# 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* (33·9%), *A. arabiensis* (63·2%), *A. melas* (0·3%) and *A. funestus* (2·5%). Similar proportions of *A. gambiae* (74·2%) and *A. arabiensis* (73·8%) contained human blood; 27·0% 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; MBOGO *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 (SAUL, 1993; SNOW & MARSH, 1995; TRAPE & ROGIER, 1996).

For this reason, 2 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 ROGIER & 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 representative of villages in this area, contrary to Dielmo which is situated on the marshy bank of a small permanent 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).

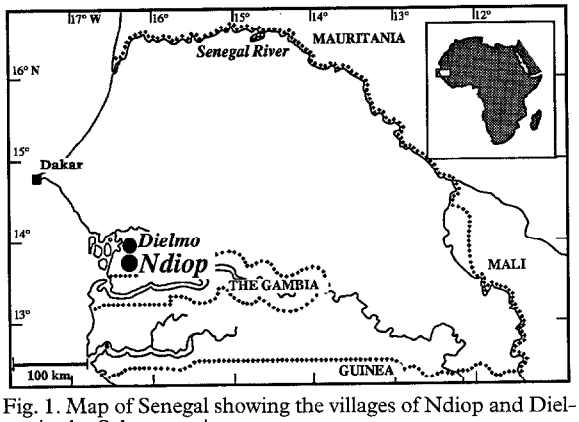


Fig. 1. Map of Senegal showing the villages of Ndiop and Dielmo in the Saloum region.

*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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(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.

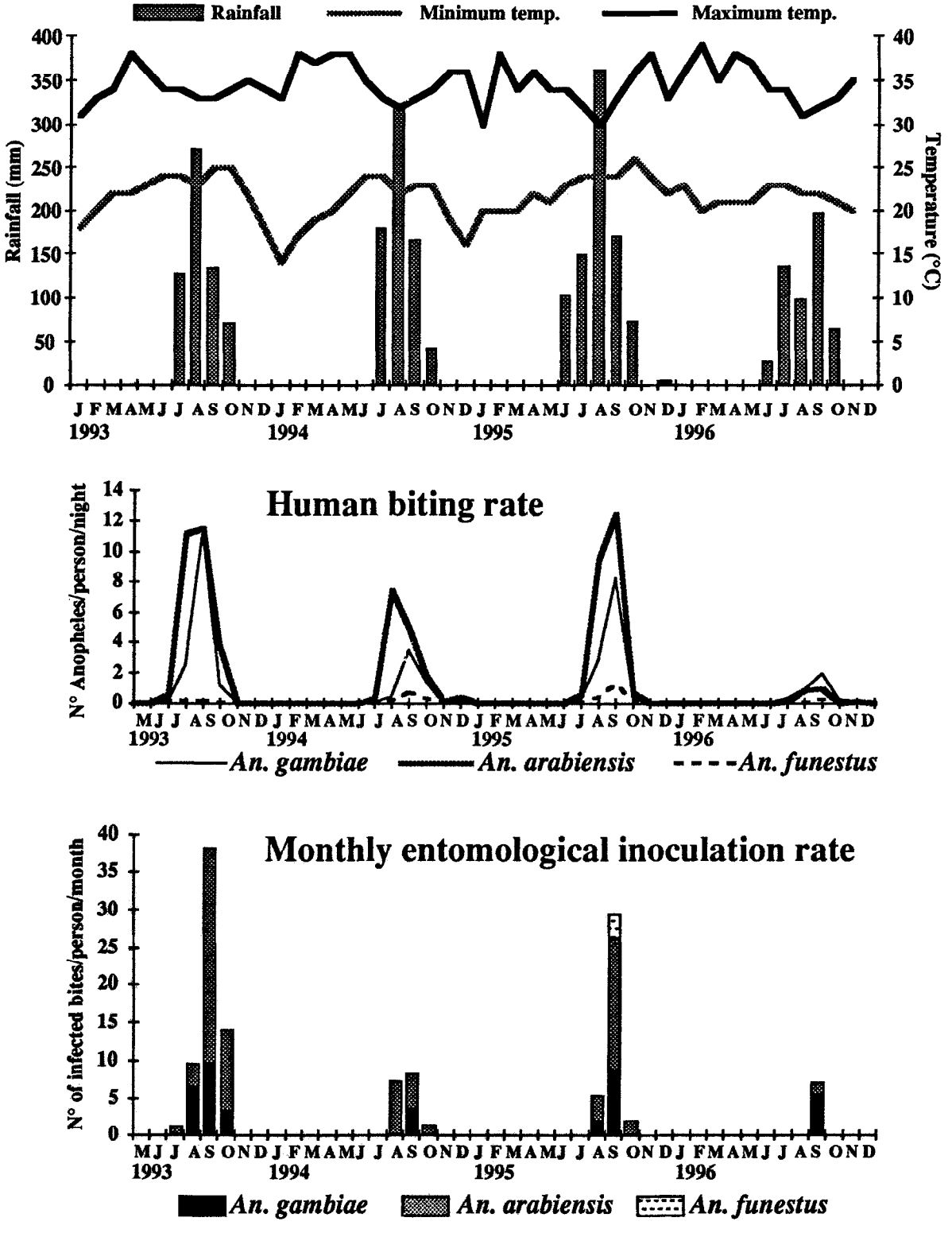


Fig. 2. Rainfall, temperature, human biting rate, and monthly entomological inoculation rate (estimated by ELISA) for each malaria vector species from May 1993 to December 1996 in Ndiop, Senegal.

**Table 1. Number of malaria vectors caught from April 1993 to December 1996 by two different methods in Ndiop, Senegal**

| | No. of mosquitoes | |
|---|---|---|
| | Feeding on human bait | Resting in bedrooms |
| Total | 1810 | 788 |
| *A. funestus* | 53 (2·9%) | 12 (1·5%) |
| *A. gambiae s.l.* | 1757 (97·1%) | 776 (98·5%) |
| *A. gambiae* | 33·0% | 36·1% |
| *A. arabiensis* | 64·0% | 61·6% |
| *A. melas* | 0·1% | 0·8% |
| Examined by PCR | 675 | 360 |

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

| | 1993 | 1994 | 1995 | 1996 |
|---|---|---|---|---|
| No. of *Anopheles* caught | 746 | 386 | 593 | 85 |
| Percentage composition | | | | |
| *A. funestus* | 0·5 | 5 | 5 | 4 |
| *A. gambiae* | 36 | 24 | 32 | 57 |
| *A. arabiensis* | 63 | 71 | 63 | 39 |
| *A. melas* | 0·4 | 0 | 0 | 0 |

*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) (BEIER *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 BURKOT *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

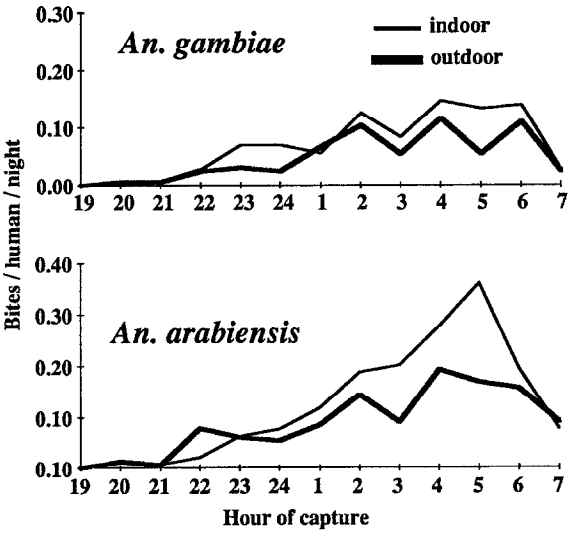


Fig. 3. Number of female *Anopheles* feeding on humans from 19:00 to 07:00; Ndiop, Senegal, 1993–1996.

*A. pharoensis* Theobald and *A. rufipes* (Gough).

*A. funestus* was rare among the specimens captured on human bait, with variations depending on the year. Overall, *A. arabiensis* formed 65·9% of the *A. gambiae* complex females captured on human bait, but in 1996 it formed only 40·2%. Only 3 *A. melas* were captured. Considerable differences were observed each year (Table 2). *A. arabiensis* formed 62·5% of the *A. gambiae* complex females captured as resting mosquitoes, and *A. gambiae* constituted 36·6%. Six *A. melas* were captured by spraying. The proportions of *A. arabiensis* and *A. gambiae* among resting mosquitoes and those captured on human volunteers did not differ significantly (*A. arabiensis*, $\chi^2$=0·22, d.f.=1, $P$=0·63; *A. gambiae*, $\chi^2$=1·49, d.f.=1, $P$=0·22).

*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 1993 (11·3), and for *A. funestus* it was in September 1995 (1·3).

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 ($\chi^2$=0·72, d.f.=1, $P$=0·40).

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* ($\chi^2$=0·87, d.f.=1, $P$=0·35). The proportion of human blood meals was 74·2% for *A. gambiae* and 73·8% for *A. arabiensis* (not significantly different: $\chi^2$=0·004, d.f.=1, $P$>0·9) (Fig. 4); 27·0% of *A. gambiae* and 28·3% of *A. arabiensis* had fed on cattle (no significant difference: $\chi^2$=0·05, d.f.=1, $P$=0·82). 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**

| | Circumsporozoite rate (%)[a] | | |
|---|---|---|---|
| | *A. gambiae* | *A. arabiensis* | *A. funestus* |
| 1993 | | | |
| No. tested | 265 | 474 | 4 |
| *P. falciparum* | 3·4 (1·6–6·3) | 4·4 (2·8–6·7) | 0 |
| *P. malariae* | 0·43 (0·001–2·1) | 0·4 (0·05–1·5) | 0 |
| *P. ovale* | 0 | 0 | 0 |
| 1994 | | | |
| No. tested | 89 | 271 | 18 |
| *P. falciparum* | 1·1 (0·03–6·1) | 3·0 (1·3–5·7) | 0 |
| *P. malariae* | 0 | 0·7 (0·1–2·6) | 0 |
| *P. ovale* | 1·1 (0·03–6·1) | 0·4 (0·001–2·0) | 0 |
| 1995 | | | |
| No. tested | 190 | 375 | 28 |
| *P. falciparum* | 3·2 (1·2–6·7) | 3·5 (1·9–5·9) | 7·1 (0·9–23·5) |
| *P. malariae* | 0 | 0·3 (0·001–3·5) | 0 |
| *P. ovale* | 0 | 0·5 (0·06–1·9) | 0 |
| 1996 | | | |
| No. tested | 49 | 33 | 3 |
| *P. falciparum* | 6·1 (1·3–16·9) | 3·0 (0·1–15·7) | 0 |
| *P. malariae* | 0 | 0 | 0 |
| *P. ovale* | 0 | 0 | 0 |

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

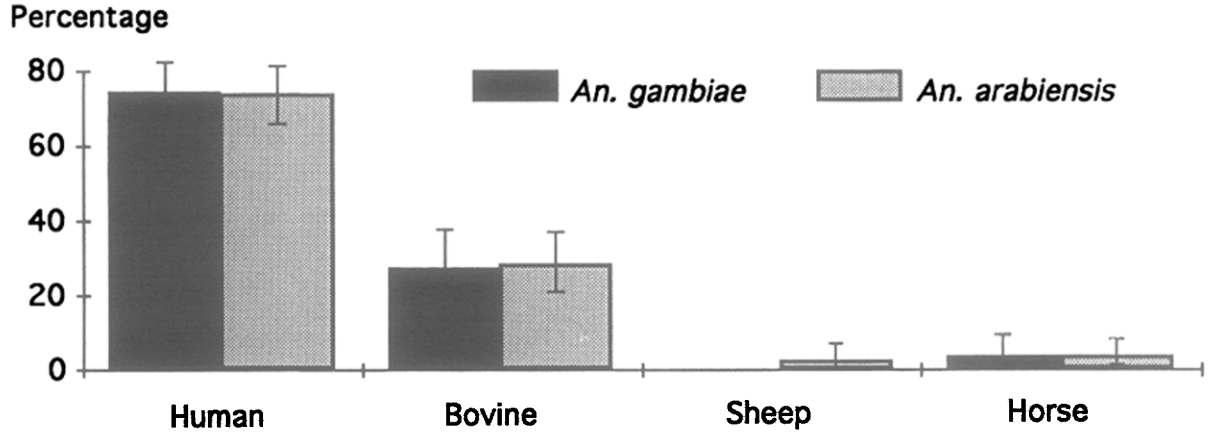


Fig. 4. Blood meal identification of indoor resting *Anopheles* spp., Ndiop, Senegal, 1993–1996.

*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* (*P*=0·49, 0·46, 0·85 and 0·65, respectively from 1993 to 1996, using $\chi^2$ 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 to be 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 typical of that in the Sahel–Sudanian region, as opposed to the nearby village of Dielmo, 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 Dielmo. The mean EIR was 31 in Ndiop and 236 in Dielmo 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 also differs between them during the same year. However, importantly, it did not vary in the same proportion in Dielmo and in Ndiop. The ratio of annual EIR in the 2 villages was 1·8 (115/63) in 1993, 9·2 (157/17) in 1994, 7·1 (263/37) in 1995 and 43·4 (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 favourable breeding site for this species around Ndiop. The 65 specimens captured during the 4 years' survey probably came from other locations, such as Dielmo where this species is abundant because of the permanent breeding sites. Only 9 *A. melas* were captured by both methods of capture: this halophilic species presumably came from the mangroves 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: savanna and bissau (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-THOMPSON (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 recolonize the region at the beginning of each rainy season from a nearby area of perennial transmission, such as Dielmo. The ratio of *A. gambiae* to *A. arabiensis* was the same (0·34) 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 Dielmo (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.*, 1972). Comparisons between these 2 species in the same location, 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 locations (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 locations (LANZARO *et al.*, 1995; LEHMANN *et al.*, 1996).

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

**Acknowledgements**

We thank Mamoudou Diallo and Henri Manga for their technical assistance, Frank Collins (CDC) for providing us with anti-*Plasmodium* monoclonal antibodies and for advice on PCR, Alison Clavier for help in the English translation, André Spiegel for very helpful suggestions, and the villagers of Ndiop for their co-operation throughout the survey. This work was supported by *Institut Français de Recherche Scientifique pour le Développement en Coopération* (ORSTOM), by the French ministry of Co-operation (*Appel d'offre Paludisme*, grant no. 950119500), and by *Centre National de la Recherche Scientifique* (CNRS–France, grant no. 96/C/08).

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*Received 2 April 1997; revised 12 May 1997; accepted for publication 13 May 1997*

## Announcement

**Tropical Health for the 21st Century: Clone, Cure and Control**
**Fourth Residential Meeting of the Royal Society of Tropical Medicine and Hygiene**
**Second European Congress on Tropical Medicine**
*Liverpool, UK*
*14–18 September 1998*

This meeting is organized jointly by the Royal Society of Tropical Medicine and Hygiene and the Federation of European Societies for Tropical Medicine and International Health; it will also mark the centenary of the foundation of the Liverpool School of Tropical Medicine, which will be hosting the meeting.

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