Four years' entomological study of the transmission of seasonal malaria in Senegal and the bionomics of *Anopheles gambiae* and *A. arabiensis*

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**Abstract**

From 1993 to 1996, an entomological survey was conducted in the village of Ndiop, Senegal, as part of a research programme on malaria epidemiology and the mechanisms of protective immunity. Mosquitoes were captured on human bait and by indoor spraying. Species from the *Anopheles gambiae* complex were identified using the polymerase chain reaction, and *Plasmodium falciparum* infections were detected by enzyme-linked immunosorbent assay for circumsporozoite protein. The vector species identified were *A. gambiae* (33.4%), *A. arabiensis* (63.2%), *A. melas* (0.3%) and *A. funestus* (2.5%). Similar proportions of *A. gambiae* (74.2%) and *A. arabiensis* (25.8%) contained human blood; 77% of *A. gambiae* and 28.3% of *A. arabiensis* had fed on cattle. The sporozoite rates were similar for *A. gambiae* (3.2%) and *A. arabiensis* (3.7%). The annual entomological inoculation rates varied greatly depending on the year. There were 63, 17, 37 and 7 infected bites per person per year in 1993, 1994, 1995 and 1996 respectively. Transmission was highly seasonal, from July to October. *A. arabiensis* was responsible for 66% of malaria transmission, *A. gambiae* for 31%, and *A. funestus* for 3%.

**Keywords:** malaria, *Plasmodium falciparum*, transmission, *Anopheles arabiensis*, *Anopheles gambiae*, *Anopheles melas*, Senegal

**Introduction**

Comparison of areas with different levels of malaria endemicity is a means of understanding the relationships between transmission, infection and morbidity of malaria and for investigating the mechanisms leading to protective immunity (Beier et al., 1994; McElroy et al., 1994; Snow et al., 1994; Beadle et al., 1995; Mboga et al., 1995). Such understanding of the effects of the intensity and seasonality of transmission is essential for a long-term prediction of the efficacy of vector control measures or malaria vaccines (Saül, 1993; Snow & Marsh, 1995; Trape & Roger, 1996).

For this reason, two Senegalese villages, Dielmo and Ndiop, only 5 km apart but with different malaria patterns, were selected for a longitudinal study of vectorial transmission, parasitaemia, clinical attacks, immunological data, and genetic diversity of *Plasmodium falciparum* (see Roger & Trape, 1995).

In Dielmo, where malaria is holoendemic and transmission continuous throughout the year, the longitudinal study began in 1990, while in Ndiop, where malaria is mesoendemic and transmission seasonal, it began in 1993. The results of the study in Dielmo have been reported by Konate et al. (1994), Trape et al. (1994) and Fontenille et al. (1997). This study presents the data obtained in Ndiop. The aims of this longitudinal study were to identify the malaria vectors, using the polymerase chain reaction (PCR), to identify species of the *Anopheles gambiae* complex, to understand their behaviour, and to evaluate the level and the seasonality of malaria transmission. These transmission data will be useful for the evaluation of the relationships of morbidity, immunity and genetic diversity of *P. falciparum* in Ndiop in successive years and between Dielmo and Ndiop during the same year.

**Materials and Methods**

**Study area**

The study was carried out in the village of Ndiop (13°14'N, 16°23'W) (Fig. 1). This village of about 350 inhabitants is situated in the Saloum, in the Sahelo-Soudanian region of Senegal. The dominant ethnic groups are Wolof and Peuhl, who are mainly farmers. The vegetation is wooded savannah, almost entirely cleared for cultivation of peanuts and millet. Most of the houses are built in the traditional style with mud walls and thatched roofs. In 18 of the 58 houses, corrugated iron has replaced the thatch, but generally a space is left between the roof and the tops of the walls. Small herds of domestic animals stay for the night within the village. Ndiop is situated on the marshy bank of a small perennial stream which permits the persistence of anopheline larval development sites throughout the year. The rainy season extends from June to October. Rainfall varies annually: 602 mm in 1993, 709 mm in 1994, 860 mm in 1995 and 521 mm in 1996. The nearest temporary ground pool, which floods during the rainy season, is 1 km from the village. The average minimum monthly temperature, recorded in Dielmo, ranged from 14° (January 1994) to 26° (October 1995). The average maximum monthly temperature ranged from 30° (January 1995) to 39° (February 1996).

**Mosquito collections**

Adult mosquitoes were captured monthly, for 3 consecutive nights, from May 1993 to April 1995, and weekly, for one night, from May 1995 to December 1996. Two collection techniques were used.

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*Fig. 1. Map of Senegal showing the villages of Ndiop and Dielmo in the Saloum region.*
(i) Hourly human bait collections were made on adult volunteers from 19:00 to 07:00 at the same 2 sites within the village. The first site, in the western part of the village, was a room with mud walls and thatched roof; the other site, situated in the eastern part of Ndiop, had a corrugated iron roof. One indoor collector and one outdoor collector were positioned at each site. A total of 12 person-nights of capture were made every month during the monthly collections, and a total of 4 every week during the weekly collections. The human biting rate (HBR) was expressed as the average number of mosquito bites per person per night during each month.

(ii) Twenty-four pyrethrum spray collections were made during the 4-year survey early in the morning inside a total of 234 bedrooms, belonging to both types of houses, in different houses from those used for human bait collections.

Fig. 2. Rainfall, temperature, human biting rate, and monthly entomological inoculation rate (estimated by ELISA) for each malaria vector species from May 1993 to December 1996 in Ndiop, Senegal.
A total of 1810 An. gambiae s.l. were captured during 604 person-night collections on human volunteers, and 788 by spray collections in bedrooms. Of the 2533 A. gambiae complex females captured, 1035 were processed using the PCR (Table 1). Non-malaria vector anophelines captured included A. funestus 53 (2.9%) and A. melas 4 (0.2%).

Field processing of anophelines

Anophelines were identified by morphological characteristics using the keys of Gillies & De Meillon (1968) and Gillies & Coetzee (1987). All mosquitoes of the A. gambiae complex were stored individually in numbered tubes with dessicant for laboratory processing in Dakar.

Laboratory processing of anophelines

The blood meals of a sample of females captured by pyrethrum spray were identified as human, bovine, ovine/caprine, or chicken using an enzyme-linked immunosorbent assay (ELISA) (Béeri et al., 1988).

The heads/thoraces of all captured female anophelines were tested for circumsporozoite protein (CSP) of P. falciparum, P. malariae and P. ovale using the ELISA described by Burkom et al. (1984) and modified by Wirtz et al. (1987). (P. vivax is not present in this region of Africa.) The entomological inoculation rate (EIR) was calculated as the product of the HBR and CSP rate of mosquitoes captured on human bait. The 95% confidence interval (95% CI) of each annual EIR was calculated according to the Poisson distribution (Liddell, 1984).

A random sample of at least 30 females (when more than 30 were captured) belonging to the A. gambiae complex was identified to species each month using the PCR described by Scott et al. (1993). A leg or wing was placed directly into the reaction mixture containing the species-specific primers, deoxynucleotide triphosphates, buffer and polymerase. The length of the amplified sequences was 315 nucleotides for A. arabiensis, 390 for A. gambiae and 464 for A. melas. This technique has been validated in West Africa (Fontenille et al., 1993). All mosquitoes giving a positive result with the CSP ELISA were processed by PCR.

Results

Mosquito collections

From May 1993 to December 1996, 1810 malaria vectors were captured during 604 person-night collections on human volunteers, and 788 by spray collections in bedrooms. Of the 2533 A. gambiae complex females captured, 1035 were processed using the PCR (Table 1). Non-malaria vector anophelines captured included A. gambiae s.l. 1757 (97.1%) and A. arabiensis 776 (98.5%). A. funestus 53 (2.9%) and A. melas 4 (0.2%).

No. of mosquitoes
Feeding on
human bait
Resting in bed rooms

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>A. funestus</td>
<td>77</td>
<td>52</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>A. gambiae</td>
<td>626</td>
<td>362</td>
<td>321</td>
<td>24</td>
</tr>
<tr>
<td>A. arabiensis</td>
<td>604</td>
<td>371</td>
<td>364</td>
<td>23</td>
</tr>
<tr>
<td>A. melas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Composition of population of Anopheles spp. caught on human volunteers in Ndiop, Senegal, 1993–1996

No. of Anopheles caught

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>A. funestus</td>
<td>77</td>
<td>52</td>
<td>16</td>
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<tr>
<td>A. gambiae</td>
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<tr>
<td>A. arabiensis</td>
<td>604</td>
<td>371</td>
<td>364</td>
<td>23</td>
</tr>
<tr>
<td>A. melas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Anopheles feeding on humans from 19:00 to 07:00; Ndiop, Senegal, 1993–1996.

Seasonality and biting cycles

The HBR for each species varied with the rainy season (Fig. 2). Each year the biting rate was maximal in September for A. gambiae, A. arabiensis and A. funestus, except in 1994 when the A. arabiensis peak was in August. The maximum rate for A. arabiensis was in September 1995, with an average peak of 12.5 bites per person per night; for A. gambiae it was in September 1995 (11.3), and for A. funestus it was in September 1995 (13).

Night biting cycles were similar for A. gambiae and A. arabiensis, with a peak between 03:00 and 06:00 (Fig. 3). All the A. funestus females were captured after 23:00.

Host-seeking behaviour

Overall, 58.2% of A. gambiae, 56.0% of A. arabiensis and 49.1% of A. funestus captured on humans were collected indoors. The proportions of A. gambiae and A. arabiensis were not significantly different (χ²=0.07, d.f.=1, P=0.80). A total of 272 blood meals from indoor resting females of the A. gambiae complex was tested using ELISA, including 89 A. gambiae and 145 A. arabiensis. Over the study period of 4 years, 4.5% of A. gambiae tested had taken mixed blood meals on 2 different host species, compared to 7.6% for A. arabiensis (χ²=0.87, d.f.=1, P=0.40). The proportion of human blood meals was 74.2% for A. gambiae and 73.8% for A. arabiensis (not significantly different: χ²=0.01, d.f.=1, P=0.91). Overall 1.1% of anophelines had fed on sheep or goats and 3.3% on horses or donkeys. No female had taken a chicken blood meal.
Table 3. Circumsporozoite rates in the three main vector species of *Anopheles*; Ndiop, Senegal, 1993–1996

<table>
<thead>
<tr>
<th>Year</th>
<th>A. gambiae</th>
<th>A. arabiensis</th>
<th>A. funestus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>265</td>
<td>474</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>P. falciparum 3·4 (1·6–6·3)</td>
<td>4·4 (2·8–6·7)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. malariae 0·43 (0·001–2·1)</td>
<td>0·4 (0·05–1·5)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. ovale 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1994</td>
<td>89</td>
<td>271</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>P. falciparum 1·1 (0·03–6·1)</td>
<td>3·0 (1·3–5·7)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. malariae 0</td>
<td>0·7 (0·1–2·6)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. ovale 1·1 (0·03–6·1)</td>
<td>0·4 (0·001–2·0)</td>
<td>0</td>
</tr>
<tr>
<td>1995</td>
<td>190</td>
<td>375</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>P. falciparum 3·2 (2·6–7)</td>
<td>3·5 (1·9–5·9)</td>
<td>7·1 (0·9–23·5)</td>
</tr>
<tr>
<td></td>
<td>P. malariae 0</td>
<td>0·3 (0·001–3·5)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. ovale 0</td>
<td>0·5 (0·06–1·9)</td>
<td>0</td>
</tr>
<tr>
<td>1996</td>
<td>49</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>P. falciparum 6·1 (1·3–16·9)</td>
<td>3·0 (0·1–15·7)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. malariae 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. ovale 0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Circumsporozoite rate calculated by ELISA from head/thoraces of female mosquitoes captured on human bait. Exact 95% confidence intervals calculated according to the binomial distribution are shown in parentheses.*

Fig. 4. Blood meal identification of indoor resting *Anopheles* spp., Ndiop, Senegal, 1993–1996.

**Circumsporozoite protein rates**

The CSP rate was calculated monthly for each species. Overall, 86·5% of those identified were *P. falciparum*, 8·1% were *P. malariae* and 5·4% were *P. ovale*. In total, 3·2% (95% CI 1·9–4·9) of *A. gambiae* and 3·7% (95% CI 2·7–5·0) of *A. arabiensis* tested were positive for *P. falciparum* (Table 3). This difference was not statistically significant ($\chi^2=0·31$, d.f.=1, $P=0·58$). No significant difference was observed each year between *A. gambiae* and *A. arabiensis* ($\chi^2$ or Fisher’s exact test). Only 53 *A. funestus* were captured on human bait and tested; 2 of 28 captured in 1995 contained CSP. The average CSP rate was 3·8% (95% CI 0·5–13·0). Six and 4 mosquitoes contained *P. malariae* and *P. ovale* CSP, respectively. Two mixed infections were found in 2 *A. arabiensis* captured on human bait, one with *P. falciparum* and *P. malariae* and one with *P. falciparum* and *P. ovale*.

**Entomological inoculation rates**

The mean annual EIR for the 4 years was 31 infected bites per human per year. The annual EIR varied greatly; in 1993, it was 63 (95% CI [Poisson distribution] 37–97), in 1994 it was 17 (95% CI 5–35), in 1995 it was 37 (95% CI 21–61), and in 1996 it was 7 (95% 2–19). Transmission took place from July to October (Fig. 2). *A. arabiensis* was responsible for 66% of malaria transmission, *A. gambiae* for 31%, and *A. funestus* for 3%.

**Discussion**

There was a nine-fold variation in the malaria transmission rate depending on the year. The EIR ranged from 7 to 63, mainly due to variation in the HBR of each vector species.

Transmission was highly seasonal, occurring for only 1–4 months, depending on the year. To limit overestimation of the CSP rate, only the heads and thoraces were tested. A comparison between dissection and ELISA in 1995 showed that ELISA detected 1·5 times more positive mosquitoes than dissection (unpublished data). These results are in accordance with other studies (ROBERT et al., 1998; BEIER et al., 1990). Thus the mean EIR of 31 which we estimated should be considered a maximum value, and the mean annual transmission rate was certainly lower—about 21 infective bites per human per year.

No correlation was observed between the HBR and rainfall or temperature. However, rainfall in 1996 was lower than in previous years and the main mosquito breeding site, a temporary ground pool 1 km west of Ndiop, dried up earlier than it usually does. Only 85
mosquitoes were captured on human bait during 180 person-nights in that year (Fig. 2).

The malaria transmission pattern in Ndiop was typi-
cal of that in the Sahel-Sudanian region, as opposed to the nearby village of Dieulom, which is an exception where mosquitoes are present even during the dry season because of breeding sites in a permanent stream and where transmission occurs throughout the year. Despite being only 5 km apart, there are very large differences between Ndiop and Dieulom. The mean EIR was 31 in Ndiop and 236 in Dieulom over 6 years. (FONTENILLE et al., 1997 and unpublished data). The transmission level is different from one year to another in both villages, and it is also different from year to year. However, importantly, it did not vary in the same proportion in Dieulom and Ndiop. The ratio of annual EIR in the 2 villages was 1:8 (115/63) in 1993, 9:2 (19/21) in 1994, 7:1 (263/37) in 1995 and 4:3 (304/7) in 1996. Such variations have to be taken into account in any study of malaria infection rates, morbidity or immunity.

A. funestus was very rare in Ndiop. There was no favourite breeding site for this species around Ndiop. The 65 specimens captured during the 4 years’ survey probably came from other locations, such as Dieulom where mosquitoes are present all year round. Only 9 A. melas were captured by both methods of capture: this halophilic species presumably came from the mangrove 14 km west of Ndiop. The 2 main malaria vectors were A. gambiae and A. arabiensis. Previous cytotaxonomic studies have revealed 2 main cytotypes of A. gambiae in this region, savannia and urubusau (BRYAN et al., 1982). A. arabiensis was more abundant than A. gambiae, except in 1996. No mosquito was captured on human bait during the dry season, as also observed in Barkedji, a village in northern Senegal in the Sahelian region (LEMASSON et al., in press). In Sudan, OMER & CLOUDSLEY-HOPKIN (1970) found that a few autochthonous females remained during the dry season. In Mali and Burkina Faso, studying gene frequencies, TAYLOR et al. (1993) suggested that populations of A. arabiensis were present continuously, but with seasonal variation in numbers. In Ndiop it remains to be seen whether some females hibernate from one rainy season to another or whether they relocalize the region at the beginning of each rainy season from a nearby area of perennial transmission, such as Dieulom. The ratio of A. gambiae to A. arabiensis was the same (0.35) among indoor resting mosquitoes as it was among host-seeking specimens. The rate of endophagy and the night biting cycles were similar for A. gambiae and A. arabiensis, as was the average CSP rate and the proportions feeding on human blood. Similar observations have also been made in Dieulom (unpublished data) and in the Senegalese Sahel (LEMASSON et al., in press), as opposed to what is classically observed in East Africa where the proportion of human blood meals in A. gambiae is higher (WHITE et al., 1992). Comparisons between these 2 species in the same species, during the same period of time, have rarely been conducted, mainly due to the difficulty of identifying species of the A. gambiae complex before the availability of PCR. These similarities in the bionomics of the 2 species, which have not been observed in some other West or East African localities (WHITE & ROSEN, 1973; COLUZZI, 1984; TAYLOR et al., 1990; FONTENILLE et al., 1997; LEMASSON et al., in press), indicate the need for more population genetic studies to compare the populations in different genetic locations (LANZARO et al., 1995; LEHMANN et al., 1996).

Our study in Ndiop, which complements those conducted in Dieulom, provided basic data on transmission useful for the evaluation of the relationship between transmission, morbidity, and immunity in malaria.

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