Trends in malaria epidemiological factors following the implementation of current control strategies in Dangassa, Mali

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Abstract

Background:
Over the past decade, three strategies have reduced severe malaria cases and deaths in endemic regions of Africa, Asia, and the Americas, specifically: 1] artemisinin-based combination therapies (ACTs), 2] insecticide-treated bed nets (ITNs), and 3] intermittent preventive treatment with sulfadoxine-pyrimethamine in pregnancy (IPTp). The rationale for this study was to examine communities in Dangassa, Mali where in 2015 two additional control strategies were implemented: ITN universal coverage and Seasonal Malaria Chemoprevention (SMC) among children less than five years.

Methods:
This was a prospective study based on a rolling longitudinal cohort of 1,401 subjects participating in biannual smear surveys for the prevalence of asymptomatic *P. falciparum* infection and continuous surveillance for the incidence of human disease (uncomplicated malaria). Entomologic collections were performed to examine the intensity of transmission based on pyrethroid spray catches, human landing catches, and enzyme-linked immunosorbent assay (ELISA) testing for circumsporozoite antigen.

Results:
A total of 1,401 participants of all ages were enrolled in the study in 2012 after random sampling of households from the community census list. Prevalence of infection was
extremely high in Dangassa varying from 9.5% to 62.8% at the start of the rainy season and from 15.1% to 66.7% at the end of the rainy season. Likewise, the number of vectors per house, biting rates, sporozoïtes rates and entomologic inoculation rates (EIR) were substantially greater in Dangassa.

Discussion:
The findings for this study are consistent with the progressive implementation of effective malaria control strategies in Dangassa. At baseline (2012-2014), prevalence of *P. falciparum* was above 60% followed by a significant year-to-year decease starting in 2015. Incidence of uncomplicated infected was greater among children < five years while asymptomatic infection was more frequent among the five to fourteen years old. A significant decrease in entomological inoculation rate was also observed from 2015 to 2020. Likewise, vectors’ density, sporozoïtes rates, and entomologic inoculation rates decrease substantially during the study period.

Conclusion:
Efficient Implementation of two main malaria prevention strategies in Dangassa substantially contribute to a reduction of both asymptomatic and symptomatic malaria in Dangassa from 2015 to 2020.

Key Words: *P. falciparum* Malaria, Control strategies, failure, Dangassa - Mali
Background:

Despite the intensive and continued implementation of evidence-based interventions, malaria remains a worldwide public health problem, particularly in Sub-Saharan Africa (SSA). With a significant geographical and seasonal variation, SSA is the most affected malaria region with respect to malaria-related deaths. Between 2000 and 2015, malaria cases declined from 238 million to 218 million detected cases [1]. Malaria deaths also decreased by from 839,000 in 2000 to 409,000 in 2019 [1]. However, this dynamic has changed over the last five years, with 11 million malaria cases in 2019 compared to 2015 [1].

During the past decade, Mali, and most of SSA countries, started the progressive implementation of proven malaria prevention and control strategies, such as the distribution of insecticide-treated bed nets (INTs), indoor residual spraying in targeted geographic areas, improved access to healthcare with early diagnosis and prompt treatment, prevention and rapid management of malaria epidemics and surveillance, provision of Intermittent Preventive Therapy to pregnant women (IPTp), and Seasonal Malaria Chemoprevention (SMC) for children less than five years. The Malian government and its partners have been offering free malaria diagnostics and treatment to children less than five years and pregnant women since 2008 [2-4].

Malaria epidemiological factors for malaria in Mali are associated with the four eco-climatic zones encountered in the country: 1) the Sahara zone, with a short rainy season where epidemics of malaria could occur; 2) the Sahel zone, with mostly irrigated rice production enhancement projects where malaria epidemiology varies according to water used and agricultural activities; 3) the Sudanese-Guinean zone, where transmission can
last up to six months a year and 4) the Inner Delta of the Niger River, where malaria transmission continues year-round.

In addition to insecticide residual spraying (IRS), which is implemented in only two of Mali’s districts, all other control and prevention strategies are present countrywide regardless of the differences in transmission dynamic. Also, as in many malaria endemic countries, one of the main gaps in control strategy planning and implementation is the lack of accurate data on the disease from local health facilities. Community-based cohort data on malaria prevalence and incidence over several consecutive years are more reliable and can provide evidence-based and area-specific interventions [5-8].

This cohort study was performed in Dangassa, located in a high malaria transmission area of Mali (Sudanese-Guinean zone) from 2012 to 2020. Dangassa is one the site of the International Center for Excellence in Malaria Research in West Africa (ICEMR-WAF) [9] defined as an intense malaria transmission area. Malaria control and prevention strategies in Dangassa are based on case management at community health centers, using rapid diagnostic tests (RDT), artemisinin-based combination therapy (ACT), and ITNs, beginning in 2008. In 2015, the first mass distribution of long-lasting insecticidal nets (LLINs) occurred in the village, achieving universal coverage (one LLIN per every two people). In the same year, SMC was first implemented for children less than five years during the high-risk season of malaria, from July to October (see table 1).

The aim of the study was to perform a year- and age-specific analysis of the change in malaria epidemiology in relation to the implementation of control and prevention strategies in the area.
Methods:

Study site

The study was carried out in the village of Dangassa, Mali, which is situated in a Sudanese-Guinean zone, where malaria transmission can persist for up to six months a year. Dangassa village is one of the health zones within the health district of Ouelessebougou, in the region of Koulikoro, Mali (Figure 1). With approximately 7,000 inhabitants, the village is located near the Niger River, approximately 80 km southwest of Bamako. The average annual temperature and rainfall are 27.5°C and 855 mm, respectively [10, 11]. Malaria transmission is highly seasonal, with more than 65% of cases observed between June and November each year. Dangassa has a community health center covering 11 surrounding villages and 47 community health workers. In Dangassa, ACT, rapid diagnostic tests (RDTs), IPTp, and ITNs, have been available since 2006 - 2008, while LLINs universal coverage and SMC for children aged less than five years were introduced in 2015 (Figure1) [12].
Study Design:

Before beginning the study, the goals and procedures were presented to the chief and elders of Dangassa, to garner community support for the project. After obtaining approval, a general meeting with the community was held, and individual presentations were made to the occupants of candidate houses according to the random selection of households as described below.

Once communities with high, intermediate, and low intensities of *P. falciparum* infection and disease had been identified by reviewing historical data on the frequency of infection and disease, a census was performed in each candidate community. This approach was done to identify occupied houses in the community and update the number and ages of the persons in the houses. After all of the currently occupied houses in Dangassa had been identified from the updated census, households were randomly selected from the database to obtain a total of 1,401 participants of all age groups. Then, members of selected households were informed about the study and asked if they would consider participating in the biannual smear surveys.

Biannual Smear Surveys for the Prevalence of *P. falciparum* Infection:

Each year, two smear surveys were performed at the beginning and the end of the malaria transmission season. Each survey was done in an average of 12 days at the community health center of Dangassa. Selected household members were screened for malaria parasitemia, and a structured questionnaire was administered. Information on the history of fever was collected, and a clinical examination was performed. Only participants with fever or reported fever within the past 48 hours were tested using malaria RDTs. The SD
BIOLINE Malaria Ag P.f/Pan test, a qualitative and differential test for the detection of histidine-rich protein II (HRP-II) antigen of *P. falciparum* and common *Plasmodium* lactate dehydrogenase (pLDH) of *Plasmodium* species in whole human blood, was used.

In addition, blood films were performed (capillary blood) for all participants, regardless of symptoms, to determine asymptomatic malaria parasitemia. Thick and thin blood film slides were prepared using 10% Giemsa solution for 30 minutes. The stained slides were then examined under a light microscope, using 100x oil immersion, by an experienced technician. Parasitemia was calculated per 200 white blood cells (WBC), assuming 8,000 WBC/µl of blood [13]. Anemia was assessed for each participant using HemoCue® Hb 301 System (HemoCue America, Danaher Company, Brea, CA 92821 USA).

Only symptomatic malaria cases, defined as a patient with fever or history of fever within the past 48 hours and RDT positive, were treated, free of charge, according to the national policy for malaria case management by the Malian National Malaria Control Program (NMPC).

Slide reading was performed, and records on infection status, malaria parasite species, and count of asexual and sexual forms for *P. falciparum* were collected.

**Continuous Surveillance for Uncomplicated *P. falciparum* Malaria:**

During the enrollment process, participants were asked to visit the clinician for any symptoms on any day of the week. Passive case detection of malaria incidence was implemented at the Dangassa community health center in January 2013. A physician and a medical student were based in the village and allocated a workspace at the community
health center to perform the daily passive case detection year-round. A weekly household visit was performed in randomly selected households to ensure that all malaria cases were seen by the research team. Once a patient visited the health center, a questionnaire was administered, followed by clinical examination and blood sample collection. Both malaria RDT and finger-prick blood samples, obtained under sterile conditions using disposable equipment, were systematically performed for patients. Parasitemia was calculated per 200 WBC assuming 8,000 WBC/µl of blood [13]. A clinical report and lab analysis form for malaria RDT, slide reading, and an anemia assessment was completed for each visit. Malaria treatment was given based on the malaria RDT result, according to the national malaria control policy by the NMPC.

**Mosquito collection and sample processing:**

The collection of Anopheline mosquitoes was performed by pyrethrum spray catches at the beginning and end of the rainy season two weeks before the smear surveys. Forty-five compounds out of the 250 of Dangassa village were randomly selected for mosquito collection. In each selected compound, one room was sprayed. A compound was defined as a group of households in which members share the same meal.

Each survey lasted 12 days during which 15 rooms were sprayed every three days, three times between 7:00 and 9:00 for indoor resting mosquitoes collection using the “Premium Insect Killer”, composed of 1.2% dichlorvos, 0.4% fenitrothion and 0.15% tetramethrin. *Anopheles gambiae s.l.* specimens were identified morphologically [14, 15] and placed in labelled vials containing 80% ethanol and transported to the laboratory for analysis and species identification. *Anopheles gambiae s.l.* infection rate (IR) and human blood index
(HBI) were established using the Enzyme Linked-Immuno-Sorbent Assay (ELISA) techniques [16, 17].

**Calculation of prevalence and incidence**

The prevalence of malaria was defined as the number of participants with microscopic positive blood smear to sexual and/or asexual *P. falciparum* divided by the number of participants seen at each visit. Incidence rate of clinical malaria defined as history of fever or a measured temperature ≥37.5°C and a microscopic positive blood smear to sexual and/or asexual *P. falciparum* is defined as the total number of incident malaria cases divided by the total person-time observed in the cohort.

**Statistical analysis**

Results were expressed as frequencies and percentages or means and standard deviations as appropriate. Data were entered in StudyTRAX database management system (version v3.2.0802, StudyTRAX, Macon, GA). The clinical data were analyzed in R-studio version 1.3.1093 and GraphPad Prism v.7 Software for Windows [18, 19]. The prevalence of malaria parasitaemia was defined as the proportion of subjects with microscopy *P. falciparum* positive smear. From the PCD data, the malaria incidence rate was estimated as the number of new malaria cases per person-weeks during the follow-up period (expressed per 1000 person-weeks and 100 person-weeks respectively for figures 4 and 5). The following entomological parameters were calculated: vector density per room, human biting rate (HBR), infection rate (IR), entomological inoculation rate (EIR), and human blood index (HBI). The density of malaria vectors was calculated as the average number of indoor resting mosquitoes per room per day; the HBR as the average number of mosquito bites received by a sleeping person per time unit (blood-fed
and half gravid mosquito/number of room sleeping people in the room); the IR corresponds to the proportion of An. gambiae s.l. carrying Plasmodium falciparum sporozoites; the HBI as the proportion of female mosquitoes having human blood in their guts. All analyses were carried out with a 5% type I error threshold

**Ethical consideration**

Ethical approvals were obtained from the National Institutes of Health (NIAID) and from the IRBs of Tulane University (FWA00002055) and the University of Sciences, Techniques and Technology of Bamako in Mali (2011/77/FMPOS). Before patients were enrolled in this study in 2012 and for those enrolled after, a written informed consent was obtained from each participant or their parent/legal guardian if under 18 years. Please note that the cohort study protocol has been reviewed and renewed annually since that time. Individual informed consent forms were obtained for PSCs and HLCs (for both room owners and data collectors) before starting mosquito collections.
**Results:**

A total of 1,401 children and adults were enrolled to the study in September 2012 with a sex ratio of 1.18 female to male. The median age was 11 years (maximum 86 years old). Adults aged over 20 years old represented 33% of the study population, while children less than five years represented 18% of the study population and children aged five to nine years represented 19% of the study population (Table 1).

The mean prevalence of *P. falciparum* parasitaemia at the start of the malaria transmission (June-July), shows a significant year to year variation from 2014 to 2020. The lowest prevalence was observed in 2019 with only 10.3% and the highest in 2014 with 58.6% of participants positive at microscopy. Overall, prevalence of *P. falciparum* infection in the study population decrease by 41.5% ($X^2 = 529.5; p < 0.0001$) from 2014 to 2020.

At the end of the malaria transmission season (October – November), *P. falciparum* infection also show a significant year-to-year variation with the lowest prevalence observed in 2019 (17%) and the highest in 2015 (62.2%). Asymptomatic malaria prevalence decreased by 27.9% ($X^2 = 856.3; p < 0.0001$) at the peak season over the study period (figure 3).
Figure 4 provides age-specific malaria infection rates per year, with August 2012 as the baseline for comparison. At the start of the malaria transmission season, a significant decrease in asymptomatic carrying of the malaria parasite was observed over multiple years, among all age groups. Parasitaemia was much higher among children less than five years (33% to 59.4%) compare to other age groups. However, in 2016 we observed an age-shift of infection prevalence with more children aged five to nine and ten to fourteen infected than all other age groups. A similar trend was observed through 2020. The year-to-year decrease in infection prevalence was observed among both age groups during the study period. However, this change was more significant among children less than five years, with a decrease of 49.3% [95%CI = 37.2 – 59.2] vs. 43% [95%CI = 35.7 – 49.6] between 2014 and 2020.

In opposition to the start of the malaria transmission season, from the beginning of the study in 2012 to the end (2020), parasitaemia at the end of the malaria transmission was always higher among children aged five to fourteen years compare to the less than five years age group with an average parasitaemia of 44.2% and 32.4% respectively. Comparison of *P. falciparum* infection prevalence at the peak malaria transmission season from 2012 to 2020 shows 24% [95%CI = 12.7 – 33.5] and 45% [95% = 38.9 – 50.7] decreases among children less than five years and children five to fourteen years respectively.
Incidence of symptomatic malaria is shown in Figure 5. This figure shows the seasonality of malaria, with incidence rates lower than 5% during the dry season, from January to June, and closer to 10% in July except in 2018 where incidence rate was closer 15 case per 1,000 persons’ weeks. Each year about 80% to 90% of all malaria cases were observed from July to November in Dangassa.

The temporal trends of malaria occurrence was similar for both years two peaks observed in 2015 and 2016 with respectively 34 and 32 cases per 1,000 person-weeks (figure 5).

Figure 6 shows that malaria is highly seasonal in Dangassa with less than 5 cases per 1,000 person-weeks from January to June each year corresponding to the dry season. A fluctuation of malaria cases started from July (7.5 malaria cases per 1,000 person-weeks), peaked in October with approximately 22.5 malaria cases per 1,000 person-weeks then decrease progressively down to 9 malaria cases per 1,000 person-weeks.

In figure 7, mean incidence rate is significantly higher among the children less than five years compared to any other age groups during that period (p<0.05). However, the other age groups did not show any statistically significant difference during the study period, in terms of incidence rates (p > 0.05).

**Density and Human blood index of Anopheline mosquitoes**

The mean density at the start of the malaria transmission season shows a significant year-to-year variation (from 2 An. gambiae s.l. per room in 2019 to to 30 An. gambiae s.l. per room in 2014) while the human biting index remain extremely high regardless the year (80% to 100%). Regardless the year, density of mosquitoes at the end of the malaria transmission season was
less than 25 mosquitoes per room). However, the human biting index remain high as at the start of the malaria transmission season varying from 78% to 90% (Figure 8)

**Mean Human Biting Rate, Infection Rate (IR) and Entomological Inoculation Rates (EIR) in *An. gambiae* s.l**

Figure 9 shows the mean monthly human biting rate (MHBR) and the entomological inoculation rate (EIR) from 2012 to 2019. At the start of the malaria transmission, the highest MHBR were observed in June 2014 with approximately 110 bites/person/month follow by 4 times decrease at the same moment of the year in 2015. MHBR was always lower at the end of the malaria transmission with the highest MHBR observed in 2012 and 2014 with respectively 45 and 43.5 105.5 bits/person/month. EIR at the start of the malaria transmission season varied from 1.1 to 1.5 infective bites/person/month except in 2015 where EIR was less than 0.5 infective bite / person / month). However, in contrast with the MHBR, EIR was significantly high at the end of malaria transmission varying from 0.01 to 4.02 infective bites / person / month in 2016 and 2012 respectively. Overall, we observed a significant decrease in both MHBR and EIR from 2015 to 2019 (p < 0.05).

**Discussion**

**Prevalence of asymptomatic malaria in the study population**

The study assesses *P. falciparum* infection prevalence in Dangassa over height years, at the start and end of the transmission season, with all age groups represented. High malaria prevalence was observed at baselines (end of malaria transmission season in 2012) and (start of the malaria transmission season in 2014) with 60.3% and 62.8% of participants having positive blood smears, respectively. A significant decrease was observed in 2015, with a 50% drop at the start of the transmission season. However, the same year prevalence at the end of the transmission season was similar to the ones
observed previous year at the same month. Since 2016, a year-to-year decrease in prevalence of asymptomatic malaria was observed at both start and end of the transmission season with the lowest prevalence in October 2018 (15.7%) and June 2019 (9.7%). This decrease can be related to the ITNs universal coverage campaign in 2015 follow by the efficient implementation of SMC in 2016 in the study area. It is worth mentioning that observations occurring in a single year may be due to random fluctuations, and those observations stable over a two-year interval are consistent with true patterns.

Data shows the presence of relevant reservoirs for malaria parasites in the study population, which can contribute to maintaining the transmission right after the onset of the rainy season and the presence of malaria vectors in the study area. A similar study in Bandiagara, Mali, shows that asymptomatic carry persisted in the community, with a ratio to clinical episodes from high to low transmission periods of about 0.5 to 5 episodes per child. Throughout the entire dry season, malaria transmission was low, and asymptomatic carriers were the only reservoir of parasites during this time [20]. The decrease in asymptomatic carry of malaria parasites in 2016 can be explained by the combined effect of universal coverage of LLINs in 2015, followed by the country-wide implementation of SMC for children less than five years in 2016 [11].

Seasonal variations of the age specific carriage of malaria parasites shows that children aged five to nine years old and 10 to 14 years old represent the main reservoir for parasites in the study area, at both the start and end of the rainy season. Similar patterns have been described in Sélingué, Mali, with a shift of both asymptomatic and symptomatic malaria prevalence and incidence among older children, who are unfortunately not yet targeted by specific malaria interventions, such as free testing and ACTs, as well as SMC [21]. The high infection rates observed at the start of the malaria transmission season suggest adoption of specific strategies, such as mass drug administration or SMC for older children starting a month or two before the rainy season in Dangassa, could help reduce malaria incidence in the region [11, 20, 22]

**Malaria seasonality and age-specific mean incidence of malaria in Dangassa**
Symptomatic malaria is highly seasonal in Dangassa, with a peak observed in August during the first two years of the study (2013 and 2014) and in October in 2015 and 2020. Relatively low incidence was observed during the dry season from January to June each year. This pattern has been described in several regions of Mali [20, 21]. However, the length of the transmission season in Dangassa is much longer than the four months described in many other regions of the country, calling for a reinforcement and extension of the duration of control interventions, such as increasing SMC from four to five months (July – November instead of July – October). In addition, as described earlier, a mass administration of malaria drugs at the end of the dry season can contribute to the decline of clinical cases during the transmission season [23, 24].

In opposition of the high prevalence of asymptomatic malaria among the older children, over the follow-up period, malaria incidence was higher among children less than five years, regardless of their benefit of all present interventions in the study area. These observations show that malaria disease is still more significant among this group than the rest of the population. Several hypotheses can explain this situation: 1] Appropriate treatment with ACTs given free of charge to children less than five years may lead to the lowest asymptomatic carriage of the parasites observed; 2] Compliance to SMC treatment (proportion of children receiving all three doses of the treatment each month) may be overestimated, since only the first dose is taken under direct observation and parents are asked to administer the second and third doses at home; 3] Experiencing many clinical episodes at an early age is known to induce premonition for the five years and older age group, leading to a decrease in the risk of presenting with symptoms in cases of low parasitaemia [25, 26].

**Malaria vector population: density, human biting index and entomological inoculation rate**

The main malaria vector in Dangassa is *An. gambiae s.l.* representing more than 90% of the anopheles' fauna, regardless of the season. Similar results have been reported by several authors in localities near Dangassa [27, 28]. Our results showed that *Anopheles gambiae s.l.* density per room varied significantly by month, season and year. These
fluctuations are related to the rain-dependency of the breeding sites of *An. gambiae s.l.* [29, 30].

*An. gambiae s.l.* was very anthropophilic. Outdoor staying of the inhabitants watching television and/or other domestic activities may have allowed mosquitoes' access to human blood sources despite of the LLIN universal coverage. In addition, outdoor resting behavior developed by malaria vector in this area may compromise the efficacy of indoor-based intervention such LLINs [31]

As expected in seasonal malaria transmission areas, our results show higher EIRs at the end of the rainy season compared to the beginning [27, 32-34]. There was a trend of decrease in both MHBR and EIR observed from 2015 to 2020 (with some fluctuation at the onset of the rainy season in 2018 and 2020), which can be related to the increase in malaria control intervention as described elsewhere [35, 36]. The finding are consistent with observation made in Mopti region of Mali from 1999 to 2006 after the large deployment of ITNs [37].

**Limitations**

The work here focused on a single rural site in Mali which may not represent suburban or urban areas. In year 2013 and 2017, we were unable to carry prevalence studies due to budget shortage causing missing data for this year. Passive case detection data are also missing for 2017 corresponding to the time in between the two rounds of ICEMR. Entomological data in 2020 are not presented because sample processing was not possible because of shortage of lab supplies as a consequence of the Covid19 pandemic.
Conclusions

Despite the decrease observed both in terms of asymptomatic and symptomatic malaria since 2015, the malaria transmission remain hyper endemic in Dangassa. Older children not targeted by many of these interventions seem to be more affected and represent the main malaria parasite reservoir in this community. High entomological inoculation rates observed can explain the rapid increase of malaria cases at the onset of rainy season in Dangassa.

Updated strategies such as extension of free diagnostics and treatment and SMC to older children, malaria mass drug administration before the start of the transmission season and Insecticide Residual Spraying could be effective in reducing the malaria burden in Dangassa.

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