

# Some evidence that seasonal malaria chemoprevention with SPAQ did not have effect on antibodies response against pre-erythrocyte stage but on the blood-stage in Niger.

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## Research

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# Abstract

**Background:** In endemic areas, children develop slowly and naturally 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 antibodies levels in Niger.

**Methods:** This survey was conducted in areas 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 total Ig G Ab against *P. falciparum* antigens, we measured antibodies levels of two *P.falciparum* asexual stage vaccine candidate antigens (Circum Sporozoid Protein and Glutamate-rich Protein R2) in children aged 3–59 months and compared these levels in Zinder, Dosso and Gaya by enzyme-linked immune-sorbent assay (ELISA) on the elution extracted from the RDTs positive and negative cassette.

**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=17.5 and GLURP-R2=14.3) median antibodies levels observed were higher than in Gaya (CSP=7.7 and GLURP-R2=6.5) and Dosso (CSP=4.5 and GLURP-R2=3.6) ( $p < 0.0001$ ).

**Conclusion:** We have some evidence in this study that suggest SMC with SPAQ did not have effect on antibodies response against pre-erythrocyte stage but on the blood-stage. Future studies are necessary to provide better understanding of SMC effect on malaria immunity.

## Introduction

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 given to 3–59 months old age children at 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].

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 have 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 Ig G anti-AMA1 and anti-MSP1<sub>–42</sub> [13]. In Mali, Mahamar et al., conclude that exposure to SMC/SPAQ reduced IgG antibody levels to AMA-1, MSP1<sub>–42</sub> and CSP [14]. Others conclusions of Malian study is that duration of exposure to SMC had no effect on antibody levels to MSP1<sub>–42</sub> 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 Ig G levels. To assess the impact of SMC on the level of the antibodies to *P. falciparum* antigens, total Ig G Ab to liver-stage vaccine candidate CSP antigen and asexual blood stage GLURP-R2 antigen were determined by ELISA using the elution extracted from RDTs.

## 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. Zinder and Gaya districts have implemented SMC with SPAQ respectively since 2014 and 2016; they were classified as mesoendemic and hyperendemic areas. Whereas SMC was not implemented in Dosso district, classified as hyperendemic and as a control district of the study. In 2016, the SMC coverage in Zinder was respectively 91% received SMC at least once, 73% at least 3 times, and 50% received 4 treatments (Unpublished Data). Both sites used ACTs as first line treatment for uncomplicated malaria and received universal coverage of bed nets.

To assess the impact of SMC on the level 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 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, date of consultation, age, gender, whether a test was performed and the result (positive or negative) were reported on the cassette. Samples have been collected in November 2015 to December 2016.

The total number of children to include was calculated at 249, based on a prevalence of antibodies to CSP at 79.8% in children that received SMC for 1, 2 or 3 years [14] a confidence level at 95% with a precision of 5%.

### Recombinant antigens

The malaria antigens used in this study included a recombinant antigen circum sporozoid protein (CSP) and glutamate-rich protein R2 (GLURP-R2). CSP-M3 and GLURP-R2 (amino acids 706-1178, F32 strain) was expressed respectively in *E. Coli* and *Pichia Pastoris*.

### Sera extraction

Sera were extracted from blood inside RDTs cassette collected [15]. RDTs have proximal, middle and distal parts according Cnops et al., description [16]. The distal part of RDT contains a filter paper component that absorbed the residual blood solution. Distal part of each RDT was cut with sterile tweezers in two or three pieces about 2 mm. Antibodies were extracted with 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. Total IgG ELISA for *P.falciparum* antigens CSP and GLURP-R2 were performed with the elution to measure Abs levels.

### **Antibody measurements**

The standard operating procedures developed by the African Malaria Network Trust was used to assess total Ig G concentrations by Enzyme Linked Immuno Sorbent Assay (ELISA) to CSP and GLURP R2 as described previously [17]. 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 1/200 for all recombinant proteins. An anti-human IgG diluted 1/3000 was used for revealing the reaction.

### **Data management**

Standard curves were established using human IgG purified proteins (Binding Site, France) to determine the concentration of specific antibodies and included in each assay. Concentrations of standard IgG were 500; 250; 125; 62.5; 31.3; 15.6; 7.8; and 3.9 ng / ml. The ADAMSEL FLP b039 software [18] was used to analyze automatically the ELISA optical density (OD) measured at 450 nm leading to antibody concentrations (µg/ml). Discordant duplicates (with a variation coefficient >15%) were technically retreated.

### **Statistical analysis**

The Median test was used to analyze differences between IgG medians concentrations. The comparisons between IgG medians concentrations in Zinder, Dosso and Gaya were performed to investigate the potential impact of SMC on Abs responses. The comparisons between IgG medians concentrations were performed by Mann-Whitney test. Data were analyzed with SPSS software version 16.0. A p-values less than or equal to 5% 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 (Fig1). The number of samples categorized by mean age and sex was comparable between districts and difference was seen in RDTs results (Table 1).

### **Figure 1: Characteristics of samples collected.**

**Table 1. Characteristics of children included in the survey**

Characteristic	ZINDER (N=71)	DOSSO (N=77)	GAYA (N=81)
<b>AGE (months)</b>			
Mean age	30.2 ± 17.5	25.4 ±14.5	25.0 ±15.4
<b>SEX (%)</b>			
M	53	50	63
F	47	50	37
<b>RDTs results (%)</b>			
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).

### **Anti-CSP and GLURP-R2 IgG antibodies medians levels measured by districts**

The Ig G antibody levels to CSP and GLURP-R2 were compared between the three (3) districts, are presented on Table 2. The medians of anti-CSP Ig G level were higher than anti-GLURP-R2 Ig G in all districts (CSP=17.5; 4.5 and 7.7 µg/ml) vs (GLURP-R2=14.3; 3.6 and 6.5 µg/ml). In Zinder where SMC was implemented since 2014 Ig G antibody levels to CSP and GLURP-R2 observed were higher than Gaya (SMC+ 2016) and Dosso (Never received SMC). A low level of Ig G antibody CSP=4.5 µg/ml and GLURP-R2=3.6 µg/ml was observed in the district that never received SMC. The medians levels of IgG anti-CSP and anti-GLURP-R2 by districts were significantly different ( $p<0.0001$  and  $p=0.0004$ ) (Table 2).

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 level of Ig G antibody responses was significantly different for the two antigens (figure 2 and 3). No significant difference in median levels of all antibodies was showed 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 levels of anti-CSP Ig G and anti-GLURP-R2 in Zinder and Gaya showed significant difference (CSP:  $p=0.008$  and GLURP-R2:  $p=0.017$ ).

**Table 2. Anti-CSP and GLURP-R2 IgG antibodies levels compared between the districts.**

Antibodies levels	Zinder (N=71)	Dosso (N=77)	Gaya (N=81)	<i>P</i>
<b>CSP</b>				
Medians	17.5 (IQR : 33.9)	4.5 (IQR : 9.8)	7.7 (IQR : 21.26)	0.0001
<b>GLURP-R2</b>				
Medians	14.3 (IQR : 37.6)	3.6 (IQR : 19.5)	6.5 (IQR : 23.5)	0.0004

D1: Zinder district, SMC+ since 2014, D2: Dosso district, No SMC and D3: Gaya district, SMC+ 2016. IQR: Interquartile range. Comparison was performed by Mann-Whitney test. The significance level was 0.05.

**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  $p < 0.05$ .**

### **Anti-CSP and GLURP-R2 IgG median levels measured by RDTs results**

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

**Table 3. Anti-CSP and GLURP-R2 IgG antibodies levels compared between RDT positive and negative**

Antibodies levels	RDT+	RDT-	<i>P</i>
			<i>0.093</i>
CSP			
Median	9.4 (IQR : 29.9)	6.5 (IQR : 16.4)	
GLURP-R2			<i>0.539</i>
Median	8.3 (IQR : 35.4)	6.0 (IQR : 20.1)	

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 level of the antibodies to *P. falciparum* antigens 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 that 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 [19]. Total IgG antibody level against liver-stage CSP antigen showed significantly higher than blood-stage GLURP-R2 antigen in all districts. SMC was primarily directed against blood-stage parasites, because SP and AQ association inhibits erythrocyte stage, this may results in a shorter exposure to GLURP-R2 antigen. This finding is contrasting with a recent study published in malaria journal by Mahamar et al [14]. Others studies demonstrated that sulfadoxine does not affect liver stages, pyrimethamine has some effect on liver stages in *P. yoelii* models [20] but there are high levels of resistance to pyrimethamine in SMC countries [21]. Amodiaquine acts on blood stage parasites [20]. In vaccine trial cohorts in the Gambia [22] 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. SMC with SPAQ is therefore not expected to affect acquisition of anti-CSP antibodies.

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 subsequent life cycle conversion by SPAQ. This is contrasting with other studies [14], [13], [23], [12] which found a decrease in the levels of antibodies after SMC delivery. Previous studies established that chemoprophylaxis conferred protective immunity against reinfections when anti-malaria drugs are not present [24],[25], [26]. The inhibitory effect of SP on pre erythrocyte stage [27],[28] and AQ on erythrocyte stage was previously described [29]. 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 [24]. The increase in antibodies levels is believe to be linked with exposure of the immune system to an attenuate hepatic stage parasites or complete suppression of subsequent blood-stage infections, thereby resulting in a higher IgG antibody levels to CSP antigen.

The comparative analysis of antibodies levels against CSP and GLURP-R2 antigens between age groups, showed 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 is some evidence of reduced acquisition of blood stage immunity in SMC areas but 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 one marker of blood stage 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.

This data suggest that SMC by SPAQ did not have effect on antibodies response against pre-erythrocyte stage but on the blood-stage; however, other factors may interfere such as intensity of transmission and exposure that have significant influence on the antibodies levels. If SMC did not impact on the liver stage antigens antibodies, that anti-CSP antibody concentration could therefore be used as a measure of

exposure to malaria in children whether they have received SMC or not. Future studies are necessary to provide better understanding of SMC impact on malaria immunity in Niger.

## 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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## Figures

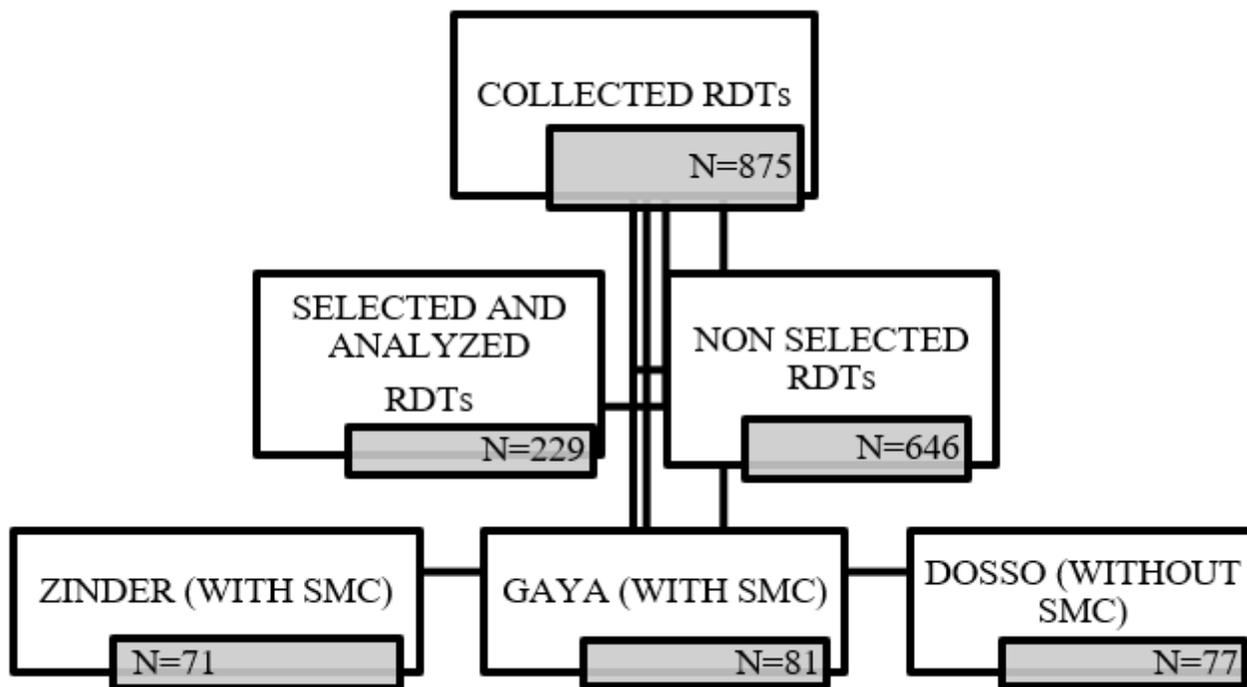
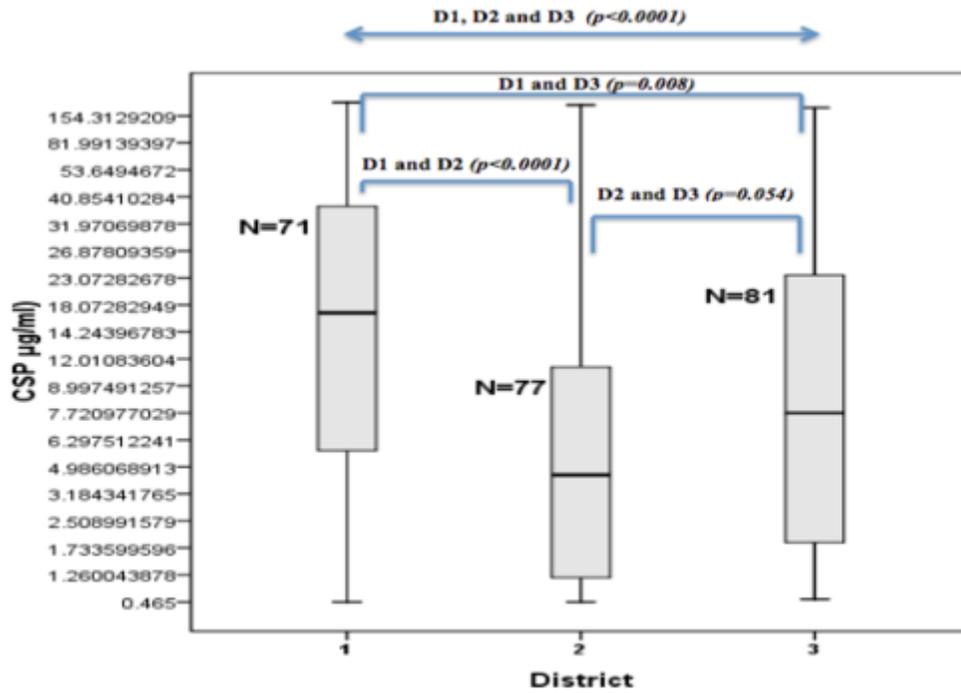


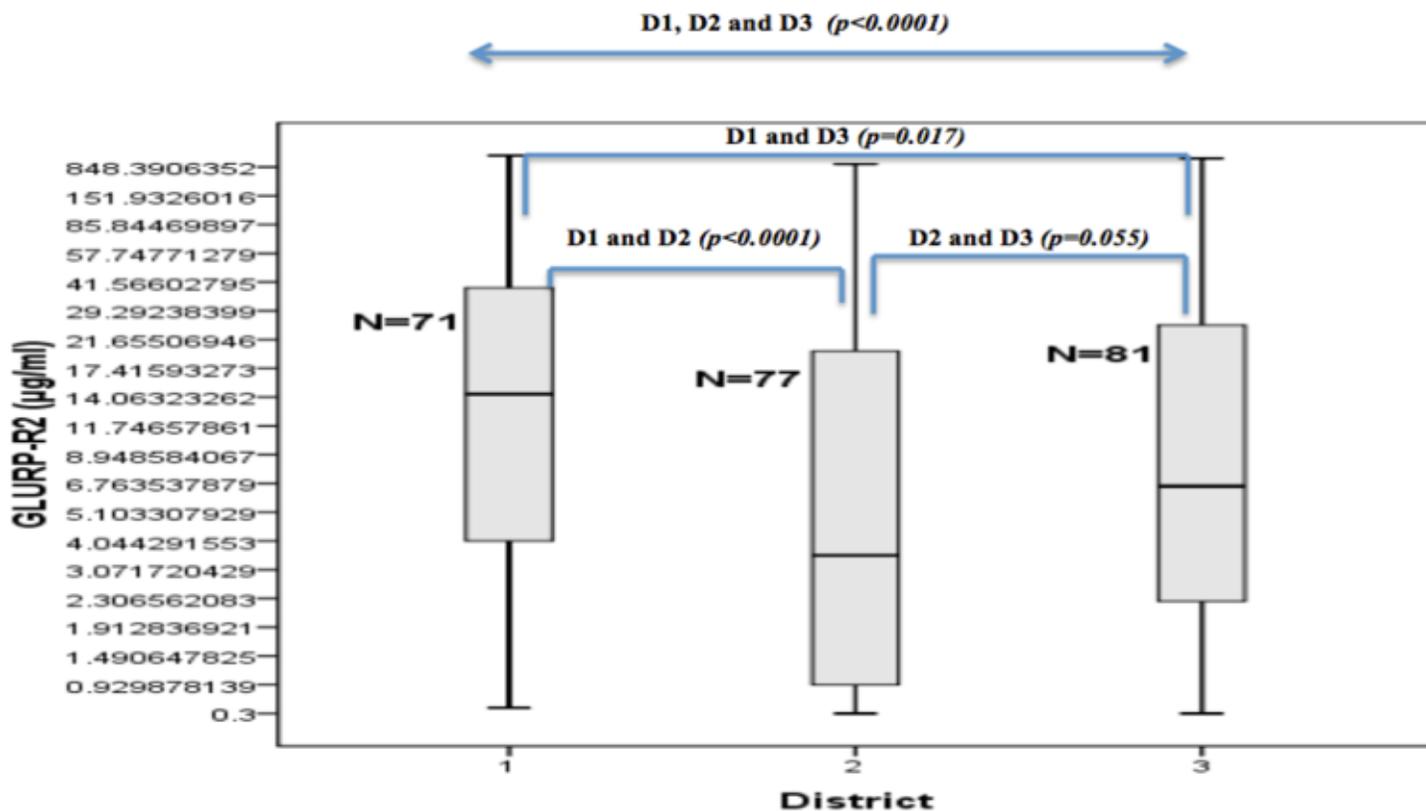
Figure 1

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  $p < 0.05$ .