Malaria Infection and Gametocyte Carriage Rates in Preparation for Transmission Blocking Vaccine Trials in Bancoumana, Mali

Mahamadoun Hamady Assadou,^{1*} Issaka Sagara,¹ Sara A. Healy,² Merepen Agnes Guindo,¹ Mamady Kone,¹ Sintry Sanogo,¹ M'Bouye Doucoure,¹ Sekouba Keita,¹ Ruth D. Ellis,³ Yimin Wu,⁴ Freda Omaswa,⁵ Patrick E. Duffy,² and Ogobara K. Doumbo¹

¹Malaria Research and Training Center, FMOS-FAPH, Mali-NIAID-ICER, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali; ²Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland; ³Biologics Consulting Group Inc., Alexandria, Virginia; ⁴PATH-Malaria Vaccine Initiative, Washington, District of Columbia; ⁵University of British Columbia, Vancouver, Canada

Abstract. The epidemiological characterization of transmission reservoirs is a critical step in preparation for interventional trials for malaria elimination/eradication. Using cluster sampling and households/compounds as units of sampling, we recruited and followed monthly, from June 2011 to June 2012, 250 volunteers 3 months to 50 years of age in Bancoumana, Mali. In July 2012, only participants 5–35 years of age ($N = 121$) were reenrolled and followed for an additional year. Malaria infection prevalence was highest in October in both 2011 (21.5%, 50/233) and 2012 (38.2%, 26/68). During both years, malaria infection prevalence was highest in children 5–14 years of age ($P = 0.01$ and $P = 0.02$, respectively). The gametocyte carriage prevalence was highest in November 2011 (7.6%, 17/225) and in October 2012 (16.2%, 11/68). Gametocyte carriage rates by age did not significantly differ in 2011 and 2012. In Bancoumana, the asexual and sexual parasite carriage rates are relatively high and highly seasonal. Seasonal variation and age differences in parasite and gametocyte carriage provide essential knowledge for the design of transmission blocking assay and vaccine studies in the field.

BACKGROUND

According to the World Health Organization 2016 Malaria World Report, global control efforts have resulted in a significant reduction in the number of malaria deaths since 2000.¹ Despite this success, 429,000 malaria deaths still occurred in 2015.²

A vaccine to interrupt malaria transmission would be a valuable tool for malaria eradication. In the past decade, various malaria vaccine candidates have succeeded in being implemented in phase 1 studies in endemic populations, including a few of these candidate vaccines reaching phase 2 trials.^{3–5} However, sustained efficacy against naturally occurring infection has been modest to none, including RTS,S, which has shown partial protective efficacy for preventing clinical malaria episodes in phase 3 studies.⁶

A *Plasmodium falciparum* transmission blocking vaccine (TBV) has been identified as a key component in the effort to eradicate malaria.^{7,8} TBVs induce the production of anti-sporogonic antibodies in the vaccinated human host that is then taken up by the mosquito during a blood meal and thereby halting transmission to another human host. Until the recent completion of the Pfs25H-EPA/Alhydrogel® vaccine study at our clinical study site, this type of vaccine had never been tested in the field.^{9,10}

Epidemiological characterization of the transmission reservoir is a critical step in the design of TBV protocols. In preparation for TBV studies at our clinical site, updated parasite and gametocyte carriage rates in the targeted population were needed for sample size estimation, vaccine administration timing, and endpoint evaluation and timing. This study hypothesis was that malaria parasite carriage rates including the gametocyte carriage in various age groups

in Bancoumana, Mali, is still high and will be appropriate for TBV studies.

In this study, we aimed to assess malaria infection and gametocyte carriage rate over time among various age groups of children and adults in a malaria-endemic area in Mali.

METHODS

Study site. The study site is located in and around Bancoumana village, Mali, West Africa. Bancoumana is located 60 km southwest of Bamako and has a population of 9,000 inhabitants. The site is situated in the South-Sudanese area of Mali. The climate is hot, with daily temperatures ranging from 19°C to 40°C. The annual rainfall varies between 600 mm and 1,200 mm and the rainy season typically occurs from June to October. Malaria transmission is hyperendemic with an intense transmission season from July to November.¹¹

Study population. Healthy volunteers between 3 months and 50 years of age were enrolled into the study. In 2011, 250 participants were enrolled and stratified into four age groups (3–11 months, 1–4 years, 5–14 years, and 15–50 years) and followed monthly through June 2012. Starting in July 2012, only subjects 5–35 years of age ($N = 121$) were continued to be followed from 2012 to 2013.

The sample size calculation was based on parameter (proportion) estimation in a population. In this case, the gametocyte carriage rate per age strata was the study interest and therefore the sample size was computed on this basis.

Ethics. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Pharmacy, and Dentistry, University of Sciences, Techniques and Technologies in Bamako, Mali, and the National Institute of Allergy and Infectious Diseases Institutional Review Board at the National Institutes of Health.

Participant recruitment. Bancoumana community leaders were approached by the study team prior to the start of

* Address correspondence to Mahamadoun Hamady Assadou, Malaria Research and Training Center, FMOS-FAPH, Mali-NIAID-ICER, University of Sciences, Techniques and Technologies of Bamako, Point G, Bamako BP1805, Mali. E-mail: mmaiga@icermali.org

the study and study procedures were explained. Once the community leaders' permission was obtained, the study team invited the volunteers or their parents to come to the study clinic to obtain individual informed consent and complete screening procedures. Pregnant women were excluded from participation. Study participants underwent clinical and laboratory examinations as described below. Final eligibility was determined by the investigator prior to enrollment.

Study procedures. Subjects were seen actively on a monthly basis for medical history, vital signs, physical examination, blood smears, and research laboratories. Unscheduled visits were completed as needed for acute illnesses with clinical evaluation and malaria diagnostics completed if indicated. Any illnesses, including malaria, were documented and treated per Mali National Guidelines.

Laboratory tests. Giemsa-stained thick and thin films were completed using approximately 10 μ L of blood and examined for asexual and sexual malaria parasites and rapid diagnostic tests were used if clinically indicated. The thick and thin films were read by two certified technicians. The parasite density was calculated per 1,000 leukocytes on a thick film. Discordant results were resolved by a third read completed by a senior technician. Between the two readers, there was a concordance of 87.8% (86.4–89.2%) for asexual parasite count and a concordance of 66.4% (58.8–73.9%) for gametocyte counts. Hemoglobin levels were assessed using HemoCue® or as part of a complete blood count. A urine β -human chorionic gonadotropin test was performed at screening and as clinically indicated at any point during the study. Study physicians were able to request additional laboratory examinations related to patient care at their discretion at any time during the course of the study.

Data collection and analysis. All data were manually reported into a case report form and faxed into iDataFax, an application used to electronically capture patient data, and analyzed using SPSS statistics software. Demographic characteristics and parasite and gametocyte carriage frequencies were summarized using descriptive statistics. Comparison between age groups and year-to-year variation regarding parasite and gametocyte carriage were done using the same SPSS statistics software.

RESULTS

In June 2011, 278 volunteers 3 months to 50 years of age were consented, of which 250 enrolled into the study. In 2012, 121 subjects (5–35 years of age) reenrolled into the study for an additional year. The majority of enrolled participants were females (60.2%) and of Malinke ethnicity (90%). Volunteers from 2011 were further stratified into four age groups: 3–11 months ($N = 51$), 1–4 years ($N = 51$), 5–14 years ($N = 73$), and 15–50 years ($N = 75$) (Table 1).

As expected, three malaria species were detected, with *P. falciparum* being the most common: *P. falciparum* only (97.8%, 229/234), *Plasmodium malariae* only (1.8%, 3/234), *Plasmodium ovale* only (0.4%, 1/234), and mixed infections (0.4%, 1/234). Only sexual forms of *P. falciparum* were assessed and reported in this study. In both October 2011 and 2012, malaria infection was highest: 21.5%, 50/233 (Table 2), and 38.2%, 26/68 (Table 3), respectively. During

TABLE 1

Demographic characteristics of study population at the enrollment in June/July 2011

Age group	Gender		Total
	Male	Female	
3–11 months	16	35	51
1–4 years	19	32	51
5–14 years	35	38	73
15–50 years	32	43	75
Total	102	148	250

the peak of malaria infection in October, both in 2011 (Table 2) and 2012 (Table 3), the infection rate was significantly higher in the age group of 5–14 years compared with the other age groups ($P = 0.01$ and $P = 0.02$). At the onset of the malaria transmission season in July, the infection rate was significantly higher in 2011 compared with in 2012 in the age group of 5–14 years: 31.4% (22/70) (Table 2) versus 13.2% (7/53) ($P = 0.02$). Parasitemia was detected throughout the year in 2012 with the lowest prevalence in May (5.6%, 7/126). As expected, a gradual increase in prevalence was seen from July to October (Table 3).

Malaria infection prevalence varied across age groups and across months both years. For example, in the age group of 5–14 years, while the malaria infection prevalence was higher in July 2011 compared with July 2012, the opposite was seen when comparing October 2011 (32%) to October 2012 (45%). Infection in adults (participants ≥ 15 years of age) was consistently $> 10\%$ during the entire transmission season both years (Tables 2 and 3, respectively).

Gametocyte carriage rates did not vary significantly between the age groups in 2011 and 2012 (Tables 4 and 5, respectively). In November 2011 (Table 4), among the four age groups, the highest gametocyte carriage rate was found in the age group of 5–14 years (13.2%, 9/68), whereas the lowest carriage rate was found in the age group of 3–11 months (4.4%, 2/45). In October 2012 (Table 5), among the two age groups, the highest carriage rate was among 15–35 years (26.6%, 4/15), versus 5–14 years (13.2%, 7/53). The gametocyte carriage rate differed slightly between the two age groups (5–14 and 15–35 years) but was not statistically significant ($P = 0.08$).

DISCUSSION

The characterization of a study site and associated targeted population for gametocyte carriage is a key step in designing trials of TBVs. This includes the determination of *P. falciparum* gametocyte carriage rates in various age groups as well as the dynamics of *P. falciparum* gametocyte carriage over time among infected individuals.¹²

At our Bancoumana site, the malaria infection rate was consistently high throughout the transmission season from July to November for two consecutive years, which was consistent with the known seasonal malaria transmission pattern in this area.¹¹ The malaria infection variation between the two years and across the two age groups (5–14 years and 15–35 years) may be explained by the fact that many of the same individuals were followed for the 2-year period and given participation in the trial, participants may have been treated more frequently for malaria

TABLE 2
Malaria infection prevalence per month and age group in Bancoumana in the study cohort in 2011

Age group	July	August	September	October	November	December
3–11 months	4.0 (2/50)	2.1 (1/47)	2.1 (1/48)	13.0 (6/46)	13.3 (6/45)	4.4 (2/45)
1–4 years	14.0 (7/50)	5.9 (3/51)	12.0 (6/50)	22.0 (11/50)	20.0 (10/50)	8.2 (4/49)
5–14 years	31.4 (22/70)	31.3 (21/67)	32.4 (22/68)	32.4 (22/68)	25.0 (17/68)	13.2 (9/68)
15–50 years	11.0 (8/73)	11.8 (8/68)	18.6 (13/70)	15.9 (11/69)	17.7 (11/62)	9.7 (6/62)
Total	16.0 (39/243)	14.2 (33/233)	17.8 (42/236)	21.5 (50/233)	19.6 (44/225)	9.4 (21/224)

Infection prevalence is in % (n/N).

TABLE 3
Malaria infection prevalence per month and age group in Bancoumana in the study cohort in 2012

Age group (years)	January	February	March	April	May	June	July	August	September	October	November	December
5–14	16.4 (11/67)	16.1 (11/68)	10.6 (7/66)	10.6 (7/66)	6.0 (4/66)	12.1 (8/66)	13.2 (7/53)	24.0 (12/50)	33.9 (18/53)	45.2 (24/53)	28.3 (15/53)	20.7 (11/53)
15–35	8.3 (5/60)	6.3 (4/63)	5.0 (3/60)	0 (0/55)	5.0 (3/60)	7.6 (4/52)	12.5 (2/16)	13.3 (2/15)	13.3 (2/15)	13.3 (2/15)	25.0 (3/12)	7.6 (1/13)
Total	12.5 (16/125)	11.4 (15/131)	7.9 (10/126)	5.7 (7/121)	5.5 (7/126)	9.3 (11/118)	13.0 (9/69)	21.5 (14/65)	29.4 (20/68)	38.2 (26/68)	27.6 (18/65)	18.1 (12/66)

Infection prevalence is in % (n/N).

TABLE 4
Gametocytes carriage prevalence per month and age category in Bancoumana in study cohort in 2011

Age group	July	August	September	October	November	December
3–11 months	0.0 (0/50)	0.0 (0/47)	2.0 (1/48)	2.1 (1/46)	4.4 (2/45)	6.6 (3/45)
1–4 years	6.0 (3/50)	0.0 (0/51)	2.0 (1/50)	0.0 (0/50)	6.0 (3/50)	10.2 (5/49)
5–14 years	5.7 (4/70)	7.4 (5/67)	7.3 (5/68)	10.2 (7/68)	13.2 (9/68)	7.3 (5/68)
15–50 years	1.3 (1/73)	2.9 (2/68)	7.1 (5/70)	7.2 (5/69)	4.8 (3/62)	3.2 (2/62)
Total	3.2 (8/243)	3.0 (7/233)	5.0 (12/236)	5.5 (13/233)	7.5 (17/225)	6.6 (15/224)

Gametocyte carriage prevalence is in % (n/N).

TABLE 5
Gametocytes carriage prevalence per month and age category in Bancoumana in study cohort in 2012

Age group (years)	January	February	March	April	May	June	July	August	September	October	November	December
5–14	7.4 (5/67)	8.8 (6/68)	4.5 (3/66)	1.5 (1/66)	1.5 (1/66)	1.5 (1/66)	5.6 (3/53)	8.0 (4/50)	11.3 (6/53)	13.2 (7/53)	7.5 (4/53)	7.5 (4/53)
15–35	1.6 (1/60)	0.0 (0/63)	1.6 (1/60)	0.0 (0/55)	1.6 (1/60)	0.0 (0/55)	6.2 (1/16)	6.6 (1/15)	13.3 (2/15)	26.6 (4/15)	0.0 (0/12)	7.6 (1/13)
Total	4.7 (6/127)	4.5 (6/131)	3.1 (4/126)	0.7 (1/136)	1.5 (2/126)	0.7 (1/126)	5.7 (4/69)	7.6 (5/65)	11.7 (8/68)	16.1 (11/68)	6.1 (4/65)	7.5 (5/66)

Gametocytes prevalence is in % (n/N).

than usual. However, other factors such as the amount of precipitation, the duration of the rainy season, and other environmental conditions that may impact vector or host behaviors should be taken into account. Detection of malaria parasites (sexual and asexual) during the dry season is likely explained by persistent asymptomatic parasitemia which can last for several months^{11–14} as well as continued low rates of mosquito biting.¹⁵ Collectively these observations guided our team to schedule our TBV series to start during the dry season to have the last vaccination received in mid-September. It has been previously shown that gametocyte density and mosquito infectivity correlate, though it has been seen that mosquito infectivity can occur even with submicroscopic gametocytemia.^{15,16} This timing permitted our team to complete transmission blocking assay assessments after receipt of last vaccination during the height of gametocyte carriage.

Our study cohort demographics is consistent with the Mali general population in terms of sex ratio (0.95 male/female) but differs in terms of ethnicity, which is expected given the study population was restricted to the Bancoumana region¹⁷ and did not enroll pregnant women. Although malaria infection and gametocyte carriage rates are consistently higher in the age group of 5–14 years across the two years, the adult population is also consistently carrying gametocytes, allowing for malaria TBVs to be assessed in this age group.

The main limitation of this study is that we do not present the infectivity data of the gametocytes in the mosquito. This is still assessed during this study and will be reported in another article. Another limitation of this study is that in July 2012, only 25% of the adults followed in 2011 were reenrolled. This was due to the fact that only those subjects 18–35 years of age were targeted for reenrollment and many of our young women became pregnant and were thus withdrawn from the study. This selective withdrawal and reenrollment may influence our results, that is, toward less precise parameter estimate in that age group for 2012 compared with 2011.

Because TBVs do not directly prevent infection of vaccinated individuals and have no direct benefit to the vaccinated individual, early trials of these products to determine safety, immunogenicity, and functional activity are needed to be performed in adults. The lower infection and gametocyte carriage rates in the age group of 3–11 months are expected and consistent with available data,^{18–20} but the notable high carriage rates in our pediatric age groups indicate that for a licensed TBV to be successful, all age groups eventually need to be included in their assessment to achieve malaria eradication.

Received November 23, 2015. Accepted for publication January 4, 2017.

Published online May 22, 2017.

Acknowledgments: This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Authors' addresses: Mahamadoun Hamady Assadou, Issaka Sagara, Merepen Agnes Guindo, Mamady Kone, Sintry Sanogo, M'Bouye Doucoure, Sekouba Keita, and Ogobara K. Doumbo, Malaria Research and Training Center, FMOS-FAPH, Mali-NIAID-ICER, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, E-mail: mmaiga@icermali.org. Sara A.

Healy and Patrick E. Duffy, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD. Ruth D. Ellis, Biologics Consulting Group Inc., Alexandria, VA. Yimin Wu, PATH-Malaria Vaccine Initiative, Washington, DC. Freda Omaswa, University of British Columbia, Vancouver, Canada.

REFERENCES

1. World Health Organization, 2016. *World Malaria Report 2016*. Geneva, Switzerland: WHO. ISBN 978-92-4-151171-1.
2. World Health Organization, 2017. *Disease and Topics*. Available at: <http://apps.who.int/tdr/svc/diseases/malaria>. Accessed January 2, 2017.
3. Sagara I, Dicko A, Ellis RD, Fay MP, Sory D, Assadou MH, Sissoko MS, Kone M, Diallo AI, Saye R, Guindo MA, Kante O, Niambele MB, Miura K, Mullen GE, Pierce M, Martin LB, Dolo A, Diallo DA, Doumbo OK, Miller LH, Saul A, 2009. A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. *Vaccine* 27: 3090–3098.
4. Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, Guindo AB, Traore K, Traore I, Kouriba B, Diallo DA, Diarra I, Daou M, Dolo A, Tolo Y, Sissoko MS, Niangaly A, Sissoko M, Takala-Harrison S, Lyke KE, Wu Y, Blackwelder WC, Godeaux O, Vekemans J, Dubois MC, Ballou WR, Cohen J, Thompson D, Dube T, Soisson L, Diggs CL, House B, Lanar DE, Dutta S, Heppner DG Jr, Plowe CV, 2011. A field trial to assess a blood-stage malaria vaccine. *N Engl J Med* 365: 1004–1013.
5. Ogutu BR, 2009. Malaria management: progress made and challenges still to face. *Malar J* 8 (Suppl 1): S1.
6. Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, Methogo BG, Doucka Y, Flamen A, Mordmüller B, Issifou S, Kremsner PG, Sacarlal J, Aide P, Lanaspa M, Aponte JJ, Nhamuave A, Quelhas D, Bassat Q, Mandjate S, Macete E, Alonso P, Abdulla S, Salim N, Juma O, Shomari M, Shubis K, Machera F, Hamad AS, Minja R, Mtoro A, Sykes A, Ahmed S, Urassa AM, Ali AM, Mwangoka G, Tanner M, Tinto H, D'Alessandro U, Sorgho H, Valea I, Tahita MC, Kaboré W, Ouédraogo S, Sandrine Y, Guiguemdé RT, Ouédraogo JB, Hamel MJ, Kariuki S, Odero C, Onoko M, Otieno K, Awino N, Omoto J, Williamson J, Muturi-Kioi V, Laserson KF, Slutsker L, Otieno W, Otieno L, Nekoye O, Gondi S, Otieno A, Ogutu B, Wasuna R, Owira V, Jones D, Onyango AA, Njuguna P, Chilengi R, Akoo P, Kerubo C, Gitaka J, Maingi C, Lang T, Olotu A, Tsofa B, Bejon P, Peshu N, Marsh K, Owusu-Agyei S, Asante KP, Osei-Kwakye K, Boahen O, Ayamba S, Kayan K, Owusu-Ofori R, Dosoo D, Asante I, Adjei G, Adjei G, Chandramohan D, Greenwood B, Lusingu J, Gesase S, Malabeja A, Abdul O, Kilavo H, Mahende C, Liheluka E, Lemnge M, Theander T, Drakeley C, Ansong D, Agbenyega T, Adjei S, Boateng HO, Rettig T, Bawa J, Sylverken J, Sambian D, Agyekum A, Owusu L, Martinson F, Hoffman I, Mvalo T, Kamthunzi P, Nkomo R, Msika A, Jumbe A, Chome N, Nyakuipa D, Chintedza J, Ballou WR, Bruls M, Cohen J, Guerra Y, Jongert E, Lapiere D, Leach A, Lievens M, Ofori-Anyinam O, Vekemans J, Carter T, Lebouilleux D, Loucq C, Radford A, Savarese B, Schellenberg D, Sillman M, Vansadia PRTS, S Clinical Trials Partnership, 2011. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med* 365: 1863–1875.
7. Greenwood B, Targett G, 2009. Do we still need a malaria vaccine? *Parasite Immunol* 31: 582–586.
8. Birkett AJ, 2010. PATH Malaria Vaccine Initiative (MVI): perspectives on the status of malaria vaccine development. *Hum Vaccin* 6: 139–145.
9. Wu Y, Ellis RD, Shaffer D, Fontes E, Malkin EM, Mahanty S, Fay MP, Narum D, Rausch K, Miles AP, Aebig J, Orcutt A, Muratova O, Song G, Lambert L, Zhu D, Miura K, Long C, Saul A, Miller LH, Durbin AP, 2008. Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. *PLoS One* 3: e2636.
10. Talaat KR, et al., 2016. Safety and immunogenicity of Pfs25-EPA/Alhydrogel(R), a transmission blocking vaccine against *Plasmodium falciparum*: an open label study in malaria naive adults. *PLoS One* 11: e0163144. doi:10.1371/journal.pone.0163144.

11. Dolo A, Poudiougou B, Modiano D, Camara F, Kouriba B, Diallo M, Bosman A, Crisanti A, Robson K, Doumbo O, 2003. Epidemiology of malaria in a village of Sudanese savannah in Mali (Bancoumana). Anti-TRAP and anti-CS humoral immunity response. *Bull Soc Pathol Exot* 96: 287–290.
12. Drakeley CJ, Eling W, Teelen K, Bousema JT, Sauerwein R, Greenwood BM, Targett GA, 2004. Parasite infectivity and immunity to *Plasmodium falciparum* gametocytes in Gambian children. *Parasite Immunol* 26: 159–165.
13. Sternberg ED, Thomas MB, 2014. Local adaptation to temperature and the implications for vector-borne diseases. *Trends Parasitol* 30: 115–122.
14. Townes LR, Mwandama D, Mathanga DP, Wilson ML, 2013. Elevated dry-season malaria prevalence associated with fine-scale spatial patterns of environmental risk: a case-control study of children in rural Malawi. *Malar J* 12: 407.
15. Schneider P, Bousema JT, Gouagna LC, Otieno S, van de Vegte-Bolmer M, Omar SA, Sauerwein RW, 2007. Sub-microscopic *Plasmodium falciparum* gametocyte densities frequently result in mosquito infection. *Am J Trop Med Hyg* 76: 470–474.
16. Drakeley C, Sutherland C, Bousema JT, Sauerwein RW, Targett GA, 2006. The epidemiology of *Plasmodium falciparum* gametocytes: weapons of mass dispersion. *Trends Parasitol* 22: 424–430.
17. CIA World Factbook. *Mali Sex Ratio*. Available at: <https://www.cia.gov/library/publications/the-world-factbook/geos/ml.html>. Accessed January 2, 2017.
18. Sogoba N, Doumbia S, Vounatsou P, Baber I, Keita M, Maiga M, Traoré SF, Touré A, Dolo G, Smith T, Ribeiro JM, 2007. Monitoring of larval habitats and mosquito densities in the Sudan savanna of Mali: implications for malaria vector control. *Am J Trop Med Hyg* 77: 82–88.
19. Mharakurwa S, Mutambu SL, Mberikunashe J, Thuma PE, Moss WJ, Mason PR, Southern Africa ICEMR Team, 2013. Changes in the burden of malaria following scale up of malaria control interventions in Mutasa District, Zimbabwe. *Malar J* 12: 233.
20. Roca-Feltre A, Carneiro I, Smith L, Schellenberg JR, Greenwood B, Schellenberg D, 2010. The age patterns of severe malaria syndromes in sub-Saharan Africa across a range of transmission intensities and seasonality settings. *Malar J* 9: 282.