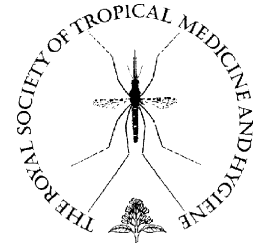




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# Malaria transmission and rice cultivation in Lagdo, northern Cameroon

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**Summary** Cross-sectional entomological surveys were carried out during the 2006 dry and rainy seasons in Lagdo, Cameroon to measure the impact of rice cultivation on malaria transmission and to monitor vector susceptibility to insecticides. Adult anopheline mosquitoes were captured on human volunteers and by pyrethrum spray collections. A total of 4740 mosquitoes was collected during the study. *Anopheles arabiensis* was the major species and the main malaria vector in all study sites, followed by *A. funestus*. Malaria transmission was high in the non-irrigated zone of Mayo Mbocki, whereas in the irrigated area of Gounougou it was below detection level during the dry season and high during the rainy season. Insecticide susceptibility tests performed on *A. gambiae* s.l. populations detected resistance to lambda-cyhalothrin and to a lower extent to deltamethrin. All survivors were *A. arabiensis*. None of the surviving mosquitoes carried the *kdr* mutation, suggesting an alternative resistance mechanism.

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## 1. Introduction

Malaria accounts for much of the disease burden in Cameroon, claiming about 30–35% of the total deaths each

year and 40–45% of morbidity cases (Same Ekobo, 2000). Although over 90% of the population is at risk of malaria, malaria transmission intensity is not uniform across the country. The disease is perennial in the southern part of the country, situated in the equatorial forest domain, while in the northern part of the country, situated in dry savannah areas, it is seasonal and transmitted by vectors such as *Anopheles arabiensis* and *A. funestus* (Antonio-Nkondjio

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et al., 2006; Fondjo, 1996; Robert et al., 1992). Most of the information on malaria transmission in Cameroon is the result of studies in the southern part of the country, and few studies have been carried out in northern Cameroon (Antonio-Nkondjio et al., 2006; Cavalie and Mouchet, 1961; Fondjo, 1996; Robert et al., 1992; Same Ekobo, 1997; Wondji et al., 2005a, 2005b) partly due to poor road infrastructure linking the south to the north.

Insecticide resistance is more widespread throughout the northern part of the country, where it has been associated with the use of pesticides in cotton-growing areas (Chouai-bou et al., unpublished data) (Etang et al., 2003). There is a growing concern that continued or increased use of insecticides associated with the high exophilic and zoophilic nature of the major malaria vector of the area, *A. arabiensis*, may result in increased resistance that could jeopardize control efforts.

In this part of the country, the creation of dams and the extension of irrigated land surface have deeply modified the epidemiology of vectorborne diseases such as malaria (Same Ekobo, 1997; Same Ekobo et al., 2001). In the subdivision of Lagdo, north Cameroon, over 800 ha are used for irrigated rice cultivation (Robert et al., 1992). During the last two decades, following the construction of the Lagdo Dam and the extension of irrigated land surface, Lagdo registered intense population migration. This migration was accompanied by an increase in human malaria cases, with malaria transmission rates reaching 49 infected bites per human per month in irrigated areas (Robert et al., 1992; Slootweg and Van Schooten, 1990). In the frame of a pilot malaria control project conducted by the non-governmental organization Care International and Sanofi-Aventis, whose goal was to train and educate communities about malaria and about means of fighting the disease, we conducted cross-sectional studies during the 2006 dry and rainy seasons to measure the impact of rice cultivation on malaria transmission. This study also presents a report on the monitoring of the insecticide susceptibility status of anophelines.

## 2. Materials and methods

### 2.1. Study sites

Lagdo (9°05'N, 13°40'E) is located in the tropical basin of River Bénoué. The vegetation of this area is made of dry savannah and belongs to the Sudanese–Sahelian domain. The climate exhibits two seasons of 6 months each: the rainy season from June to November and the dry season from December to May. Mean annual rainfall ranges from 900 to 1000 mm. Lagdo is bordered eastward by a hydroelectric dam constructed on River Bénoué, the water of which is also used for the practice of irrigated rice cultivation on over 800 ha.

Mosquito collections were carried out in three villages of Lagdo subdivision: Gounougou, Djipporde and Mayo Mbocki (Figure 1). Gounougou is situated in an area of irrigated rice fields; beside the rice, large areas are used for cotton, maize and groundnut farms. Cattle rearing and fishing are also practised. The village Djipporde is situated in the urban area of Lagdo, along the banks of the River Bénoué

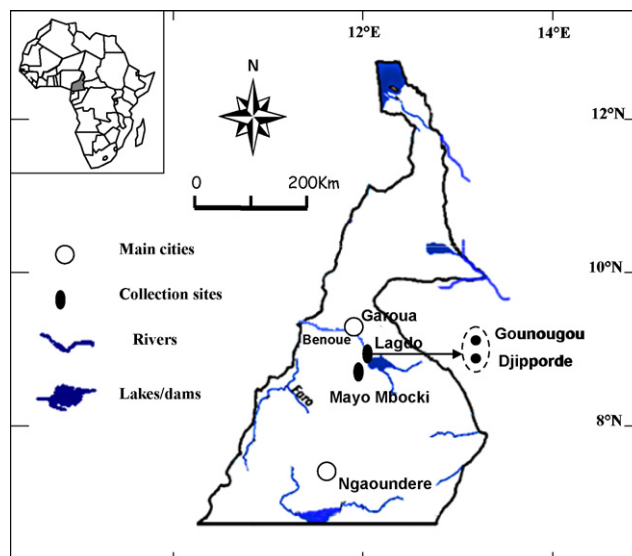


Figure 1 Map of northern Cameroon, showing the geographic location of collection sites.

dam. Gounougou and Djipporde are about 4 km apart. Mayo Mbocki is situated 50 km away, south of the two villages along the highway connecting Ngaoundéré to Garoua. Maize, millet and groundnuts are the main crops cultivated by the inhabitants of Mayo Mbocki; cattle rearing is also practised.

The three villages of Gounougou, Djipporde and Mayo Mbocki were chosen among the other communities because parasitological data were available from local health care centres.

### 2.2. Adult mosquito collections

Adult mosquitoes were collected during the months of May and October 2006. Two sampling methods were used. (1) Capture of blood-seeking females after landing on the legs of a human volunteer from 19:00 h to 06:00 h. Collections were done in eight randomly selected sites in each village (four outdoors and four indoors), twice each month in Djipporde, twice during the month of May and once in October in Gounougou and once in both months in Mayo Mbocki. All volunteers gave free and informed consent for capturing mosquitoes and were given appropriate malaria prophylaxis. (2) Indoor pyrethrum spray catches of resting females were carried out in the morning and in the afternoon in human dwellings. A total of 20–30 houses was sprayed per village during the study period.

### 2.3. Field processing of mosquitoes

Mosquitoes were identified to the genus or species level. Anophelines were identified to species using morphological characteristics according to the identification keys of Gillies and Coetsee (1987) and Gillies and De Meillon (1968). Each anopheline specimen was stored individually in a numbered tube containing desiccant, archived and kept at  $-20^{\circ}\text{C}$  until processed in the laboratory in Yaoundé.

## 2.4. Laboratory processing of anophelines

Members of the *A. gambiae* complex were identified using molecular diagnostic tools (Favia et al., 2001; Scott et al., 1993). DNA extracted from a leg or a wing was used for this analysis. Blood-meal sources of all blood-fed females captured by pyrethrum spray were identified by an ELISA (Beier et al., 1988). The technique identified human, bovine, ovine (sheep and goat), equine (horse and donkey), pig or chicken hosts. The head and thorax of female anophelines were tested for the presence of the circumsporozoite proteins (CSP) of *Plasmodium falciparum* (Welch) by ELISA, as described in Fontenille et al. (2001). The CSP rate was calculated as a ratio of mosquitoes infected to mosquitoes tested after ELISA. The entomological inoculation rate (EIR) was calculated by multiplying the human biting rate estimated from landing catches by the CSP rate. The anthropophilic rate was calculated as a ratio of mosquitoes fed on humans to total mosquitoes tested for blood-meal source after ELISA.

## 2.5. Insecticide susceptibility tests

Bioassays were carried out using WHO test kits for adult mosquitoes (WHO, 1998). Impregnated papers were provided by the Laboratoire de Lutte Contre les Insectes Nuisibles LIN-IRD (Montpellier, France), a WHO collaborative centre. The following diagnostic concentrations of insecticides were tested: 4% DDT, 1% permethrin, 0.05% deltamethrin, 0.05% lambda-cyhalothrin, 1% propoxur and 1% fenitrothion.

Susceptibility tests were carried out with 2- to 4-day-old unfed *A. gambiae* s.l. females obtained from larvae collected in rainwater accumulations, marshes around taps or broken water pipes, non-harvested rice fields and stagnant water within the irrigation system. Batches of 20 to 30 females were exposed to impregnated papers for 1 h. The number of mosquitoes knocked down was recorded every 5 min during exposure. The knockdown times for 50% (KdT50) and 95% (KdT95) of tested mosquitoes were calculated using WINDL V.2.0 software according to Giner et

**Table 1** Number of mosquitoes collected and human biting rate in the three study sites in the dry and rainy seasons, Lagdo, northern Cameroon, 2006

	Djipporde			Gounougou			Mayo Mbocki		
	Resting	Feeding	HBR <sup>a</sup>	Resting	Feeding	HBR <sup>a</sup>	Resting	Feeding	HBR <sup>a</sup>
<b>Dry season</b>									
<i>A. arabiensis</i>	0	0	0	137	93	5.81	111	64	8
<i>A. gambiae</i>	0	0	0	0	0	0	3	3	0.38
<i>A. funestus</i>	0	0	0	7	0	0	8	9	1.12
<i>A. rufipes</i>	1	0	0	0	1	0.06	5	0	0
<i>A. pharoensis</i>	0	0	0	0	2	0.12	0	0	0
<i>A. nili</i>	0	0	0	0	0	0	0	1	0.12
Total <i>Anopheles</i>	1	0	0	144	96	6	127	77	9.63
<i>Culex</i> spp.	5	11	0.69	10	4	0.25	30	2	0.25
<i>Mansonia</i> spp.	0	0	0	0	5	0.31	0	7	0.87
<i>Aedes</i> spp.	0	0	0	0	2	0.12	0	22	2.75
<b>Rainy season</b>									
<i>A. arabiensis</i>	23	46	2.87	30	133	16.63	52	160	20
<i>A. gambiae</i>	0	0	0	2	1	0.13	5	29	3.63
<i>A. funestus</i>	0	3	0.19	109	122	15.25	47	61	7.62
<i>A. rufipes</i>	0	0	0	0	31	3.87	7	46	5.75
<i>A. ziemanni</i>	0	1	0.06	0	33	4.12	0	18	2.25
<i>A. coustani</i>	0	0	0	0	0	0	0	10	1.25
<i>A. squamosus</i>	0	0	0	0	1	0.12	0	22	2.75
<i>A. pharoensis</i>	0	11	0.69	0	26	3.25	0	5	0.62
<i>A. nili</i>	0	0	0	0	0	0	0	8	1
<i>A. moucheti</i>	0	0	0	0	0	0	0	1	0.12
Total <i>Anopheles</i>	23	61	3.81	141	347	43.37	111	360	45
<i>Culex</i> spp.	355	1864	116.5	37	112	14	32	120	15
<i>Mansonia</i> spp.	0	14	0.87	0	517	64.62	0	50	6.25
<i>Aedes</i> spp.	0	8	0.5	0	19	2.37	0	28	3.5
Total	384	1958	61.19	332	1102	45.92	300	666	41.62
Overall per village	2342			1434			966		

<sup>a</sup> HBR: human biting rate = number of bites per person per night.

al. (1999). The mortality rate was recorded after 24 h. Tests with untreated papers were systematically run as controls. Mortality rate in tested samples was corrected using Abbott's formula (Abbott, 1925) when the mortality rate of control was between 5 and 20%. WHO (1998) criteria were used to evaluate the resistance and susceptibility status of the tested mosquito populations. The resistance status was indicated by a mortality rate below 80%, whereas mortality rates greater than 98% were indicative of susceptibility and mortality rates between 80–98% suggested increased tolerance, although resistance should be confirmed. The presence of the *kdr* gene was screened using the PCR-based diagnostic test of Martinez-Torres et al. (1998).

### 3. Results

#### 3.1. Mosquito composition and abundance

Mosquitoes were collected in May 2006 during the dry season and in October 2006 during the rainy season. A total of 4742 mosquitoes was collected during this study: 3726 by 72 human-night collections (1958 by 32 human-nights in Djipporde, 1102 by 24 human-nights in Gounougou and 666 by 16 human-nights in Mayo Mbocki) and 1016 after morning or afternoon spraying of 20 to 30 rooms per village. *Anopheles*

spp., *Culex* spp., *Aedes* spp. and *Mansonia* spp. were collected in all study sites. *Culex* spp. were the most abundant (Table 1).

The anopheline species caught were: *A. gambiae*, *A. arabiensis*, *A. funestus*, *A. moucheti*, *A. nili*, *A. ziemanni*, *A. pharoensis*, *A. squamosus*, *A. coustani* and *A. rufipes*. Most of these species (70% of the anopheline fauna) were collected during the rainy season. *Anopheles arabiensis* was the most abundant species, representing over 50% of the anophelines caught in the three study sites. In Mayo Mbocki, *A. arabiensis* was the most abundant malaria vector in both the dry and rainy season, accounting for more than 50% of the anophelines caught. By contrast, in Gounougou *A. arabiensis* was most abundant in the dry season (95.8%), while *A. funestus* (47.3%) was predominant during the rainy season. Compared to Mayo Mbocki and Gounougou, anopheline catches from Djipporde were poor in both the dry and rainy seasons (Table 1). All the *A. gambiae* s.s. identified by PCR were of the S molecular form.

#### 3.2. Biting behaviour

The average human biting rate (HBR) varied temporally, depending on the season (Table 1). In the urban area of Djipporde, the HBR varied from 0.69 to 116.5 bites/person/night

**Table 2** Blood meal source from anopheline mosquitoes collected in the three study sites in the dry and rainy seasons, Lagdo, northern Cameroon, 2006

	Blood meal source						
	Tested	Human	Cow	Sheep	Horse	Pig	Mixed <sup>a</sup>
<b>Djipporde</b>							
Rainy season							
<i>A. arabiensis</i>	16	4	3	1	0	3	5
<b>Gounougou</b>							
Dry season							
<i>A. arabiensis</i>	100	56	11	1	1	0	29
<i>A. funestus</i>	5	0	3	2	0	0	0
Total	105	56	14	3	1	0	29
Rainy season							
<i>A. arabiensis</i>	20	15	4	0	0	0	1
<i>A. gambiae</i>	2	2	0	0	0	0	0
<i>A. funestus</i>	84	81	0	0	0	1	2
Total	106	98	4	0	0	1	3
<b>Mayo Mbocki</b>							
Dry season							
<i>A. arabiensis</i>	64	59	5	0	0	0	0
<i>A. gambiae</i>	2	2	0	0	0	0	0
Total	66	61	5	0	0	0	0
Rainy season							
<i>A. arabiensis</i>	37	25	5	0	1	0	6
<i>A. gambiae</i>	5	3	1	0	0	0	1
<i>A. funestus</i>	24	14	3	0	0	1	6
<i>A. rufipes</i>	7	4	2	0	0	0	1
Total	73	46	11	0	1	1	14
<b>Overall total</b>	<b>366</b>	<b>265</b>	<b>37</b>	<b>4</b>	<b>2</b>	<b>5</b>	<b>51</b>

<sup>a</sup> Blood meal taken on at least two different hosts.

**Table 3** Infective rate of *Plasmodium falciparum* calculated by circumsporozoite protein (CSP) ELISA from head and thoraxes of mosquitoes captured in Lagdo, northern Cameroon, 2006

	Djipporde		Gounougou		Mayo Mbocki	
	<i>n</i> tested ( <i>n</i> infected)	CSP rate (95% CI)	<i>n</i> tested ( <i>n</i> infected)	CSP rate (95% CI)	<i>n</i> tested ( <i>n</i> infected)	CSP rate (95% CI)
Dry season						
<i>A. arabiensis</i>	0 (0)	ND	230 (0)	0	175 (2)	1.14 (0.31–4.08)
<i>A. funestus</i>	0 (0)	ND	7 (0)	0	17 (3)	17.65 (3.8–43.4)
Rainy season						
<i>A. arabiensis</i>	68 (2)	2.94 (0.36–10.2)	163 (3)	1.84 (0.62–5.3)	212 (10)	4.72 (2.6–8.5)
<i>A. gambiae</i>	0	ND	3 (0)	0	34 (3)	8.82 (1.86–23.68)
<i>A. funestus</i>	3 (0)	0	231 (2)	0.87 (0–6.2)	108 (2)	1.85 (0.05–9.89)
<i>A. pharoensis</i>	11 (0)	0	26 (2)	7.69 (0.95–25.1)	5 (0)	0
<i>A. coustani</i>	0 (0)	ND	0 (0)	ND	10 (1)	10 (0.25–44.5)

ND: not determined.

in the dry and rainy season, respectively, and was mainly due to *Culex* spp. Despite an increase in mosquito burden during the rainy season, the HBR of the anopheline fauna was still low (3.81 bites/person/night). In Gounougou, situated in the irrigated rice field area, the anopheline HBR varied from 6 to 43.37 bites/person/night. This variation was mainly due to an increase in *A. arabiensis* and *A. funestus* HBRs of 5.81 to 16.63 and 0 to 15.25 bites/person/night, respectively. In Mayo Mbocki, the anopheline HBR varied from 9.63 to 45 bites/person/night and for the major vector, *A. arabiensis*, the HBR increased from 8 to 20 bites/person/night.

In Gounougou and Mayo Mbocki, 37.5% (36/96) and 51.9% (40/77) of anophelines were collected indoors during the dry season. The rates of endophily were 49.3% (171/347), 53.3% (192/360) and 59.02% (36/61) during the rainy season in Gounougou, Mayo Mbocki and Djipporde, respectively. These rates did not vary significantly between seasons in Gounougou and Mayo Mbocki ( $P > 0.05$ ).

### 3.3. Feeding behaviour

In total, 366 blood meals from resting anopheline females collected in the three collection sites were tested by ELISA to determine their origin. Of those, 265 were taken on humans (Table 2) and 51 were found with mixed blood meals: bovine–ovine ( $n = 20$ ), human–bovine ( $n = 16$ ), human–ovine ( $n = 13$ ) and human–pig ( $n = 2$ ). In Gounougou, the anthropophilic rate without mixed blood meals varied from 53.3% during the dry season to 92.4% in the rainy season and was significantly different ( $\chi^2 = 40.9$ ; d.f. = 1;  $P < 0.001$ ). This increase in the anthropophilic rate was due to the predominance of *A. funestus*, which was found to be more anthropophilic than *A. arabiensis* ( $\chi^2 = 7.65$ ; d.f. = 1;  $P = 0.006$ ). In Mayo Mbocki, where *A. arabiensis* was the most abundant species during both seasons, the anthropophilic rate varied from 92.4% in the dry season to 63.01% in the rainy season and was significantly different ( $\chi^2 = 16.9$ ; d.f. = 1;  $P < 0.001$ ). No significant difference was found between *A. gambiae* and *A. arabiensis* anthropophilic rates ( $\chi^2 = 0.01$ ; d.f. = 1;  $P = 0.72$ ).

### 3.4. Circumsporozoite protein rate

A total of 1485 female anopheline specimens belonging to 10 species collected in Djipporde ( $n = 82$ ), Gounougou ( $n = 728$ ) and Mayo Mbocki ( $n = 675$ ) was processed by ELISA. Twenty-six anophelines belonging to five species were tested positive for *P. falciparum* infections: *A. gambiae*, *A. arabiensis*, *A. funestus*, *A. pharoensis* and *A. coustani* (Table 3).

The CSP rate was significantly different between infected anopheline species found in these collection sites ( $\chi^2 = 10.4$ ; d.f. = 4;  $P = 0.03$ ). It was, however, not significantly different between *A. arabiensis* and *A. gambiae* ( $\chi^2 = 0.98$ ; d.f. = 1;  $P = 0.46$ ). No significant difference was found when the two collection methods were compared ( $P = 0.79$ ).

### 3.5. Entomological inoculation rate

In Mayo Mbocki, malaria transmission was estimated at 3.9 infected bites per human during the month of May 2006 (dry season). No infected mosquito was found during the May surveys in both Djipporde and Gounougou. In the rainy season, the entomological inoculation rate was 2.7, 19.4 and 36.5 infective bites per human during the month of October in Djipporde, Gounougou and Mayo Mbocki, respectively.

### 3.6. Susceptibility to insecticides

Two susceptibility tests were carried out with DDT, lambda-cyhalothrin and permethrin (one during each season). Susceptibility to deltamethrin, propoxur and fenitrothion was assessed only during the rainy season. For each test, 90 to 120 mosquitoes were used.

Complete mortality was recorded with fenitrothion and propoxur. The lowest mortality rates (78 and 80%) were recorded with lambda-cyhalothrin, which is the insecticide used for bed net impregnation in the area. Knockdown times recorded during this study were always higher than their corresponding values with the *A. gambiae* Kisumu reference strain, except for permethrin (Table 4). Of the 70 mosquitoes that survived exposure, none were found with the *kdr* Leu–Phe mutation. All were *A. arabiensis*.

**Table 4** Knockdown times and mortality rates of *Anopheles gambiae* s.l. field populations of Gounougou to 4% DDT, 0.05% lambda-delta-cyhalothrin, 0.05% deltamethrin, 1% fenitrothion and 1% propoxur

Insecticide	Season	n	Knockdown times			Mortality <sup>d</sup> %
			KdT50 <sup>b</sup> (95% CI) min	KdT95 <sup>c</sup> (95% CI) min	KdT50R <sup>a</sup>	
4% DDT	Dry season	92	38.93 (37.7–40.1)	61.54 (58.3–65.7)	2.07	95
	Rainy season	97	37.25 (35.05–39.3)	58.7 (53.9–66.3)	1.98	95
1% permethrin	Dry season	111	12.27 (10.3–13.9)	25.34 (22.5–29.9)	1.33	98
	Rainy season	130	8.32 (7.1–9.33)	14.72 (12.9–17.9)	0.9	95
0.05% lambda-delta-cyhalothrin	Dry season	121	25.96 (24.3–27.5)	41.64 (38.5–46.3)	2.76	78 <sup>e</sup>
	Rainy season	97	18.8 (17.2–20.4)	41.7 (37.5–47.7)	2	80 <sup>e</sup>
0.05% deltamethrin	Rainy season	107	11.1 (10.4–11.8)	21.34 (19.9–23.2)	1.18	89
1% fenitrothion	Rainy season	115	44.5 (42.9–46.1)	59.02 (55.9–63.8)	ND	100
1% propoxur	Rainy season	100	ND	ND	ND	100

ND: not determined.

<sup>a</sup> KdT50R, KdT50 of tested population divided by KdT50 of Kisumu strain.<sup>b</sup> KdT50, Kd time in minutes for 50% mosquitoes.<sup>c</sup> KdT95, Kd time in minutes for 95% mosquitoes.<sup>d</sup> Mortality rate 24 h post-exposure.<sup>e</sup> Resistant populations.

#### 4. Discussion

Malaria transmission intensity was lower in the rice cultivation area of Gounougou, despite high anopheline burden. *Anopheles arabiensis* and *A. funestus* were the main malaria vectors. This observation is consistent with the distribution of these species in Cameroon and in dry savannah areas of West Africa (Antonio-Nkondjio et al., 2006; Dossou-Yovo et al., 1995; Koudou et al., 2005; Robert et al., 1985). However, the number of *A. arabiensis* caught outnumbered *A. funestus* by far. *Anopheles arabiensis* was present in both seasons, benefiting from the availability of stagnant water sources such as marshes around the irrigated area, water taps or broken water pipes, stream-bed pools during the dry season and numerous puddles during the rainy season. Biting *A. funestus* densities were high during the rainy season, particularly in the irrigated area of Gounougou. The presence of this species was closely related to rice cultivation, as most of its larvae were collected in rice fields. Its typical breeding site consists of more-or-less permanent water with emergent vegetation (Gillies and De Meillon, 1968), and its scarcity throughout the dry season was probably in relation to the stoppage of rice cultivation due to interruption in the irrigation system. Overall, higher anopheline densities were recorded in Gounougou and Mayo Mbocki compared with the much more urban village of Djipporde. Although people mainly slept outdoors during the dry season, anophelines were equally endophagous independently of the season. However, mosquito feeding preference varied from one season to the other, depending on the succession of species with different host preference (Gounougou) or the availability of cattle as alternative host (Mayo Mbocki). Indeed, the presence of cattle can have a major influence on host choice for mosquitoes (Dolo et al., 2004; Garrett-Jones et al., 1980). In Senegal, seasonal migration of cattle was reported to influence vector anthropophily (Vercruysse, 1985).

Five species were found to contain the *P. falciparum* CSP: *A. gambiae*, *A. arabiensis*, *A. funestus*, *A. pharoensis* and *A. coustani*. Infections in *A. pharoensis* and *A. coustani* have been reported several times in Cameroon (Antonio-Nkondjio et al., 2006; Robert et al., 1992). Despite their contribution to malaria transmission in several sites across the country, it is improbable that these species could maintain parasite transmission on their own in the absence of major vectors. Malaria transmission was lower in the irrigated rice cultivation area of Gounougou compared with Mayo Mbocki, situated in a non-irrigated zone. This observation was similar to those of many studies conducted in irrigated rice cultivation areas in Africa and suggests no, or only a small, impact of irrigated rice cultivation on malaria transmission intensity and malaria prevalence (Henry et al., 2003; Ijumba and Lindsay, 2001; Keiser et al., 2004). Yet even though no infected mosquito was found in Gounougou during the dry season, malaria transmission might not have been totally absent during this period, owing to the presence of malaria vectors and new cases of malaria recorded in the health care centre (data not shown). Altogether, this showed that proximity of irrigated areas to households is a great risk factor (Afrane et al., 2004). In rice cultivation areas with low malaria transmission it is assumed that good access to effective antimalarial drugs and coverage with personal protective measures, such as sleeping under insecticide-treated nets (ITNs), sleeping in houses with ceilings and use of insecticide, aerosols and burning mosquito repellents, reduces malaria prevalence (Ijumba and Lindsay, 2001; Lindsay et al., 1990). Despite the fact that a programme of ITNs was put in place 2 years ago by the Ministry of Health, bed nets were used by less than 20% of the 120 households visited in the frame of this study. Moreover, bed nets were not used during warm weather when people slept outdoors. Although it was reported that bed net coverage could have a great effect on the reduction of malaria transmission at the community level (Maxwell et al., 2002), such

a low proportion of net utilization is not a good explanation of the low transmission rate in Gounougou. It is likely that the association of high mosquito density to a relatively young mosquito population described earlier by Robert et al. (1992) could be the main factor responsible for that. Similar situations were highlighted by various studies across Africa (Coosemans, 1989; Dolo et al., 2004; Robert et al., 1991).

Increased resistance levels of *A. gambiae* s.l. populations to lambda-delta-cyhalothrin, and to a lower extent to deltamethrin, were detected during this study in agreement with previous findings from the area by the national malaria control programme (Ndjemai, 2004). This situation was attributed to the extensive use of insecticides in cotton-growing areas. In Cameroon, insecticides used during cotton treatment include endosulfan (cyclodiene), profenofos (organophosphate) and cypermethrin (pyrethroid), while only small quantities of pyrethroids are used on rice farms. However, the fact that rice farms are located next to cotton fields could have exposed them to residual insecticide or pollutants driven by rain runoff. Our findings of relatively high susceptibility levels of *A. gambiae* s.l. populations to DDT and permethrin were not in accordance with the increased levels of resistance to these compounds in neighbouring villages (Etang et al., 2003) (Chouaibou et al., unpublished data) and reflects the heterogeneous pattern of resistance within the area. However, the expansion of resistant *A. gambiae* s.l. populations is of major concern, as this could hamper the efficacy of ITN programmes implemented across the country. Analysis of knockdown times and the absence of *kdr* mutation definitively ruled out the presence of the *kdr* resistance mechanism. Moreover, no resistance to propoxur (Carbamate) and fenitrothion (organophosphate) was found, suggesting the absence of the acetylcholinesterase resistance mechanism (Hemingway et al., 2004). However, the presence of this gene in West Africa (Corbel et al., 2007; N'Guessan et al., 2003) underlines the need to carefully monitor its extension to Cameroon. With regard to the high gene flow between *A. arabiensis* populations situated at this latitude (Wondji et al., 2005b), it is possible that other resistance mechanisms, such as the metabolic detoxification reported in Pitoa (Etang, 2003), situated only 50 km away from Lagdo, could be responsible for the low susceptibility to pyrethroids of these mosquitoes. However, biochemical assays still need to be done to confirm this assumption.

This study confirms the high malaria endemicity in the area of Lagdo and the rise of resistant mosquito populations. With the expansion of agricultural practices in the area and the extension of irrigated land surface, the amount of pesticide being applied in agriculture is expected to increase significantly in the following years. This may favour the emergence and spread of resistance within malaria vectors and may constitute an obstacle for future success of malaria control programmes based on ITNs or indoor residual spraying.

**Authors' contributions:** CAN and FS conceived the study; DF and EF designed the study protocol; CAN, JA, PAA and CN carried out field and laboratory studies; CAN analysed and interpreted the data; CAN drafted the manuscript; CAN, JA, PAA, CN, DF, EF and FS revised the manuscript. All authors

read and approved the final manuscript. FS and DF are guarantors of the paper.

## Uncited reference

Abbott (1987).

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