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ORIGINAL PAPER

Malaria transmission in two rural communities in the forest zone of Ghana

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Abstract Malaria transmission was assessed in two rural communities, Kona and Afamanaso in Sekyere South district, Ashanti Region, in the forest zone of Ghana to provide baseline data for ongoing clinical studies and the evaluation of the effect of interventions. Altogether, 3,479 *Anopheles gambiae* and 1,157 *Anopheles funestus* were caught by human landing catches. Sporozoite rates determined by either microscopy of salivary glands or enzyme-linked immunosorbent assay (ELISA) for *Plasmodium falciparum* in the two villages were 6.6% vs. 8.9% for the main vector *A. gambiae* and 3.2% vs. 6.3% for *A. funestus*. ELISA tests of dissected specimens compared to microscopy of salivary glands were 1.3 and 2.0 times more positive for *A. gambiae* and *A. funestus*, respectively. *Plasmodium* infections of 122 microscopically positive salivary glands of *A. gambiae* were identified by real-time PCR as 95 (77.9%) *P. falciparum*, 7 (5.7%) *Plasmodium malariae*, 7 (5.7%) *Plasmodium ovale* and 1 (0.8%) mixed infection of *P. falciparum* and *P. malariae*. Transmission in the area was found to be intense and perennial with some seasonal variations during the study period from Dec. 2003 to Aug. 2005. Although the two villages were only 10 km apart from each other, Annual Biting Rates (ABRs) and Annual Entomological Inoculation Rates (AEIRs) were much higher at Afamanaso (11,643 vs. 866) than at Kona (5,329 vs. 490). Most of the transmission (91.4%) occurred during bedtime hours from 21 to 6 h. It is important to note that there was still a substantial transmission before 21 h with AEIRs of 57.3 at Afamanso and 38.7 at Kona. The distribution of impregnated bednets alone, therefore, may not be sufficiently effective.

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Introduction

Malaria is the leading cause of mortality among children under 5 years old and pregnant women in Ghana and accounts for 40–60% outpatient attendance and for more income and workdays lost than any other disease (Asante et al. 2004). In the forest zone of the Ashanti Region, parasitaemia peaks at a prevalence of 93% in 11-year-old children and declines to a plateau of 20% in adults, as reported by Browne et al. (2000). Intensities of transmission, as defined by Entomological Inoculation Rates (EIRs), have so far been determined in the northern savanna areas (Appawu et al. 2004), in the coastal forest and coastal savannah (Appawu et al. 2001) and the forest-savanna transitional zone (Owusu-Agyei et al. 2009), but not in the main forest region of Ghana.

The present study aimed to describe malaria transmission in two communities in the forest zone by analysing monthly (MBR) and annual biting rates (ABRs) and EIRs by the two vectors *Anopheles gambiae* and *Anopheles funestus*. A clinical study conducted in parallel had shown that in this homogeneous forest environment malaria incidences of 3 to 15-month-old babies were highly heterogeneous between villages (Kreuels et al. 2008).

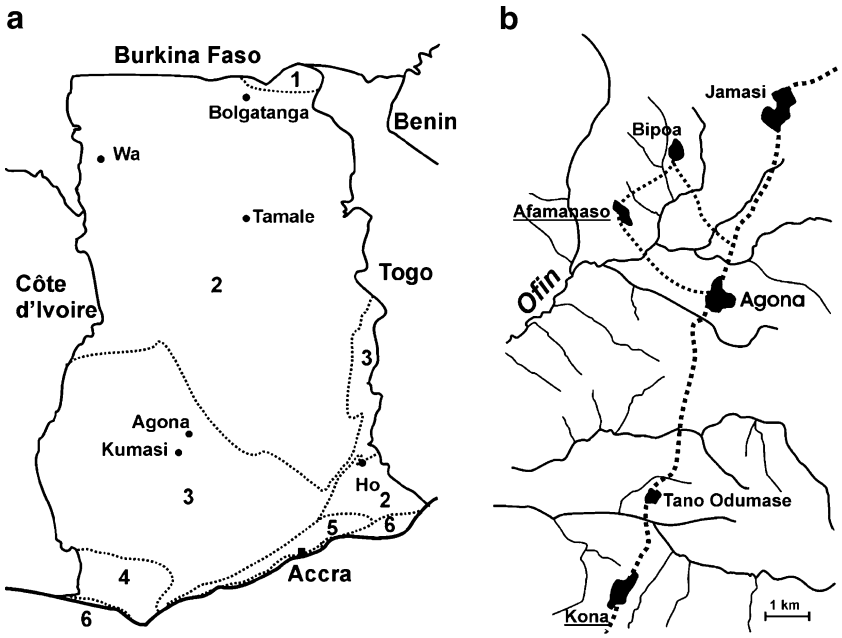

Fig. 1 Maps of Ghana (**a**) with vegetation zones: *1* Sudan savannah, *2* interior wooded savanna, *3* semi-deciduous forest, *4* rainforest, *5* coastal savannah, *6* strand and mangrove; and **b** the study area in Afigya Sekyere District with its capital Agona and the two towns Afamanaso and Kona

Materials and methods

Study sites

Studies were carried out in Afamanaso and Kona, two towns in the Afigya Sekyere District (714 km^2, population 131,658, Ghana Statistical Service, 2000) in the north-eastern part of the Ashanti Region in the forest zone of Ghana. The district capital is Agona, located 27 km north of Kumasi (Fig. 1a). The area is characterised by semi-deciduous forest and farmland. The river Ofin with its tributaries meanders through the district including a forest reserve, providing pools of water and flooded marshy areas in the rainy season (Fig. 1b).

Afamanaso (6° 56′ N, 1° 30′ W), a rural village in the plain, 290 m above sea level and 2.5 km off the main road, is a typical farming village with a population of 2,508 (Ghana Statistical Service 2000). Most of the houses are made of mud with thatched roofs or covered with corrugated iron. Kona (6° 52′ N, 1° 30′ W) is a fast-developing town, 305–320 m above sea level, situated on a small mountain crest on the Kumasi–Ejura Road, with a population of 5,853, engaged in crafts and trading. Most of

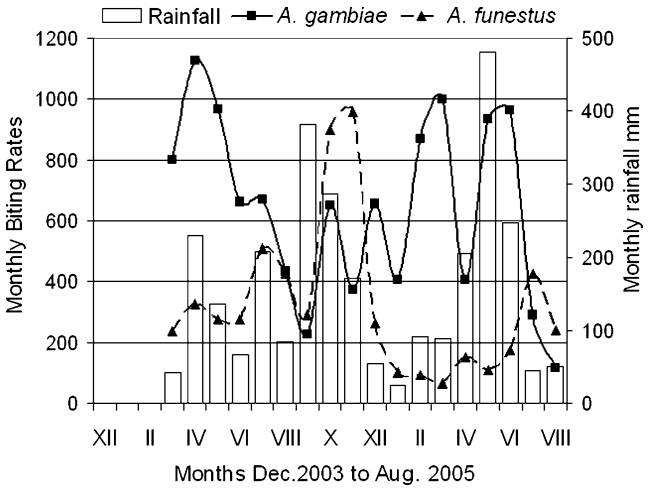

Fig. 2 Monthly biting rates (MBR) of *A. gambiae* and *A. funestus* in Afamanaso from Mar 2004 to Aug 2005 and monthly rainfall (mm) measured in Kona (Mar 2004 to Aug 2005)

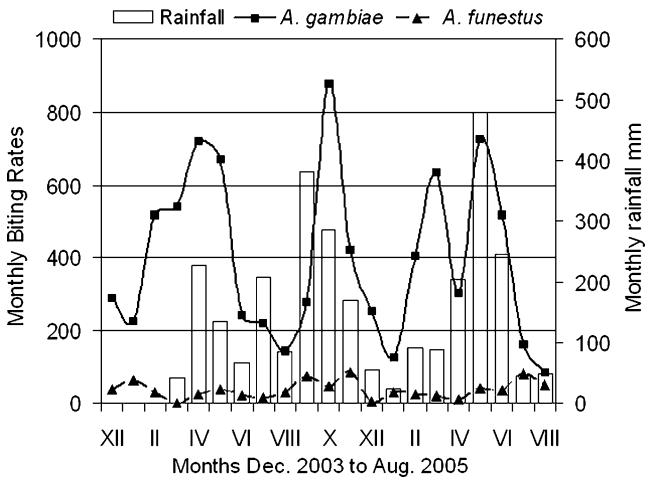

Fig. 3 Monthly biting rates (MBR) of *A. gambiae* and *A. funestus* in Kona from Mar 2004 to Aug 2005 and monthly rainfall (mm) measured in Kona (Mar 2004 to Aug 2005)

Table 1 Mean annual biting rates (ABR), bites per person per night (b/p/n), annual entomological inoculation rates (AEIR), entomological inoculation rates per person per night (EIR/p/n) and sporozoite rates of *A. gambiae* and *A. funestus* in Afamanaso and Kona for the study period XII.2003 to VIII.2005

Afamanaso			
	A. gambiae	*A. funestus*	Total
ABR	7,451	4,192	11,643
b/p/n	20.4	11.5	31.9
Annual EIR	638	228	866
EIR/p/n	1.7	0.6	2.3
Sporozoite rate %	8.6	5.4	7.4
Kona			
ABR	4,896	433	5,329
b/p/n	13.4	1.2	14.6
Annual EIR	457	33	490
EIR/p/n	1.3	0.1	1.4
Sporozoite rate %	9.3	7.5	9.2

the houses are made of cement bricks. The peasant farmers of both villages mainly cultivate cocoa, plantain, maize, palm oil and fruit. Some farmers rear livestock, such as poultry, goats and sheep, but not cattle.

Three seasons can be distinguished: a minor rainy season from Mar to Jun, a major rainy season from July to Oct, and a dry season from Nov to Feb (Meteorological Service, Kumasi). Monthly rainfall was measured in Kona from Mar 2004 to Aug 2005 (Figs. 2, 3). Total rainfall for 1 year (Sept 2004 to Aug 2005) was 2,124 mm, with a minimum of 25 mm in Jan 2005 and a maximum of 480 mm in May 2005. The average monthly rainfall was 177 mm. It was exceptional that the minor rainy season in 2005 had more rainfall than the previous major rainy season of 2004.

Mosquito collection

Human landing catches (HLC) were performed twice a month at three sites in each town beginning Dec 2003 in Kona and Mar 2004 in Afamanaso until the end of Aug 2005. One mosquito collector caught from 1800 h to midnight and a second one from midnight to 0600 h. Collectors rotated from site to site to compensate for possible differences in individual attraction to mosquitoes. The mosquitoes collected were kept in cool boxes and transported to the laboratory for further processing the next morning.

Identification and processing of mosquitoes

Mosquitoes were sorted into *Anopheles* species and *Culicinae*. The former were further identified using the keys of Gillies and Coetzee (1987); culicines were counted and discarded. From *A. gambiae* s.l., legs and wings were removed before dissection and retained for species identification by polymerase chain reaction (PCR), following the protocol of Scott et al. (1993). *Anopheles* females were dissected under a stereomicroscope, their midgut and ovaries were removed, and the latter were examined under a compound microscope to determine parity by inspection of the ovarian tracheoles (Detinova 1962). Salivary glands were examined for sporozoites, using a compound microscope. Sporozoite positive salivary glands were stored at −80°C for later determination of *Plasmodium* species by real-time PCR (Mangold et al. 2005). Head and thorax of all *Anopheles* females were examined for the presence of circumsporozoite (CS) *P. falciparum* antigen, using the enzyme-linked immunosorbent assay (ELISA) (Wirtz 1987). Head and thorax of nulliparous females were used as negative control. A mosquito was considered infective if it was found positive by salivary gland dissection and/or ELISA.

Statistics

Statistica for Windows, 1993, StatSoft Inc., Tulsa, OK, USA, was used for the statistical analysis of the results. Differences between percentages and chi^2 values were analysed with the Quick Probability Calculator of this programme.

Results

Vector collection

Altogether, 4,636 *Anopheles* mosquitoes were collected during 217 full-night HLCs in the two villages, 63.6% (2,948) in Afamanaso and 36.4% (1,688) in Kona. Morpho-

Table 2 Comparison of results of ELISA tests of *A. gambiae* and *A. funestus* from which salivary glands had been removed for microscopy with those with salivary glands

Glands	Removed		Not removed		Significance
	No. examined	No. +ve (%)	No. examined	No. +ve (%)	*P* value
A. gambiae	2,302	163 (7.1)	1,175	105 (8.9)	0.052
A. funestus	554	19 (3.4)	602	38 (6.3)	0.024

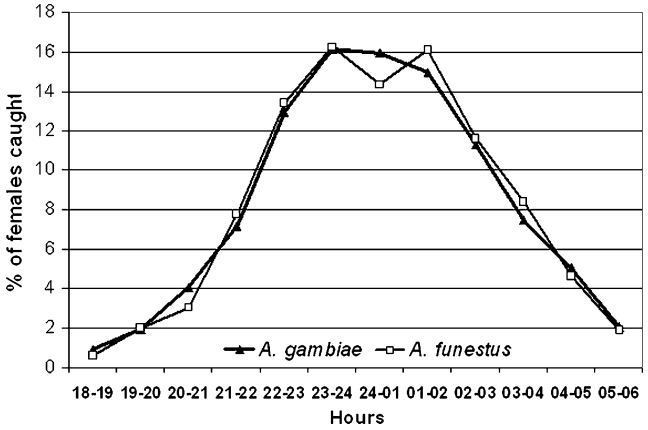

Fig. 4 Hourly biting activities in % of all *Anopheles gambiae* (*N*=3479) and *A. funestus* (*N*=1157) caught at Kona and Afamanaso

logically, 75% (3,479) were identified as *A. gambiae* s.l. and 25% (1,157) as *A. funestus*. One hundred thirty-five *A. gambiae* were classified by PCR as *A. gambiae* s.s.; DNA of three specimens did not amplify.

Parous rates

Parous rates of the *Anopheles* females were high throughout. Mean parous rates of *A. gambiae* and *A. funestus* were 85.8% vs. 85.2% at Afamanaso and 84.0% vs. 82.2% at Kona. Parous rates of *A. gambiae* were significantly higher (*P*=0.0029) in the dry season (90%) than in the major rainy season (83%). Parous rates of *A. funestus* of the dry and the major rainy season did not vary significantly at 85.3% vs. 84.3%, respectively (*P*=0.71).

Annual and seasonal biting activities

Anopheles biting activities were perennial but varied seasonally. When MBRs of months with more or less than 100 mm rainfall were compared, means of MBRs were always lower in months with less rain. Differences, however, were only significant for *A. gambiae* in Kona (*P*=0.024, Mann–Whitney *U* test). *A. funestus* contributed 36% of the bites in Afamanaso but only played a minor role of 8.9% of bites in Kona (Figs. 2 and 3, Table 1). A person passing a night at Afamanaso received an average 31.9 bites per night (b/p/n), more than twice as much as a person in Kona at 14.6 b/p/n.

Infection rates

Altogether, 4,634 *Anopheles* females were tested for the presence of *P. falciparum* CS protein. Salivary glands were removed from 2,858 mosquitoes and were examined microscopically for the presence of sporozoites; 6.6% of 2,280 *A. gambiae* and 3.2% of 570 *A. funestus* turned out to be positive. Of the non-dissected mosquitoes (*N*=1174), 8.9% of *A. gambiae* and 6.3% *A. funestus* were positive in ELISA, indicating that the ELISA was 1.3 times more sensitive for *A. gambiae* and 2.0 times more sensitive for *A. funestus*. Differences were significant (*P*=0.00084 vs. *P*=0.023). When ELISA results for mosquitoes with and without salivary glands were compared, differences were only significant for *A. funestus* (Table 2). When microscopically negative mosquitoes were retested with ELISA, 3.1% of 2,154 *A. gambiae* and 1.9% of 536 *A. funestus* became positive. For the calculation of infection rates and EIRs, mosquitoes, either positive in microscopy and or ELISA, were used (*A. gambiae* 9.1%, *A. funestus* 5.8%). Infection rates of *A. funestus* were always lower than those of *A. gambiae* (Table 1).

Entomological inoculation rates (EIR)

Annual entomological inoculation rate (AEIR) was 866 in Afamanaso and 490 in Kona. The contribution of *A.*

Table 3 Total numbers, numbers (%) of infected *A. gambiae* and *A. funestus* caught before (1800–2100 h), during bedtime (2100-0400 h) and in early morning hours (0400–0600 h) in Afamanaso and Kona, and percentages of all females and all infected females caught during these time intervals

Time interval hours	*A. gambiae*		*A. funestus*		*A. gam. + A. fun.*		% of all caught	% of+ves caught
	No.	No.(%)+ve	No.	No. (%)+ve	No.	No.(%)+ve		
Afamanaso								
1800–2100	139	15 (10.8)	54	5 (9.3)	194	20 (10.3)	6.6	8.7
2100–0400	1,678	153 (9.1)	868	44 (5.1)	2,546	197 (7.7)	86.4	85.7
0400–0600	138	10 (7.2)	71	3 (4.2)	209	13 (6.2)	7.1	5.7
Kona								
1800–2100	104	12 (11.5)	29	1 (3.5)	133	13 (9.8)	7.9	8.4
2100–0400	1,311	117 (8.9)	130	14 (10.8)	1,441	131 (9.1)	85.4	84.5
0400–0600	109	11 (10.1)	5	0 (0)	114	11 (9.6)	6.8	7.1

Table 4 *Plasmodium* species from infected salivary glands in *A. gambiae* and *A. funestus*

	P. falciparum No. (%)	*P. malariae* No. (%)	*P. ovale* No. (%)	*P. falciparum* & *P. malariae* No. (%)	Not amplified No. (%)	Total
A. gam.	95 (77.9)	7 (5.7)	7 (5.7)	1 (0.8)	12 (9.8)	122
A. fun.	11 (64.7)	0	0	0	6 (35.3)	17
Total	106 (76.3)	7 (5.0)	7 (5.0)	1 (0.7)	18 (13.0)	139

funestus reached 35.7% in Afamanao but only 7.2% in Kona (Table 1).

Hourly biting activities and risk of transmission

Biting activities of both *A. gambiae* and *A. funestus* started as early as 1800 h, peaked between 2300 and 0200 h, and persisted until 0600 h in the morning (Fig. 4). There were no significant differences between the two species (chi^2 test of homogeneity by Brandt–Snedecor, Sachs 1999, P=0.75). The percentage of infected *A. gambiae* and *A. funestus* caught before, during bedtime and in the early morning hours were assessed to calculate the risk to be bitten by mosquitoes or to pick up an infection during different time intervals (Table 3). In both communities, 85% of all bites and infected bites occurred during bedtime hours between 2100 h in the evening and 0400 h in the morning, and about 91% between 2100 and 0600 h in the morning. Infection rates of both species did not change significantly (chi^2= 1.91, P=0.38 for *A. gambiae*, chi^2=0.71, P=0.38 for *A. funestus*) during the three time intervals (Table 3). It is important to note that high transmission rates occurred before bedtime from 1800 to 2100 h with an EIR of 57.3 at Afamanaso and 38.7 at Kona.

Identification of *Plasmodium* species

Plasmodium infections in 121 of 139 microscopically positive salivary glands (110 *A. gambiae*, 11 *A. funestus,* 18 did not amplify) could be identified by real-time PCR (Table 4); the distribution of *Plasmodium* species was 87.6% *P. falciparum*, 5.8% *P. malariae* and 5.8% *P. ovale*. As expected, no *P. vivax* infection was detected. One *A. gambiae* contained a mixed infection of *P. falciparum* with *P. malariae* (Table 4).

The kdr gene in *Anopheles gambiae* in the study area

The knock down resistance (kdr) gene was highly prevalent in the *A. gambiae* populations of the two study villages and only absent in 3 of the 109 successfully amplified specimens (Table 5).

Discussion

Altogether, 3,479 *A. gambiae* and 1,157 *A. funestus*, which had been caught by HLCs in the two study villages Kona and Afamanaso, were examined. Samples of *A. gambiae* from both villages were identified as *A. gambiae* sensu stricto, which agrees with the results of Tuno et al. (2010), who furthermore recorded a high human blood ratio and strong endophilic behaviour of this species. The genotype of *A. gambiae* was not determined. It can be assumed that it was the S form which predominates in the forest region of Ghana and is positively associated with malaria (De Souza et al. 2010). All 52 specimens collected at Kumasi were identified as S form and carried the kdr mutation. (Yawson et al. 2004). This was in accordance with the high kdr (89.9% homozygous) detected in our material from Kona and Afamanaso.

The sporozoite rates for *A. gambiae* were always higher than those of *A. funestus* which corroborates with the findings of Owusu-Agyei et al. (2009) from the forest transitional zone in Brong Ahafo, north of Kumasi. This is in contrast to the coastal forest, the coastal savannah and

Table 5 Presence of the kdr gene in *Anopheles gambiae* in the study area

Town	No. amplified	Homozygous resistant (RR)	Heterozygous resistant (RS)	Homozygous susceptible (SS)	Not amplified
Afamanaso	61	53 (86.9%)	6 (9.8%)	2 (3.3%)	5
Kona	48	45 (93.8%)	2 (4.2%)	1 (2.1%)	13
Totals	109	98 (89.9%)	8 (7.3%)	3 (2.8%)	18

also to the northern savannah of Ghana where infection rates of *A. funestus* were higher than those of *A. gambiae* (Appawu et al. 2001, 2003; Okoye et al. 2005).

The malaria transmission in both study villages was perennial and intensive with annual EIRs of 866 for Afamanaso and 490 for Kona. This was comparable with the transmission in the Sudan savannah (418) of northern Ghana (Appawu et al. 2004) and higher than that measured in the forest savannah transitional zone around Kintampo (269) and the coastal forest at Dodowa (21.9) (Owusu-Agyei et al. 2009; Appawu et al. 2001).

Biting rates and transmission varied in both villages with rainfall. *A. gambiae* was the main vector contributing 81% of the transmission. *A. funestus* was the secondary vector. However, the contribution of *A. funestus* differed in the two villages, 36% in Afamanaso but only 7.7% in Kona. Similarly *A. funestus* was found to be the secondary vector also in Dodowa (Appawu et al. 2001), the area around Kintampo (Owusu-Agyei et al. 2009) and in the Kassena Nankana District of the northern savannah (Appawu et al. 2004). Differences in the amount of transmission in Kona and Afamanaso were in parallel with the number of malaria episodes per year of small children of 2.2 and 1.1, respectively (Kreuels et al. 2008). The larger population in Kona (5,853, Afamanaso 2,508) might lead to a dilution of man vector contact and a main reason for the difference in biting rates and EIRs determined in both villages. Differences of transmission and species composition of the vector populations are further influenced by the distinct topographies of the two study sites. Afamanaso is a village in the plain surrounded by the Ofin river and swampy areas, while Kona is located on a ridge with only small streams in the valley.

The hourly biting activities were similar for both vectors with peaks from 23.00-02.00 h as described by Gillies and de Meillon (1968). It was important to note that essential biting and transmission occurred before bedtime from 1800–2100 h in both villages with AEIRs of 57.3 in Afamanaso and 38.7 in Kona. These AEIRs were even higher than the AEIR of 22 measured for whole nights at Dodowa in the coastal forest (Appawu et al. 2004), but malaria prevalences in the human population of 42.2% in April and 51.3% in August (Afari et al. 1995) were comparable with 50.7% determined in Ashanti Region (Browne et al. 2000). It is to be feared that the use of impregnated long-lasting bednets may not be effective in preventing transmission in areas with such high early-evening transmission rates: i.e. before bedtime. Children are only sufficiently protected by bednets in areas with no transmission in the early evening hours, e.g. Dodowa of the coastal forest (Appawu et al. 2001). It is another problem that in Afamanaso 15.9% of women and 37% of men sleep outside (Tuno et al. 2010).

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