Some evidence that seasonal malaria chemoprevention with SPAQ has an effect on blood stage antibody responses and pre-erythrocytic stage of Plasmodium falciparum infections in Niger.

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Research

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Abstract

**Background:** In endemic areas, children develop slowly and naturally develop *anti-Plasmodium* antibodies and become semi-immune. Seasonal Malaria Chemoprevention (SMC) with sulfadoxine-pyrimethamine + amodiaquine (SPAQ) is a new strategy to reduce malaria morbidity in young children in West Africa. However, SMC may impact on the natural acquisition of anti-*Plasmodium* immunity. We evaluated the effect of SMC with SPAQ on malaria antibody concentration in Niger.

**Methods:** This survey was conducted in areas targeted with SMC since 2014 (Zinder district), without SMC (Dosso district), and with one year SMC 2016 (Gaya district). To assess the relationship between SMC and *P. falciparum* IgG antibody responses, we compared total antibody concentrations against two *P. falciparum* asexual stage vaccine candidate antigens, circumsporozoite protein (CSP) and glutamate-rich protein R2 (GLURP-R2), in children aged 3-59 months across the three sites. Antibody concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) on the elution extracted from positive and negative RDT cassettes.

**Results:** A total of 229 children aged 3-59 months were included in the analysis: 71 in Zinder, 77 in Dosso, and 81 in Gaya. In Zinder (CSP=17.5µg/ml and GLURP-R2=14.3µg/ml) median antibody concentration observed were higher than in Gaya (CSP=7.7 µg/ml and GLURP-R2=6.5 µg/ml) and Dosso (CSP=4.5 µg/ml and GLURP-R2=3.6 µg/ml) (p<0.0001).

**Conclusion:** We have some evidence that seasonal malaria chemoprevention with SPAQ has an effect on blood stage antibody responses and pre-erythrocytic stage of *Plasmodium falciparum* infections in Niger. Future studies are necessary to provide better understanding of the effect of on malaria immunity.

**Background**

Malaria caused by *Plasmodium falciparum* remains the major cause of morbidity and mortality in children under 5 years in Sub-Saharan Africa [1]. It is the main public health problem in Niger [2]. The national malaria control program of Niger has implemented complementary malaria control strategies based on WHO recommendations, including seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine + amodiaquine (SPAQ) [3],[4, 5]. SMC is an administration of full therapeutic doses of these drugs to children aged 3-59 months as monthly interval during period of greatest malaria risk in endemic areas [6]. In these areas, children develop slowly and naturally *anti-Plasmodium* antibodies and become semi-immune [7],[8].

Sulfadoxine is an antibacterial and antimalarial of the chemical class of sulfonamides. It is a dihydropteroate synthetase (dhps) inhibitor, a key enzyme in the biosynthesis of folate. It acts by competitive inhibition of para amino benzoic acid (PABA) to block the synthesis of folic acid and *Plasmodium* nucleotides (purines and pyrimidines). The pyrimethamine associated with sulfadoxine is a competitive inhibitor of dihydrofolate reductase (dhfr), a key enzyme in the redox cycle for the production
of tetrahydrofolate, a cofactor necessary for the biosynthesis of DNA and proteins. SP is acts on the asexual forms of the hepatic and erythrocytic stage of *Plasmodium*.

Amodiaquine is an antimalarial with antipyretic and anti-inflammatory properties. It is a related 4-aminoquinoline related in structure and activity with chloroquine. Amodiaquine is active on the erythrocyte form of *Plasmodium*.

Malaria immunity is partial, short-lived, and requires exposure to infected mosquitoes bites to be maintained [7]. Monthly given SMC reduce malaria morbidity in young children in West Africa [5, 9–11]. However, SMC may impact on the natural acquisition of anti-*Plasmodium* immunity. In Senegal, Ndiaye et al., suggested that long-term SMC by SPAQ has limited impact on the development of acquired immunity [12]. In the same country, Sylla et al., showed that SMC with SPAQ can induce decreasing of IgG anti-AMA1 and anti-MSP1$_{42}$ [13]. In Mali, Mahamar et al., concluded that exposure to SMC/SPAQ decreasing of anti-AMA-1, MSP1$_{42}$ and CSP titers [14]. Other Malian study conclude that duration of exposure to SMC had no effect on antibody to MSP1$_{42}$ and CSP [14].

The hypothesis of this study was that SMC/SPAQ could reduce immunity to erythrocyte stage antigens but not to liver-stage malaria antigens, and RDTs materials could be used to measure IgG titers. To assess the relationship between SMC and *P. falciparum* antibody responses, we compared total IgG concentrations against two *P. falciparum* asexual stage vaccine candidate antigens, circumsporozoite protein (CSP) and glutamate-rich protein R2 (GLURP-R2), in children aged 3-59 months across the three sites. Antibody concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) on the elution extracted from positive and negative RDT cassettes.

**Methods**

**Study design and sample collection**

The data presented here were generated from the malaria morbidity sentinel surveillance sites within the SMC program in Zinder, Dosso and Gaya districts located in western Niger, where malaria transmission is seasonal[15, 16]. Zinder and Gaya districts have implemented SMC with SPAQ respectively since 2014 and 2016; they were classified as mesoendemic and hyperendemic areas[15, 17]. SMC was not implemented in Dosso district, which was classified as hyperendemic and as a control district of the study[15, 17]. In 2016, SMC coverage in Zinder received once, at least 3 times and 4 times was 91%, 73% and 50%, respectively (Unpublished data). The coverage of Gaya district were 77.72%, 81.56%, 71.26%, and 69.47 respectively for round 1, 2, 3 and 4 (Unpublished data). All the 3 sites used ACTs as first line treatment for uncomplicated malaria and received universal coverage of bed nets. The seasonality of malaria transmission in these 3 sites is the same.

To assess the impact of SMC on the titer of antibodies to two asexual *P. falciparum* stage antigens, 6 health facilities in Zinder, Dosso and Gaya were selected. In this health facilities, malaria RDTs (SD Bioline) of randomly selected children aged 3-59 months were collected from symptomatic cases (fever +
positive or negative RDTs) for serological analysis. For all RDTs collected, the date of consultation, the age, the gender, whether a test was performed and the result (positive or negative) were reported on the cassette. Samples were collected all three month at the same time in all sites between November 2015 to December 2016. The RDTs was stored at room temperature. The analyses were performed in April 2017. The average time of the RDT before testing across the sites was: 06 month for Zinder, 05 month for Dosso and 7 month for Gaya.

The total number of children to include was 249, and calculated based on 78% circumsporozoite protein antibody prevalence in children that received SMC for 1, 2 or 3 years [14] (95% CI) with a precision of 5%.

**Recombinant antigens**

The malaria antigens used in this study included a recombinant antigen circumsporozoite protein (CSP) and glutamate-rich protein R2 (GLURP-R2). CSP antigen was a 44-aa NANP repeat-sequence peptide of the *P. falciparum* circumsporozite protein synthesised by Sygma Genosys, while GLURP-R2 (amino acids 706-1178, F32 strain) was produced at the Statens Serum Institutes of Copenhagen (Denmark) and was expressed in *Escherichia coli*.

**Sera extraction**

Sera were extracted from filter paper inside RDTs cassette collected [18]. RDTs have proximal, middle and distal parts according Cnops et al., description [19]. The distal part of RDT contains a filter paper component that absorbed the residual blood solution. The cassettes was opened by sterile tweezers and distal part of each RDT was cut with sterile scissors in two or three pieces about 2 mm and eluted (all pieces obtained) into 300 µl of Phosphate Buffered Saline (PBS) from this fragment placed in 1.5ml Eppendorf tubes. The solution was stored at 4°C overnight. The solution is equivalent to 1/100 dilution of whole blood, with about half of the concentration of antibodies in plasma or serum resulting to a dilution of 1/200 [18]. The elations CSP and GLURP-R2 total IgG antibody responses were quantified using ELISA [20].

**Antibody measurements**

The standard operating procedure developed by the African Malaria Network Trust was used to assess total IgG concentrations by Enzyme Linked Immuno Sorbent Assay (ELISA) to CSP and GLURP R2 as described previously [21]. Briefly, recombinant proteins (0.1 µg/well) diluted in Phosphate Buffered Saline (PBS) were coated on MaxiSorp Nunc plates (Thermo Fisher Scientific, Denmark) and blocked with 3% powdered-milk + 0.1% of PBS-Tween 20. Sera samples were diluted at 1:200 for all recombinant proteins. Polyclonal goat anti-human IgG (Gamma) (Caltag) conjugated to HRPO diluted 1:3000 (Skybio, France) was used for revealing the reaction with 3,3',5,5'-tétraméthylbenzidine TMB as substrate and 0.2 M H$_2$SO$_4$ to stop the reaction. Standard curves were established using human IgG purified proteins (Binding Site, France) to determine the concentration of specific antibodies. Each point was tested in duplicate. Concentrations of standard IgG were: 500; 250; 125; 62.5; 31.3; 15.6; 7.8; and 3.9 µg/ml. The ADAMSEL
FLP b039 software [22] was used to analyse the optical density (OD) of the plates at 450nm and interpolate the standard curve (µg/ml). Discordant duplicates (with a variation coefficient >15%) were dropped.

**Statistical analysis**

The Median test was used to analyze differences between IgG medians concentrations. The comparisons between IgG median concentrations in Zinder, Dosso and Gaya were performed to investigate the potential impact of SMC on Antibody responses. The comparison between IgG median concentrations were performed by Mann-Whitney test. Data were analyzed with SPSS software version 16.0. P-values ≤ 0.05 were considered were considered statistically significant.

**Results**

**Population characteristics**

A total of 229 children aged 3-59 months were included in the analysis: 71 from Zinder, 77 from Dosso, and 81 from Gaya (Figure 1). The number of samples categorized by mean age and sex was comparable between districts and differences were seen between RDT results (Table 1).

| Characteristic               | ZINDER (N=71) | DOSSO (N=77) | GAYA (N=81) |
|-----------------------------|---------------|--------------|-------------|
| **AGE (months)**            |               |              |             |
| Mean age                    | 30.2 ± 17.5   | 25.4 ±14.5   | 25.0 ±15.4  |
| **SEX (%)**                 |               |              |             |
| M                           | 53            | 50           | 63          |
| F                           | 47            | 50           | 37          |
| **RDTs results (%)**        |               |              |             |
| Positive                    | 60.3          | 66.2         | 44.44       |
| Negative                    | 39.7          | 33.8         | 55.56       |

Zinder district (SMC since 2014), Dosso district (No SMC) and Gaya district (SMC 2016).

Using ANOVA test no statistical difference of mean age between the sites was showed (p=0.11).

**Anti-CSP and GLURP-R2 IgG median antibody concentrations by districts**
In Zinder where SMC (D1) has been implemented since 2014, anti-CSP and GLURP-R2 IgG antibodies titers were higher. A low concentration of IgG antibody CSP=4.5 µg/ml and GLURP-R2=3.6 µg/ml was observed in Dosso that never received SMC (D2).

In Zinder with SMC since 2014 (CSP=17.5 µg/ml and GLURP-R2=14.3 µg/ml) and Dosso with no SMC (CSP=4.5 µg/ml and GLURP-R2=3.6 µg/ml), median concentration of IgG antibody responses was significantly different for the two antigens by districts (figure 2 and 3). No significant difference in median concentration of all antibodies was shown between Dosso (CSP=4.5 µg/ml and GLURP-R2=3.6 µg/ml) and Gaya (CSP=7.7 µg/ml and GLURP-R2=6.5 µg/ml) ($p=0.05$ and $p=0.05$).

Analysis of the differences between the median concentration of anti-CSP IgG and anti-GLURP-R2 in Zinder and Gaya (D3) showed a significant difference (CSP: $p=0.008$ and GLURP-R2: $p=0.017$).

**Anti-CSP and GLURP-R2 IgG median antibody concentrations by RDT results**

The median concentrations of anti-CSP and GLURP-R2 IgG antibodies by RDTs results are in table 3. When subdividing the groups into those that were RDTs positive or negative and compared the differences between the median concentrations of antibodies responses against CSP and GLURP-R2 in each groups, no significant differences were observed ($p=0.093$ and $p=0.539$).

| Antibodies  | RDT+ | RDT- | $P$   |
|-------------|------|------|-------|
| CSP         | 9.4 (IQR : 29.9) | 6.5 (IQR : 16.4) | 0.093 |
| GLURP-R2    | 8.3 (IQR : 35.4) | 6.0 (IQR : 20.1) | 0.539 |

RDT+= Positive rapid diagnostic test and RDT-= Negative rapid diagnostic test. IQR: Interquartile range.

Comparison was performed by Mann-Whitney test. The significance limit was $p<0.05$.

**Discussion**

This study assessed the impact of SMC on the antibody titers to *P. falciparum* antigens CSP and GLURP-R2 in children aged 3-59 months in areas that received SMC for different time period. The total IgG antibodies to liver-stage vaccine candidate antigen CSP and blood stage antigen GLURP-R2) were significantly higher in Zinder where SMC was implemented since 3 years, than Gaya where SMC has been implemented for one year, and Dosso, which has never received SMC. This is consistent with a previous
intriguing finding that demonstrated sustained protection during one year of follow-up in children who had receive malaria intermittent preventive treatment [23]. SMC was primarily directed against blood-stage parasites, because sulfadoxine-pyrimethamine and amodiaquine association inhibits the erythrocyte stage and liver-stage, which may contribute to increase the titer of IgG against the antigen of these stages. This finding is contrasting with a recent study published SMC reduced antibody against liver-stage antigens MSP-142 and CSP in malaria journal by Mahamar et al [14]. Others studies demonstrated that sulfadoxine does not affect liver stages, pyrimethamine has some inhibitory effect on liver stages in P. yoelii models [24] but there are high levels of resistance to pyrimethamine in SMC countries [25]. In Niger a high prevalence (> 60%) of mutations N51I, C59R and S108N in Pfdhfr gene, known to be associated with resistance to pyrimethamine was observed [26]. Amodiaquine acts on blood stage parasites [24]. In vaccine trial cohorts in the Gambia [27] SP did not affect the incidence of low level P. falciparum infections detected by PCR, consistent with SP affecting blood stage and not liver stage parasites.

The concentrations of both antibodies against CSP and GLURP-R2 showed an increase with SMC implementation probably as a result of decrease of either liver-stage maturation or erythrocyte stage by SPAQ. This is contrasting with other studies [14], [13], [28], [12] which found a decrease in the titers of antibodies after SMC delivery. Previous studies established that chemoprophylaxis conferred protective immunity against reinfections when anti-malaria drugs are not present [29],[30], [31]. The inhibitory effect of SP on pre erythrocyte stage [32],[33] and AQ on erythrocyte stage was previously described [34]. Friesen J et al., showed in the redone malaria model induction of antimalarial immunity by pyrimethamine prophylaxis during exposure to sporozoites and attenuation by pyrimethamine permits hepatocyte invasion but appears to block intrahepatocytic replication [29]. The increase in antibodies concentration is believe to be linked with exposure of the immune system to an attenuate hepatic stage parasites or complete suppression of blood-stage parasites, thereby resulting in an increase of IgG antibody concentration to CSP and GLURPR2 antigen in the sites where SMC was implemented.

This is the first used of antibody elution from RDT filter paper for the assessment of CSP and GLURPR2 antibody concentration in Niger. As demonstrated by Amrish Baidjoe et al, antibody elution from filter paper is an operationally attractive approach[18]. RDT cassette can be use to monitor molecular markers of malaria drugs resistance and study antimalarial immunity in Niger.

The comparative analysis of antibodies concentration against CSP and GLURP-R2 antigens between age groups by district, observed no significant difference (CSP: $p= 0.6813$; GLURP-R2: $p= 0.0760$). This difference was not statically significant, perhaps because of the small number of samples and the short period of following, which is a limitation of this study. There are some important limitations, we do not know have confirmation of SMC status of all children included in the study, the sample size is small, and from only three districts, and only two marker of immunity has been measured. Also we are not able to interpret the immune responses in terms of implications for the risk of malaria. But this approach might be useful for monitoring rates of acquisition of immunity in older children who have stopped receiving SMC.
**Conclusion**

This data suggest that SMC by SPAQ have an effect on antibody responses against pre-erythrocytic stage and blood-stage antigens. However, other factors that have a significant influence on antibody titers, such as transmission intensity, may confound this. Future studies are necessary to provide a better understanding of the impact of SMC on malaria immunity in Niger. In summary, duration of SMC administration may increased the antibody concentration of *P. falciparum* blood stage antigen GLURP-R2 and pre-erythrocytic stage antigen CSP. RDT filter paper serum elution methodology can significantly reduce the workload and cost in large-scale epidemiological and immunological studies.

**Declarations**

**Ethics approval and consent to participate**

All the study participants provide informed consent before their enrollment. Ethical approval was obtained from the Ethics Committee of Niger (Deliberation N°024/2015/CCNE).

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

MML and RA carried out the ELISA processing, the analysis and interpretation of data, and contributed to the drafting of the manuscript; ALPM and DA participated to the analysis and interpretation of data; IML participated to the conception of the study and the field samples collection and identification; DC, JT and NJL coordinated the study, contributed to the analysis, interpretation of data and the drafting of the manuscript. All authors read and approved the final manuscript.
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References

1. mondiale de la Santé O. Rapport sur le paludisme dans le monde 2015: résumé. 2016. http://apps.who.int/iris/handle/10665/205422. Accessed 12 Oct 2016.

2. SNIS - Systeme National d'Information Sanitaire - Niger: http://snis.cermes.net/donnees.php. Accessed 13 Mar 2016.

3. OMS | Recommandation de politique générale de l’OMS: Chimioprévention du paludisme saisonnier pour lutter contre le paludisme à Plasmodium falciparum en zone de forte transmission saisonnière dans la sous-région du Sahel en Afrique. WHO. http://www.who.int/malaria/publications/atoz/who_smc_policy_recommendation/fr/. Accessed 23 Nov 2014.

4. Salissou I, Moustapha LM, Yerima B, Alkassoum I, Hadiza D, Ibrahim ML. Perception de la chimioprévention du paludisme saisonnier au Niger. Int J Biol Chem Sci. 2016;10:2710–5. doi:10.4314/ijbcs.v10i6.24.

5. Salissou I, Moustapha LM, Alkassoum I, Hadiza D, Ibrahim ML. Estimation de l’impact en santé publique de la chimioprévention du paludisme saisonnier au Niger. Int J Biol Chem Sci. 2017;11:685–93. doi:10.4314/ijbcs.v11i2.12.

6. OMS | Chimioprévention du paludisme saisonnier par administration de sulfadoxine-pyriméthamine et d’amodiaquine aux enfants: guide de terrain. WHO. http://www.who.int/malaria/publications/atoz/9789241504737/fr/. Accessed 23 Nov 2014.

7. Noland GS, Jansen P, Vulule JM, Park GS, Ondigo BN, Kazura JW, et al. Effect of transmission intensity and age on subclass antibody responses to Plasmodium falciparum pre-erythrocytic and blood-stage antigens. Acta Trop. 2015;142:47–56. doi:10.1016/j.actatropica.2014.10.011.

8. Malaguarnera L, Musumeci S. The immune response to Plasmodium falciparum malaria. Lancet Infect Dis. 2002;2:472–8. doi:10.1016/S1473-3099(02)00344-4.

9. Cairns M, Roca-Felttrer A, Garske T, Wilson AL, Diallo D, Milligan PJ, et al. Estimating the potential public health impact of seasonal malaria chemoprevention in African children. Nat Commun. 2012;3:881. doi:10.1038/ncomms1879.

10. Dicko A, Diallo Al, Tembine I, Dicko Y, Dara N, Sidibe Y, et al. Intermittent Preventive Treatment of Malaria Provides Substantial Protection against Malaria in Children Already Protected by an Insecticide-Treated Bednet in Mali: A Randomised, Double-Blind, Placebo-Controlled Trial. PLoS Med. 2011;8:e1000407. doi:10.1371/journal.pmed.1000407.

11. Cissé B, Sokhna C, Boulanger D, Milet J, Bâ EH, Richardson K, et al. Seasonal intermittent preventive treatment with artemesunate and sulfadoxine-pyrimethamine for prevention of malaria in Senegalese
children: a randomised, placebo-controlled, double-blind trial. The Lancet. 2006;367:659–67. doi:10.1016/S0140-6736(06)68264-0.

12. Ndiaye M, Faye B, Tine R, Cisse B, Abiola A, Sow D, et al. Potential Impact of Seasonal Malaria Chemoprevention on the Acquisition of Antibodies Against Glutamate-Rich Protein and Apical Membrane Antigen 1 in Children Living in Southern Senegal. Am J Trop Med Hyg. 2015;93:798–800. doi:10.4269/ajtmh.14-0808.

13. Sylla K, Koully Tine RC, Sow D, NDiaye M, Sarr A, Tshibola Mbuyi ML, et al. Effect of Seasonal Malaria Chemoprevention (SMC) with SulfadoxinePyrimethamine (SP) and Amodiaquine (AQ) on the Acquisition of antiAMA1 and anti-MSP1_42 Antibodies among Children under 10 Years Living in the Southern part of Senegal (Velingara). Malar Control Elimin. 2017;06. doi:10.4172/2470-6965.1000155.

14. Mahamar A, Issiaka D, Barry A, Attaher O, Dembele AB, Traore T, et al. Effect of seasonal malaria chemoprevention on the acquisition of antibodies to Plasmodium falciparum antigens in Ouelessebougou, Mali. Malar J. 2017;16:289. doi:10.1186/s12936-017-1935-4.

15. Doudou MH, Mahamadou A, Ouba I, Lazoumar R, Boubacar B, Arzika I, et al. A refined estimate of the malaria burden in Niger. Malar J. 2012;11:89. doi:10.1186/1475-2875-11-89.

16. Guillebaud J, Mahamadou A, Zamanka H, Katzelma M, Arzika I, Ibrahim ML, et al. Epidemiology of malaria in an area of seasonal transmission in Niger and implications for the design of a seasonal malaria chemoprevention strategy. Malar J. 2013;12:379.

17. Guillebaud J, Mahamadou A, Zamanka H, Katzelma M, Arzika I, Ibrahim ML, et al. Epidemiology of malaria in an area of seasonal transmission in Niger and implications for the design of a seasonal malaria chemoprevention strategy. Malar J. 2013;12:379. https://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-12-379. Accessed 21 Feb 2017.

18. Baidjoe A, Stone W, Ploemen I, Shagari S, Grignard L, Osoti V, et al. Combined DNA extraction and antibody elution from filter papers for the assessment of malaria transmission intensity in epidemiological studies. Malar J. 2013;12:272. doi:10.1186/1475-2875-12-272.

19. Cnops L, Boderie M, Gillet P, Van Esbroeck M, Jacobs J. Rapid diagnostic tests as a source of DNA for Plasmodium species-specific real-time PCR. Malar J. 2011;10:67. doi:10.1186/1475-2875-10-67.

20. Adamou R, Chénou F, Sadissou I, Sonon P, Dechavanne C, Djilali-Saïah A, et al. Plasmodium falciparum infection and age influence parasite growth inhibition mediated by IgG in Beninese infants. Acta Trop. 2016;159 Supplement C:111–9. doi:10.1016/j.actatropica.2016.03.020.

21. Courtin D, Oesterholt M, Huisms H, Kusi K, Milet J, Badaut C, et al. The quantity and quality of African children’s IgG responses to merozoite surface antigens reflect protection against Plasmodium falciparum malaria. PloS One. 2009;4:e7590.

22. Cavanagh DR, Dubois PM, Holtel A, Kissar A, Leroy O, Locke E, et al. Towards validated assays for key immunological outcomes in malaria vaccine development. Vaccine. 2011;29:3093–5. doi:10.1016/j.vaccine.2011.01.070.
23. Schellenberg D, Menendez C, Aponte JJ, Kahigwa E, Tanner M, Mshinda H, et al. Intermittent preventive antimalarial treatment for Tanzanian infants: follow-up to age 2 years of a randomised, placebo-controlled trial. The Lancet. 2005;365:1481–3. doi:10.1016/S0140-6736(05)66418-5.

24. Delves M, Plouffe D, Scheurer C, Meister S, Wittlin S, Winzeler EA, et al. The Activities of Current Antimalarial Drugs on the Life Cycle Stages of Plasmodium: A Comparative Study with Human and Rodent Parasites. PLOS Med. 2012;9:e1001169. doi:10.1371/journal.pmed.1001169.

25. NAIDOO I, ROPER C. Drug resistance maps to guide intermittent preventive treatment of malaria in African infants. Parasitology. 2011;138:1469–79. doi:10.1017/S0031182011000746.

26. Grais RF, Laminou IM, Woi-Messe L, Makarimi R, Bouriema SH, Langendorf C, et al. Molecular markers of resistance to amodiaquine plus sulfadoxine-pyrimethamine in an area with seasonal malaria chemoprevention in south central Niger. Malar J. 2018;17:98.

27. IMOUKHUDE EB, ANDREWS L, MILLIGAN P, BERTHOUD T, BOJANG K, NWAKANMA D, et al. LOW-LEVEL MALARIA INFECTIONS DETECTED BY A SENSITIVE POLYMERASE CHAIN REACTION ASSAY AND USE OF THIS TECHNIQUE IN THE EVALUATION OF MALARIA VACCINES IN AN ENDEMIC AREA. Am J Trop Med Hyg. 2007;76:486–93. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3836239/. Accessed 27 Jul 2018.

28. Staalsoe T, Shulman CE, Dorman EK, Kauuondo K, Marsh K, Hviid L. Intermittent Preventive Sulfadoxine-Pyrimethamine Treatment of Primigravidae Reduces Levels of Plasma Immunoglobulin G, Which Protects against Pregnancy-Associated Plasmodium falciparum Malaria. Infect Immun. 2004;72:5027–30. doi:10.1128/IAI.72.9.5027-5030.2004.

29. Friesen J, Borrmann S, Matuschewski K. Induction of Antimalaria Immunity by Pyrimethamine Prophylaxis during Exposure to Sporozoites Is Curtailed by Parasite Resistance. Antimicrob Agents Chemother. 2011;55:2760–7. doi:10.1128/AAC.01717-10.

30. Palakkod Govindan V. Protection after Malaria Therapy: A Step-up to Immunity. Malar Control Elimin. 2016;5. doi:10.4172/2470-6965.1000148.

31. White NJ. Intermittent presumptive treatment for malaria. PLoS Med. 2005;2:e3.

32. Bray RS, Burgess RW, Fox RM, Miller MJ. Effect of pyrimethamine upon sporogony and pre-erythrocytic schizogony of Laverania falciparum. Bull World Health Organ. 1959;21:233.

33. Most H, Herman R, Schoenfeld C. Chemotherapy of sporozoite- and blood-induced Plasmodium berghei infections with selected antimalarial agents. Am J Trop Med Hyg. 1967;16:572–5.

34. Famin O, Ginsburg H. Differential effects of 4-aminoquinoline-containing antimalarial drugs on hemoglobin digestion in Plasmodium falciparum-infected erythrocytes. Biochem Pharmacol. 2002;63:393–8.
Figure 1

Characteristics of samples collected.
Figure 2

Comparison of anti-CSP IgG antibody medians levels. D1: Zinder district (SMC since 2014), D2: Dosso district (No SMC) and D3 Gaya district (SMC since 2016). Comparison of the three districts simultaneous was performed by Median test and two by two with Mann-Whitney test. The significance limit was p<0.05.
Figure 3

Comparison of anti-GLURP-R2 IgG antibody medians levels. D1: Zinder district (SMC since 2014), D2: Dosso district (No SMC) and D3 Gaya district (SMC since 2016). Comparison of the three districts simultaneous was performed by Median test and two by two with Mann-Whitney test. The significance limit was p<0.05.