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Effects of Irrigated Rice Fields and Seasonality on *Plasmodium* Transmission in West Africa, Particularly in Central Côte d'Ivoire

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1. Introduction

The transmission of the parasites that cause human malaria is influenced by myriad environmental factors, including changes in agricultural practices, deforestation, and water-resources development and management (Ijumba and Lindsay, 2001; Ijumba et al., 2002; Keiser et al., 2005; Guerra et al., 2006), climatic factors such as rainfall, humidity and temperature (Reiter, 2008), and various cultural, economic, political and social factors, including health-seeking behaviour, urbanization, armed conflict and war (Esse et al., 2008; Baragatti et al., 2009). Drug resistance in the causative parasites and insecticide resistance in the mosquito vectors are also important factors that now influence malaria transmission (Reiter, 2008). Each year, in many parts of Africa, the local populations of anopheline mosquitoes build up rapidly and peak shortly after the onset of the rainy season (Mbogo et al., 1995). In two studies on the relationships between mosquito abundance, malaria transmission and rainfall in West Africa, 70%–90% of the children investigated were found infected with *Plasmodium spp*. after the rainy season (Bonnet et al., 2002; Koudou et al., 2009). It is particularly during and at the end of the rainy season that malaria becomes one of the leading causes of mortality and health-seeking at dispensaries and hospitals in this region (Rey et al., 1987). Not only season but also changing patterns of agriculture, particularly irrigated rice farming, influence malaria transmission in Africa (Ijumba & Lindsay, 2001; Diuk-Wasser et al., 2007; Sogoba et al., 2007).
Additionally, malaria transmission, *Plasmodium* prevalence rates, the proportion of presumptive and clinically-confirmed malaria episodes have been studied in two villages of central Côte d'Ivoire: one with irrigated rice farming (Zatta) and one without (Tiémélékro) (Koudou et al., 2009). Due to a farmers' conflict over land and socio-political issues, irrigated rice farming was interrupted in Zatta in 2003. The goal of this contribution to a book chapter is to evaluate the relationship between *Plasmodium* transmission, seasonality and agriculture practices.

2. Methods

2.1 Study sites

The study described here was carried out in the villages of Tiemelekro (geographical coordinates: 6˚500 N, -4˚170 W) and Zatta (6˚880 N, -5˚390 W), located in central Côte d'Ivoire (Figure 1). A detailed description of Tiemelekro, including climatic conditions, current health care delivery structures and key demographic and socioeconomic indicators, has been presented recently (Girardin et al., 2004). Zatta is located 7 km north-west of Yamoussoukro, the capital city of Côte d'Ivoire. The mean annual temperature in this village is 26.5˚C and the mean annual precipitation is 1280 mm. There is a long rainy season between April and July and a shorter one in October/November. A dispensary, run by two local nurses, is located in Zatta and also covers nearby settlements. Two small dams were constructed in this village in the mid-1970s. Since 1997, a very large irrigated rice field has been cultivated on an estimated surface area of 36 ha, in close proximity to human habitations. However, due to unstable socio-political conditions and a farmers' conflict over land, rice irrigation was interrupted in 2000 and again in 2003/2004.

Living conditions and several of the investigated household characteristics are comparable between the two study villages. For example, similar proportions of houses utilized iron-corrugated sheets as roofing material (93.8% in Tiemelekro vs. 92.9% in Zatta), and had running water at home (74.1% vs. 65.4%). On the other hand, improved sanitation facilities were less prominent in Tiemelekro than in Zatta (17.0% vs. 47.6%). With regard to personal protective measures against mosquito bites, the proportion of people sleeping under a bednet was similarly low in both villages (8.4–11.2%), whereas use of fumigating coils was much more pronounced in Zatta (47.3%) when compared to Tiemelekro (9.1%).

2.1.1 Rainfall data collection

The Ivorian "Societe d'Exploitation et de Developpement Aeroportuaire et Meteorologique" (SODEXAM) holds rainfall data for the study area, from 1971 onwards. For the present study, monthly rainfall data from 2002 to 2005 were extracted from the society's records.

2.1.2 Adult mosquitos' collection

Overall, 13 entomological surveys were carried out: seven in the long rainy seasons (in the April and June of 2002, the April, May, June and July of 2003, and the May of 2005), and six in dry seasons (in the February and August of 2002, 2003 and 2005). Adult mosquitoes were collected by means of human-bait night catches. The surveys in 2002 and 2005 were each conducted between 18.00 and 06.00 hours, both inside and outside sentinel houses. Each of the six surveys carried out in 2003, however, covered shorter time periods (from 22:00 to 06:00 hours) and the collectors were only stationed inside the sentinel house because of the unstable socio–political situation at the time. Overall, 96, 48 and 32 night catches were carried out in 2002, 2003 and 2005, respectively. No surveys could be undertaken in 2004, as it was then considered too dangerous to reach the study villages.

2.1.3 Laboratory procedures

Adult mosquitoes were brought to a laboratory and processed. Firstly, the physiological age of each female *Anopheles* and the corresponding parity rate (i.e. the proportion of female mosquitoes that had laid eggs at least once) were determined by dissection of ovaries and examination of tracheoles (Detinova, 1962). For quality control, a random sample of 10% of the mosquitoes investigated were re-examined by a senior technician. Secondly, a proportion of *An. gambiae* female having laid eggs at least once was checked for *P. falciparum* infection, in an ELISA for detecting the parasite's circumsporozoite protein (Beier et al.,

Fig. 1. Vegetation mapping of Côte-d'Ivoire presenting both study sites (Zatta and Tiemelekro) located in the central part of the country.

1988). Thirdly, some of the females belonging to the *An. gambiae* complex were identified to species level, in a PCR-based assay (Scott et al., 1993). Finally, some of the mosquitoes belonging to the *An. funestus* group were further identified using an assay based on a multiplex PCR (Koekemoer et al., 2002; Cohuet et al., 2003).

2.1.4 Clinical and Parasitological surveys

Repeated cross-sectional surveys were carried out in the study villages to assess malaria parasitaemia and clinical malaria in children aged ≤15 years. The first survey was done in June 2002. In 2003, two surveys were carried out in Zatta and three in Tiémélékro. The research team first worked in the primary schools and all children aged between 7 and 15 years from randomly selected classes were invited for a finger prick blood sample. Next, mothers and caregivers of under 7-year-old children were invited to accompany their children to a designated community location where a blood sample was taken from each child.
Thick and thin blood films were prepared on microscope slides. The slides were air-dried prior to transfer to a nearby laboratory where they were stained with Giemsa for 45 min. The slides were examined by the same experienced laboratory technician throughout the study under a microscope at high magnification. *Plasmodium* species and gametocytes were identified and counted against 200 leucocytes. When less than 10 parasites were found, reading was continued for a total of 500 leucocytes. Parasitaemia was expressed by the number of parasites per µl of blood, assuming for a standard count of 8000 leucocytes/µl blood. For quality control, 10% of the slides were randomly selected and re-examined by a second senior technician.
In our study, fever was defined when an individual had an axillary temperature >37.5 ◦C. Clinical malariawas defined as fever plus parasitaemia (Smith et al., 1994). Particular emphasis was placed on clinical cases with a parasitaemia >5000 parasites/_l blood. The latter threshold has been chosen after comparing the proportions of fever cases and asymptomatic carriers for different classes of parasite density (Gaye et al., 1989). Subjects with malaria-related symptoms (e.g. headache) plus axillary temperature >37.5 ◦C were given artesunate plus amodiaquine (the respective first-line antimalarial treatment at the time of the study) and paracetamole.

2.1.5 Ethical issues

The study protocol was approved by the institutional research commission of the Centre Suisse de Recherches Scientifiques (Abidjan, Côte d'Ivoire). Ethical clearance was obtained from the Ivorian Ministry of Public Health and National Malaria Control Programme. People who acted as bait and collectors in the mosquito collections were all volunteers and signed informed consent forms. During the study, Patients with malaria-related symptoms who presented at the dispensaries and mosquitoes' collectors were treated and protected for free against malaria by artesunate–amodiaquine chemoprophylaxis (artesunate–amodiaquine being the recommended, first-line treatment for malaria in Côte d'Ivoire at the time of the present study) and all mosquitoes' collectors were immunized against yellow fever. The heads of household in both study sites were informed and the parents or legal guardians of participating children signed a written informed consent sheet.

3. Results

3.1 Species composition of *An. gambiae* complex and *An. funestus* group

A total of 110 mosquitoes were identified to species level by PCR: 60 from Tiémélékro and 50 from Zatta. Within *Anopheles* spp. morphologically identified as *An. gambiae* complex, 100% were *An. gambiae s. s.* With regard to the *An. funestus* group, it consisted of 100% *An. funestus s. s.*

3.2 Effects of agricultural practices (irrigated rice fields & vegetable farming) on *Plasmodium* transmission

Comparison between years revealed that the biting rate of *An. gambiae s.l.* in Zatta decreased several-fold from 49.3 bites per person per night (b/p/n) in 2002 to 7.9 b/p/n in 2003 (likelihood ratio test (LRT=1072.66; P<0.001). In Tiemelekro, the biting rates recorded in 2002 and 2003 remained fairly constant. These observations were paralleled by a marked decrease in the infective rate of *An. gambiae s.l.* in Zatta (4.6–1.2%), and an increase in Tiemelekro (3.1–7.6%). Meanwhile, the entomological inoculation rate (EIR) of *An. gambiae s.l.* decreased 21-fold in Zatta, from 789 to 38 infective bites per person per year (ib/p/y), whereas it remained high in Tiemelekro (233 vs. 342 ib/p/y). In Zatta, the return to irrigated rice farming in January 2005 was paralleled by a significant increase of the EIR ranging from 38 infective bites per person per year (ib/p/y) in 2003 to 295 ib/p/y in 2005. In Tiémélékro high EIRs were found in 2003 (342 ib/p/y) and 2005 (572 ib/p/y).

3.3 Effects of agricultural practices (irrigated rice fields & vegetable farming) on *Plasmodium* prevalence and clinical malaria cases

3.3.1 Irrigated rice fields and *Plasmodium* prevalence

In both villages, the peak prevalence of *P. falciparum* was generally observed in children aged 3-6 years. There were three exceptions: in Tiémélékro, the peak prevalence of *P. falciparum* during the May 2005 survey was found in the youngest age group (≤ 2 years), whereas in Zatta, the highest prevalence in the baseline survey (June 2002) and the second last survey (May 2005) was observed in children aged 7-15 years.

In June 2002, similarly high *P. falciparum* prevalence rates were observed in Zatta (85.4%) and Tiémélékro (86.1%). In Zatta, a significant decrease in the mean *P. falciparum* prevalence rate occurred from 2002 to 2003 (58.4%; $\chi^2 = 42.33$, degree of freedom (df) = 1; $P < 0.001$). There was a significant increase from 2003 to 2005 (66.0%; $\chi^2 = 14.78$, df = 1, $P = 0.012$). In Tiémélékro, the *P. falciparum* prevalence rate in June 2003 (78.2%) was significantly lower than during the June 2002 survey ($\chi^2 = 4.92$, df = 1; $P = 0.027$). The annual *P. falciparum* prevalence rate decreased significantly from 2003 (70.7%) to 2005 (60.4%; $\chi^2 = 17.27$, df = 1; $P < 0.001$).

3.3.2 Fever cases and asymptomatic carriers, stratified by parasite density

Table 1 shows how many of the children examined with parasitaemia in the 2003 surveys were either asymptomatic carriers or presented with a fever. There was a strong seasonal variation in the proportion of fever cases among individuals with parasitaemia. In Zatta, for example, the proportion of fever cases among *Plasmodium*-positive individuals was significantly higher towards the end of the rainy season (August) when compared to the dry season (March) (22.1% **versus** 9.9%; $\chi^2 = 9.90$, df = 1; $P = 0.002$). In Tiémélékro, considerably higher frequencies of fever cases among *Plasmodium*-positive individuals were recorded during the peak rainy season in June (27.3%) and towards the end of the rainy season in August (25.5%) when compared to the dry season in March (15.9%; $P < 0.05$ for both comparisons).

In Zatta, all individuals with a high level of parasitaemia (≥ 5000 parasites/µl blood) presented with fever, accounting for a highly significant difference between the proportion of asymptomatic carriers and fever cases in this parasitaemia class ($P < 0.001$). Similarly, there was a highly significant association between the fever cases and high parasitaemia in the three surveys carried out in 2003 in Tiémélékro ($P < 0.05$). No statistically significant difference was found in children with lower parasitaemias (1000-5000 parasites/µl of blood), neither in Zatta (March: $\chi^2 = 1.53$; df = 1; $P = 0.068$ and August: $\chi^2 = 0.116$; df = 1; $P = 0.733$) nor in Tiémélékro (March: $\chi^2 = 0.18$; df = 1; $P = 0.671$, June: $\chi^2 = 2.23$; df = 1; $P = 0.135$ and August: $\chi^2 = 0.001$; df = 1; $P = 0.973$).

3.3.3 Annual variation of presumptive cases and malaria transmission

In Zatta, 966, 812, 693 and 884 presumptive cases were recorded in 2002, 2003, 2004 and 2005, respectively. The annual number of presumptive malaria cases decreased significantly by 15.1% and 14.7%, respectively, from 2002 to 2003 (IRR = 0.841, $P < 0.001$) and from 2003 to 2004 (IRR = 0.853, $P = 0.002$). An opposite trend was observed from 2004 to 2005; the number of presumptive malaria cases increased significantly by 27.5% (IRR = 1.276, $P < 0.001$). The monthly number of presumptive cases was not related to the monthly number of infective bites per person (IRR = 0.994, $P = 0.827$).

Date of survey	*P. falciparum* parasitaemia (parasites/µl blood)	Tiémélékro		Zatta	
		No. (%) of asymptomatic carriers	No. (%) of children with fever	No. (%) of asymptomatic carriers	No. (%) of children with fever
March 2003	< 1000	69 (54.8%)	13 (10.3%)	135 (73.4%)	9 (4.2%)
	1000-5000	37 (29.4%)	6 (4.8%)	57 (26.8%)	9 (4.2%)
	≥ 5000	0 (0)	1 (0.8%)	0 (0)	3 (1.4%)
	Total	106 (84.1%)	20 (15.9%)	192 (90.1%)	21 (9.9%)
June 2003[a]	< 1000	86 (52.1%)	34 (20.6%)	n.a.	n.a.
	1000-5000	32 (19.4%)	7 (4.2%)	n.a.	n.a.
	≥ 5000	2 (1.2%)	4 (2.4%)	n.a.	n.a.
	Total	120 (72.7%)	45 (27.3%)	n.a.	n.a.
August 2003	< 1000	99 (50.5%)	22 (11.2%)	78 (57.4%)	12 (8.8%)
	1000-5000	46 (23.5%)	16 (8.2%)	28 (20.6%)	7 (5.1%)
	≥ 5000	1 (0.5%)	12 (6.1%)	0 (0)	11 (8.1%)
	Total	146 (74.5%)	50 (25.5%)	106 (77.9%)	30 (22.1%)
Overall 2003 (number of positive children/ number of total children)		54.0% (372/689)	16.7% (115/689)	49.8% (298/598)	8.5% (51/598)

n.a.: not assessed

[a] No survey carried out in June 2003 in Zatta due to unstable sociopolitical situation

Table 1. Number (%) of children infected with *P. falciparum* who were asymptomatic carriers or presented with fever, stratified by different levels of parasitaemia, in the two study villages of Tiémélékro and Zatta, central Côte d'Ivoire.

In Tiémélékro, the yearly numbers of presumptive malaria cases were 2089, 1858, 1655 and 1541. Thus, we observed significant decreases in the yearly number of presumptive cases by 11.1% from 2002 to 2003 (IRR = 0.889, $P < 0.001$), 9.0% from 2003 to 2004 (IRR = 0.910, $P = 0.005$) and 8.9% from 2004 to 2005 (IRR = 0.911, $P = 0.008$).
As in the case of Zatta, the monthly number of presumptive cases was not related to the monthly number of infective bites per person (IRR = 1.007; $P = 0.776$).

3.4 Effects of seasonality on *Plasmodium* Transmission

Tables 2 and 3 summarise the mean biting rate, infection rate and the entomological inoculation rate (EIR) of *An. gambiae* and *An. funestus* in the two study villages in 2002, 2003 and 2005.

3.4.1 Relationship between season and biting and infection rates

In Zatta, significantly higher *An. gambiae s. s.* biting rates were recorded in the dry seasons of 2002 and 2005 when irrigated rice farming was practiced, compared to the dry season of 2003 when irrigated rice farming was interrupted (LRT comparing 2002 with 2003: 13.79, LRT comparing 2005 with 2003: 20.50; both $P < 0.001$). In 2003, there was no seasonal difference in the biting rate of *An. gambiae s. s.* (LRT = 0.13; $P = 0.900$) and *An. funestus s. s.* (LRT = 0.17, $P = 0.879$). In Tiémélékro, in 2002 (LRT = 1.84; $P = 0.069$) and 2005 (LRT = 0.56; $P = 0.455$), there were no significant differences in *An. gambiae s. s.* biting rates between the dry and the rainy season. In 2003, the biting rate was significantly higher in the long rainy season (LRT = 3.87, $P < 0.001$). Regarding *An. funestus s. s.* biting rates, those recorded in the dry season of 2002 (LRT = 6.15) and 2003 (LRT = 4.50) were significantly higher than those recorded in the rainy season (both $P < 0.001$). The difference in the biting rates between the dry and rainy season in 2005 also showed statistical significance (LRT = 3.26; $P = 0.031$).

3.4.2 Relationship between season and *Plasmodium* transmission

In both villages, higher EIRs of *An. gambiae s. s.* were usually recorded in the rainy season. For example, in Zatta, the EIR of *An. gambiae s. s.* recorded in the rainy seasons of 2002 and 2005 were 458 and 365 infective bites per person per season (ib/p/s), respectively. In 2003, when irrigated rice farming was interrupted in Zatta, *P. falciparum* transmission by *An. gambiae s. s.* and *An. funestus s. s.* only occurred during the rainy season. In Tiémélékro, in the rainy seasons of 2003 and 2005, the number of infective bites recorded for *An. gambiae s. s.* (357 and 208 ib/p/s, respectively) were 3-14 times higher than in the dry seasons (25 and 77 ib/p/s in the respective years). In 2002, in contrast, the EIR of *An. gambiae s. s.* recorded in the dry season was 2.5 times higher than the one recorded in the rainy season.
The highest EIRs of *An. funestus s. s.* were usually noted during the dry season. In Tiémélékro, this species was the primary *P. falciparum* transmitter during the dry season of 2005 when 207 ib/p/s were recorded. With regard to infection rates, with the exception of the 2005 infection rate of *An. funestus s. s.* recorded in Tiémélékro ($\chi^2 = 4.47$, $P = 0.035$), no significant differences were observed between seasons, neither for *An. gambiae s. s.* nor for *An. funestus s. s.* in any village.

Malaria vector	Entomological parameter	Dry season		Rainy season		χ^2 or LRT	P value
		Mean (n)	95% CI	Mean (n)	95% CI		
An. gambiae							
2002[a]	Biting rate	38.7	36.3-41.1	59.9	56.9-68.2	3.79	<0.001
	Infection rate	4.8 (1,120)	3.6-6.1	4.2 (978)	3.0-5.5	0.33	0.564
	Parity rate	40.4 (673)	36.7-44.1	31.1 (1,157)	28.4-33.8	16.28	<0.001
	Total EIR	338	-	458	-		
2003[b]	Biting rate	7.1	5.4-9.3	8.3	5.0-11.6	0.13	0.900
	Infection rate	0.0 (65)	0.0-0.2	1.7 (176)	0.0-3.6	1.12	0.290
	Parity rate	61.9 (63)	49.8-74.2	36.7 (139)	28.6-44.8	11.16	<0.001
	Total EIR	0	-	26	-		
2005[c]	Biting rate	18.3	11.8-24.7	58.6	23.8-93.4	20.50	<0.001
	Infection rate	2.3 (127)	0.2-5.8	3.4 (136)	1.7-5.2	0.37	0.542
	Parity rate	52.9 (194)	45.2-60.6	46.2 (199)	40.6-52.0	1.71	0.191
	Total EIR	77		365			
An. funestus							
2002[a]	Biting rate	0.0	0.0-0.2	0.0	0.0	0	0
	Infection rate	0.0 (0)	0.0	0.0 (0)	0.0	0	0
	Parity rate	0.0 (0)	0.0	0.0 (0)	0.0	0	0
	Total EIR	0		0			
2003[b]	Biting rate	1.4	0.7-2.4	1.2	0.6-2.1	0.17	0.879
	Infection rate	0.0 (18)	0.0-0.2	2.3 (44)	0.0-6.8	0.42	0.519
	Parity rate	0.0 (9)	0.0-0.2	58.3 (12)	25.6-91.1	7.88	0.005
	Total EIR	0	-	5	-		
2005[c]	Biting rate	2.7	1.1-4.4	0.6	0.0-1.5	0.61	0.435
	Infection rate	8.3 (8)	0.0-28.4	0.0 (3)	0.0	0.87	0.824
	Parity rate	70.9	24.3-84.3	60.0	0.0-100.0	0.58	0.216
	Total EIR	41		0			0

In brackets are the number of malaria vectors analyzed; LRT (likelihood ratio test)
[a]Irrigated rice farming performed in a synchronized manner
[b]Interruption of rice cultivation
[c]Irrigated rice farming performed in a synchronized manner

Table 2. Monthly average biting rate, infection rate, parity rate and entomological inoculation rate (EIR) of *An. gambiae* and *An. funestus* during the dry season and the rainy season in 2002, 2003 and 2005 in Zatta, central Côte d'Ivoire

Malaria vector	Entomological parameter	Dry season		Rainy season		χ^2 or LRT	P value
		Mean (n)	95% CI	Mean (n)	95% CI		
An. gambiae							
2002*	Biting rate	19.6	18.1-21.1	12.6	11.2-14.1	1.84	0.069
	Infection rate	4.1 (268)	1.7-6.5	2.6 (531)	1.3-4.0	1.27	0.260
	Parity rate	72.5 (240)	66.8-78.2	52.4 (597)	48.4-56.4	28.34	<0.001
	Total EIR	146	-	60	-		
2003**	Biting rate	5.2	3.8-7.0	24.7	21.6-28.3	3.87	<0.001
	Infection rate	2.6 (35)	0.0-8.7	7.9 (467)	5.4-10.4	1.19	0.274
	Parity rate	59.0 (39)	42.8-75.1	58.6 (449)	54.0-63.1	0.002	0.961
	Total EIR	25	-	357	-		
2005**	Biting rate	3.9	2.1-5.7	16.7	9.1-24.4	10.56	<0.001
	Infection rate	10.9 (52)	3.7-17.3	6.8 (163)	2.6-10.6	1.47	0.226
	Parity rate	48.3 (103)	33.3-63.4	81.1 (201)	75.5-86.7	30.19	<0.001
	Total EIR	77	-	208	-		
An. funestus							
2002	Biting rate	5.0	4.1-5.9	0.7	0.4-1.1	6.15	<0.001
	Infection rate	3.1 (97)	0.0-6.6	7.7 (26)	0.0-18.7	1.11	0.292
	Parity rate	65.4 (185)	58.5-72.3	50.0 (22)	27.3-72.7	2.02	0.155
	Total EIR	28	-	9	-		
2003	Biting rate	8.4	6.6-10.5	4.0	2.9-5.4	4.50	<0.001
	Infection rate	3.6 (55)	0.0-8.7	9.1 (66)	1.9-16.2	1.45	0.229
	Parity rate	75.0 (60)	63.7-86.3	69.0 (42)	54.4-83.6	0.44	0.507
	Total EIR	55	-	67	-		
2005	Biting rate	29.9	20.9-38.9	1.0	0.6-1.9	3.26	0.031
	Infection rate	3.8 (131)	1.2-5.6	17.6 (11)	0.0-37.8	4.47	0.035
	Parity rate	65.1 (203)	50.6-73.2	91.7 (12)	73.3-100.0	3.61	0.057
	Total EIR	207	-	32			

In brackets are the number of malaria vectors analyzed; LRT (likelihood ratio test)
*Vegetable farming is performed intensively with 2 production cycles per year
**Vegetable farming is performed intensively with 1 production cycle per year

Table 3. Monthly average biting rate, infection rate, parity rate and entomological inoculation rate (EIR) of *An. gambiae* and *An. funestus* during the dry season and the rainy season in 2002, 2003 and 2005 in Tiémélékro, central Côte d'Ivoire

4. Discussion

The interruption of irrigated rice farming due to a farmers' dispute over land property rights, coupled with an unstable socio-political situation in the face of the 2002-2004 armed conflict (Betsi et al., 2006; Fürst et al., 2009) offered a unique opportunity to study the dynamics of malaria transmission. Our analyses complement previous publications

(Girardin et al., 2004; Koudou et al., 2005, 2007, 2009), now with an explicit focus on the effect of seasonality on malaria transmission under changing agro-ecological conditions. The following points are offered for discussion.

Firstly, biting rates of *An. gambiae* in both villages were usually significantly higher in the rainy season than in the dry season. When irrigated rice farming was interrupted in Zatta in 2003, much lower biting rates were observed than in the preceding year and in 2005, but there were no seasonal differences. Hence, the interruption of irrigated rice farming appeared to have hidden the effect of season on *An. gambiae* biting rate. These findings are in agreement with previous investigations in the humid savannah of Côte d'Ivoire: in an area characterised by intensive agriculture, the biting rate of *An. gambiae* increased significantly a few weeks after the beginning of the rainy season, whereas it decreased and became lowest towards the end of the dry season (Doannio et al., 2006). Moreover, the blunting of seasonal differences in biting rates due to changing patterns in irrigated rice farming has been documented previously for the savannahs of Senegal (Faye et al., 1993) and Mali (Dolo et al., 2004). In contrast to *An. gambiae* with the highest biting rates usually observed in the rainy season, the highest biting rates of *An. funestus* were consistently recorded in the dry season regardless of the prevailing agricultural activity. Moreover, interruption of irrigated rice farming in Zatta showed no effect.

Secondly, with the only exception of a significantly higher infection rate of *An. funesuts* in Tiémélékro in the rainy season compared to the dry season of 2005, infection rates of both *An. gambiae* and *An. funestus* showed no clear seasonal patterns. Different results were reported from Dielmo, a holoendemic area in Senegal, where the infection rate of malaria vectors showed considerable seasonal variation (Fontenille et al., 1997). The observations made in Senegal corroborated previous findings obtained in the savannah area in the north of Côte d'Ivoire (Dossou-Yovo et al., 1995), and other findings documenting a high infection rate of *An. funestus* at the beginning of the dry season in an irrigated rice area compared to a non-irrigated rice farming area (Dossou-Yovo, 2000). It should be noted, however that the mean annual infection rate of *An. gambiae* in Zatta was significantly higher when irrigated rice farming was in place (in 2002 and 2005) compared to a year with interrupted irrigated rice farming (Koudou et al., 2005).

Thirdly, the influence of changing patterns of irrigated rice farming on the *An. gambiae*-specific EIR in Zatta has been discussed elsewhere (Koudou et al., 2005, 2007). In brief, interruption of irrigated rice farming resulted in several-fold lower EIRs compared to normal years. Here, we now document that seasonal patterns of transmission remained. Indeed, considerably higher EIRs were observed for *An. gambiae* in the rainy season compared to the dry season. Of note, the EIR of *An. gambia* in the dry season of 2003 in Zatta dropped to zero. In Tiémélékro, high EIRs were recorded throughout the study period for *An. gambiae* and, in general, EIRs were higher in the rainy season compared to the dry season. *An. funestus* seemed to play an important role in the transmission of malaria, particularly in the dry season. Our results therefore confirm previous observations made elsewhere in the northern savannah of Côte d'Ivoire (Dossou-Yovo, 2000) and in southern Cameroon (Bonnet et al., 2002). Whilst *An. gambiae* was the key *P. falciparum* transmitter mainly during the rainy season, *An. funestus* was the main vector species during the dry season. It is interesting to note that a previous study focusing on climatic models for suitable malaria transmission in Africa, based on monthly rainfall and temperature data, concluded that an average of 80 mm rainfall per month, for at least 3-5 months, is a minimum to ascertain stable malaria transmission (Craig et al., 1999). Usually, a rapid rise in the

An. gambiae population at the beginning of the short rainy season was followed by an increase in the EIR (Bonnet *et al.*, 2002).
With regard to *An. funestus*, the highest EIRs were usually observed during the dry season. Indeed, *An. funestus* is often abundant and has high EIR during dry season compared to the rainy season (Fontenille et al., 1997; Manga et al., 1997). *An. funestus* was identified as the main malaria vector in the Guinean climatic region, in East Africa and Madagascar (Robert et al., 1985; Severini et al., 1990). As shown in our study, despite the presence of irrigated rice field, there is a great variability in the annual EIR values and seasonality would seem to play a key role (Mabaso et al., 2007).
Finally, an important finding of our study is that in Zatta, where irrigated rice farming was interrupted in 2003/2004, *Plasmodium* prevalence rates and the number of presumptive malaria cases decreased. This observation is corroborated by a significant decrease in the EIR from 2002 to 2003 (Koudou et al., 2005) and a significant increase from 2003 to 2005 (Koudou et al., 2007). This study demonstrated also that irrigated rice cultivation is associated with elevated malaria prevalence rates, as well as high numbers of presumptive malaria cases, as seen in Burundi (Coosemans, 1985), Kenya (Githeko et al., 1993) and Madagascar (Marrama et al., 1995). However, research carried out in Tanzania showed that irrigated rice farming was not associated with a higher risk of malaria. One important reason for this observation is that farmers engaged in irrigated rice have the opportunity to gain some extra money, part of which is spent for protective measures against malaria. A reduced risk of malaria despite enhanced rice production has been termed 'paddies paradox' (Ijumba & Lindsay, 2001).

5. Conclusion

In conclusion, analyses of our entomological data revealed that malaria transmission in two different agro-ecological settings of central Côte d'Ivoire is very high, but there are clear seasonal patterns. Whilst the interruption of irrigated rice farming in one of the two study villages resulted in a highly significant reduction in the EIR, seasonal patterns of transmission remained. Hence, even in intensive agriculture areas, the effect of season on malaria transmission must be taken into consideration for the design of integrated interventions and their monitoring.
Additionally, in Zatta, from 2002 to 2003, the highly significant reduction in the annual EIR was paralleled by a significant reduction in the *Plasmodium* prevalence rate, and the proportions of presumptive and clinically-confirmed malaria cases. Once irrigated rice farming was resumed, there was an increase in entomological and parasitological parameters of malaria. In Tiémélékro, despite the significant increase in the EIR from the year 2002 to 2005 (Koudou et al., 2005, 2007), malaria prevalence rates, and the presumptive and clinical malaria cases decreased. Hence, the reduction of malaria transmission in endemic areas does not necessary reduce the incidence of clinical malaria episodes (Charlwood et al., 1998), highlighting the complex relationship between these parameters.

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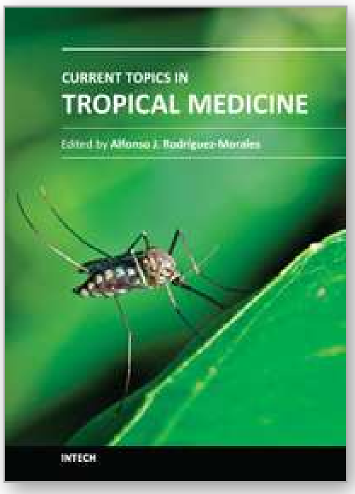

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