Marked Decline in Malaria Prevalence among Pregnant Women and Their Offspring from 1996 to 2010 on the South Kenyan Coast

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Abstract. Expanded malaria control in Kenya since the early 2000s has resulted in marked reduction in hospital admissions for malaria; however, no studies have reported changes in malaria infection rates in the same population over this period. Randomly selected archived blood samples from four cohorts of pregnant women and their children from 1996 to 2010 in Kwale District, Coast Province, Kenya, were examined for *Plasmodium falciparum* (Pf), *P. malariae*, *P. ovale*, and *Plasmodium vivax* by quantitative polymerase chain reaction (PCR) and microscopy. Maternal delivery Pf prevalence by PCR declined from 40% in 2000–2005 to 1% in 2009–2010, concordant with increased bed net and malaria chemoprophylaxis use. Individual risk of Pf infection in children from birth to 3 years in serial longitudinal cohort studies declined from almost 100% in 1996–1999 to 15% in 2006–2010. Declines in *P. malariae* and *P. ovale* infections rates were also observed. These results show a profound reduction in malaria transmission in coastal Kenya.

BACKGROUND

A substantial decrease in hospital admissions for malaria in coastal regions of East Africa has been reported over the past decade.^{1–5} This decrease has been attributed to expanded malaria control initiatives, including intermittent preventive treatment of pregnant women during pregnancy, insecticide-treated bed net (ITN) usage, and availability of more effective antimalarial drugs (initially, sulfadoxine-pyrimethamine [SP] and more recently, artemisinin combination therapies [ACTs]) for case management of malaria illness.^{6–10} Presumably, these changes contributed to reduced malaria transmission and consequent reduced hospital admissions for malaria, although this finding has not been systematically examined. Few studies have examined population-based changes in malaria infection rates in pregnant women, and no studies have examined pregnant women in serial birth cohorts in the same population before and after the introduction of malaria control measures.

To investigate this question, a random sample of archived blood obtained from four birth cohorts of pregnant women and their children between 1996 and 2010 at the Msambweni District Hospital located in Coast Province, Kenya, was examined using the same molecular diagnostic assay to assess multispecies malaria infections. Non-immune infants and young children represent a good marker of ongoing transmission in a community. Over this period, we observed an approximately fivefold decline in incidence of malarial infection among young children and a similar decline in pregnant women at delivery. These results suggest that a profound reduction in malaria transmission has been a major contributor to the overall reduction in the burden of malaria disease in coastal regions of Kenya.

MATERIALS AND METHODS

Study population and blood sample collection. Pregnant women and children were recruited from the antenatal clinic (ANC) of Msambweni District Hospital, Msambweni (formerly Kwale) District in Coast Province, Kenya, in four separate birth cohorts in March of 1996, February of 2000, April of 2006, and

November of 2009 (Table 1). Samples reported in the current study were obtained through 2010. Peripheral venous blood samples were available from pregnant women at delivery for all birth cohorts as well as the first ANC visit for the 2006 and 2009 cohorts. Peripheral venous blood samples were obtained from offspring of pregnant women beginning at 6 months of age and at approximately 6-month intervals thereafter until 3 years of age for the first three birth cohorts (Table 2). At each examination period, approximately 5 mL peripheral venous blood were collected in (ethylenedinitrilo) tetraacetic acid vacutainers, plasma separated, and stored at -80°C as previously described.^{11,12} Informed consent was obtained from all participants in the study in accordance with the Case Western Reserve School of Medicine Institutional Review Board (No. 11-05-42) and Kenyan Ministry of Health policies (KEMRI ERC No. SSC 1040).

During this 14-year period, the recommended first-line antimalarial drugs for case management and malaria prophylaxis during pregnancy changed considerably. In 1998, SP replaced chloroquine as the Kenyan Ministry of Health (KMOH) recommended first-line treatment of uncomplicated malaria in children.¹³ At the same time, SP prophylaxis was adopted as a Kenyan national policy for control of malaria in pregnancy, with 1,500 mg sulfadoxine and 75 mg pyrimethamine administered one time at the beginning of the second trimester and again at the beginning of the third trimester.¹⁴ Between 2000 and 2006, SP was the only available antimalarial drug. In April of 2004, KMOH again officially changed the first-line antimalarial treatment of uncomplicated illness to artemether-lumefantrine (AL), but the new policy was not fully implemented until September of 2006.^{5,9}

Antimalarial treatment during pregnancy. During the cohorts beginning in 1996 and 2000, pregnant women were enrolled at delivery, and prophylaxis taken during pregnancy could not be precisely determined, although most women reported that they had not taken any antimalarial chemoprophylaxis. Women occasionally reported having fevers and self-treating with chloroquine. In the cohorts beginning in 2006 and 2009, women received SP prophylaxis in accordance with KMOH guidelines.

DNA extraction and blood smear diagnosis. Blood smear diagnosis was performed on all samples as previously described.^{12,15} DNA was extracted from a 200- μL red cell

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TABLE 1
Characteristics of pregnant women in the study population by cohort

Cohort	1996–1997	2000–2005	2006–2010	2009–2010
Women, <i>n</i>	107	374	441	181
Median age, years (range)	24 (15–41)	26 (15–46)	25 (14–44)	25 (15–41)
Gravidity (%)				
0	30 (28)	100 (27)	109 (26)	51 (28)
1	19 (18)	70 (19)	83 (19)	33 (18)
> 2	58 (54)	203 (54)	235 (55)	97 (53)

pellet obtained from venous blood samples after Ficoll–Hypaque centrifugation using the QIAamp 96 DNA blood kit or individual spin blood kits (Qiagen, Valencia, CA). In the cohort from 1996 to 2000, DNA was extracted from whole blood.

Polymerase chain reaction amplification. Amplification of a *Plasmodium* genus-specific small-subunit ribosomal RNA (rRNA) gene fragment (491–500 bp) was performed as previously documented.^{16,17} Reaction master mix (25 μ L) included 10 pmol each upstream and downstream primers (Invitrogen Corporation, Carlsbad, CA), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 10 mM total deoxyribonucleotide triphosphates (dNTPs), and 5 units/ μ L *Taq* DNA Polymerase (New England Biolabs, Beverly, MA). Amplification conditions were 92°C for 2 minutes (1 time), 92°C for 30 seconds and 63°C for 2 minutes (40 times), and 63°C for 5 minutes. All polymerase chain reactions (PCRs) were performed using a Peliter Thermal Cycler PCT-100 (MJ Research, Watertown, MA).

***Plasmodium* species-specific post-PCR multiplex ligase detection reaction.** The ligase detection reaction (LDR) analysis used to identify the *P. falciparum* (*Pf*), *P. vivax*, *P. malariae*, and *P. ovale* amplicons was performed as described previously.^{16,17} All LDR reactions were performed in solution (15 μ L) containing 20 mM Tris-HCl (pH 7.6), 25 mM potassium acetate, 10 mM magnesium acetate, 10 mM dithiothreitol, 10 mM nicotinamide adenine di nucleotide+ (NAD⁺), 0.1% Triton X-100, 10 nM each LDR probe, 1 μ L each PCR product, and 2 units *Taq* DNA ligase (New England Biolabs, Beverly, MA). Reactions conditions were 95°C for 1 minute (one time), 95°C for 15 seconds (denaturation), and 58°C for 2 minutes (annealing/ligation for 32 cycles).

Labeling and detection of LDR products. Detection of the LDR products was performed as previously described.¹⁷ Detection of species-specific LDR:bead-labeled anti-TAG hybrid complexes was performed using a Bio-Plex array reader (Bio-Rad Laboratories, Hercules, CA).

Samples from 2000 to 2005 analyzed by real-time quantitative PCR. Although molecular diagnostic assays that varied somewhat in protocol had been previously performed on samples for detection of *Pf* infection, the assays were repeated using the same molecular diagnostic test on samples performed at the same time to assure consistency.^{12,15,18} Rather than repeating an assay on all samples from this cohort, approximately one-half of the available samples were examined for all

species of malaria using the described protocol (PCR-LDR). Concordance for *Pf* infection comparing results obtained in both assays (real-time quantitative PCR [RTQ-PCR] and PCR-LDR) was approximately 86% (data not shown). All other samples presented (1996–2000, 2006–2010, and 2009–2010) were analyzed using the same PCR-LDR assay as described.

RESULTS

Decline in malaria prevalence in pregnant women at delivery. Because early malaria control measures often targeted pregnant women attending ANC, we examined the rates of malaria infection among women at delivery. From 1996 to 2010, the prevalence of *Pf* infection among pregnant women at delivery declined from a high of 41% by PCR in the cohort from 2000–2005 to 1% in 2009–2010 (Figures 1 and 2). The prevalence of *P. malariae* infection declined from 12% in 2000–2005 to 1% in 2009–2010, and the prevalence of *P. ovale* infection declined from 3.5% in 2000–2005 to no detectable infections in 2009–2010. The decline in PCR positivity paralleled the decline observed for blood smear positivity. Although the presence of malaria was not systematically measured at the first ANC visit in the 1996–1997 and 2000–2005 cohorts, a subset of women was examined for malaria infection at the first ANC clinic in 2000–2005 and again at delivery. In this group of randomly selected women (*N* = 37, paired samples), the average PCR positivity for *Pf* was 45% at the first ANC clinic visit and 43% at delivery, indicating that malaria control measures during pregnancy were limited.¹²

Decline in prevalence of malaria infection among pregnant women by year. To further appreciate the declines in the prevalence of *Pf* infection among pregnant women at delivery, all samples selected for PCR diagnosis are presented by year of delivery (Tables 3 and 4). Although the number of women evaluated for malaria was low or absent for some years, a consistent decline can be appreciated: 37–48% *Pf* infections each year from 1996 to 2002 and 0–6% infections from 2003 to 2010.

Increased use of bed nets and malaria chemoprophylaxis in pregnant women. To evaluate changes in use of bed nets and malaria chemoprophylaxis, we queried women from the 2000–2005 cohort of pregnant women about antimalarial prophylaxis at delivery and monitored its use in ANCs in later cohorts. Surveys from the 2000–2005 cohort indicated

TABLE 2
Characteristics of children in the study population

Cohort	Children, <i>n</i>	Follow-up duration	Median age, months (range)	Average visits per child
1996–1997	119	June of 1996 to April of 1998	11 (6–23)	2.4
2000–2005	374	September of 2000 to February of 2005	16 (4–35)	2.9
2006–2010	460	July of 2007 to March of 2010	12 (2–35)	2.5

FIGURE 1. Prevalence of *Pf* infections among pregnant women at delivery at the Msambweni District Hospital in Kenya over a 14-year period. BS = blood smear.

that ~32% of pregnant women recalled taking some malaria chemoprophylaxis during pregnancy (usually chloroquine [often obtained from local pharmacies or private nurses] or SP from the ANC at Msambweni). At that time, SP was only intermittently available at ANC clinics at Msambweni. Reported bed net use among these women was 13–17%.¹² In later cohorts of pregnant women attending the ANC at Msambweni District Hospital, there was a marked increase in the use of intermittent preventive treatment with SP and bed net usage (Table 3).^{7,10}

Increased use of bed nets and malaria chemoprophylaxis correlated with a marked decline in malaria prevalence during pregnancy. To examine the impact of increased usage of malaria chemoprophylaxis and bed nets during pregnancy on malaria infection rates at delivery (Table 3), pregnant women provided a venous blood sample at their first ANC visit (median gestational age = 27.4 months [range = 14–33 months]) and again at delivery in cohorts spanning 2006–2010 and 2009–2010. In the period of observation from 2006 to 2009, there was a 54% decrease in PCR positivity and 68% decline in blood smear positivity. For the period from 2009 to 2010, there was an 82% decline in PCR positivity and an absence of detectable malaria by blood smear at delivery. Thus, increased

FIGURE 2. Prevalence of *Pf* infections declined from the first ANC enrollment visit compared with delivery. PCR data: 2006–2009, $N = 437$ ($P = 0.03$); 2009–2010, $N = 133$ ($P = 0.005$). There was no significant difference for BS results. * Indicates the differences in % prevalence between the ANC and delivery time points that were statistically significant.

TABLE 3
Community level of usage of SP and bed nets

Characteristics	2001–2002 ¹⁰	2006–2007 ⁷	2006–2010
Women, <i>n</i>	537	357	441
Attended ANC ≥ 1 (%)	401 (75)	263 (74)	441 (100)
Used bed net (%)	92 (17)	231 (65)	317 (75)*
Took any SP (%)	111 (21)	172 (48)	423 (100)†
Took more than one dose of SP (%)	36 (7)	67 (19)	312 (71)

* Bed net usage in 2006–2010 was available for 422 women.

† SP usage in 2006–2010 was available for 423 women.

usage of bed nets and malaria chemoprophylaxis in pregnant women seems to have contributed to the increased clearance of malaria infection during pregnancy.

Changes in malaria risk in children from birth to 3 years of age from 1996 to 2010. To estimate risk of malaria infection in infants and young children between 2000–2005 and 2006–2010, the proportion of children with one or more malaria infections during the follow-up period (referred to as cumulative infection rate) (Table 2) was calculated as shown in Figure 3. The cumulative infection rate dropped 89% by PCR ($P < 0.001$) (Figure 3) and 97% by blood smear (38% in the 2000–2005 cohort and 1% in the 2006–2010 cohort, $P < 0.001$). In the same cohorts, the cumulative rates of *P. malariae* dropped by 66% and *P. ovale* dropped by 50%. Mixed infections were included in the analysis.

To further evaluate the malaria risk in three cohorts of children from 1996 to 2010, we examined the effect of cohort and age on the likelihood of infection over the first 3 years of life. The crude prevalence of *Pf* infection in each age group is shown in Figure 4, left panel, whereas the cumulative risk of *Pf* infection as a function of age is calculated over the same period using a generalizing estimating equation that adjusted for season, location, and missing follow-up of children (Figure 4, right panel). In the 1996–2000 cohort, almost all children were at risk of being infected with falciparum malaria during the first 3 years of life, whereas the adjusted risk for falciparum malaria per child was over 50% in the 2000–2005 cohort ($P = 0.053$ compared with the 1996–2000 cohort). By contrast, the estimated risk per child was about 15% from birth to 3 years of age in the 2006–2010 cohort ($P = 0.003$ compared with the 1996–2000 cohort and $P < 0.001$ compared with the 2000–2005 cohort). The infection rates for *P. malariae* and *P. ovale* were too low in the cohort for a similar analysis, although unadjusted infection rates also declined (Figure 3). Thus, the risk of *Pf*

TABLE 4
Declines in the prevalence of infection with *Pf* among pregnant women at delivery by year (disaggregated from cohorts)

Year	Deliveries, <i>n</i>	<i>Pf</i> infections, <i>n</i> (%)
1996	107	40 (37)
2000	108	52 (48)
2001	141	52 (37)
2002	106	42 (40)
2003	19	1 (5)
2006	8	0 (0)
2007	165	9 (5)
2008	230	13 (6)
2009*	102	4 (4)
2010	117	0 (0)

* Samples analyzed from 2009 were aggregated from two different birth cohorts: between cohort 2006–2010 and cohort 2009–2010.

FIGURE 3. Cumulative percent *Pf*, *P. malariae* (*Pm*), and *P. ovale* (*Po*) species infections among children between 2000–2005 and 2006–2010 cohorts.

infection dropped dramatically in serial cohort studies in children residing in the same community over a 14-year period.

DISCUSSION

Over the last 10–15 years, hospital admissions for malaria in coastal regions of East Africa have declined substantially, and there is reduced prevalence of malaria parasitemia in communities.^{3,5} Malaria vector populations have also markedly declined.^{19,20} The increasing use of ITNs and greater availability of effective antimalarial medications have been thought to be major contributors to this marked decrease in malaria in coastal regions of East Africa, although a direct association with these interventions and reduction in malaria transmission has not been firmly established. Indeed, the decline in malaria vector populations and hospital admissions preceded widespread use of bed nets and drug distribution, suggesting that other factors, such as changes in local ecology, economic development, and better use of health facilities, have contributed to these declines.¹⁹ By studying serial birth cohorts in the same community over the past 14 years, the current study shows an 85% decrease in malaria transmission among young children and a similar drop in prevalence in pregnant women at deliv-

ery. These decreases in malaria transmission at Msambweni occurred sometime between 2003 and 2006 and were temporally related to increased use of bed nets and malaria chemoprophylaxis in pregnancy and better availability of effective antimalarial drugs in local hospitals and clinics. Our study was not designed to assess whether these malaria control interventions are causally related to the observed decline in malaria transmission. Changes in rainfall patterns over this same period could have contributed to the drop in malaria transmission. There were droughts in 1999/2000 and 2004/2005²¹; however, these changes in rainfall do not consistently track with the observed changes in malaria transmission.

There was also reduced transmission of non-falciparum malaria in the communities, although the magnitude of the decrease was less striking than the magnitude observed for falciparum malaria. Reduced malaria vector populations will impact all malaria species; however, non-falciparum malaria infections are less likely to cause moderate to severe illness, and therefore, individuals may not seek antimalarial treatment, thereby allowing persistent gametocytes carriage in some individuals. This result may account for the less pronounced reduction in transmission of non-falciparum malaria. A few samples were PCR-positive for *P. vivax*, confirmed by PCR amplification of *PvdhpfII*, and present in Duffy-negative individuals (data not shown); however, none of the samples were blood smear-positive, thus making it difficult to determine if they represent true blood-stage infections. The presence of low levels of *P. vivax* infection is consistent with other observations of *P. vivax* infections in predominantly Duffy-negative populations in East Africa.^{22–24}

Malaria prevalence in pregnant women at delivery dropped dramatically from 37% and 41% PCR positivity to *Pf* in 1996–1997 and 2000–200, respectively, to 1% in 2009–2010. This decrease corresponded to increased usage of bed nets and expanded use of malaria chemoprophylaxis during pregnancy in the population (Table 3) and throughout the Coast Province of Kenya, where 34% and 10% of households had any bed nets and long-lasting insecticidal nets (LLINs), respectively, in 2003 compared with a boost in bed net usage in 2008–2009 to 71% and 66%, respectively.^{25,26} Importantly, these changes led to a marked reduction in the incidence of malaria infection in

FIGURE 4. Prevalence (left panel) and adjusted risk (right panel) of *Pf* infection in children. Adjusted risk of *Pf* infection was calculated as a function of age using a generalizing estimating equation that adjusted for season, location, and missing follow-up of children.

children, with the cumulative prevalence of any species of *Plasmodium* infection declining from 71% in 2000–2005 to 8% in 2006–2010 as determined by PCR. Additionally, blood smear-diagnosed *Plasmodium* infections declined from 38% in 2000–2005 to 1% in 2006–2010. This result also corresponded to a 99% decline in the entomological inoculation rate over approximately the same span of time in the same area, emphasizing that these control measures are likely responsible for the observed changes.²⁰

There were limitations to the present study. Children were followed approximately every 6 months, and it is likely that intervening infections occurred, resulting in an underestimation of the incidence of malaria infections. The incidence of clinical malaria and complexity of malaria infections (i.e., the number of unique clones with which an individual is infected) were not systematically measured over the span of the study. However, all samples from 1996 to 2010 analyzed for this study were taken from individuals presenting at the same ANC at the Msambweni District Hospital, and the same sampling techniques were used in recruitment of pregnant women in each cohort, providing a unique serial cross-sectional study of this population.

Although hospital admissions for malaria have dropped markedly in endemic areas on the Kenyan coast, potential hot spots remain. For example, monthly admissions for putative cases of malaria in 2012 at the Msambweni District Hospital ranged from 32 to 95 per month, with a median of 42 cases. Most cases were in children. Although these numbers are slightly lower than a decade earlier, they still represent a significant number of admissions. This finding contradicts the marked reduction in prevalence and transmission in the current study. This discrepancy may have arisen, because admissions may come from different communities not monitored in the current study or the presumptive malaria cases are misdiagnosed. It is also possible that careful monitoring and treatment of individuals according to the Kenyan MOH guidelines suppressed malaria infections in study subjects to a greater degree compared with the general community.

With the dramatic drop in malaria transmission over the past 5 years, it is likely that an increasing proportion of children and adults have limited or no exposure to malaria during this period. This finding affects natural acquisition of immunity and could markedly increase their risk of malaria morbidity with subsequent infections.²⁷ The low but still ongoing malaria transmission, changes in mosquito behavior from indoor to more outdoor biting behavior,²⁸ increasing drug resistance, and possible complacency in malaria control could all lead to increased malaria transmission, with potentially severe effects in this increasingly immunologically naïve population.

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