33	42	76	77	109	119
	% (95% CI)	2.8 (1.9;3.9)	3.4 (2.5;4.6)	2.5 (2.0;3.1)	2.6 (2.1;3.2)	2.6 (2.1;3.1)	2.8 (2.4;3.4)

*Pf*infected = Individuals infected with *P. falciparum*parasitemia measured by microscopy.
Pf not infected = Individuals not infected with *P. falciparum*parasitemia measured by microscopy.
N = total number of individuals overall or per site.
n = number of individualsin a given category.
95% CI = Exact 95% confidence limits.
BF = Burkina Faso; GH = Ghana; KE = Kenya; SN = Senegal; TZ = Tanzania.

5.5. *P. falciparum* infection risk factors analysis

Supplemental Table 11 Risk factors of being infected with *P. falciparum* (as assessed by microscopy)derived from the fitted logistic regression model with study site as cluster (Survey 1)

Characteristics	Category	Reference category	Odds ratio (OR)	95% CI of OR
Age (in years)*	Continuous		1.141	[1.048;1.242]
Antimalarial drugs consumed in the last 14 days	Yes	No	0.614	[0.376;1.002]
Antimalarial or any other medication within 14 days	Yes	No	1.033	[0.772;1.383]
Main house construction material: Nets	Nets present on all windows	Nets not present	0.729	[0.631;0.842]
	Nets present on some windows	Nets not present	1.003	[0.688;1.463]
	Other	Nets not present	1.130	[1.019;1.252]
Main house construction material: Roof	Iron sheet	Grass/Palm	0.888	[0.753;1.046]
	Tiles	Grass/Palm	1.090	[0.786;1.513]
	Clay	Grass/Palm	1.824	[1.593;2.088]
	Other	Grass/Palm	1.175	[1.063;1.300]
Main house construction material: Walls	Brick	Mud	0.860	[0.747;0.990]
	Cement/Plaster	Mud	0.811	[0.685;0.961]
	Cement /Paint	Mud	0.842	[0.638;1.113]
	Clay	Mud	0.612	[0.214;1.752]
	Other	Mud	0.622	[0.476;0.812]
Main house construction material: Windows/eaves	Closed	Open	0.984	[0.845;1.144]
	No Windows	Open	0.959	[0.810;1.137]
	Partially open	Open	0.987	[0.794;1.228]
	Other	Open	1.374	[1.234;1.530]
Number of holes	< 5	≥5	0.921	[0.712;1.191]
	No bednet	≥5	1.044	[0.740;1.473]
	No pierced bednet	≥5	1.055	[0.813;1.369]
Number of persons living in the same part of the house	4-5	≤3	1.168	[1.000;1.364]
	>5	≤3	1.317	[1.165;1.488]
Pierced/torn bednet	No bednet	No	1.000	[1.000;1.000]
	Yes	No	1.000	[1.000;1.000]
Presence of electricity	Yes	No	0.752	[0.611;0.926]
Use of traditional repellents over 7 days	Yes	Missing/No	0.994	[0.907;1.088]

Note: Odds ratios were adjusted by a cluster variable.
95% CI = Exact 95% confidence limits.
The fitted logistic regression model was only applied on significant variables produced by the backward selection.
The variables 'Floor', 'number of persons enrolled into the study' and 'localisation' were not included in the model due to convergence issue.
*Age was introduced in the model as a continuous variable. The corresponding OR is given for a 1 year increase of age.

Supplemental Table 12 Risk factors of being infected with *P. falciparum* (as assessed by microscopy)derived from the fitted logistic regression model with study site as cluster (Survey 2)

Characteristics	Category	Reference category	Odds ratio (OR)	95% CI of OR
Age (in years)*	Continuous		1.087	[1.018;1.161]
Antimalarial drug consumed in the past 14 days	Yes	No	0.457	[0.324;0.645]
Impregnated Bednet	No Bednet	No	1.000	[1.000;1.000]
	Yes	No	1.093	[0.887;1.345]
Localization	Semi- Rural Area	Rural area	0.741	[0.419;1.308]
	Urban area	Rural area	0.185	[0.109;0.314]
Main house construction material: Nets	Nets present on all windows	Nets not present	0.787	[0.660;0.939]
	Nets present on some windows	Nets not present	0.947	[0.901;0.995]
	Other	Nets not present	1.007	[0.886;1.143]
Main house construction material: Roof	Iron sheet	Grass/Palm	0.953	[0.818;1.111]
	Tiles	Grass/Palm	0.995	[0.703;1.407]
	Clay	Grass/Palm	1.011	[0.913;1.120]
	Other	Grass/Palm	1.236	[0.786;1.943]
Main house construction material: Walls	Brick	Mud	0.922	[0.783;1.085]
	Cement/Plaster	Mud	0.866	[0.757;0.992]
	Cement /Paint	Mud	0.990	[0.853;1.148]
	Clay	Mud	1.197	[1.120;1.278]
	Other	Mud	0.658	[0.415;1.044]
Main source of drinking water	Closed water source†	Open water source††	0.921	[0.835;1.015]
Malaria or fever treatment sought for in the past 14 days	Yes	No	1.227	[1.047;1.439]
New net (less than 1 year)	No Bednet	No	1.095	[0.901;1.330]
	Yes	No	0.929	[0.839;1.029]
Pierced/torn bednet	No Bednet	No	1.000	[1.000;1.000]
	Yes	No	0.912	[0.798;1.042]
Presence of electricity	Yes	No	0.885	[0.803;0.976]
Use of traditional repellents over 7 days	Yes	Missing/No	0.910	[0.747;1.109]
No use of mosquito coils–insecticide sprays–commercial or traditional repellents over 7 days	Yes	Missing/No	0.879	[0.782;0.988]

Note: Odds ratios (ORs) were adjusted by a cluster variable.
95% CI = Exact 95% confidence limits.
The fitted logistic regression model was only applied on significant variables produced by the backward selection.
The variables 'Number of persons living in the same part of the house', 'Type of location', 'Floor', 'Windows/eaves' and 'Use of Insecticide sprays over 7 days' were not included in the model due to convergence issue.
*Age was introduced in the model as a continuous variable. The corresponding OR is given for a 1 year increase of age.
†Closed water source (piped water, tube well, dug well, protected well).
††Open water source (unprotected well, spring water, rainwater, tanker truck, surface water).

REFERENCES

1. World Health Organization. Basic Malaria Microscopy Part I: Learner's Guide. Available at http://whqlibdoc.who.int/publications/1991/9241544309.pdf.
2. Hermsen CC, Telgt DS, Linders EH, van de Locht LA, Eling WM, Mensink EJ, Sauerwein RW, 2001. Detection of Plasmodium falciparum malaria parasites in vivo by real-time quantitative PCR. Mol Biochem Parasitol. 118:247-251.
3. Schneider P, Schoone G, Schallig H, Verhage D, Telgt D, Eling W, Sauerwein R, 2004. Quantification of Plasmodium falciparum gametocytes in differential stages of development by quantitative nucleic acid sequence-based amplification. Mol Biochem Parasitol. 137:35-41.
4. World Health Organization. Indoor residual spraying: An operational manual for IRS for malaria transmission, control and elimination. Available at https://www.who.int/malaria/publications/atoz/9789241508940/en/.