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# Bionomics of malaria vectors and relationship with malaria transmission and epidemiology in three physiographic zones in the Senegal River Basin

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**Abstract**

Following the implementation of two dams in the Senegal River, entomological and parasitological studies were conducted in three different ecological zones in the Senegal River Basin (the low valley of Senegal River, the Guiers Lake area and the low valley of Ferlo) every 3 month in June 2004, September 2004, December 2004 and March 2005. The objective of this work was to study the influence of environmental heterogeneities on vector bionomics and malaria epidemiology. Mosquitoes were collected when landing on human volunteers and by pyrethrum spray catches. In the parasitological survey, blood samples were taken from a cohort of schoolchildren under 9 years during each entomology survey. Seven anopheline species were collected: *Anopheles arabiensis*, *Anopheles gambiae* M form, *Anopheles funestus*, *Anopheles pharoensis*, *Anopheles coustani*, *Anopheles wellcomei* and *Anopheles rufipes*. *A. arabiensis*, *A. funestus* and *A. pharoensis* were predominant in the low valley of the Senegal River, *A. funestus* in the Guiers Lake area and *A. arabiensis* in the low valley of Ferlo. Mosquito populations' dynamics varied temporally depending on the rainy season for each zone. The anthropophilic rates varied between 6 and 76% for *A. gambiae* s.l. and 23 and 80% for *A. funestus*. Only 4/396 *A. pharoensis* and 1/3076 *A. funestus* tested carried *Plasmodium falciparum* CS antigen. These results suggest the implication of *A. pharoensis* in malaria transmission. The related entomological inoculation rates were estimated to 10.44 in Mbilor and 3 infected bites in Gankette Balla and were due, respectively, to *A. pharoensis* and *A. funestus*. Overall, 1636 thick blood smears were tested from blood samples taken from schoolchildren with, respectively, a parasite and gametocyte average prevalence of 9 and 0.9%. The parasite prevalence was uniformly low in Mbilor and Gankette Balla whereas; it increased in September (16%) and then remained stable in December and March (22%) in Mboula where malaria transmission was not perceptible. However, significant differences were observed over time for parasite prevalence in Mbilor and Mboula villages whereas; it was only in Gankette Balla village where gametocyte prevalence was significantly different over time. Our study demonstrates the influence of ecological changes resulted from dams implementation in the Senegal River on the composition of vectorial system, malaria transmission and epidemiology. Such changes should be thoroughly surveyed in order to prevent any possible malaria outbreak in the Senegal River Basin.


*Keywords:* *Anopheles arabiensis*; *Anopheles gambiae*; *Anopheles funestus*; *Anopheles pharoensis*; Senegal River Basin; Malaria transmission; Environmental heterogeneity

## 1. Introduction

Because of several cycles of drought events that have occurred during the 1970s, many African countries were themselves constrained to develop sustainable hydro-agricultural politics in order to reach food self-sufficiency. This strategy

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is related on a policy of development of irrigated growing areas based on water control. As a consequence, such projects for water-resources development have direct effects on the transmission and epidemiology of water-related diseases. In addition, these transformations have major consequences because changes in the patterns of water-borne diseases can result to a rapid evolution in diseases endemicity (Molyneux, 1997) by a dramatic reappearance or increase of these water-related diseases (Mouchet and Brengues, 1990). For instance, the implementation of dams can worsen the incidence of schistosomiasis and in some extent malaria (Ghebreyesus et al., 1999; Tetteh et al., 2004; Singh et al., 1999).

In the Senegal River Basin, in order to develop and better use the natural resources, an integrated development program has been designed, including the construction of two dams: (i) the Diama dam near the mouth of the River to stop the inflow of saltwater, to allow the availability over year of freshwater and the development of irrigated agriculture along the river valley and (ii) the Manantali dam in the high basin in Mali, as a reservoir, 2000 km upstream for stabilising the river flow, the production of energy and an improvement of inland navigation. These implementations were followed by an increase of *Schistosoma mansoni* and *Schistosoma haematobium* infections cases near the town of Richard Toll in the low valley of the River and neighbouring localities (Talla et al., 1990). The irrigation schemes have increased significantly the number of mosquitoes breeding sites and also provided an extension of duration and period of presence that can then cover the dry season usually without mosquitoes in these sahelian zones The first evidence of the impact of these ecological changes concerning vector-borne arboviral diseases, is the occurrence of Rift valley fever epidemic in 1987 (Jouan et al., 1990) and the establishment of this disease in the area regarding the subsequent outbreaks recorded (Thonnon et al., 1999; Diallo et al., 2000). For malaria, early studies following the construction of the dams showed an increase of the incidence (Handschumacher et al., 1992) in parallel to an increase of anophelines vectors densities but with any significant effect on the level of malaria transmission (Faye et al., 1993).

The rare entomological studies conducted before the implementation of the dams showed that, *Anopheles gambiae* s.s. and *Anopheles arabiensis* were responsible of the whole malaria transmission in the Senegal River Basin with a predominance of *A. gambiae* (Vercruysse, 1985; Petrarca et al., 1987). Because of the large developments of rice-irrigation schemes following the implementation of the Diama dam, *Anopheles pharoensis* was found to be the prevalent man-biting anopheline mosquito in the Delta of the Senegal River where it was subsequently incriminated in *Plasmodium falciparum* transmission (Carrara et al., 1990).

Besides the local ecological modifications resulting from the extension of agricultural surfaces in the Delta of the Senegal River, the Diama dam has also permitted the maintenance of less salted water bodies upstream so that many aquatic freshwater plants developed there (Thiam, 1998). A major result of this environmental change is the comeback of *Anopheles funestus* populations in the low valley of the Senegal River (Konate et al., 2001). Within the framework of a large program, a multidisciplinary study was carried out to study the influence of the ecological changes following the implementation of two dams in the Senegal River on malaria transmission and its factors of variations. Within the entomological part, a preliminary study was carried out in the low valley of the Senegal River, the zone of the Guiers Lake and the low valley of Ferlo in order to study the northern distribution limits of the newly established *A. funestus* populations and their possible involvement in malaria transmission. This survey has shown, in addition to the implantation of the new populations of *A. funestus* in the low valley of the Senegal River, a heterogeneous distribution of the anopheline populations with *A. pharoensis* being the prevalent species in the upper and middle delta of the River and at low frequencies along the eastern shore of the Guiers Lake. *A. gambiae* s.l. represented mainly by *A. arabiensis* was present in the three zones and the only one species observed in the low valley of Ferlo (Konate et al., unpublished data). The main area recolonized by *A. funestus* was the Guiers Lake and the nearby localities of the low valley of Ferlo. The present work was undertaken in this context in order to study the bionomics of the anopheline species present and their exact role in malaria transmission in three different evolutionary ecosystems; the low valley of Senegal River, the Guiers Lake area and the low valley of Ferlo.

## 2. Materials and methods

### 2.1. Study sites

Three villages belonging to three different ecological settings were prospected: Mbilor in the low valley of the Senegal River, Gankette Balla situated in the shores of the Guiers Lake and Mboula in the Ferlo area.

Mbilor (16°29′N, 15°33′W) is about 1100 inhabitants and is situated in the low valley of the Senegal River, 20 km from Richard-Toll town (Fig. 1). The dominant ethnic groups are Peulh and Wolofs, who are mainly farmers. The dwellings are of varied types with traditional style with mud walls and thatched roofs and modern ones with cement walls and corrugated iron.

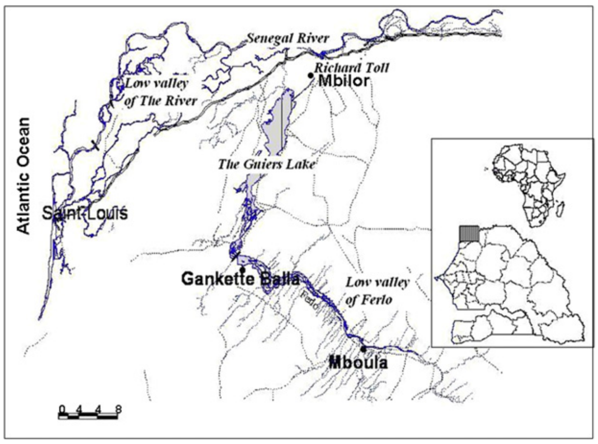


Fig. 1. Localisation of the study sites.

People spent generally the night outside the rooms under generally non-impregnated bed nets. The rainy season lasts from July to September. In 2004, an average of 254 mm of rainfall was recorded. A retention basin made by the Senegalese Sugarcane Company and derived from the River represents the unique source of permanent water that is used for agricultural purposes. This permits rice cultivations practices with two different cycles per year. Domestic animals rearing are fairly developed and livestock consists of sheep, goats and cows. A parasitologic study carried out between July 1995 and November 1996 has shown that malaria is hypoendemic in this village (Mbaye, 1997).

Gankette Balla (15°58′N, 15°55′W) is a village situated on the shores of the Guiers Lake, in its southern part. A population of 1400 inhabitants from the Wolof ethnic group lives in the village. Most of the houses are made of cement walls and corrugated iron. The rainy season extends from July to September with an average rainfall amount of 223 mm recorded in 2004. Millets, beans and watermelons represent the main cultures. Breeding is little developed and cattle (cows, goats and sheep) are taken during daytime in pasture and brought back in the village during the night where they are parked in enclosures inside houses. Entomological studies carried out by Sy (1998) in the northern part of the Guiers Lake showed that malaria transmission level is low and is maintained mainly by *A. gambiae* s.l.

Mboula (15°40′N, 15°25′W) is a village located in the southern part of the low valley of the Ferlo area, in the sahelian belt. This village of 1200 inhabitants is situated in the fossil valley river bed of the Ferlo area that is subjected to revitalization since 1988. The village is surrounded by clay hollows, which collect water as soon as the rains start. The dominant ethnic group is Wolof, mainly cattle and sheep farmers that live in houses of modern styles made of cement walls and corrugated iron. Houses of traditional styles are rare. A parasitological study carried out by Faye et al. (1998) showed that malaria is mesoendemic in the revitalized zone of the fossil valley of the Ferlo area.

### 2.2. Field sampling and processing of mosquitoes

In each village, entomological surveys were conducted in June 2004, September 2004, December 2004 and March 2005 corresponding to the beginning, the middle and the end of the rainy season and the dry season, using two classical methods: capture of females landing on human volunteers from three households at six collection sites half indoors half outdoors at the frequency of two consecutive nights, and pyrethrum spray collection of resting females in randomly selected dwellings, the morning following the second night collection. After collection, mosquitoes were sorted, counted and identified morphologically to *Anopheles* species (Gillies and De Meillon, 1968). A random sample of females from each species were then dissected to extract ovaries and to determine the mosquito reproductive age using the method of Detinova (1962). The blood meals from freshly fed females collected by pyrethrum spray collections were squashed and dried onto filter paper for host source identification in the laboratory.

All mosquitoes were stored individually in numbered vials with desiccant for laboratory processing.

### 2.3. Parasitology survey

In each village, blood samples were taken from a cohort of schoolchildren under 9 years during each entomology survey. Were included only the children for which an informed consent was obtained from the parents. Thick smears were constituted from a drop of blood from the fingertip, Giemsa-stained and microscopically examined by experienced microscopists at the Association Espoir Pour la Santé and quality control performed at the Department of Parasitology, University Cheikh Anta Diop, Dakar. A total of 200 microscopic fields (about 0.5 μl of blood) were examined on each slide. The ratio of parasites to leukocytes was estimated by counting the parasites from the total number of parasites observed on the 200 fields based on a mean leukocytes number of 8000 per microliter of blood.

The present study followed ethical principles according to the Helsinki Declaration, and were approved by the ethical committees of the Ministry of Health of Senegal (June 2004).

### 2.4. Laboratory processing of mosquitoes

The origin of blood meals from freshly fed indoor resting females collected after pyrethrum spray collections, was identified as human, bovine, ovine and horse using an enzyme-linked immunosorbent assay (ELISA) from the procedure of Beier et al. (1988). The heads and thoraces of all anopheline females were tested by ELISA for circumsporozoite protein (CSP) of *P. falciparum* diagnostics from the procedures of Wirtz et al. (1987). The mosquitoes from the *A. gambiae* complex were identified using the PCR technique described by Scott et al. (1993). If more than 20 anophelines were collected on human, a random sample of 20 specimens was selected for each month. All specimens identified as *A. gambiae* s.s. were subsequently identified to molecular form according to Favia et al. (2001).

### 2.5. Data analysis

The human biting rate (HBR) was defined as the ratio of the total mosquitoes captured for a period to the total person-night used for the same period. The endophagous rate was defined as the proportion of indoors biting among the total females captured from human landing collections. The circumsporozoite protein (CSP) rate was calculated as the proportion of females found to contain the CS protein. The anthropophilic rate was calculated as the proportion of human blood among the total blood meals determined. The entomological inoculation rate (EIR) was calculated as the product of the human biting rate (HBR) and the CSP rate of mosquitoes collected on night catches.

For the parasitological survey in human population, the parasite (whatever the stage) and gametocyte prevalence (percentage of positive sample) were determined.

Table 1
Species and number of anophelines collected in Mbilor, Gankette Balla and Mboula in June 2004, September 2004, December 2004 and March 2005

| Anopheline species | Mbilor (Senegal River) | | | Gankette Balla (Guiers Lake) | | | Mboula (Ferlo Valley) | | |
|---|---|---|---|---|---|---|---|---|---|
| | HLC[a] | | PSC[b] | HLC[a] | | PSC[b] | HLC[a] | | PSC[b] |
| | Indoor | Outdoor | Indoor | Indoor | Outdoor | Indoor | Indoor | Outdoor | Indoor |
| *A. gambiae* s.l. | 299 | 258 | 116 | 6 | 4 | 48 | 21 | 19 | 258 |
| *A. funestus* | 137 | 85 | 66 | 1813 | 1083 | 3415 | 0 | 0 | 6 |
| *A. pharoensis* | 140 | 202 | 5 | 29 | 31 | 1 | 2 | 0 | 0 |
| *A. coustani* | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. wellcomei* | 9 | 10 | 0 | 104 | 61 | 0 | 0 | 0 | 0 |
| *A. rufipes* | 0 | 0 | 35 | 0 | 0 | 8 | 0 | 0 | 4 |
| Total | 587 | 558 | 222 | 1952 | 1179 | 3472 | 23 | 19 | 268 |

[a] Human-landing catches.
[b] Pyrethrum spray collections.

## 3. Results

### 3.1. Mosquito collections

During the 48 person-nights collections made in June 2004, September 2004, December 2004 and March 2005, 1145, 3131 and 42 anopheline specimens were collected, respectively, in Mbilor, Gankette Balla and Mboula. Anopheline species collected are presented in Table 1. The anopheline fauna was less diversified in the village of Mboula in the low valley of Ferlo area in comparison with the low valley of the Senegal River (Mbilor) and the Guiers Lake (Gankette). Very rare in the Guiers Lake area, *A. gambiae* s.l. was the prevalent species in the low valley of the Senegal River and almost the only species collected in the low valley of Ferlo. *A. funestus* was collected only in Mbilor and Gankette Balla where it was largely predominant. *A. pharoensis* was collected in large numbers only in Mbilor.

The 37, 23 and 40 bedrooms visited for pyrethrum spray collections have permitted the collection of 222, 3472 and 268 anopheline specimens, respectively, in Mbilor, Gankette Balla and Mboula. In Mbilor and Gankette Balla villages, the same species were collected. The presence of *Anopheles rufipes* was noted in the three villages contrary to *Anopheles wellcomei* that was not collected by this method. Inversely, *A. pharoensis* was rare contrary to landing catches.

Of the 607 *A. gambiae* s.l. females captured on human (557 in Mbilor, 10 in Gankette Balla and 40 in Mboula), 109 (75 from Mbilor, 10 from Gankette Balla and 24 from Mboula) were processed using the PCR technique. Only *A. gambiae* M form and *A. arabiensis* were present in the three villages. *A. arabiensis* was predominant and represented 97, 100 and 92% in Mbilor, Gankette Balla and Mboula, respectively. The proportions of the two species were not statistically different between the three villages ($\chi^2 = 2.07$, d.f. = 1, $p = 0.35$).

### 3.2. Mosquito population dynamics

For each village, the human biting rate (HBR) varied temporally depending on the rainy season (Fig. 2). The mean number of anopheline bites per human per night (BHN) calculated from the four surveys was estimated to 26.4 (consisting of 11.6 for *A. gambiae*, 4.6 for *A. funestus* and 7.1 for *A. pharoensis*) in Mbilor, 72.6 (0.2 for *A. gambiae*, 60.3 for *A. funestus* and 1.2 for *A. pharoensis*) in Gankette Balla and 0.9 (0.8 for *A. gambiae* and 0.04 for *A. pharoensis*) in Mboula. The highest human biting rate was observed in September in each village except for *A. pharoensis* in Mbilor (June) and Gankette Balla (March). The mean biting rate was uniformly low in Mboula. Contrary to Mboula and Gankette Balla, biting females of *A. gambiae* s.l. were captured in each sampling survey in Mbilor. A regular decrease of anopheline abundance was observed towards

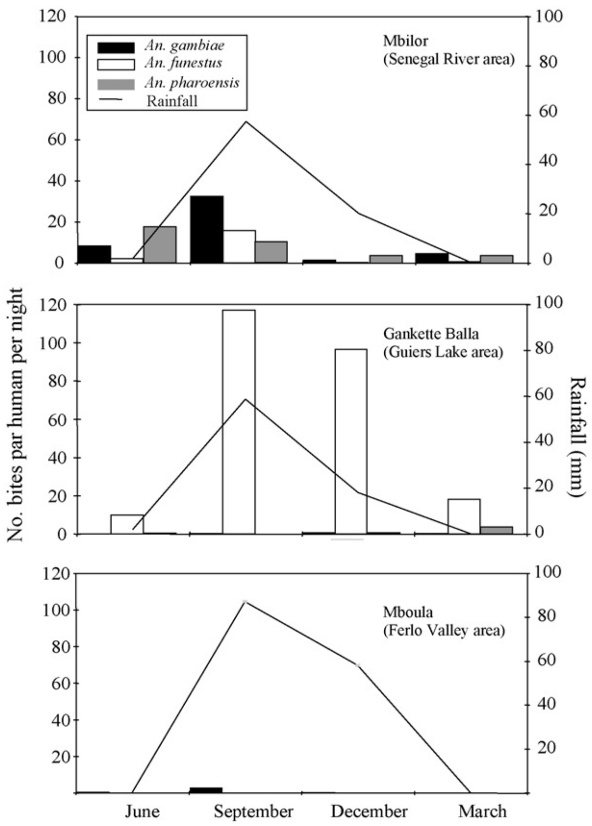


Fig. 2. Temporal dynamics of *A. gambiae*, *A. funestus* and *A. pharoensis* in relation to monthly rainfall in Mbilor, Gankette Balla and Mboula villages from June to March 2005.

Table 2
Percentage of *A. gambiae*, *A. funestus* and *A. pharoensis* fed on each vertebrate host in Mbilor, Gankette Balla and Mboula among resting mosquitoes

| Location | Species | Number tested | Vertebrate hosts (%) | | | | Mixed |
|---|---|---|---|---|---|---|---|
| | | | Human | Bovine | Ovine | Equine | |
| Mbilor (Senegal River) | *A. gambiae* | 105 | 67.6 | 19 | 1.9 | 11.4 | 6.7 |
| | *A. funestus* | 55 | 80 | 9.1 | 3.6 | 7.3 | 9.1 |
| | *A. pharoensis* | 15 | 93.3 | 6.7 | 0 | 0 | 0 |
| Gankette Balla (Guiers Lake) | *A. gambiae* | 31 | 6.5 | 35.5 | 0 | 58.1 | 3.2 |
| | *A. funestus* | 256 | 23 | 23 | 5.9 | 48 | 15.2 |
| | *A. pharoensis* | – | – | – | – | – | – |
| Mboula (Ferlo Valley) | *A. gambiae* | 91 | 75.8 | 3.3 | 8.8 | 12.1 | 3.3 |
| | *A. funestus* | – | – | – | – | – | – |
| | *A. pharoensis* | – | – | – | – | – | – |

the end of the rainy season. *A. funestus* females were collected during the whole period of study in both Mbilor and Gankette Balla with a similar population dynamics. A peak of 15.7 BHN and 117 BHN, respectively, observed in Mbilor and Gankette Balla was followed by regular decrease in the abundance of mosquitoes reaching a mean of 0.6 BHN in Mbilor and 18.2 BHN in Gankette Balla.

### 3.3. Host-seeking behaviour

Overall, 53.7% of *A. gambiae*, 61.7% of *A. funestus* and 40.9% of *A. pharoensis* captured on human in the village of Mbilor were collected indoors. The endophagous rates of these three sympatric species were significantly different in Mbilor ($\chi^2 = 25.6$, d.f. = 2, $p < 0.001$) while they were not different in Gankette Balla ($\chi^2 = 5.1$, d.f. = 2, $p = 0.07$). There was also no significant difference between the endophagous rate of *A. gambiae* and *A. pharoensis*, the two only species captured in Mboula ($\chi^2 = 1.7$, d.f. = 1, $p = 0.18$). No significant differences were observed neither for both *A. gambiae* and *A. pharoensis* between the three villages ($\chi^2 = 0.2$, d.f. = 2, $p = 0.91$ and $\chi^2 = 3.9$, d.f. = 2, $p = 0.14$) nor for *A. funestus* between Mbilor and Gankette Balla villages ($\chi^2 = 0.07$, d.f. = 1, $p = 0.08$).

A total of 553 bloodmeals from resting females collected in the three villages were tested by ELISA to determine the source (Table 2). The three species have taken their bloodmeals from Human, Bovine, Ovine and Equine hosts. The mean anthropophilic rates were highly significant for *A. gambiae* ($\chi^2 = 49.6$, d.f. = 2, $p < 0.001$) between the three villages and for *A. funestus* ($\chi^2 = 66.3$, d.f. = 1, $p < 0.001$) between Mbilor and Gankette Balla. Within villages, the anthopophilic rate was similar for Gankette Balla village ($\chi^2 = 4.55$, d.f. = 1, $p = 0.05$) and for Mbilor ($\chi^2 = 6.11$, d.f. = 2, $p = 0.05$).

### 3.4. Parity rates

Samples of 469, 1108 and 34 females, respectively, collected during the four surveys in Mbilor, Gankette Balla and Mboula were dissected for parity determinations (Table 3). There was no significant differences in parity rates between the three species in the village of Mbilor in the low valley of the Senegal River valley whereas highly significant difference was documented in the village of Gankette Balla in the Guiers Lake area ($\chi^2 = 15.5$, d.f. = 2, $p = 0.0004$) where a particularly low parity rate was noted for *A. funestus* ($\chi^2 = 16.5$, d.f. = 1, $p = 0.0001$). Highly significant differences were observed between the four surveys for the three species in Mbilor and for *A. gambiae* s.l. and *A. funestus* in Gankette Balla whereas no significant difference was observed for *A. pharoensis* in Gankette Balla ($\chi^2 = 4.34$, d.f. = 2, $p = 0.11$) and *A. gambiae* s.l. in Mboula village (Fisher exact test, $p = 0.40$).

Table 3
Mean parity rate of *A. gambiae*, *A. funestus* and *A. pharoensis* in Mbilor, Gankette Balla and Mboula during the four surveys

| Location | Species | June | | September | | December | | March | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N[a] | PR[b] | N[a] | PR[b] | N[a] | PR[b] | N[a] | PR[b] | N[a] | PR[b] |
| Mbilor (Senegal River) | *A. gambiae* s.l. | 79 | 62 | 111 | 83.8 | 15 | 86.7 | 43 | 88.4 | 248 | 77.8 |
| | *A. funestus* | 18 | 22.2 | 67 | 62.7 | 3 | 66.7 | 6 | 83.3 | 94 | 56.4 |
| | *A. pharoensis* | 25 | 44 | 49 | 95.7 | 19 | 89.5 | 34 | 61.8 | 127 | 71.7 |
| Gankette Balla (Guiers Lake) | *A. gambiae* s.l. | 0 | 0 | 2 | 100 | 5 | 60 | 2 | 0 | 9 | 55.6 |
| | *A. funestus* | 102 | 60.8 | 390 | 44.6 | 423 | 27 | 148 | 16.9 | 1063 | 35.3 |
| | *A. pharoensis* | 6 | 50 | 0 | 0 | 11 | 27.3 | 19 | 10.5 | 36 | 22.2 |
| Mboula (Ferlo Valley) | *A. gambiae* s.l. | 2 | 100 | 32 | 90.6 | 0 | 0 | 0 | 0 | 34 | 91.2 |
| | *A. funestus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | *A. pharoensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Number dissected.
[b] Parity rate.

Table 4
Mean infection rate calculated by enzyme-linked immunosorbent assay for *P. falciparum* for *A. gambiae*, *A. funestus* and *A. pharoensis* in Mbilor, Gankette Balla and Mboula

| Location | Species | Number tested | Positive | CSP rate (95% CI) |
|---|---|---|---|---|
| Mbilor | *A. gambiae* s.l. | 548 | 0 | 0 |
| | *A. funestus* | 219 | 0 | 0 |
| | *A. pharoensis* | 334 | 4 | 1.2% (0–2.8) |
| Gankette Balla | *A. gambiae* s.l. | 10 | 0 | 0 |
| | *A. funestus* | 2857 | 1 | 0.04% (0–0.11) |
| | *A. pharoensis* | 60 | 0 | 0 |
| Mboula | *A. gambiae* s.l. | 37 | 0 | 0 |
| | *A. funestus* | 0 | 0 | 0 |
| | *A. pharoensis* | 2 | 0 | 0 |

### *3.5. Circumsporozoite protein and entomological inoculation rates*

A total of 1101 anophelines collected in Mbilor, 2927 in Gankette Balla and 39 in Mboula were processed by ELISA for *P. falciparum* circumsporozoite antigen detection. All specimens giving positive ELISA results were retested. In total, 1.2% [CI 95% = 0–2.8] of *A. pharoensis* collected in Mbilor and 0.04% [CI 95% = 0–0.11] of *A. funestus* collected in Gankette Balla were positive for *P. falciparum* circumsporozoite antigen. No positive mosquito was identified in Mboula (Table 4).

Due to very low positive mosquitoes observed, it was difficult to evaluate the exact entomological inoculation rate (EIR). It was, however, estimated to 10.44 and 3.00 infected bites, respectively, in Mbilor and Gankette Balla.

### *3.6. Malaria prevalence*

Overall 1636 thick blood smears (500 from Mbilor, 604 from Gankette Balla and 532 from Mboula) were obtained from 486 children (149 in Mbilor, 173 in Gankette Balla and 164 in Mboula). Among the included schoolchildren, the percentage of those who were involved in the subsequent three surveys varied from 75 to 80% in Mbilor, 78 to 90% in Gankette Balla and 72 to 78% in Mboula. Overall, 147 thick smears were positive. Only *P. falciparum* was observed. The parasite and gametocyte prevalence for each village during the different months surveyed are presented in Table 5. The parasite prevalence was uniformly low in Mbilor and Gankette Balla villages whereas; it increased in September (16%) and then remained stable in December and March (22%) in Mboula. Significant differences were, however, observed over time for parasite prevalence in Mbilor ($\chi^2 = 10.5$, d.f. = 3, $p = 0.01$) and Mboula villages ($\chi^2 = 20.5$, d.f. = 3, $p < 0.001$) and for gametocyte prevalence only in Gankette Balla village ($\chi^2 = 8.6$, d.f. = 3, $p = 0.03$).

## 4. Discussion

During this study, seven anopheline species out of the 20 already recorded in Senegal (Diagne et al., 1994) were collected by the two sampling methods in the Senegal River Basin. This observation contrasts with that obtained in the south-east part of Senegal where up to 12 anopheline species were described (Dia et al., 2003).

Within the studied area, the anopheline fauna was more diversified in the low valley of the Senegal River valley (7 species) in comparison with the Guiers Lake and the low valley of the Ferlo area (4 species each). The presence of *A. gambiae*, *A. arabiensis*, *A. pharoensis* and at lesser extent that of *A. rufipes* was previously described in the low valley of the Senegal River (Faye et al., 1998). In addition to these species, our study has permitted to record *A. wellcomei*, *Anopheles coustani* and *A. funestus*. Indeed, this latter species was not predominant as it was already observed in Keur Mbaye village, 5 km apart from Mbilor village (Konate et al., 2001). In contrast, *Anopheles ziemanni*, a species already observed in this region were not found in our study. The observed discrepancies about the species observed could be linked to the ecological changes resulting from the implementation of the Diama dam. Indeed, for *Anopheles ziemanni*, the breeding occurs abundantly in coastal swamps, sometimes in slightly saline-water. For *A. wellcomei* and *A. pharoensis*, which are abundantly found, aquatic stages breed in vegetated swamps with vertical or horizontal vegetation (Hamon et al., 1956), usually in confined fresh water (Lariviere and Abonnenc, 1957), that were the predominant breeding opportunities found in the prospected areas.

*A. arabiensis* was found to be the prevalent species of the *A. gambiae* complex in the whole studied area. This observation is in agreement with the distribution of this species which is located in dry to humid savannah areas (Coetzee et al., 2000). However, it is noteworthy that *A. gambiae* was the predominant species of the *A. gambiae* complex before the implementation of the dams in the Senegal River (Vercruysse, 1985; Petrarca et al., 1987).

A differential distribution of *A. gambiae*, *A. funestus* and *A. pharoensis* was documented in this study. *A. pharoensis* was the prevalent species in the Senegal River valley where it was sympatric with both *A. gambiae* s.l. and *A. funestus*. In the Guiers Lake area, *A. funestus* was the principal species while *A. gambiae* was the only species present in the low valley of Ferlo area. This heterogeneous spatial distribution of malaria vectors could be related to the great ecological differences observed in the prospected areas. Indeed, the stop of

Table 5
Parasite and gametocyte prevalence of schoolchildren under 9 years from Mbilor, Gankette Balla and Mboula during the four surveys

| Locality | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | | | September | | | December | | | March | | |
| | N[a] | PP[b] | GP[c] | N[a] | PP[b] | GP[c] | N[a] | PP[b] | GP[c] | N[a] | PP[b] | GP[c] |
| Mbilor (Senegal River) | 149 | 8.4 (4–12.8)[d] | 2 (0–4.8)[d] | 120 | 9.2 (4–14.4)[d] | 2.5 (0–5.3)[d] | 112 | 2.7 (0–5.7)[d] | 0 | 119 | 1.6 (0–3.8)[d] | 0 |
| Gankette Balla (Guiers Lake) | 173 | 4 (1.1–6.9)[d] | 0 | 135 | 7.4 (3–11.8)[d] | 3 (0.1–5.9)[d] | 156 | 7 (2.9–11.1)[d] | 1.3 (0–3)[d] | 140 | 5.7 (1.9–9.5)[d] | 0 |
| Mboula (Ferlo Valley) | 164 | 5.5 (2–9)[d] | 0 | 118 | 16.1 (9.5–22.7)[d] | 0.8 (0–1.4)[d] | 128 | 21.9 (14.7–29.1)[d] | 1.3 (0–3.2)[d] | 122 | 22 (14.7–29.3)[d] | 0.9 (0–2.5)[d] |

[a] Number of thick smears examined.
[b] Parasite prevalence.
[c] Gametocyte prevalence.
[d] 95% confidence interval.

saltwater intrusion from the Atlantic Ocean into both the Senegal River and the Guiers Lake band by the Diama dam has permitted the availability of fresh-water for hydro-agricultural implementations and the development of fresh-water plants (Thiam, 1998; Sarr et al., 2001) that allowed the restoration of the larval habitats for *A. funestus*. However, salted water forwarded from the south part of the Guiers Lake for refreshment (SGPRE, 1999) makes the village of Mboula and the whole southeast part of the low valley of the Ferlo unfavourable to *A. funestus*.

In all the studied areas, anopheline specimens were collected in every month. Thus, Anopheles larval breeding sites are present in these areas year round. However, *A. gambiae*, *A. funestus* and *A. pharoensis* were abundant only in the village of Mbilor where they were also sympatric, allowing hence the comparison of their population dynamics. The dynamics of *A. gambiae* and *A. funestus* was similar to what is usually observed for these species in sahelian zones (Gillies and De Meillon, 1968; Brengues et al., 1979). However, for *A. funestus*, the peak of activity is usually observed at the end of the rainy season and the beginning of the dry season (Fontenille et al., 1997) but not in September as observed in both Mbilor and Gankette Balla villages. This atypical situation can be related to insecticides treatments during the rainy season 2004. In fact, the States bordering the Senegal River were constrained to use insecticides treatments during this period, for the control of an invasion of migratory locusts. These campaigns used mainly pyrethroid (Cyhalothrine, Tralométhrine) and organophosphate (Chlorpyriphos-ethyl, Fenitrothion, Malathion) insecticides in the whole infested area including our study area (PAN, 2006). The decrease of anopheline densities was less marked in Gankette Balla where the treatments were made lately. For *A. pharoensis*, the peak was observed in June with a regular decrease during the following months. This dynamics is correlated with the cycle of rice growing as already observed in this area (Faye et al., 1995).

The measure of parasitic prevalence in schoolchildren indicated that this area has a typical hypo to mesoendemic situation. Malaria transmission was perceptible only in the low valley of the Senegal River and the Guiers Lake area with, respectively, 10.44 and 3 infected bites in Mbilor and Gankette Balla, with *A. pharoensis* and *A. funestus* being, respectively, responsible of the whole transmission. The possible implication of *A. funestus* in malaria transmission in the Guiers Lake area was recently reported by Konate et al. (unpublished data). However, this is the first time that specimens of *A. pharoensis* are involved in malaria transmission apart from the delta of the Senegal River. Indeed, *A. pharoensis* was already incriminated in malaria transmission in the delta of the Senegal River (Carrara et al., 1990). Even if the ELISA evidence is not conclusive for vector incrimination, some indication can support the role of *A. pharoensis* in malaria transmission. The infection was observed twice during this study, respectively, in June (3 specimens) and September 2004 (1 specimen). Elsewhere in Africa, an implication of this species in malaria transmission was observed particularly in irrigated rice cultivation areas in Cameroon (Robert et al., 1992) and in Kenya (Ijumba et al., 1990) with the respective infection rates of 2.1 and 0.1%. However, a study carried out thereafter by Faye et al. (1995), 5 years after that of Carrara and collaborators did not confirm the previous results obtained by these authors. It is nevertheless important to note that, contrary to the present study; very low parity rates had been observed during the study of Faye et al. (1995) in the delta of the Senegal River. It may suggest that the populations of *A. pharoensis* underwent an adaptation to the new ecology of the low valley of the Senegal River having a more anthropophilic behaviour as demonstrated by the study of the trophic behaviour. The paradox between low or no transmission and high malaria parasite prevalence as observed in Mboula have been previously found elsewhere in Africa (Beier et al., 1999; Robert et al., 2006; Hay et al., 2005). Such an observation raises again the question about the relationships between the EIR and the prevalence of malaria infection. However, the fact that *A. arabiensis* was already proven to be a good malaria vector in this area (Lemasson et al., 1997) and that an infection rate of 0.43% was observed in the low valley of Ferlo previously in our preliminary study (Konate et al., unpublished data), confirm that the level of malaria transmission is low in this area.

*A. funestus* was the only species involved in malaria transmission in Gankette Balla village where the highest densities of this species were observed. The infection rate observed was low compared to those generally obtained from this species in Senegal and elsewhere in Africa (Dia et al., 2003; Fontenille et al., 1997; Coetzee and Fontenille, 2004). This observation could be the result of the relative low parity and anthropophilic rates observed during this study. Another plausible hypothesis not mutually exclusive is the low parasite prevalence observed in the three villages studied. In turn, low gametocytes carriers that did not exceed 3% could explain the low infection rate observed in mosquitoes. A similar situation was already observed in the middle valley of the Senegal River by Faye et al. (1993). This situation contrasts with a recent observation made by Robert et al. (2006) who demonstrated the presence of a moderate transmission associated with high malaria prevalence in Madagascar. A third possible assumption of such a phenomenon could be related to a differential adaptation of *A. funestus* adults and aquatic stages to the new ecological conditions of the Guiers Lake area. Indeed, because of the reconstitution of favourable breeding sites, the aquatic stages have probably found the appropriate conditions for their development towards the emergence of adults. Thus, this situation produces high adult population densities that do not find, however, the optimal conditions allowing them to have a sufficient longevity to be infected and to transmit malaria parasites as previously stressed (Konate et al., unpublished data). Such a situation was already observed previously for *A. pharoensis* in the zone of the delta of the Senegal River by Faye et al. (1995). However, due to the evolution of the ecology of the area and the environmental changes, it could not be excluded a positive evolution of this situation tending to a good adaptation of the adult females being able to lead to a great risk for malaria transmission in human populations. Thus, such changes should be thoroughly surveyed in order to prevent any possible malaria outbreak in this area.

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