International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 3, p. 40-49, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Infectious potential of *Plasmodium falciparum* in the Commune of Aguegues in Benin

Gouissi Fadéby Modeste[1,4*], Salifou Sahidou[2], Edorh Aléodjrodo Patrick[1, 3], Djenontin Armel[4], Hounkpatin Armelle sabine Yélignan[1], Montcho Sabine Afiavi[1], Akogbeto Martin[4], Boko Michel[1]

[1]*Laboratory of Toxicology and Environmental Health, CIFRED, University of Abomey- Calavi (UAC), 03 BP 1463 Jericho, Cotonou, Benin*

[2]*Polytechnic School of Abomey-Calavi (EPAC), Department of Animal Production, University of Abomey-Calavi (UAC), 01 BP 2009 Cotonou, Benin*

[3]*Department of Biochemistry and Cell Biology, Faculty of Science and Technology (FAST), University of Abomey-Calavi (UAC), 01 BP 526, Cotonou, Benin*

[4]*Entomological Research Center of Cotonou (CREC), 06 BP 2604 Cotonou, Benin*

Received: 16 February 2012
Revised: 26 February 2012
Accepted: 27 February 2012

Key words: Malaria, *Plasmodium falciparum*, hemoglobin, anaemia, Aguégués .

Abstract

Malaria continues to be the most important parasitic disease of humans. It is of major public health importance in the tropics, especially in Africa south of the Sahara, where it remains a leading cause of morbidity and mortality. That publication consist to study the transmission of *Plasmodium falciparum* in the commune of Aguégués. We proceeded to a blood test for hemoglobin level and the test of malaria parasitemia among all children from 6 to 59 months of all households surveyed. 35% of children aged 6-59 months were found positive to RDT. Malaria prevalence among children aged 6-59 months is estimated at 38% with microscopic test. The prevalence of malaria parasitemia increases with age children, the arrondissement and the flood. *An. Melas* which is the major vector in the area, is a minor vector of malaria transmission in the commune of Aguégués. This study aims to know the infectious potential of *Plasmodium falciparum* to assess its resistance against drug control strategies in the area.

*Corresponding Author: Gouissi Fadéby Modeste ✉ gouissi@yahoo.fr

Introduction

Aguégués is a fluvial, lagoon, coastal and lacustrine commune of Bénin. This ecosystem characterized by brackish waters is favorable for the development of *An. melas* which is one of malaria vector in the area. Our study aims to investigate the infectious potential of *Plasmodium falciparum*, the vector parasite.

Plasmodium falciparum infections are responsible for more than 200 million episodes of clinical malaria, which result in at least one million deaths per year (Snow *et al.,* 1999). Malaria continues to be the most important parasitic disease of humans. It is of major public health importance in the tropics, especially in Africa south of the Sahara, where it remains a leading cause of morbidity and mortality. Between 400 and 900 million acute febrile episodes occur yearly in African children under 5 years of age living in endemic areas (Breman, 2001). At a minimum, 0.7-2.7 million deaths occur yearly from malaria, about one million in children below 5 years (Breman, 2001; World Health Organization, 1996). Over 75% of these deaths occur in sub-Saharan Africa (World Health Organization, 1997). In Africa, 25-30% of all deaths among children under 5 years of age are attributed to malaria (Molineaux, 1985; Greenwood *et al.,* 1987; Payne *et al.,* 1976; Snow *et al.,* 1999), but less than 20% of these deaths come to the attention of any formal health system. Severe anaemia due to malaria is now believed to occur in between 1.5 to 6.0 million African children per year with case fatality rate of nearly 15% (Murphy *et al.,* 2001). Respiratory distress, hypoglycemia and overlapping conditions contribute another 1-2 million cases with mortality nearing 20% (Murphy *et al.,* 2001). Malaria reduces the capacity for children to learn. (World Health Organization, 2000). One reason is the growing spread of *Plasmodium falciparum* resistance to Chloroquine and other anti-malarials. From data collected in Senegal, Trape (2001) demonstrated a two- to three-fold increase in hospital admissions and deaths, and a six-fold increase in child malaria mortality when Chloroquine resistance emerged in the late 1980s and early 1990s. Anaemia is one of the major complications of malaria in children (Murphy *et al.,* 2001; Newton *et al.,* 1997). Anaemia is a common cause of paediatric mortality and morbidity in sub-saharan africa (de Maeyer *et al.*, 1985; Desai *et al.*, 2005). In highly endemic areas, susceptibility to severe malarial anaemia increases during the age periods when parasite density and the frequency of uncomplicated episodes of malaria are highest (Mc Elroy *et al.*, 2000). Malaria, in turn is a leading cause of anaemia in endemic regions. The use of insecticide-treated bed nets and of chemoprophylaxis has been shown to increase mean haemoglobin concentrations in children (Alonso *et al.*, 1993). Implementation of malaria control interventions is therefore expected to increase. The correct and timely application of current malaria control strategies (personal protection, drug use and vector control), can result in significant decrease in malaria-specific and overall mortality. This study was designed to examine the relationship between *Plasmodium falciparum*, anaemia and malaria in children under 5 years living in the commune of Aguégués.

Study area

The commune of Aguégués is situated in the Ouémé department between 6°29'00" N and 2°32'00"E. Its population is 26,650 inhabitants in 2002. Its living space is 52 km². The commune of Aguégués is located on the lake and fluvial route 15 km from Cotonou and 6 km from Porto-Novo. This lagoon chain continues without interruption up to Badagry in Nigeria. Thus, it is important circuit lagoon and river traffic. It consist of a set of islands of alluvial accumulation accommodated in the lower part of the river Ouémé. The commune is marked by seasonal flooding characterized by a flood of three to five months a year (August-December). Climatically, Aguégués has a humid tropical climate characterized by two (02) dry seasons and two (02) rainy seasons of unequal importance: a long rainy season from April to July, a short dry season from August to September, a short

rainy season from October to November and a long dry season from December to March.

Materials and methods

The study was performed in order to investigate the transmission of *Plasmodium falciparum* in the commune of Aguégués. The study was conducted from 23rd January to 17th November 2010.

Test of anaemia

Eligible population for the test of anemia is the population of children from 6 to 59 months living in the households surveyed, and who slept in the household the night before the interview. We proceeded to a blood test for hemoglobin level and the test of malaria parasitemia among all children from 6 to 59 months of all households surveyed. For the test of hemoglobin level, the sample was taken as follows: capillary blood was first sampled by a finger prick made with a small retractable lancet (Tenderlett); We collected one or two drops of blood in a miniature bowl that is then placed in a portable haemoglobinometer (HemoCue), a device that, in less than a minute, gives an accurate measure of the level (in grams) of hemoglobin per blood decilitre. We classified the level of anemia provided by the haemoglobinometer (HemoCue) into three levels depending on the concentration of hemoglobin in the blood. This classification was developed by researchers of the WHO (DeMaeyer *et al.,* 1989). Thus, in the context of malaria parasitemia, anemia is considered severe in children if the level of hemoglobin per decilitre of blood is less than 8.0 g/dl. It will then be considered moderate if the hemoglobin level is between 8.0 and 9.9 g/dl and, last qualified as mild if the measure is between 10.0 and 10.9 g/dl.

Test of malaria

We collected blood drops from children aged 6-59 months to estimate the prevalence of malaria parasitaemia in children 6-59 months from a representative sample of households. The thick for microscopic reading and rapid diagnostic test (RDT) are the methods of malaria diagnosis used in household surveys. The microscopic reading is the "gold standard" for detection and identification of the parasite species in the blood. By against, RDT detects the parasite secreted antigens in the blood. The microscopic reading is carried out on blood smears stained, thick or thin.

Collection procedures and blood test

The blood collection area was scrubbed with alcohol swab before puncture. The puncture was made with a retractable needle, not reusable. After taking blood, blood flow was stopped by applying pressure to the puncture site with gauze. Once the bleeding stopped, the puncture site was protected by a bandage. After taking the drops of blood, we performed successively RDT and the preparation of thick films of blood on two slides was read after in laboratory. The RDT results obtained after 15 minutes were immediately given to the parent or other adult caregiver of the children who participated in the test. All children from 6 to 59 months have been first tested using a rapid diagnostic test (RDT - Paracheck) that is specific for malaria P. falciparum. Children with positive results to RDT were treated according to national guidelines for treatment of malaria. Thus, children with positive RDT, received immediately free treatment based artemether-lumefantrine (AL), according to WHO guidelines for the treatment of uncomplicated malaria in children under five years. Information was also given to parents about the against-indications and potential side effects of treatment.

Statistical analysis

Significant tests were carried out using the analysis of variance (ANOVA) of the Statistical Package for Social Sciences (SPSS) computer programme. Means were separated using the Duncan Multiple Range Test. Statistical analysis (ANOVA) showed significant differences ($P < 0.05$).

Results

The Table 1 shows the participation rate in malaria testing by sex and arrondissement of residence and the reason for which blood sampling was not performed. It is found that coverage was very high, since the blood sample was taken and tested for almost all (98%) of 2898 children who were eligible for the test (Table 1). Overall, the coverage rates of malaria test show no significant variations by sex and arrondissement of residence.

Table 1. Distribution in% of children from 6 to 59 months eligible for the blood test by sex and arrondissement.

	Test coverage of malaria			
Characteristics	Blood sample tested	Absent at the time of blood collection	**Total**	**Effectif Unweighted**
Sex				
male	97.80	2.20	100.0	1405
female	98.20	1.80	100.0	1435
Arrondissements				
Avagbodji	99.32	0.68	100.0	970
Houédomè	99.40	0.60	100.0	918
Zoungamè	99.28	0.72	100.0	952
Total	98.00	2.00	100.0	2840

Table 2. Percentage of children from 6 to 59 months whose result of TDR is positive by age and arrondissement.

Characteristics	Percentage of children whose RDT is positive	Effectif Unweighted
Age in months		
6-11	20	368
12-23	35.4	560
24-35	38.5	570
36-47	45.5	635
48-59	50	707
Sex		
Male	35.5	1405
Female	34.5	1435
Arrondissements		
vagbodji	49	970
Houédomè	32.5	918
Zoungamè	23.5	952
Total	35	2840

RDT

The results in Table 2 indicate that 35% of children aged 6-59 months were found positive to RDT of Paracheck that is specific for malaria *Plasmodium falciparum*. The proportion of girls positive to RDT is not very different from that of boys (35.5% for boys against 34.5% for girls). Moreover, the proportion of positive RDT increases with age children, from 20% for children 6-11 months and 50% for those 48-59 months. Moreover, the proportion of children whose result was positive in TDR is significantly higher in Avagbodji than two other arrondissements.

Coverage rate test of anemia

The coverage rate is very high (98.2%) for 2840 eligible children. The coverage rate is higher among boys as girls (98.4% against 97.9%) (Table 3).

Table 3. Distribution in % of children from 6 to 59 months eligible for the hemoglobin test by sex and arrondissement.

	Test coverage of anaemia			
Characteristics	Blood sample tested	Absent at the time of blood collection	**Total**	**Effectif Unweighted**
Sex				
Male	98.4	1.6	100.0	1405
Female	97.9	2.1	100.0	1435
Arrondissements				
Avagbodji	98.2	1.8	100.0	970
Houédomè	98.0	2.0	100.0	918
Zoungamè	98.4	1.8	100.0	952
Total	98.2	1.8	100.0	2840

Prevalence of anemia in among children

Over 79% of children from 6 to 59 months suffer from anemia. Of these, 22.23% suffer of severe anemia and 12.73% are with a mild form (Table 4). The results show differences by age. Indeed, it is mainly children aged 12-23 months who are most affected by anemia (91.5%).

Prevalence of malaria parasitemia

Malaria prevalence among children aged 6-59 months is estimated at 38%. The prevalence of malaria parasitemia increases with age children. Thus, it passes from 16% for young children who have not passed their first birthday (6-11 months) to 45% among older children, that is to say, those of 48-59 months (Fig 1).

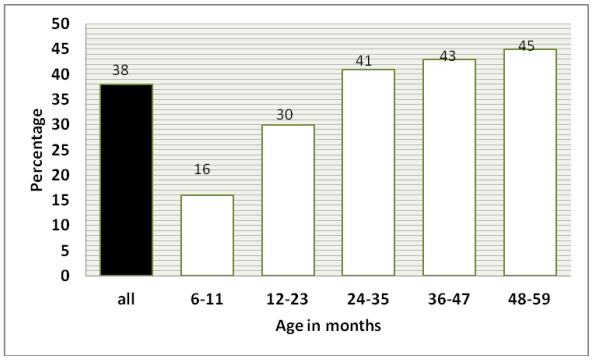

Fig 1. Prevalence of malaria parasitaemia in children 6-59 months according age.

Thus, we see that compared to younger children aged 6-11 months, the prevalence of malaria parasitaemia was 1.8 times higher among those aged 12-23 months, 2.5 times higher among those 24 - 35 months, 2.6

times higher among those 36-47 months and 2.8 times higher among those of 48-59 months (Fig1). If we examine the prevalence by sex, there shall be no significant difference (39% males against 36% for females) (Table 5). From the perspective of the district, it is noted that the lowest prevalence concern Houédomè (36%) and Zoungamè (28%). The highest prevalence is observed in Avagbodji (50%) (Fig. 2).

The microscopic test gives good result than the TDR (38% and 35%). X^2 = 0.5 (Yates corrected), P= 0.81. There are more anemic children in Avagbodji than in the other two arrondissements. Furthermore, the rate of anemic children and those suffering from malaria is higher in flood period (juillet-octobre) (71%) than in recession period (january-juin) (5%) X^2 = 019.05 (Yates corrected), P= 12.10^{-6}.

Table 4. Percentage of children from 6 to 59 months with anemia by age, sex and arrondissement.

	Anemia according to hemoglobin level				
Characteristics	mild (10.0-10.9 g/dl)	moderate (8.0-9.9 g/dl)	Severe <8.0g/dl	**All of children with anaemia <11.0g/dl**	**Number of children**
Age in months					
6-11	17.6	40.8	24.9	83.3	368
12-23	18.6	39.3	33.6	91.5	560
24-35	10.9	49.6	22.1	82.6	570
36-47	18.4	38.2	27.9	84.5	635
48-59	20.2	39.1	12.9	72.2	707
Sex					
Male	17.6	41.6	23	82.2	1405
Female	18.4	40.7	24	83.1	1435
Arrondissements					
Avagbodji	20.6	40.4	26	87	970
Houédomè	13.6	43.6	22.9	80.1	918
Zoungamè	10	43.9	17.8	71.7	952
Total	12.73	42.8	22.23	79.6	2840

Discussion

Improving the understanding of chilhood malarial anaemia may help in the design of appropriate management strategies. Data from 2.840 febrile children up to five years old were analyzed. A high prevalence of anaemia (91.5%) was found in the commune. This prevalence is comparable to that observation was the marked dependence of the prevalence of anaemia on age as reported from other malaria endemic areas (Desai *et al.*, 2005; Schellenberg *et al.*, 2003; Eliades *et al.*, 2006). In Libreville, the highest prevalence rate of anaemia (89.1) occurred among the 12 to 35 months old children (more than 60%), compared to that reported for similarly aged children elsewhere (Desai *et al.*, 2005; Eliades *et al.*, 2006; Cornet, 1998). Moreover, the mean Hb concentration, which was generally low across the whole population (8.6 g/dl), increased with age from four years, but did not reach normal level. Moderate to severe anaemia occurred more frequently in the first three years of life, described by other (Desai *et al.*, 2005; Dicko *et al.*, 2004; Menendez *et al.*, 1997).

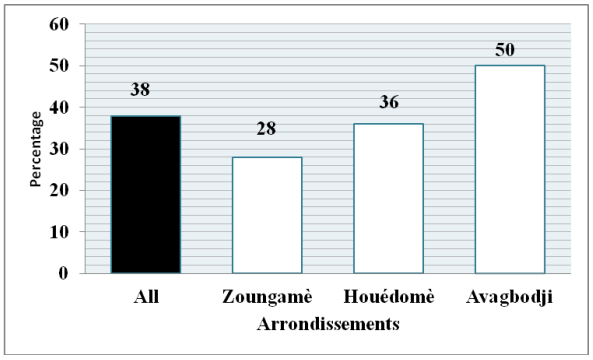

Fig 2. Prevalence of malaria parasitaemia in children 6-59 months according arrondissements.

Table 5. Prevalence of malaria parasitaemia in children 6-59 months by sex.

Characteristics	Percentage of children whose parasitemia test is positive	Number of children
Sex		
Male	38.6	1405
Female	37.4	1435
Total	38	2840

All these observation highlight the burden of anaemia in the commune. The prevalence of *Plasmodium falciparum* infection detected by microscopy was 38%, less than that reported for children from another hospital, in urban area from Ghana and Congo Democratic Republic, but indistinguishable from that noticed in Cameroon and Togo (Desai *et al.*, 2005; Cornet *et al.*, 1998; Koko *et al.*, 1997; Hedberg *et al.*, 1993).The prevalence of malaria was also age-related, increasing from birth to 47 months with children between 24 and 59 months having the highest risk of infection. More than 40% of febrile children over the age of three years were parasitaemic, this is not in agreement with the epidemiology of malaria in a hyperendemic region. Aguégués is a rural, fluvial, lagoon, coastal and lacustrine area in which a low and high malaria transmission coexists. Urbanization impedes malaria transmission and would increase the number of non immune individuals, possibility delaying premunition in non-immune individuals (Robert *et al.*, 2003). Malaria infection during the first few months of life is rare. In children living in a highly endemic area of western Kenya the mean time between birth and detectable parasitaemia was 3.4 months (Mc Elroy *et al.*, 2000). One of the 26 febrile children less than one month old was *Plasmodium falciparum*. The magnitude of the impact of *Plasmodium falciparum* infection on anaemia was age-related and more pronounced in children between six months and five years of age, as previously reported (Schellenberg *et al.*, 2003; Eliades *et al.*, 2006; Ekvall *et al.*, 2001; Kitua *et al.*, 1997). Asymptomatic parasitaemia, which accounts for 7% to 23% of all *Plasmodium falciparum* detected by microscopy, is also an independent factor influencing Hb levels (Ekvall *et al.*, 2001; Missinou *et al.*, 2003; Kurtzhals *et al.*, 1999). The prevalence of mild anaemia did not vary with infection and was not age-related; on the other hand, moderate to severe malarial anaemia were strongly associated with parasitaemia in children less than four years old. Several studies performed in intense malarial anaemia in children less than three years old (Desai *et al.*, 2005; Dzeing-Ella *et al.*, 2005; Eliades *et al.*, 2006; Dicko *et al.*, 2004; Slutsker *et al.*, 1994; Koram *et al.*, 2003). However, few of them found a correlation between parasite density and severe anaemia in young children, severe malarial anaemia is age-related and the low prevalence of severe malarial anaemia in older children and adults may result from the capacity to control parasitaemia. The high level of anaemia and parasitaemia rate is accompanied by the presence of *An. gambiae* in the commune during the flood period. This leads us to say that *An. melas*, the second vector in the commune which is found in recession period is a minor vector of malaria transmission in the commune of Aguégués. Malaria transmission is caused by *An. gambiae*. These results confirm the observations of Akogbéto in 1992.

Acknowledgements

We thank the people of Aguégués for their participation in the study. Special thanks go to medical team of Aguégués. The study received financial support from the Ministry of Higher Education and Scientic Research of Bénin.

References

Akogbeto M. 1992. Malaria transmission in the lagoon area of Cotonou, Bénin. Parasitological **34,** 147-154.

Alonso PL, Lindsay SW, Armstrong Schellenberg JR, Gomez P, Hill AG, David PH, Fegan G, Cham K, Greenwood BM. 1993 A malaria control trial using insectide-treated bed nets and targeted chemoprophylaxis in a rural area of the Gambia, west Africa: 2. Mortality and morbidity from malaria in the study area. Trans R Soc Trop Med Hyg **87**, 13-17.

Breman JG. 2001. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am J Trop Med Hyg* **64,** 1-11.

Cornet M, Le Hesran JY, Fievet N, Cot M, Personne P, Gounoue R, Beyeme M, Deloron P. 1998. Prevalence of and risk factors for anemia in young children in southern Cameroon. Am J Trop Med Hyg **58,** 606 611.

de Maeyer E, Adiels-Tegman M. 1985. The prevalence of anaemia in the world. World Health Stat Q **38,** 302-316.

Desai MR, Terlouw DJ, Kwena AM, Phillips-Howard PA, Karluld SK, Wannemuehler KA, Odhacha A, Hawley WA, Shi YP, Nahlen BL, ter Kuile TO. 2005. Factors associated with hemoglobin concentrations in pre-school children in western Kenya : Cross-Sectional studies. Am J Trop Med Hyg **72,** 47-59.

Dicko A, Klion AD, Théra MA, Sagara I, Yalcouyé D, Niambele MB, Sogoba M, Dolo G, Dao A, Diallo DA, Doumbo OK, Miller LH. 2004. The etiology of severe anemia in a village and a periurban area in Mali. Blood **104,** 1198-1200.

Dzeing-Ella A, Nze Obiang PC, Tchoua R, Planche T, Mboza B, Mbounja M, Muller-Roemer U, Jarvis J, Kendjo E, Ngou-Milama E, Kremsner PG, Krishna S, Kombila M. 2005. Severe *falciparum* malaria in Gabonese children: clinical and laboratory features. Malar J. **9,** 1.

Ekvall H, Premji Z, Bennett S, Bjorkman A. 2001. Hemoglobin concentration in children in a malaria holoendemic area is determined by cumulated *Plasmodium falciparum* parasite densities. Am J Trop Med Hyg **64,** 58-66.

Eliades MJ, Wolkon A, Morgah K, Crawford SB, Dorkenoo A, Sodahlon Y, Hawley WA, Hightower AW, Kuile FO, Terlouw DJ. 2006. Burden of malaria at community level in children less than 5 years of age in Togo**.** Am J Trop Med Hyg **75,** 622-629.

Greenwood BM, Bradley AK, Greenwood AM, Byass P, Jammeh K, Marsh K, Tulloch S, Oldfield FS, Hayes R. 1987. Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. Trans R Soc Trop Med Hyg **81,** 478-486.

Hedberg K, Shaffer N, Davachi F, Hightower A, Lyamba B, Paluku KM, Nguyen-Dinh P, Breman JG. 1993. *Plasmodium falciparum*-associated anaemia in children at a large urban hospital in Zaire. Am J Trop Med Hyg **48,** 365-371.

Kitua AY, Smith TA, Alonso PL, Urassa H, Masanja H, Kimario J, Tanner M. 1997. The role of low level *Plasmodium falciparum* parasitaemia in

anaemia among infants living in an area of intense and perennial transmission. Trop Med Int Health **2,** 325-333.

Klein Klouwenberg PM, Oyakhirome S, Schwarz NG, Gläser B, Issifou S, Kiessling G, Klöpfer A, Kremsner PG, Längin M, Lassmann B, Necek M, Pötschke M, Ritz A, Grobusch MP. 2005. Malaria and asymptomatic parasitaemia in Gabonese infants under the age of 3 months. Acta Trop **95,** 81-85.

Koko J, Dufillot D, Zima-Ebeyard AM, Duong TH, Gahouma D, Kombila M. 1997. Aspects of malaria in the hospitalized child in Gabon**.** Med Trop **57,** 177-180.

Koram KA, Owusu-Agyei S, Fryauff DJ, Anto F, Atuguba F, Hodgson A, Hoffman SL, Nkrumah FK. 2003. Seasonal profiles of malaria infection, anaemia, and bednet use among age groups and communities in northern Ghana. Trop Med Int Health **8,** 793-802.

Kurtzhals JA, Addae MM, Akanmori BD, Dunyo S, Koram KA, Appawu MA, Nkrumah FK, Hviid L. 1999. Anaemia caused by asymptomatic *Plasmodium falciparum* infection in semi-immune African schoolchildren. Trans R Soc Trop Med Hyg **93,** 623-627.

Mc Elroy PD, ter Kuile FO, Lal AA, Bloland PB, Hawley WA, Oloo AJ, Monto AS, Meshnick SR, Nahlen BL. 2000. Effect of *Plasmodium falciparum* parasitemia density on hemoglobin concentrations among full-term, normal birth, weight children in western Kenya. IV. The Asembo bay cohort project. Am J Trop Med Hyg **62,** 504-512.

Menendez C, Kahigwa E, Hirt R, Vounatsou P, Aponte JJ, Font F, Acosta CJ, Schellenberg DM, Galindo CM, Kimario J, Urassa H, Brabin B, Smith TA, Kitua AY, Tanner M, Alonso PL. 1997. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. Lancet **350,** 844-850.

Missinou MA, Lell B, Kremsner PG. 2003. Uncommon asymptomatic *Plasmodium falciparum* infections in Gabonese children. Clin Infect Dis **36,** 1198-1202.

Molineaux L. 1985. The pros and cons of modelling malaria transmission. Trans R Soc Trop Med Hyg **79,** 743-747.

Murphy SC, Breman JG. 2001 Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. Am J Trop Med Hyg **64,** 57-67.

Newton CR, Warn PA, Winstanley PA, Peshu N, Snow RW, Pasvol G, Marsh K. 1997. Severe anaemia in children living in a malaria endemic areas of Kenya. Trop Med Int Health **2,** 165-178.

Payne D, Grab B, Fontaine RE, Hempel JH. 1976. Impact of control measures on malaria transmission and general mortality. Bull.World Health Organ **54,** 369-377.

Snow RW, Craig M, Deichmann U, Marsh K. 1999. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. Bull. World Health Organ **77,** 624-640.

Robert V, Macintyre K, Keating J, Trape JF, Duchemin JB, Warren M, Beier JC. 2003**. Malaria transmission in urban sub-Saharan Africa.** Am J Trop Med Hyg **68,** 169-176.

Schellenberg D, Schellenberg JR, Mushi A, Savigny D, Mgalula L, Mbuya C, Victora CG. 2003. The silent burden of anaemia in Tanzanian children: a community-based study. Bull World Health Organ **81,** 581-590.

Slutsker L, Taylor TE, Wirima JJ, Steketee RW. 1994. In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. Trans R Soc Trop Med Hyg **88,** 548-551.

Trape JF. 2001. The public health impact of Chloroquine resistance in Africa. *Am J Trop Med Hyg* **64,** 12-17.

World Health Organisation. 1996. The World Health Report. Fighting disease, fostering development. **137**. Geneva, WHO.

World Health Organization. 1997. Guidelines for the evaluation of *Plasmodium falciparum* vaccines in populations exposed to natural infections. TDR/MAL/VAC/97. Geneva, Switzerland.

World Health Organization. 2000. Economic costs of malaria are many times higher than previously estimated. WHO/28. Geneva, WHO.