Serological signatures of declining exposure following intensification of integrated malaria control in two rural Senegalese communities

Ronald Perraut, Marie-Louise Varela, Cheikh Loucoubar, Oumy Niass, Awa Sidibé, Adama Tall, Jean-François Trape, Amele Wotodjo, Babacar Mbengue, Cheikh Sokhna, et al.

To cite this version:
Ronald Perraut, Marie-Louise Varela, Cheikh Loucoubar, Oumy Niass, Awa Sidibé, et al.. Serological signatures of declining exposure following intensification of integrated malaria control in two rural Senegalese communities. PLoS ONE, Public Library of Science, 2017, 12 (6), pp.e0179146. 10.1371/journal.pone.0179146. pasteur-01635035

HAL Id: pasteur-01635035
https://hal-riip.archives-ouvertes.fr/pasteur-01635035
Submitted on 14 Nov 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Serological signatures of declining exposure following intensification of integrated malaria control in two rural Senegalese communities

Ronald Perraut, Marie-Louise Varela, Cheikh Loucoubar, Oumy Niass, Awa Sidibé, Adama Tall, Jean-François Trape, Amele Nyedzie Wotodjo, Babacar Mbengue, Cheikh Sokhna, Inès Vigan-Womas, Aissatou Touré, Vincent Richard, Odile Mercereau-Puijalon

Institut Pasteur de Dakar, Unité d’Immunologie, Dakar, Sénégal, Institut Pasteur de Dakar, G4 Biostatistiques Bioinformatiques et Modélisation, Dakar, Sénégal, Institut Pasteur de Dakar, Unité d’Épidémiologie, Dakar, Sénégal, Institut de Recherche pour le Développement (IRD), URMITE, Dakar, Sénégal, Institut Pasteur de Dakar, Unité d’Immunogénétique, Dakar, Sénégal, Institut Pasteur de Madagascar, Unité d’Immunologie des Maladies Infectieuses, Antananarivo, Madagascar, Institut Pasteur, Department of Parasitology and Insect Vectors, 25 Rue du Dr Roux, Paris, France

These authors contributed equally to this work.

Abstract

Recent control scale-up has reduced malaria in many areas but new tools are needed to monitor further progress, including indicators of decreasing exposure to parasite infection. Although serology is considered a promising approach in this regard, the serological impact of control interventions has been so far studied using indirect quantification of exposure. Cohort surveys concomitantly recording entomological and malarialometric indices have been conducted in two Senegalese settings where supervised control intensification implemented in 2006 shifted malaria from historically holoendemic in Dielmo and mesoendemic in Ndiop to hypoendemic in both settings by 2013. We analyse here serological signatures of declining transmission using archived blood samples. Responses against ten pre-erythrocytic and erythrocytic antigens from Plasmodium falciparum and P. malariae alongside an Anopheles gambiae salivary gland antigen were analysed. Cross-sectional surveys conducted before (2002) and after (2013) control intensification showed a major impact of control intensification in both settings. The age-associated prevalence, magnitude and breadth of the IgG responses to all antigens were village-specific in 2002. In 2013, remarkably similar patterns were observed in both villages, with marginal responses against all parasite antigens in the 0-5y children and reduced responses in all previously seropositive age groups. Waning of humoral responses of individuals who were immune at the time of control intensification was studied from 2006 to 2013 using yearly samplings. Longitudinal data were analysed using the Cochran-Armitage trend test and an age-related reversible catalytic conversion model. This showed that the antigen-specific antibody declines were more rapid in older children than adults. There was a strong association of antibody decline with the declining entomological inoculation rate. We thus identified serological markers of declining
exposure to malaria parasites that should help future monitoring of progress towards malaria elimination.

**Introduction**

Intensification of integrated control interventions have considerably reduced the global burden of malaria [1]. There remain however multiple challenges to reaching elimination, including developing indicators of declining exposure to infection to monitor progress [2]. Indeed, collection of age-associated case distribution, which varies by transmission intensity, becomes logistically challenging in the context of declining malaria and moreover overlooks the asymptomatic infection reservoir [3]. Screening campaigns using sensitive molecular assays only provide a snapshot record of infection. Entomological methods lack sensitivity when transmission is low. Serology provides a signature of exposure to parasites overcoming many of these caveats [4]. A large number of parasite antigens elicit robust humoral responses and numerous assays have been used, based on parasite preparations [5–8] recombinant proteins or peptides in monoplex or mutiplex formats [8–19]. Seroprevalence and antibody levels to *P. falciparum* antigens are associated with transmission intensity but the relationship is non-linear and antigen- and age-dependent [4, 9, 12, 19–23]. However, these conclusions stem from comparison of sites with differing socioeconomic and/or climatic conditions, being either located distantly [21, 23–26] or at different altitudes [11, 12, 19, 27–29], from short-term follow up [7, 9, 13, 21, 22, 30–34] or from studies focussing on specific age groups [9, 21, 24, 31, 32]. Decreased antibody responses are observed several years after successful control intervention(s) [5, 7, 8, 35–39]. Some antibody responses seem short-lived [9, 13, 26], while others persist after extended periods of interrupted exposure [40, 41]. Analysis of the temporal dynamics of antibody responses in communities after supervised interventions and quantified impact on entomological transmission is lacking.

To gain insight into the consequences of control interventions and transmission decline on the antibody responses, we analyse here two communities living in a rural area of Senegal under the same climatic conditions albeit distinct historical malaria endemicity [42–46]. In both settings, *P. falciparum* dominates, but *P. malariae* and *P. ovale* are endemic as well [42, 47, 48]. High coverage, supervised control interventions have been introduced simultaneously in both communities. In 2006, an artemisinin-based combination therapy (ACT) was introduced as first-line treatment. Long-lasting insecticide-treated nets (LLINs) were provided to each household in July 2008 and renewed in 2011 [46]. The incidence of malaria attacks decreased after 2006 and dropped to low levels after 2008 [46, 47, 49]. Some rebound was observed in 2011, the highest incidence being in the older children and young adults [42, 46]. Importantly, parasite rates dramatically declined a few months after LLIN deployment, and transmission of parasites dropped markedly after LLIN renewal in 2011 [46, 48, 49].

We profile here the IgG responses of archived blood samples using a multistage, multispecies approach. We first analyse cross-sectional blood samplings made at the end of the dry season in the years 2002 and 2013 and monitor responses to a panel of antigens used in previous studies as inducing high seroprevalence in malaria-exposed communities [10, 14, 34]. We then analyse on a yearly basis the waning of responses to three antigens during the years 2006–2013 in previously immune age groups. This data shows that control intensification induced major changes in the age-stratified antibody profiles, reflecting age-dependent seroreversion of previously immune age groups and minimal responses in young children. The similar response profiles of both communities in 2013 provide serological signatures of efficacious transmission
decline in the previous years that should help future monitoring of declining immunity in communities during the transition to elimination.

Material and methods

Study sites, procedures and ethics

Dielmo and Ndiop are located 5 km apart in the district of Fatick, Senegal, and exposed to the same climatic environment but distinct historical transmission/endemicity conditions, with perennial and intense transmission in Dielmo situated on the banks of a stream [44, 46, 48], and moderate and highly seasonal in Ndiop [42–45]. The long-term observational studies of malaria implemented in these communities uninterruptedly record for more than 20 years the episodes of fever, clinical malaria, malaria infection, parasite and vector species and entomological inoculation rate in both settings. The protocol was approved by the National Senegalese Health Research Ethics Committee. Informed consent was obtained from all participants or their parents or guardians in the presence of an independent witness.

The procedures, similar for both villages, have been described in detail [42, 43, 45, 46]. Briefly, clinical cases were monitored daily by active and passive case detection and parasite rates were measured quarterly. Prompt diagnosis and treatment were provided on site. In October 2003, chloroquine was replaced as first-line treatment of falciparum malaria for the amodiaquine /sulfadoxine-pyrimethamine combination itself replaced in May 2006 for the artesunate/ amodiaquine combination. Transmission monitoring included measurement of vector density by human-landing catches. The monthly human biting rate and the proportion of infected mosquitoes identified by ELISA for the P. falciparum circumsporozoite protein were used to determine the entomological inoculation rate (EIR), i.e. the mean number of infected mosquito bites per person per year [45, 46]. In July 2008, vector control was massively implemented with provision of long-lasting insecticide-treated nets (LLINs) to each household. New LLINs were provided in July 2011 [46].

The protocol includes a monthly systematic and a yearly cross-sectional blood sampling. Samples are frozen and archived. Two cross-sectional samplings, conducted in July 2002 and July 2013 at the end of the dry season (Table 1) as well as monthly capillary samples withdrawn from 2006 to 2013 have been studied here.

Blood stages and parasite species were numerated using microscopy [46, 48]. Plasmodium DNA was detected in blood samples by nested, species-specific PCR [50].

Antigens

The P. falciparum schizont extract (SE) of the 07/03 Dielmo strain was prepared as described [5, 15, 51, 52]. PF13, the recombinant NTS-DBL1α1 domain encoded by the 3D7/PF13_0003 P. falciparum var gene was produced in Escherichia coli as reported [53]. PfMSP1p19, the 19 kDa C-terminal domain of the P. falciparum Merozoite Surface Protein-1 [54], produced using a baculovirus-insect cell expression system, was a kind gift of S. Longacre (Vaximax, Genopole Evry, France). Peptide synthesis and coupling to Bovine Serum Albumin (BSA) were custom made by GenScript HK Inc., Hong Kong, China, or Genecust, France. Purity of each BSA-peptide was estimated >85% by HPLC and mass spectrometry. The sequence of the peptides used [10, 14] is displayed in Table 2.

ELISA

Indirect ELISA was used to analyse the response against SE in the cross-sectional and longitudinal studies, as well as the longitudinal response against the LSA141 peptide and the
recombinant MSP1p19. Procedures were performed using sera diluted 1/200 as described [5, 15, 16]. A pool of sera from immune adults from Dielmo and a pool of European and African non-immune sera were included in each assay as positive and negative controls, respectively. IgG Levels were expressed as OD ratio = OD_{sample} / mean OD_{naive pool}. Sera showing an OD ratio >2, corresponding to the mean OD_{naive pool} + 3 SD were considered positive.

Bead-based multiplex assay

A multiplex assay was used to monitor the response the panel of individual antigens listed in Table 2. Covalent coupling of antigens to carboxylated magnetic Luminex beads and the custom magnetic bead-based Luminex multiplex assay including the antigens listed in Table 2 were done as described [10, 14, 16, 55]. IgG levels were expressed as Mean Fluorescence Intensity (MFI). The background signal against BSA was negligible (around 50–80 for all antigens) and was subtracted from all MFI values (net value). The positivity cut-off was set as above the net MFI + 3 SD of naive controls.

### Statistical analysis

Categorical variables were compared using the Fisher exact test, continuous variables of antibody responses were analysed using the Kruskal Wallis and the Spearman rank correlation test for non-normally distributed data. Cochrane-t linear and logistic regressions adjusted for age groups or age were used for comparison of categorical and continuous variables. P values <0.05
were considered significant. Longitudinal data were analysed using the Cochran-Armitage trend test. Statistical analyses were performed with R and Statview 5.0 software.

Serocoversion (SCR) and seroreversion (SRR) rates were calculated using an age-specific reversible catalytic conversion model [12]. The model was constructed based on the longitudinal data stratified into pools of 2-year for a given period and for a given antigen.

### Results

In 2002, malaria was typically holoendemic in Dielmo [46] and mesoendemic in Ndiop [42, 44, 45]. Between 2002 and 2013, malaria case incidence decreased by 14.7-fold in Dielmo and 6.3-fold in Ndiop (Table 1). Asymptomatic, microscopically-positive parasite carriage decreased from 46.1% in Dielmo and 19.8% in Ndiop in 2002 to undetectable levels in both settings in 2013. Sub-microscopic infections were detected in 2013 by PCR in 4.6 and 2.9% blood samples from Dielmo and Ndiop, respectively.

Two cross-sectional surveys conducted before the rainy season (July 2002 and July 2013) included 184 and 196 villagers in Dielmo, and 202 and 216 villagers in Ndiop, respectively. In Dielmo and Ndiop, 77 (3.8–80 yo in 2002) and 87 (3.4–68.1 yo in 2002) individuals were commonly present in 2002 and 2013, they were considered independently in their respective age-groups as function of the year. Age distribution and mean age were similar for both surveys in each village and did not differ between the villages (Table 3). The mean number of infected bites per person during the previous 12-month was 215.5 in Dielmo and 171.8 in Ndiop in 2002 (an unusually high transmission was recorded in Ndiop during August-December 2002, contrasting with a mean of 37.1 from 1993 to 2001 and 37.8 from 2003 to 2007). It dropped to 7.5 and 2.5 in 2013 in Dielmo and Ndiop, respectively (Table 1).
Decreased seroprevalence in all age groups in 2013

We first analysed the cross-sectional blood samplings made in the years 2002 and 2013. A multiplex bead-based approach was used to monitor the responses against individual antigens derived from erythrocytic and exoerythrocytic \textit{P. falciparum} stages, \textit{P. malariae} exoerythrocytic stages as well as an \textit{A. gambiae} salivary gland antigen (Table 2). This panel included peptides and recombinant antigens known to elicit high seroprevalence in these settings [10, 15, 16, 53]. In parallel, we monitored the responses against the complex pool of erythrocyte stage antigens included in SE using ELISA also previously used in these communities [5, 52, 56–58] and providing interesting information in a previous study of immunity decay in Dielmo and Ndiop [5].

From 2002 to 2013, the community seroprevalence decreased in both villages by 26% to 66% depending on the antigen, and this was significant for all antigens (Table 4). Between-village comparison showed higher community seroprevalence for SE, PfCSP, LSA1\textsubscript{41}, GLURP, PF13 and PmCSP in Dielmo in 2002, but no difference in 2013, apart from PmCSP.

For both Dielmo and Ndiop, stratification by age (<7, 7–14, 15–29 and >30 years) showed quite different age-associated profiles in 2002 and 2013 (Fig 1, details in S1 Table). Seroprevalence against all antigens except gSG6 was very low (0 to <20%) in the 0-6y children in 2013. Seroprevalence against PfCSP, SALSA, GLURP, AMA1, PF13 and PmCSP was much lower in 2013 than in 2002 in the 7-14y old children. The age-associated profiles, which differed between the villages in 2002 for SE, LSA1\textsubscript{41}, PF13 and PmCSP (Chi-square test P<0.01 for each comparison), were similar in 2013, except for PmCSP.

Decreased magnitude and breadth of the responses

In each village, the age-associated antibody levels differed between 2002 and 2013 (P<10\textsuperscript{-3} for all antigens, except gSG6) (Fig 2, see details S2 Table). In both villages, age was weakly correlated (correlation coefficient R = 0.30, P<10\textsuperscript{-2}) in 2002 with antibody levels against SE, MSP1p19, GLURP, AMA1, PfCSP as well as against LSA1\textsubscript{41} and PmCSP in Ndiop. In 2013, a stronger correlation with age was observed for all responses except against gSG6 (R = 0.55 to 0.62, depending on the antigen P<10\textsuperscript{-2}).
The 2013 profiles were characterised by marginal IgG levels to all parasite antigens in the <6y children, low in the 7-14y children, higher in the 15-29y individuals and apart from PF13, further increased in the oldest group. Specific profiles were observed for gSG6. In Dielmo, a wide range of IgG levels was observed in the 0-6y olds and a low response in the older age groups. In Ndiop, the IgG levels against gSG6 tended to decrease with age and to be lower in 2013 compared to 2002.

The breadth of the response decreased between 2002 and 2013. The mean number of individual antigens recognised per person dropped in Dielmo from 6, 5, 6, 7 to 1, 2, 4 and 5 in the <7y, 7-14y, 15–29, >30y old groups, respectively (Mann Whitney test, P<10^{-3} for all age groups) and in Ndiop from 3, 4, 6, 6 to 1, 2, 4, 5 in the <7y, 7-14y, 15–29, >30y old groups, respectively (idem, P<10^{-3} for the first three age groups, P = 0.014 for the oldest age group).

Longitudinal analysis of antibody decay in previously immune age groups

To investigate how the changes of transmission after control intensification affected the acquired antibody responses, we focused on the period from 2006 to 2013 in Dielmo. The EIR declined substantially after LLINs deployment in 2008, rebounded in 2010 and declined to low levels after new LLINs were distributed in 2011 (Fig 3). Samples from age groups that were immune in 2006, namely 17 children aged 7-14y and 47 adults aged 15–63.5 y in 2006 were analysed on a yearly basis. IgG responses against LSA1_{41}, MSP1p19 as well as SE were studied in parallel assays by ELISA. ELISA was used here to homogenise the experimental procedures in this study and to provide a basis of comparison with published studies [4, 5, 9, 12, 15, 16, 19, 26–30, 32, 38, 39, 52, 56, 58].

Antibody levels against the three antigens fluctuated with transmission and overall declined with time (Kruskal Wallis test within each age group, P<10^{-3}) (Fig 3A). The magnitude of the responses to the three antigens decreased in both age groups after each LLIN implementation (Fig 3B). There was a significant and strong (R>0.9, P<10^{-3}) correlation between the EIR

Table 4. Community seroprevalence for the set of antigens in two cross-sectional studies conducted in Dielmo and Ndiop in July 2002 and July 2013.

| Antigen   | Dielmo 2013 vs 2002 | Ndiop 2013 vs 2002 | Inter-village comparison P |
|-----------|---------------------|--------------------|---------------------------|
|           | seroprevalence      | seroprevalence     |                           |
|           | year 2002 year 2013 | year 2002 year 2013|                           |
| Schizontextract | 93.4 64.9 |-30% <10^{-3} | 78.7 56.3 -29% <10^{-3} | <10^{-4} 0.085 |
| P/CSP     | 70.1 39.8 -43% <10^{-3} | 58.9 39.8 -32% <10^{-3} | 0.02 0.840 |
| LSA1_{41} | 84.2 62.2 -26% <10^{-3} | 72.8 52.3 -28% <10^{-3} | 0.006 0.046 |
| SALSA     | 53.8 29.1 -46% <10^{-3} | 51.9 30.6 -41% <10^{-3} | 0.759 0.748 |
| GLURP     | 78.3 50.5 -35% <10^{-3} | 67.3 48.6 -28% <10^{-3} | 0.017 0.767 |
| AMA1      | 53.8 18.4 -66% <10^{-3} | 50.9 19.4 -62% <10^{-3} | 0.611 0.802 |
| PF13      | 87.5 44.9 -49% <10^{-3} | 64.4 43.1 -33% <10^{-3} | <10^{-4} 0.765 |
| MSP1p19   | 79.3 57.7 -27% <10^{-3} | 74.3 52.3 -30% <10^{-3} | 0.278 0.321 |
| PmCSP     | 79.3 49.0 -38% <10^{-3} | 42.1 29.6 -30% 0.01 <10^{-4} <10^{-4} |
| gSG6      | 26.6 17.9 -33% 0.04 | 35.1 16.6 -53% <10^{-3} | 0.557 0.066 |

* Percent seroprevalence observed in 2002 no longer observed in 2013
* Comparison of antigen-specific seroprevalence in 2002 vs 2013 (Chi-square test)
* Between-village comparison of antigen-specific community seroprevalence observed at each cross-sectional survey (i.e Dielmo vs Ndiop in July 2002, Dielmo vs Ndiop in July 2013) (Chi-square test)

https://doi.org/10.1371/journal.pone.0179146.t004
values and the magnitude of the response (i.e. the geometric mean of the OD ratios) against
the three antigens in both age groups evidenced by polynomial regression analysis (Fig 4A).
The decreases were antigen- and age-specific, more rapid and larger in children than in adults
for the three antigens. The decrease of antibody levels over the 8-year period was confirmed
using the Cochran-Armittage trend test, which showed a general significant decreasing trend
\( P < 10^{-4} \). However, when restricted to adults, the \( P \) values were lower for MSP1p19 (\( P = 0.015 \)) and met borderline significance for SE (\( P = 0.055 \)). These data showed different kinetics
of antibody decline in previously immune children and adults depending on the antigen. The
best association with EIR was observed for the anti-MSP1p19 response in children (\( R = 0.99, \)
polynomial regression, \( P < 10^{-3} \)).

Mathematical modelling of the seroprevalence data using an age-specific reversible catalytic
conversion model [12] using a 2-year stratification combining both age groups indicated anti-
gen-specific and period-specific seroconversion (SCR) and seroreversion (SRR) rates (Fig 4B).
The individual SCRs and SRRs were unrelated to the mean EIR of the corresponding 2y period
but interestingly, the SRR/SCR ratio for LSA1\(_{41}\) and for MSP1p19 increased as the mean EIR
decreased.
Fig 2. Distribution of antibody levels against ten antigens in Dielmo and Ndiop during the cross-sectional surveys conducted in July 2002 and July 2013, by age group. Antibody levels against the ten antigens observed in four age groups <7 y (white), 7–14 y (light grey), 15–29 y (dark grey) and ≥30 y (black) in Dielmo and Ndiop are shown as box-and-whisker plots. The box shows the first to the third quartile range, the band inside represents the median, the ends of the whiskers represent the 95% percentiles. Asterisks above the
Discussion

The Dielmo and Ndiop cohort surveys, which uninterruptedly recorded entomological and malarial indices using a common methodology for more than twenty years, allow informative comparisons of the effect of control intensification on the immune responses at the community level. Seroprevalence, magnitude and breadth of the responses had decreased in 2013 in all age groups and the age-associated profiles were similar in both settings for many antigens. Thus, the humoral profiling of the communities in 2013 reflected the significant progress in malaria control in the recent years resulting in low incidence of cases, uncommon asymptomatic carriage and low transmission level [5, 44, 46, 49].

The low seroprevalence and marginal levels of antibodies to parasite antigens, including SE, in the youngest age group in 2013, is in line with observations several years after control intensification in The Gambia [36, 38] and after eradication of malaria in Mauritius [35]. These children were born after introduction of ACT as first line treatment and for many of them after LLINs implementation. Their response profile reflects the cumulative effects of reduced exposure in the first years of life as they slept under nets early at night, rare occurrence of clinical malaria, which was rapidly and efficiently drug cured [46] and short half-life of the antibody response in this age group [9, 26].

The marked drop in seroprevalence, magnitude and breadth of antibody responses in the 7-15y old children between 2002 and 2013 confirms observations about the anti-MSP1p19 response in The Gambia [36, 38]. By 2013, the children 7-15y old had lost a good fraction of the responses against SE, LSA141, MSP1p19, SALSA, PF13, AMA1, PfCSP and gSG6 observed in the youngest age group 11 years earlier. This occurred in both settings where acquired immunity was different before control intensification, likely reflecting the short half-life of the responses and the similar exposure/transmission conditions in the few years preceding the 2013 blood sampling. At the community level, seroprevalence, magnitude and breadth of the responses declined. This included a decreased response to MSP1p19, consistent with observations in The Gambia [38], but conflicting with the very long half-life (about 50 years) of anti-MSP1p19 antibodies estimated from comparisons of cross-sectional studies in settings with differing transmission conditions [29].

The longitudinal data provide interesting insights into the temporal decay of responses. Cases and parasite prevalence dropped in 2009 but a dramatic reduction of transmission occurred after 2011 only [46]. The responses against LSA141 and MSP1p19 and to a lesser extent against SE correlated with the EIR in the preceding 12 months (Fig 4A) suggesting that they are affected by recent exposure to parasite infection and hence more labile than appreciated. Mathematical modelling showed that the SCRs and SRRs were antigen-specific and poorly related to the mean EIR in the previous 24 months, but interestingly the SRR/SCR ratio increased as the EIR collapsed. The sample size of the longitudinal study did not allow modelling the antibody decay on a yearly basis in the two age groups considered. Additional studies are needed to clarify whether the SRR/SCR ratio is age-specific as suggested by both the cross-sectional data and the relationship of antibody levels with EIR (Figs 3 and 4A).

Limitations of this study include the timing of the cross-sectional surveys. We studied the antibodies present after 5–7 months of dry season, and consequently short-lived responses and responses elicited by recent infections could remain undetected. This limitation should
Fig 3. Temporal distribution of entomological inoculation rate and antibody levels to three *P. falciparum* antigens in two age groups from Dielmo during the 2006–2013 period. The entomological inoculation rate (EIR, the mean number of infected mosquito bites per person per year) is shown for the 12 month-period extended from July 1st till June 30th of the following year. In part (A), the antibody levels against SE, LSA1, and MSP1p19 are shown as box-and-whisker plots of the OD ratios (left y axis) each year from 2006 to 2013 for young.
however have little impact in Dielmo where breeding sites and mosquitoes are present all the year [46, 48]. It would thus be interesting to investigate in Dielmo the responses to antigens identified recently as signing exposure in the past 3–6 months [24], and to carry out additional surveys at the end of the rainy season to track possible recent infections. A second limitation is the uncertainty about residual asymptomatic carriage, known to affect the immune response [3, 34]. Parasitological surveillance showed uncommon microscopy-positive smears after 2008

**Fig 4. Correlation between entomological inoculation rate and mean IgG levels against three *P. falciparum* antigens observed in two age groups from Dielmo during the years 2006–2013.** A. Polynomial regression curves for the relationship between the median antibody responses to SE (black circles), LSA141 (grey squares), MSP1p19 (grey triangles) and EIR (log scale) for the two age groups ($y = y_0 + ax + bx^2 + cx^3$). B. Seroconversion (SCR) and seroreversion (SRR) rates calculated using a 2-year stratification illustrated as bar plot for each antigen (black = SCR, grey = SRR). The temporal evolution of the SRR/SCR ratio is plotted as the plain line connecting the dot point values. The mean EIR (right y axis) for each period is plotted as the step vertical dashed red line. The black crosses indicate the mean prevalence of antibody responses for a given period (the scale used for 100% and 50% is 3.0 and 1.5, respectively on the left y axis).

https://doi.org/10.1371/journal.pone.0179146.g004
none were detected in 2013 and very few sub-microscopic infections were detected using PCR (which in our hands detects less than 1 parasite per microliter of blood). This does not exclude possible infections with lower densities or that were not detected by our snapshot samplings. However, the low responses observed in 2013 in the 7-15y old children who historically had a high rate of asymptomatic infections [59], is consistent with uncommon asymptomatic carriage in the recent years.

A limited panel of individual antigens was studied here compared to published high-throughput studies [6, 11, 13, 24]. We studied the responses to SE and to an array of individual antigens known to elicit responses reaching high seroprevalences in these settings [5, 10, 15, 16, 53] such that any decay would embrace a large dynamic range. Apart from the variant PF13 [53], the antigens used here are well conserved. Temporal variation in seroprevalence/levels against these conserved antigens is thus unlikely due to temporal genetic variation of the parasites circulating in the villages. Data regarding anti-SE responses are consistent with the observed decrease from 2000 to 2010 in the 5–19 year old villagers from Dielmo and Ndiop [5]. We show here that by 2013 the decay had occurred in all previously immune groups, including adults and importantly, that the children under 6 years were essentially seronegative against SE indicating quite limited exposure to parasites in this age group. The information gathered using SE is reminiscent of observations using parasite-based assays such as immunofluorescence [35] or indirect haemagglutination [7] assays. SE has the advantage of containing a broad panel of parasite antigens, which moreover are presented in their native conformation and native post-translational modifications. The high historical seroprevalence and levels observed in both villages is consistent with previous studies [5, 56] and with the high responses observed against the various erythrocytic antigens studied here but contrasts with the low figures reported in a Kenyan setting [22], possibly reflecting differences in extract preparation. The sensitive SE-assay provides a rapid mean to monitor the anti-parasite-response, but the exact specificity of the observed signals is unknown, precluding identification of specific conversion and reversion events.

The 2002 survey confirms the high historical seroprevalence against the recombinant antigens and peptides used here [10, 15, 16, 53, 55]. All responses apart from gSG6 had declined by 2013. The response against PmCSP followed a pattern similar to PfCSP, consistent with the marked drop in P. malariae cases and infections after control scale-up [47].

The response against gSG6 presented specific age-stratified patterns and temporal trends. The peptide allows to capture of antibodies elicited by An. gambiae [17] as well as An. funestus [60], which are the main vectors in Dielmo and Ndiop [43, 46]. The lower community seroprevalence in 2013 compared to 2002 is consistent with the published association with transmission intensity [27, 61, 62]. The lower responses in the older age groups observed in both villages in 2013 are in line with the reported tolerance response in the older subjects [33, 63, 64]. The large heterogeneity of antibody levels observed in the young children in Dielmo in 2013 is surprising, as antibodies to gSG6 have been reported to be negatively associated with the use of LLIns in young children [65]. It contrasts with the more homogeneous response observed in the same age group 11 years earlier and suggests substantial heterogeneity of exposure of young children to Anopheles bites in 2013, as reported in low transmission conditions [62]. Presence of antibodies against gSG6 indicates continued exposure to mosquito bites in 2013 is consistent with the recorded vector density in the villages, and probably indicates exposure to Anopheline bites at a time when children are not using LLINs. Indeed, the low/nil responses of these young children against parasite antigens reflect the combined benefit of limited/nil exposure to infectious bites thanks to LLINs and limited exposure to parasites thanks to the use of efficacious antimalarial treatments.

The panel of antigens analysed here provided a consistent picture of declining immunity and allowed to identify clear signatures of progress towards malaria elimination. The list of
potential biomarkers is not closed and could obviously be completed with additional antigens identified recently in other settings as associated with recent infections [24]. Our data illustrate the value of community surveys for documenting malaria decline. A few consecutive years of reduced exposure decreased specific immunity against pre-erythrocytic and blood stage antigens whatever the pre-existing level of immunity in the community. The young children born after control intensification had quite limited if any acquired immunity against parasite antigens. This age group is particularly vulnerable to possible failures in control and highly informative in case of such circumstances, but is poorly informative for monitoring further progress towards elimination. The older children have lost a good fraction of their pre-existing immunity as transmission declined. There was clear evidence of waning immunity in adults, with declining antibody levels but still substantial seroprevalence levels, consistent with the residual local transmission and the high antibody levels before control intensification. The previously immune age groups thus appear sensitive indicators of declining transmission and it will be important to continue monitoring in the future years to study how their immune responses evolve as transmission further declines.

**Supporting information**

S1 Table. Mean antibody prevalence against ten antigens in four age groups in Dielmo and Ndiop during the cross-sectional surveys conducted in July 2002 and July 2013. (DOCX)

S2 Table. Age-associated distribution of antibody levels against ten antigens observed during the cross-sectional surveys conducted in Dielmo and Ndiop in July 2002 and July 2013. (DOCX)

**Acknowledgments**

We are particularly grateful to Dr Shirley Longacre (Vaximax, Paris) who generously provided the PfMSP1p19. We thank Micheline Guillotte-Blisnick for characterizing purity and stability of the recombinant antigens, Pr Alioune Dieye for laboratory support, Dr Makhtar Niang for PCR results and Joseph Faye for Dielmo/Ndiop database management. We thank gratefully all villagers from Dielmo and Ndiop for their generous and committed long-term participation to the study.

**Author Contributions**

**Conceptualization:** OMP RP.

**Data curation:** MLV CL ANW CS VR JFT A. Tall.

**Formal analysis:** RP CL OMP ON BM MLV VR IVW.

**Funding acquisition:** RP OMP.

**Investigation:** MLV ANW AS.

**Methodology:** RP OMP VR CL MLV ON.

**Project administration:** RP OMP MLV.

**Resources:** VR CS JFT ANW VW BM A. Touré.

**Software:** CL RP VR OMP.
Supervision: RP OMP MLV VR.
Validation: RP OMP MLV VR.
Visualization: CL RP OMP VR.

Writing – original draft: RP OMP.
Writing – review & editing: RP OMP CL MLV JFT ON A. Tall CS A. Touré BM.

References

1. WHO. WHO World Malaria Report 2015. http://www.who.int/malaria/publications/world-malaria-report-2015/en/; 2015.(Dec, 2015).
2. Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: new strategies for new challenges. Lancet. 2013; 382(9895):900–11. Epub 2013/04/19. S0140-6736(13)60310-4 [pii] https://doi.org/10.1016/S0140-6736(13)60310-4 PMID: 23594387.
3. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nat Rev Microbiol. 2014; 12(12):833–40. Epub 2014/10/21. nrmi-3364 [pii] https://doi.org/10.1038/nrmi3364 PMID: 25329408.
4. Drakeley C, Cook J. Chapter 5. Potential contribution of sero-epidemiological analysis for monitoring malaria control and elimination: historical and current perspectives. Adv Parasitol. 2009; 69:299–352. Epub 2009/07/23. https://doi.org/10.1016/S0065-308X(09)69005-9 PMID: 19622411.
5. Diop F, Richard V, Diouf B, Sokhna C, Diagne N, Trape JF, et al. Dramatic declines in seropositivity as determined with crude extracts of Plasmodium falciparum schizonts between 2000 and 2010 in Dielmo and Ndiop, Senegal. Malar J. 2014; 13:83. Epub 2014/03/08. https://doi.org/10.1186/1475-2875-13-83 PMID: 24602390; PubMed Central PMCID: PMC3975713.
6. Felgner PL, Roestenberg M, Liang L, Hung C, Jain A, Pablo J, et al. Pre-erythrocytic antibody profiles induced by controlled human malaria infections in healthy volunteers under chloroquine prophylaxis. Sci Rep. 2013; 3:3549. Epub 2013/12/20. https://doi.org/10.1038/srep03549 PMID: 24351974; PubMed Central PMCID: PMC4894385 Discovery Inc. that has licensed the technology.
7. Mathews HM, Fisher GU, Kagan IG. Persistence of malaria antibody in Tobago, West Indies, following eradication, as measured by the indirect hemagglutination test. Am J Trop Med Hyg. 1970; 19(4):581–5. Epub 1970/07/01. PMID: 4913003.
8. Migot F, Chougnet C, Raharimalala L, Astagneau P, Lepers JP, Deloron P. Human immune responses to the Plasmodium falciparum ring-infected erythrocyte surface antigen (Pf155/RESA) after a decrease in malaria transmission in Madagascar. Am J Trop Med Hyg. 1993; 48(3):432–9. Epub 1993/03/01. PMID: 8470778.
9. Akpogheneta OJ, Duah NO, Tetteh KK, Dunyo S, Lanar DE, Pinder M, et al. Duration of naturally acquired antibody responses to blood-stage Plasmodium falciparum is age dependent and antigen specific. Infect Immun. 2008; 76(4):1748–55. Epub 2008/01/24. https://doi.org/10.1128/IAI.01333-07 PMID: 18212081; PubMed Central PMCID: PMC2292892.
10. Ambrosino E, Dumoulin C, Orlandi-Pradines E, Remoue F, Toure-Balde A, Tall A, et al. A multiplex assay for the simultaneous detection of antibodies against 15 Plasmodium falciparum and Anopheles gambiae saliva antigens. Malar J. 2010; 9:317. Epub 2010/11/01. 1475-2875-9-317 [pii] https://doi.org/10.1186/1475-2875-9-317 PMID: 21059221; PubMed Central PMCID: PMC2992071.
11. Baum E, Badu K, Molina DM, Liang X, Felgner PL, Yan G. Protein microarray analysis of antibody responses to Plasmodium falciparum in western Kenyan highland sites with differing transmission levels. PLoS One. 2013; 8(12):e82246. Epub 2013/12/07. https://doi.org/10.1371/journal.pone.0082246 PMID: 24312649; PubMed Central PMCID: PMC3846730.
12. Corran P, Coleman P, Riley E, Drakeley C. Serology: a robust indicator of malaria transmission intensity? Trends Parasitol. 2007; 23(12):575–82. Epub 2007/11/09. https://doi.org/10.1016/j.pt.2007.08.028 PMID: 17988945.
13. Crompton PD, Kayala MA, Traore B, Kayentao K, Ongoiba A, Weiss GE, et al. A prospective analysis of the Ab response to Plasmodium falciparum before and after a malaria season by protein microarray. Proc Natl Acad Sci U S A. 2010; 107(15):6958–63. Epub 2010/03/31. 1001323107 [pii] https://doi.org/10.1073/pnas.1001323107 PMID: 20351286; PubMed Central PMCID: PMC2872454.
14. Koffi D, Toure AO, Varella ML, Vigan-Womas I, Beourou S, Brou S, et al. Analysis of antibody profiles in symptomatic malaria in three sentinel sites of ivory Coast by using multiplex, fluorescent, magnetic, bead-based serological assay (MAGPIX). Malar J. 2015; 14(1):509. Epub 2015/12/23. https://doi.org/10.1186/s12936-015-1043-2 PMID: 26692284; PubMed Central PMCID: PMC4687342.
15. Perraut R, Marrama L, Diouf B, Sokhna C, Tall A, Nabeth P, et al. Antibodies to the conserved C-terminal domain of the Plasmodium falciparum merozoite surface protein 1 and to the merozoite extract and their relationship with in vitro inhibitory antibodies and protection against clinical malaria in a Senegalese village. J Infect Dis. 2005; 191(2):264–71. Epub 2004/12/21. https://doi.org/10.1086/426398 PMID: 15609237.

16. Perraut R, Richard V, Varella ML, Trape JF, Guillotte M, Tall A, et al. Comparative analysis of IgG responses to Plasmodium falciparum MSP1p19 and PF13-DBL1alpha1 using ELISA and a magnetic bead-based duplex assay (MAGPIX(R)-Luminex) in a Senegalese meso-endoemic community. Malar J. 2014; 13:410. Epub 2014/10/19. https://doi.org/10.1186/1475-2875-13-410 PMID: 25326042; PubMed Central PMCID: PMC4221706.

17. Poinsignon A, Cornelie S, Mestres-Simon M, Lanfrancotti A, Rossignol M, Boulanger D, et al. Novel peptide marker corresponding to salivary protein gSG6 potentially identifies exposure to Anopheles bites. PLoS One. 2008; 3(6):e2472. Epub 2008/06/26. https://doi.org/10.1371/journal.pone.0002472 PMID: 18575604; PubMed Central PMCID: PMC2427200.

18. Sarr JB, Orlandi-Pрадines E, Fortin S, Sow C, Cornelie S, Rogerie F, et al. Assessment of exposure to Plasmodium falciparum transmission in a low endemicity area by using multiplex fluorescent microsphere-based serological assays. Parasit Vectors. 2011; 4:212. Epub 2011/11/09. https://doi.org/10.1186/1756-3305-4-212 PMID: 22059951; PubMed Central PMCID: PMC3223141.

19. Stewart L, Gosling R, Griffin J, Gesase S, Campo J, Hashim R, et al. Rapid assessment of malaria transmission using age-specific sero-conversion rates. PLoS One. 2009; 4(6):e6083. Epub 2009/06/30. https://doi.org/10.1371/journal.pone.0006083 PMID: 19562032; PubMed Central PMCID: PMC2698122.

20. Cook J, Kleinschmidt I, Schwabe C, Nseng G, Bousema T, Corran PH, et al. Serological markers suggest heterogeneity of effectiveness of malaria control interventions on Bioko Island, equatorial Guinea. PLoS One. 2011; 6(9):e25137. Epub 2011/10/08. https://doi.org/10.1371/journal.pone.0025137 PMID: 21980368; PubMed Central PMCID: PMC3181341.

21. Okebe J, Affara M, Correa S, Muhammad AK, Nwakanma D, Drakeley C, et al. School-based country-wide seroprevalence survey reveals spatial heterogeneity in malaria transmission in the gambia. PLoS One. 2011; 4(6):e2198036; PubMed Central PMCID: PMC3223141.

22. Ondigo BN, Hodges JS, Ireland KF, Magak NG, Lanar DE, Dutta S, et al. Estimation of recent and long-term malaria transmission in a population by antibody testing to multiple Plasmodium falciparum antigens. J Infect Dis. 2014; 210(7):1123–32. Epub 2014/04/17. https://doi.org/10.1093/infdis/jiu225 PMID: 24737801; PubMed Central PMCID: PMC4168304.

23. Shelton JM, Corran P, Risley P, Silva N, Hubbart C, Jeffreys A, et al. Genetic determinants of anti-malarial acquired immunity in a large multi-centre study. Malar J. 2015; 14:333. Epub 2015/09/01. https://doi.org/10.1186/s12936-015-0833-x PMID: 26314886; PubMed Central PMCID: PMC4552443.

24. Helb DA, Tetteh KK, Felgner PL, Skinner J, Hubbard A, Arinaitwe E, et al. Novel serologic biomarkers provide accurate estimates of recent Plasmodium falciparum exposure for individuals and communities. Proc Natl Acad Sci U S A. 2015; 112(32):E4438–47. Epub 2015/07/29. https://doi.org/10.1073/pnas.1501705112 PMID: 26216993; PubMed Central PMCID: PMC458641.

25. Razakandrainibe R, Thonier V, Ratsimbasonia A, Rakotomalala E, Ravaoarisoa E, Raherinjafy R, et al. Epidemiological situation of malaria in Madagascar: baseline data for monitoring the impact of malaria control programmes using serological markers. Acta Trop. 2009; 111(2):160–7. Epub 2009/04/21. https://doi.org/10.1016/j.actatropica.2009.04.007 PMID: 19378076.

26. White MT, Griffin JT, Akpogheneta O, Conway DJ, Koram KA, Riley EM, et al. Dynamics of the antibody response to Plasmodium falciparum infection in African children. J Infect Dis. 2014; 210(7):1115–22. Epub 2014/04/11. https://doi.org/10.1093/infdis/jiu199 PMID: 24719471.

27. Badu K, Siangla J, Larbi J, Lawson BW, Afrane Y, Ong’echa J, et al. Variation in exposure to Anopheles gambiae salivary gland peptide (gSG6-P1) across different malaria transmission settings in the western Kenya highlands. Malar J. 2012; 11:318. Epub 2012/09/12. https://doi.org/10.1186/1475-2875-11-318 PMID: 22963464; PubMed Central PMCID: PMC3541077.

28. Baidjoe AY, Stevenson J, Knight P, Stone W, Stresman G, Osoji V, et al. Factors associated with high heterogeneity of malaria at fine spatial scale in the Western Kenyan highlands. Malar J. 2016; 15:307. Epub 2016/06/05. https://doi.org/10.1186/s12936-016-1362-y PMID: 27259286; PubMed Central PMCID: PMC4893321.

29. Drakeley CJ, Carneiro I, Reyburn H, Malima R, Lusingu JP, Cox J, et al. Altitude-dependent and independent variations in Plasmodium falciparum prevalence in northeastern Tanzania. J Infect Dis. 2005; 191(10):1589–98. Epub 2005/04/20. https://doi.org/10.1086/429669 PMID: 15838785.

30. Badu K, Gyan B, Appawu M, Mensah D, Dodoo D, Yan G, et al. Serological evidence of vector and parasite exposure in Southern Ghana: the dynamics of malaria transmission intensity. Parasit Vectors.
31. Cornille-Brogger R, Mathews HM, Storey J, Ashkar TS, Brogger S, Molineaux L. Changing patterns in the humoral immune response to malaria before, during, and after the application of control measures: a longitudinal study in the West African savanna. Bull World Health Organ. 1978; 56(4):579–600. Epub 1978/01/01. PMID: 365386; PubMed Central PMCID: PMC2395642.

32. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A. 2005; 102(14):5108–13. Epub 2005/03/29. https://doi.org/10.1073/pnas.0408725102 PMID: 15792998; PubMed Central PMCID: PMC555970.

33. Drame PM, Poirson A, Besnard P, Cornelie S, Le Mire J, Toto JC, et al. Human antibody responses to the Anopheles salivary gSG6-P1 peptide: a novel tool for evaluating the efficacy of ITNs in malaria vector control. PLoS One. 2010; 5(12):e15556. Epub 2010/12/24. https://doi.org/10.1371/journal.pone.0015596 PMID: 21179476; PubMed Central PMCID: PMC301874.

34. Kerkhof K, Sluydts V, Willen L, Kim S, Canier L, Heng S, et al. Serologic markers to measure recent changes in malaria at population level in Cambodia. Malar J. 2014; 13:451. Epub 2014/11/25. https://doi.org/10.1186/1475-2875-13-451 PMID: 25416454; PubMed Central PMCID: PMC4258276.

35. van den Hoogen LL, Griffin JT, Cook J, Sepulveda N, Corran P, Conway DJ, et al. Serology describes a profile of declining malaria transmission in Farafenni, The Gambia. Malar J. 2015; 14(1):416. Epub 2015/10/27. https://doi.org/10.1186/s12936-015-0939-1 PMID: 26492873; PubMed Central PMCID: PMC4618886.

36. Ceesay SJ, Casals-Pascual C, Nwakanma DC, Walther M, Gomez-Escobar N, Fulford AJ, et al. Human antibody responses to Plasmodium falciparum sporozoite surface antigens reflect malaria transmission rates and are persistent in the absence of reinfection. Infect Immun. 1986; 53(2):393–7. Epub 1986/08/01. PMID: 3525412; PubMed Central PMCID: PMC260888.

37. Hsiang MS, Hwang J, Kunene S, Drakeley CJ, Kandula D, Novotny J, et al. Surveillance for malaria elimination in Swaziland: a national cross-sectional study using pooled PCR and serology. PLoS One. 2012; 7(1):e29550. Epub 2012/01/13. https://doi.org/10.1371/journal.pone.0029550 PONE-D-11-16427 [pii]. PMID: 22238621; PubMed Central PMCID: PMC3253098.

38. Cornille-Brogger R, Mathews HM, Storey J, Ashkar TS, Brogger S, Molineaux L. Changing patterns in the humoral immune response to malaria before, during, and after the application of control measures: a longitudinal study in the West African savanna. Bull World Health Organ. 1978; 56(4):579–600. Epub 1978/01/01. PMID: 365386; PubMed Central PMCID: PMC2395642.

39. Kerkhof K, Sluydts V, Willen L, Kim S, Canier L, Heng S, et al. Serologic markers to measure recent changes in malaria at population level in Cambodia. Malar J. 2014; 13:451. Epub 2014/11/25. https://doi.org/10.1186/1475-2875-13-451 PMID: 25416454; PubMed Central PMCID: PMC4258276.

40. Druilhe P, Pradier O, Marc JP, Miltgen F, Mazier D, Parent G. Levels of antibodies to Anopheles gambiae Salivary gSG6-P1 peptide: a novel tool for evaluating the efficacy of ITNs in malaria vector control. PLoS One. 2010; 5(12):e15556. Epub 2010/12/24. https://doi.org/10.1371/journal.pone.0015596 PMID: 21179476; PubMed Central PMCID: PMC301874.

41. Moncunill G, Mayor A, Jimenez A, Nhabomba A, Puyol L, Manaca MN, et al. Cytokine and Antibody Responses to Plasmodium falciparum in Naive Individuals during a First Malaria Episode: Effect of Age and Malaria Exposure. PLoS One. 2013; 8(2):e55756. Epub 2013/02/26. https://doi.org/10.1371/journal.pone.0055756 PONE-D-12-32333 [pii]. PMID: 23437061; PubMed Central PMCID: PMC3578867.

42. Diene Sarr F, Niang M, Thiam D, Dia N, Badiane A, Ndao AB, et al. Acute Febrile Illness and Influenza Disease Burden in a Rural Cohort Dedicated to Malaria in Senegal, 2012–2013. PLoS One. 2015; 10(12):e0143999. Epub 2015/12/19. https://doi.org/10.1371/journal.pone.0143999 PMID: 26679177; PubMed Central PMCID: PMC4682973.

43. Laneri K, Paul RE, Tall A, Faye J, Diene-Sarr F, Sokhna C, et al. Dynamical malaria models reveal how immunity buffers effect of climate variability. Proc Natl Acad Sci U S A. 2015; 112(28):8786–91. Epub 2015/07/01. https://doi.org/10.1073/pnas.1419047112 PMID: 26124134; PubMed Central PMCID: PMC4507245.

44. Trape JF, Pison G, Spiegel A, Enel C, Rogier C. Combating malaria in Africa. Trends Parasitol. 2002; 18(5):224–30. Epub 2002/05/02. PMID: 1193604.

45. Trape JF, Tall A, Sokhna C, Ly AB, Diagne N, Ndiath O, et al. The rise and fall of malaria in a West African rural community, Dielmo, Senegal, from 1990 to 2012: a 22 year longitudinal study. Lancet Infect
Drame PM, Diallo A, Poinsignon A, Boussari O, Dos Santos S, Machault V, et al. Evaluation of the effectiveness of malaria vector control measures in urban settings of Dakar by a specific anophelines salivary peptide. PLoS One. 2014; 9(2):e87169. Epub 2014/02/13. https://doi.org/10.1371/journal.pone.0087169 PMID: 24520325; PubMed Central PMCID: PMC3919715.

Trape JF, Rogier C, Kone D, Diagne N, Bouganali H, Canque B, et al. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. Am J Trop Med Hyg. 1994; 51(2):123–37. Epub 1994/08/01. PMID: 8074247.

Roucher C, Rogier C, Dieye-Ba F, Sokhna C, Tall A, Trape JF. Changing malaria epidemiology and diagnostic criteria for Plasmodium falciparum clinical malaria. PLoS One. 2012; 7(9):e46188. Epub 2012/10/03. https://doi.org/10.1371/journal.pone.0046188 PMID: 23094333; PubMed Central PMCID: PMC3460864.

Niang M, Loucoubar C, Sow A, Diagne MM, Faye O, Diallo M, et al. Genetic diversity of Plasmodium falciparum isolates from concurrent malaria and arbovirus co-infections in Kedougou, southeastern Senegal. Malar J. 2016; 15(1):155. Epub 2016/03/13. https://doi.org/10.1186/s12936-016-1208-7 PMID: 26996623; PubMed Central PMCID: PMC4788873.

Roucher C, Rogier C, Dieye-Ba F, Sokhna C, Tall A, et al. Allelic diversity of the site. Microbes and infection. 2002; 4(1):31–5. Epub 2002/02/05. PMID: 11825772.

Roucher C, Rogier C, Dieye-Ba F, Sokhna C, Tall A, et al. Pattern of immunoglobulin isotype response to Plasmodium falciparum erythrocyte membrane protein 1 entails variant-specific red cell surface epitopes. PLoS One. 2011; 6(1):e16544. Epub 2011/02/08. https://doi.org/10.1371/journal.pone.0016544 PMID: 21298021; PubMed Central PMCID: PMC3029348.

Bonnet S, Petres S, Holm I, Fontaine T, Rosario S, Roth C, et al. Soluble and glyco-lipid modified baclovirus Plasmodium falciparum C-terminal merozoite surface protein 1, two forms of a leading malaria vaccine candidate. Vaccine. 2006; 24(33–34):5997–6008. Epub 2006/07/04. https://doi.org/10.1016/j.vaccine.2006.04.069 PMID: 16814434.

Perraut R, Varela ML, Mbengue B, Guillotte M, Mercereau-Puijalon O, Vigan-Womas I. Standardization of a multiplex magnetic bead-based assay for simultaneous detection of IgG to Plasmodium antigens. J Immunol Tech Infect Dis. 2015; 4(2):1–8. http://dx.doi.org/10.4172/2329-9541.1000134.

Aribot G, Rogier C, Sarthou JL, Trape JF, Balde AT, Druilhe P, et al. Pattern of immunoglobulin isotype response to Plasmodium falciparum blood-stage antigens in individuals living in a holoendemic area of Senegal (Dielmo, west Africa). Am J Trop Med Hyg. 1996; 54(5):449–57. Epub 1996/05/01. PMID: 8644897.

Badu K, Afrane YA, Larbi J, Stewart VA, Waitumbi J, Angov E, et al. Marked variation in MSP-119 antibody responses to malaria in western Kenyan highlands. BMC Infect Dis. 2012; 12:50. Epub 2012/03/03. https://doi.org/10.1186/1471-2334-12-50 PMID: 22380785; PubMed Central PMCID: PMC3036741.

Perraut R, Marrama L, Diouf B, Fontenille D, Tall A, Sokhna C, et al. Distinct surrogate markers for protection against Plasmodium falciparum infection and clinical malaria identified in a Senegalese community after radical drug cure. J Infect Dis. 2003; 188(12):1940–50. Epub 2003/12/16. https://doi.org/10.1086/379838 PMID: 14673775.

Zwetyenga J, Rogier C, Spiegel A, Fontenille D, Trape JF, Mercereau-Pujol J. A cohort study of Plasmodium falciparum diversity during the dry season in Ndiop, a Senegalese village with seasonal, mesoendemic malaria. Trans R Soc Trop Med Hyg. 1999; 93(4):375–80. Epub 2000/02/16. https://doi.org/10.1016/S0037-929X(99)80125-X PMID: 9991385; PubMed Central PMCID: PMC1207184.

Drame PM, Diallo A, Poinsignon A, Bousari O, Dos Santos S, Machault V, et al. Evaluation of the effectiveness of malaria vector control measures in urban settings of Dakar by a specific anophelines salivary biomarker. PLoS One. 2013; 8(6):e66354. Epub 2013/07/11. https://doi.org/10.1371/journal.pone.0066354 PMID: 23840448; PubMed Central PMCID: PMC3688790.

Drame PM, Machault V, Diallo A, Cornelle S, Poinsignon A, Lalou R, et al. IgG responses to the gSG6-P1 salivary peptide for evaluating human exposure to Anopheles bites in urban areas of Dakar region,
Senegal. Malar J. 2012; 11:72. Epub 2012/03/20. https://doi.org/10.1186/1475-2875-11-72 PMID: 22424570; PubMed Central PMCID: PMC3337805.

63. Rizzo C, Lombardo F, Ronca R, Mangano V, Sirima SB, Nebie I, et al. Differential antibody response to the Anopheles gambiae gSG6 and cE5 salivary proteins in individuals naturally exposed to bites of malaria vectors. Parasit Vectors. 2014; 7:549. Epub 2014/11/28. https://doi.org/10.1186/s13071-014-0549-8 PMID: 25428638; PubMed Central PMCID: PMC4253619.

64. Rizzo C, Ronca R, Fiorentino G, Verra F, Mangano V, Poinsignon A, et al. Humoral response to the Anopheles gambiae salivary protein gSG6: a serological indicator of exposure to Afrotropical malaria vectors. PLoS One. 2011; 6(3):e17980. Epub 2011/03/26. https://doi.org/10.1371/journal.pone.0017980 PMID: 21437289; PubMed Central PMCID: PMC3060095.

65. Drame PM, Poinsignon A, Dechavann C, Cottrell G, Farce M, Ladekpo R, et al. Specific antibodies to Anopheles gSG6-P1 salivary peptide to assess early childhood exposure to malaria vector bites. Malar J. 2015; 14:285. Epub 2015/07/23. https://doi.org/10.1186/s12936-015-0800-6 PMID: 26198354; PubMed Central PMCID: PMC4511589.