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HIGH ANNUAL AND SEASONAL VARIATIONS IN MALARIA TRANSMISSION BY ANOPHELINES AND VECTOR SPECIES COMPOSITION IN DIELMO, A HOLOENDEMIC AREA IN SENEGAL

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Abstract. We conducted a three-year entomologic study in Dielmo, a village of 250 inhabitants in a holoendemic area for malaria in Senegal. Anophelines were captured on human bait and by pyrethrum spray collections. The mosquitoes belonging to the *Anopheles gambiae* complex were identified using the polymerase chain reaction. Malaria vectors captured were *An. funestus, An. arabiensis,* and *An. gambiae. Anopheles funestus* was the most abundant mosquito captured the first year, *An. arabiensis* in the following years. The annual entomologic inoculation rates calculated by enzyme-linked immunosorbent assay were 238, 89, and 150 for the first, second, and third years, respectively. Each year there was a peak of transmission at the end of the rainy season, but transmission occurred year round. The heterogeneity of transmission was found at four different levels: 1) the relative vector proportion according to the place and method of capture, 2) the human biting rate and relative proportion of vectors by month and year, 3) the infection rate of each vector by year, and 4) the number of infected bites for all vectors, and for each species, for the year. Our data show that even in areas of intense and perennial transmission, there exist large longitudinal variations and strong heterogeneity in entomologic parameters of malaria transmission. It is important to take these into account for the study of the variations in clinical and biological parameters of human malaria, and to evaluate this relationship, a very thorough investigation of transmission is necessary.

The interpretation of malaria parameters such as parasitemia, morbidity, mortality, and associated immune responses depends on having precise information and close follow-up of variations in malaria transmission.[1–3] It is very well known that the transmission of malaria in Africa is not homogeneous.[4] The vector species and density, the *Plasmodium* species, the number of infective bites per human per year (also called the annual entomologic inoculation rate [EIR]), and the monthly EIR, are changeable. Many studies have compared transmission between villages in the same area,[5–8] but few have shown that significant differences may also occur within the same location over a several year follow-up study. Such variations have to be taken into account in longitudinal studies on the development of malaria immunity, but so far these have been studied very little. A longitudinal study began in 1990 to evaluate malaria infections and the mechanisms of protective immunity in a population living in Dielmo, a village in a holoendemic area of Senegal. During a four-month follow-up of the entire population conducted during the 1990 rainy season, the cumulative prevalence of *P. falciparum, P. malariae,* and *P. ovale* were 98.6%, 50.5%, and 40.3%, respectively.[9] The preliminary studies showed that the transmission was continuous throughout the year and that the vectors were *Anopheles funestus* and mosquitoes of the *An. gambiae* complex.[10, 11] Three species of this complex were noted in the study area: *An. gambiae, An. arabiensis,* and *An. melas.*

Malaria transmission by these different vectors was studied from 1992 to 1995 using the polymerase chain reaction (PCR), which identifies the species of the *An. gambiae* complex.[12] In this paper, we have investigated the relative frequencies of the different vector species (*An. funestus, An. gambiae, An. arabiensis,* and *An. melas*) collected within the study area according to the time, season, place, and method of capture, and the role of each vector in the transmission of the different *Plasmodium* species during a three-year longitudinal survey. This is the first study in Africa that provides data describing variation of the EIR according to *An. gambiae* complex species.

MATERIALS AND METHODS

Study site. The village of Dielmo (13°45′N, 16°25′W) is situated in an area of Sudan-type savanna in the Sine-Saloum region of Senegal, 280 km southeast of Dakar and approximately 15 km north of the Gambian border (Figure 1). The rainy season lasts from June to mid-October. Over the last 20 years, the average annual rainfall has been approximately 700 mm. It was 583 mm, 721 mm, and 657 mm in 1992, 1993, and 1994, respectively. Dielmo is situated on the marshy bank of a small permanent stream that permits the persistence of anopheline larval development sites year round. A population of 250 inhabitants live in the village. The study site was described in detail in a previous article.[9]

Mosquito collections. Adult mosquitoes were collected monthly from April 1992 to March 1995 using the following methods. The first was hourly outdoor and indoor human bait catches from 7:00 PM to 7:00 AM. Captures were conducted by the same male, adult volunteers for 12–18 person-nights each month, with half of the volunteers staying outdoors and half staying indoors, always at the same locations in the village. The second was pyrethrum spray collections in selected bedrooms and in storehouses. The human biting rate (HBR), which is the number of mosquito bites per person per night, was calculated as the number of mosquitoes captured on human bait during the month divided by the number of person-nights.

Anopheline identification. The identification of the ano-

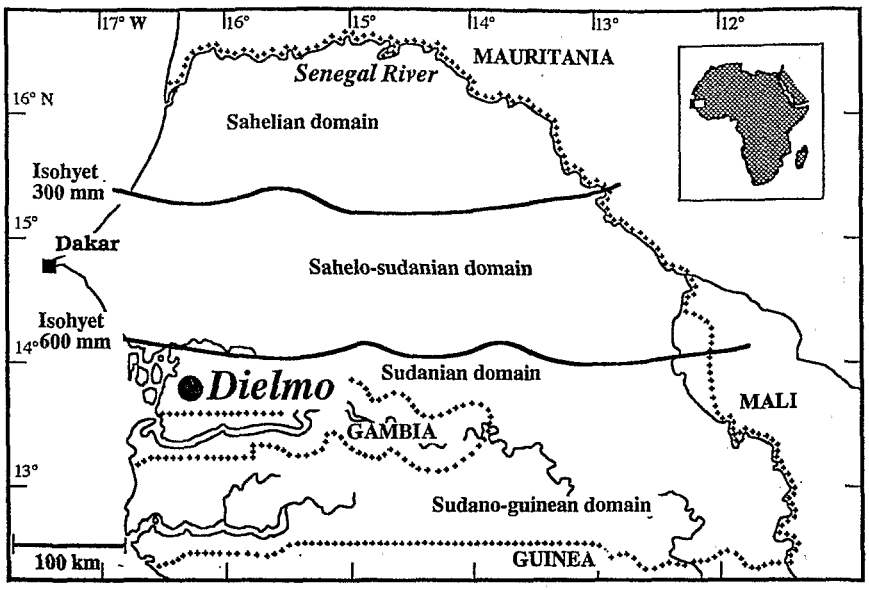

FIGURE 1. Map of Senegal showing the study area.

phelines was made in the field following the Gillies and DeMeillon morphologic identification keys.[13] The mosquitoes of the *An. gambiae* complex were stored for further identification by PCR in Dakar.

Field processing of anophelines. The salivary glands of mosquitoes collected from April 1992 to March 1994 on volunteers were dissected and examined for malaria sporozoites. When more than 40 anophelines were caught in an hour, only 40 were randomly selected for dissection. Due to the lack of time, mosquitoes collected from April 1994 to March 1995 were not dissected. All the anophelines caught (dissected or not) were stored in 1.5-ml tubes with desiccant for further laboratory analysis. These tubes were stored at −20°C in Dakar.

Laboratory processing of anophelines. The heads and thoraces of all anopheline specimens were tested for circumsporozoite (CS) protein of *P. falciparum, P. malariae,* and *P. ovale* using an enzyme-linked immunosorbent assay (ELISA) described by Burkot and others[14] and modified by Wirtz and others,[15] and the CS protein rates were calculated. *Plasmodium vivax* is not present in the area. The EIR was calculated by multiplying the HBR calculated monthly by the monthly CS protein rate (also referred to in this article as the infection rate). The mosquitoes belonging to the *An. gambiae* complex were identified using the PCR technique described by Scott and others, with minor modifications.[16] Briefly, DNA was extracted from mosquito legs in the Dakar laboratory, and the reactions were performed in 50 µl of PCR mixture using *An. gambiae* complex ribosomal DNA intergenic spacer species-diagnostic primers. Some specimens were processed by putting one leg directly in the reaction mixture without extraction of the DNA. The length of the amplified sequences was 315 nucleotides for *An. arabiensis,* 390 for *An. gambiae,* and 464 for *An. melas.* For technical reasons, if more than 30 anophelines from the *An. gambiae* complex were caught each month using each method (outdoor human bait, indoor human bait, bedroom-resting mosquitoes, and storehouse resting mosquitoes), only 30–50 were randomly selected for each of the methods for processing. If less than 30 specimens were caught, all were tested. Thus, the likely number of individuals per species captured by method each month was calculated by extrapolation. Also, all mosquitoes found positive after salivary gland dissection or CS protein ELISA were processed using the PCR.

RESULTS

Captures of mosquitoes. From April 1992 to March 1995, 17,370 anophelines belonging to malaria vector species were collected in resting sites and during 470 person-night of captures on human volunteers. A total of 5,744 *An. funestus* and 11,626 anophelines from the *An. gambiae* complex were collected, of which 2,337 were processed by the PCR. *Anopheles arabiensis, An. gambiae,* and *An. melas* were present in the village. Nonvector species, such as *An. coustani, An. freetownensis, An. pharoensis, An. rufipes, An. ziemanni,* and species from *Aedes, Culex* and *Mansonia* genera were also captured. The percentage of females by species captured by each method is presented in Table 1 using extrapolation for specimens belonging to the *An. gambiae* complex. Approximately the same number of anophelines vectors were captured on humans indoors and outdoors.

Anopheles arabiensis accounted for the majority (56.1%) of the mosquitoes collected and was the species collected most on human bait. *Anopheles arabiensis* was more exophagic than *An. gambiae* and *An. funestus.* It was also more exophilic: 59% of the vector females caught feeding inside on human bait were *An. arabiensis* versus only 26.9% of the females resting in bedrooms and caught by indoor spraying. *Anopheles funestus,* which represented 33.1% of the total captures, was the most frequent species among the endophilic vectors captured, particularly in bedrooms. *Anopheles gambiae* represented only 10.8% of the total vectors captured. Only 12 *An. melas* were caught, of which only one was caught on human bait.

TABLE 1
Number and percentage of malaria vectors caught from April 1992 to March 1995 by different methods in Dielmo, Senegal*

	Feeding†		Resting		
	Outdoors	Indoors	Bedrooms	Storehouses	Total
No. of *An. funestus* captured	1,783	2,099	1,630	232	5,744
No. of *An. gambiae* s.l. captured	5,286	5,039	930	371	11,626
Total number of vectors captured	7,069	7,138	2,560	603	17,370
No. of *An. gambiae* s.l. PCR-tested	898	773	461	205	2,337
% *An. funestus*	25.2	29.4	63.7	38.5	33.1
% *An. gambiae*	9.4	11.6	9.2	24.4	10.8
% *An. arabiensis*	65.4	59.0	26.9	36.3	56.1
% *An. melas*	0	0.0‡	0.2	0.8	0.1
Total %	100	100	100	100	100

* *An.* = *Anopheles*; PCR = polymerase chain reaction.
† Caught on human bait.
‡ Only one female was captured on human bait indoors (0.01%).

Variation of the HBR. The number and the relative proportion of each of the vector species strongly varied during the three year follow-up study (Figure 2). Among captures on human bait, *An. funestus* was much more abundant than the other vectors during the first year, while *An. arabiensis* dominated the two following years. *Anopheles gambiae* was the least captured vector each year.

Figure 3 shows the variations of the HBR, rainfall, and temperature for the three-year period. *Anopheles arabiensis* was captured during all 36 months, but showed a peak of abundance each year from June to October. It was very abundant in 1993 and 1994, with its HBR reaching 103 bites per person per night in July 1993 and 164 in August 1994. Unlike *An. arabiensis,* more *An. gambiae* females were captured the first year. The maximum HBR for this species was observed in September 1992 with 25 bites per person per night. Despite the presence of apparently favorable larval development sites, this species was rare or absent during each dry season (December to June).

Anopheles funestus was present throughout the survey, but showed great variation. It had a classic peak of abundance each year during the dry season (December to March).[13] However, the first year of the survey it was also very abundant in July and August, reaching 50 bites per person per night in July 1992. This species became less common in the second year but the number of specimens captured increased again at the end of the 1994 dry season.

Vector infection rates. The CS protein rate was calculated for each species each month. The ELISA method detected 1.9 times more *Plasmodium*-positive mosquitoes than did dissection. *Plasmodium falciparum* was found in 92% of the positive mosquitoes, *P. malariae* in 7.3%, and *P. ovale* in 1.7%. One *An. arabiensis* was positive for *P. falciparum* and *P. malariae,* and one was positive for *P. ovale* and *P. malariae*. The infection rate for each species varied greatly, not only according to the season, as it classically does, but also according to the year.

Each year *An. funestus* had the highest infection rate for *P. falciparum* (Table 2). Its mean rate calculated over the three years was 2.62%; it decreased by a factor of 2.5 between the first and the third year. These differences are highly significant ($P = 0.001$, by χ^2 test). The mean rate for *An. gambiae* was 1.46%. It was higher the first year and lower the second year. *Anopheles arabiensis* had a mean rate of 0.6% and decreased between the first and the third year. However, for *An. gambiae* and *An. arabiensis* the variations in the infection rate observed, depending on the year, were not significant ($P = 0.18$ and $P = 0.73$, respectively).

Variation of the EIR. The *P. falciparum* total EIR, calculated as described in the Materials and Methods, for the

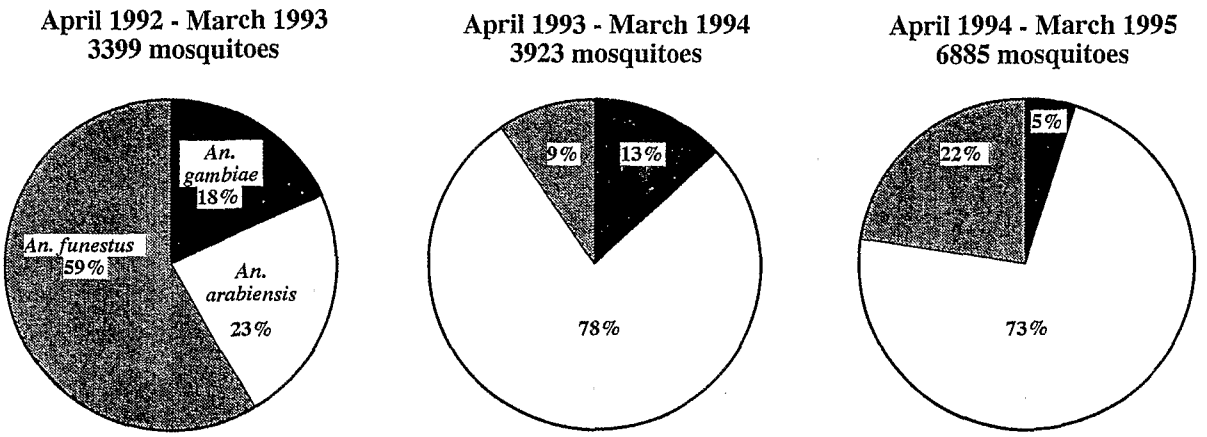

FIGURE 2. Relative proportions of the three vectors collected by outdoor and indoor human bait catches during a three-year period in Dielmo, Senegal.

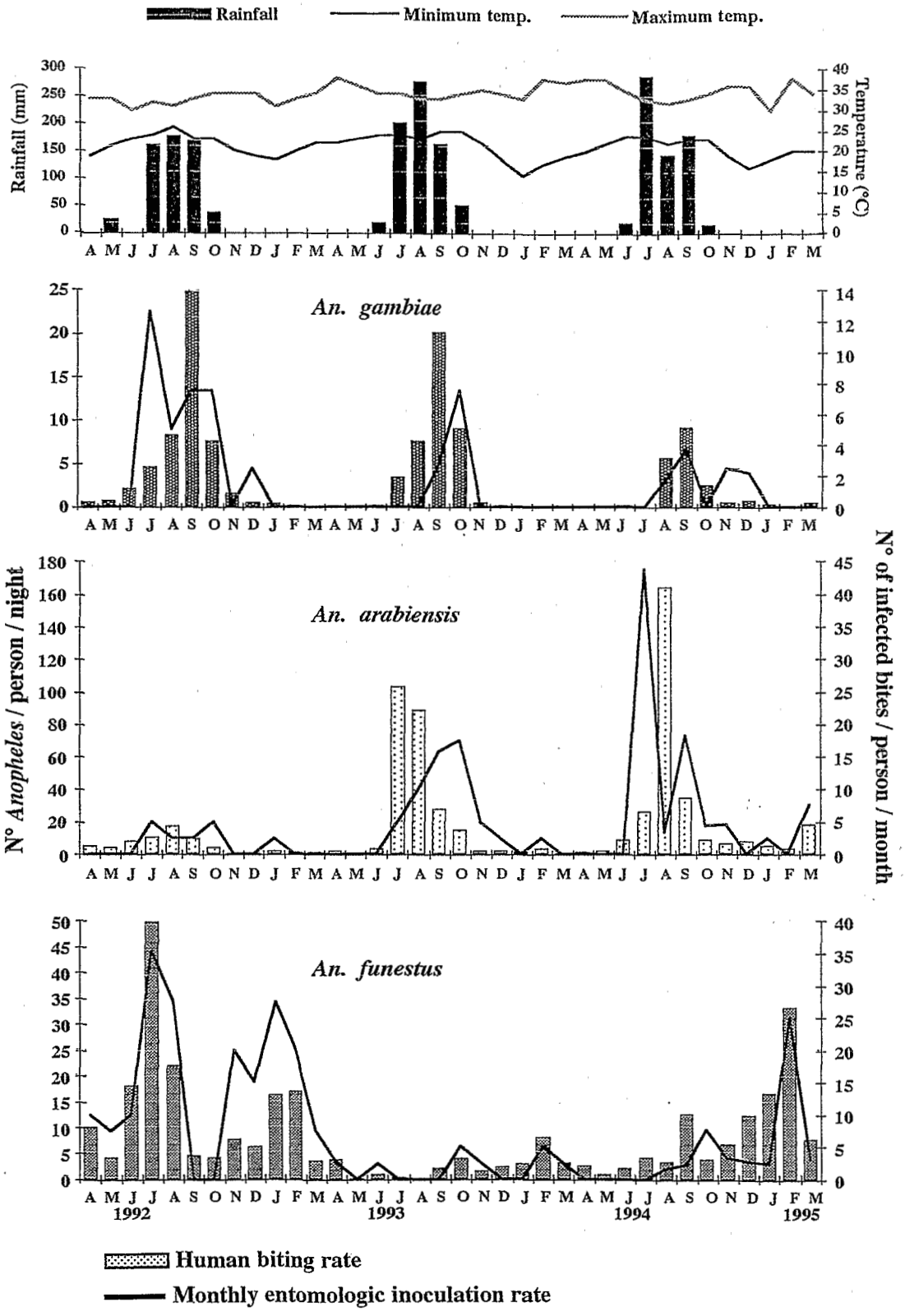

FIGURE 3. Rainfall, temperature (temp.), monthly human biting rate, and monthly entomologic inoculation rate calculated by enzyme-linked immunosorbent assay for all three *Plasmodium* species for each vector from April 1992 to March 1995 in Dielmo, Senegal.

TABLE 2

Infection rate, calculated by enzyme-linked immunosorbent assay from the head-thoraces of mosquitoes captured on human bait, for each *Plasmodium* species and each vector species by year*

	First year				Second year				Third year			
	No. tested	*P.f.*	*P.m.*	*P.o.*	No. tested	*P.f.*	*P.m.*	*P.o.*	No. tested	*P.f.*	*P.m.*	*P.o.*
An. gambiae	618	2.10	0.16	0	509	0.78	0	0	314	1.27	0.32	0
An. arabiensis	797	0.75	0.13	0.13	2,993	0.63	0.10	0.03	4,410	0.54	0.09	0
An. funestus	1,977	3.49	0.1	0.05	363	2.20	0	0	1,277	1.40	0.08	0

* *P.f.* = infection rate for *P. falciparum*; *P.m.* = infection rate for *P. malariae*; *P.o.* = infection rate for *P. ovale*; *An.* = *Anopheles*.

three-year period was 437 infected bites per person: 223 the first year, 79 the second year, and 135 the third year. Despite the low infection rate for *P. malariae* and *P. ovale,* their mean annual EIRs were 10.8 and 2.5 infected bites per person, respectively (Table 3). Table 4 shows the total EIR for *P. falciparum* for each of the 12 quarters, and the confidence interval calculated following the Poisson distribution.[17] The maximum EIR observed was approximately 95 (95% confidence interval = 69–133) infected bites during the rainy season from July to September 1992, while the EIR was 0 from April to June 1994 during the dry season when no ELISA-positive mosquitoes were found. For each season, large variations in the EIR were observed during the different years.

Figure 3 shows the variation in the EIR by month for each of the three vectors. The EIR was calculated by ELISA for each vector species. During the first year, transmission was higher, reaching 53 infected bites per person in July 1992. The comparative role of each vector according to each month is clearly identified. The first year, *An. funestus* was the main vector, accounting for 76% of all transmission, with 180 infected bites per person. At the end of the rainy season, *An. gambiae* also played a prominent role, whereas *An. arabiensis* was only a secondary vector. In contrast, during the following two years, *An. arabiensis* was responsible for the most transmission, sometimes with sudden variations from one month to another, as in June and July 1994, when the monthly EIR for *An. arabiensis* increased from 0 to 44 (Figure 3).

DISCUSSION

This study shows the high annual and seasonal variations of malaria transmission in Dielmo. Mosquitoes were present throughout the year because of the permanent stream and the presence of different kinds of anopheline larval development sites. One aim of the study was to learn as accurately as possible the rate of transmission of malaria by the different vectors to determine the relationship between the variations in transmission and the variations in clinical incidence, parasitemia, and immunologic parameters. Great care must be taken in the analysis of these variations in clinical and biological data due to the great variation in transmission found at four different levels: 1) the relative vector frequency according to the place and method of capture, 2) the abundance and relative frequency of vectors according to the month, as is classically observed, but also according to the year, 3) the infection rate of each vector by year, and 4) the number of infected bites for all vectors, and for each species, for the year.

The identification of all mosquito specimens captured, particularly those caught on humans, is necessary. Since 1990, a PCR that identifies five species of the *An. gambiae* complex from individual dried mosquitoes has been used.[12, 16] This technique makes it possible to determine the species of every specimen collected, particularly those caught on humans, and not just the half-gravid ones, as is the case with cytogenetic methods. Prior to this study, we demonstrated that the PCR technique gave the same identification as cytogenetics on West African mosquitoes of the *An. gambiae* complex.[18] Previous cytotaxonomic studies have shown two main cytotypes in the *An. gambiae* specimens of this region: savanna and bissau.[19]

Comparative studies on transmission using only cytogenetics on half-gravid females captured as indoor resting mosquitoes for the determination of specimens of the *An. gambiae* complex are often biased because this type of sample leads to an overrepresentation of *An. gambiae,* which is well known for its endophilic behavior, as our results clearly show. Among specimens from the *An. gambiae* complex, only 14.4% of the mosquitoes captured on humans were *An. gambiae,* whereas this rate was 25.4% and 39.6% for mosquitoes collected by spraying in bedrooms and storehouses, respectively.

There are few studies on the transmission of malaria that have analyzed differences in transmission according to the

TABLE 3

Annual entomologic inoculation rate calculated by enzyme-linked immunosorbent assay for the three *Plasmodium* species by vector species*

	First year			Second year			Third year		
	P.f.	P.m.	P.o.	P.f.	P.m.	P.o.	P.f.	P.m.	P.o.
An. gambiae	32.5	2.5	0	10.1	0	0	8.4	1.7	0
An. arabiensis	17.5	2.5	2.5	48.2	7.6	2.6	81.0	10.7	0
An. funestus	172.8	5	2.5	20.2	0	0	45.8	2.5	0
Total	222.8	10	5	78.5	7.6	2.6	135.2	14.9	0

* *P.f.* = *P. falciparum*; *P.m.* = *P. malariae*; *P.o.* = *P. ovale*; *An.* = *Anopheles*.

TABLE 4

Entomologic inoculation rate for *Plasmodium falciparum* calculated by enzyme-linked immunosorbent assay with 95% confidence intervals (values in parentheses), by quarter from April 1992 to March 1995 in Dielmo, Senegal

	April to June	July to Sept	Oct to Dec	Jan to March	Total
April 1992 to March 1993	27.5 (14–50)	95.3 (69–133)	47.5 (29–75)	52.5 (33–80)	222.8 (179–274)
April 1993 to March 1994	5.2 (1–18)	23.1 (11–44)	40.1 (23–66)	10.1 (3–26)	78.5 (53–111)
April 1994 to March 1995	0 (0–9)	68.8 (49–93)	25.7 (15–43)	40.7 (23–65)	135.2 (106–173)

month by the different vector species. In Africa. the only published work is that of Taylor and others,[20] but the determination of the species of the *An. gambiae* complex was done only on anophelines caught resting indoors by radiolabeled DNA probes. In our study, only 12 *An. melas* were caught. The larval development sites of this halophilic and zoophilic species are located near the mangrove forest 6 km west of Dielmo, and probably very few specimens reached the village. The infection rate was calculated by ELISA because this method allowed the processing of all mosquitoes and the comparison of the results using the same supplies and technique. To limit the overestimation of the sporozoite index by this method, only the head-thoraces were tested. While dissection in the field was done by different people and sensitivity might have varied, the results of the ELISA always remained the same throughout the survey and made possible the comparison from month to month. In our study, the ELISA detected 1.9 times more positive mosquitoes than dissection. This higher value is similar to the results of other studies.[8, 21]

Using a standardized protocol for collecting and analyzing mosquitoes for three years, we observed very large variations in malaria transmission in Dielmo. As is classically observed in Africa, the EIR for the three vectors varied greatly according to the month, with a peak of transmission during and at the end of the rainy season from July to September. More surprising were the great variations in the entomologic components of transmission, such as the HBR and the mosquito infection rate, as well as the number and relative proportion of the three vectors over the three years.

From April to June 1994, no mosquitoes carrying CS protein were found in 179 captured, whereas two years before during the same season, 28 infected bites were recorded. Likewise, transmission was higher from January to March 1993 in the dry season than during the following rainy season from July to September. These data show that in Dielmo, where larval development sites are permanent, there is no justification for dividing the year into only two seasons (dry and rainy) to assess transmission and to correlate it with clinical and immunologic data because there are too many variations between the years.

The role of each vector species was different for each of the three years. The first year, *An. funestus* had a much greater effect on transmission than *An. gambiae* and *An. arabiensis*. This was due to two factors: a higher HBR and a higher mosquito infection rate due to this species longer life expectancy and its higher anthropophilic rate (Fontenille D, unpublished data). Its role in transmission in Dielmo was particularly significant since it was the main vector during the dry season and it ensured continuous transmission throughout the year. For these reasons, this species probably plays an important role in maintaining immunity in Dielmo. In the second and the third years, *An. arabiensis,* which is generally considered to be a less efficient vector, was the major vector due to its very high HBR, despite its low infection rate. *Anopheles arabiensis* accounted for 10%, 66%, and 61%, respectively, of the transmission over the three-year period. Transmission by *An. gambiae* was the lowest except in the first year, during which its EIR was twice that of *An. arabiensis*. Over the three-year period, *An. gambiae* was responsible for only 12% of the transmission.

These data differ from those obtained in other regions of West Africa, where it is generally considered that *An. gambiae* and *An. funestus* are more efficient vectors than *An. arabiensis*.[22] In the Gambia, 30 km south of Dielmo, studies carried out during the rainy season between 1979 and 1981 and in 1988 showed that *An. gambiae* was largely dominant.[6, 19] This was also the case in Burkina Faso in the region of Bobo Dioulasso.[23] To our knowledge, no study has found such an important role for *An. funestus* in West Africa, as was found in Dielmo. Some years, for as yet unidentified reasons, *An. funestus* can be very abundant and transmission can be higher during the dry season than during the rainy season. This species is, however, present in the entire Sudanese and Guinean climatic region, and is an important vector.[24] In East Africa and Madagascar, it can play a major role.[8, 20, 25]

During the three-year study, we were unable to find any explanation, for example, temperature or rainfall, that could predict the variation of the observed transmission and the involvement of different vector species.

These detailed data on transmission are necessary to correlate with data on parasitemia, incidence of clinical attacks, associated immune responses, human genetic factors, and variation of *Plasmodium* polymorphism. Recent studies show that several different *Plasmodium* genotypes exist in Dielmo.[26, 27] These genotypes could have varying pathogenicities and may not be transmitted with equal efficiency by the different vector species. For these reasons, only a very thorough study of transmission would provide useful data for associated research.

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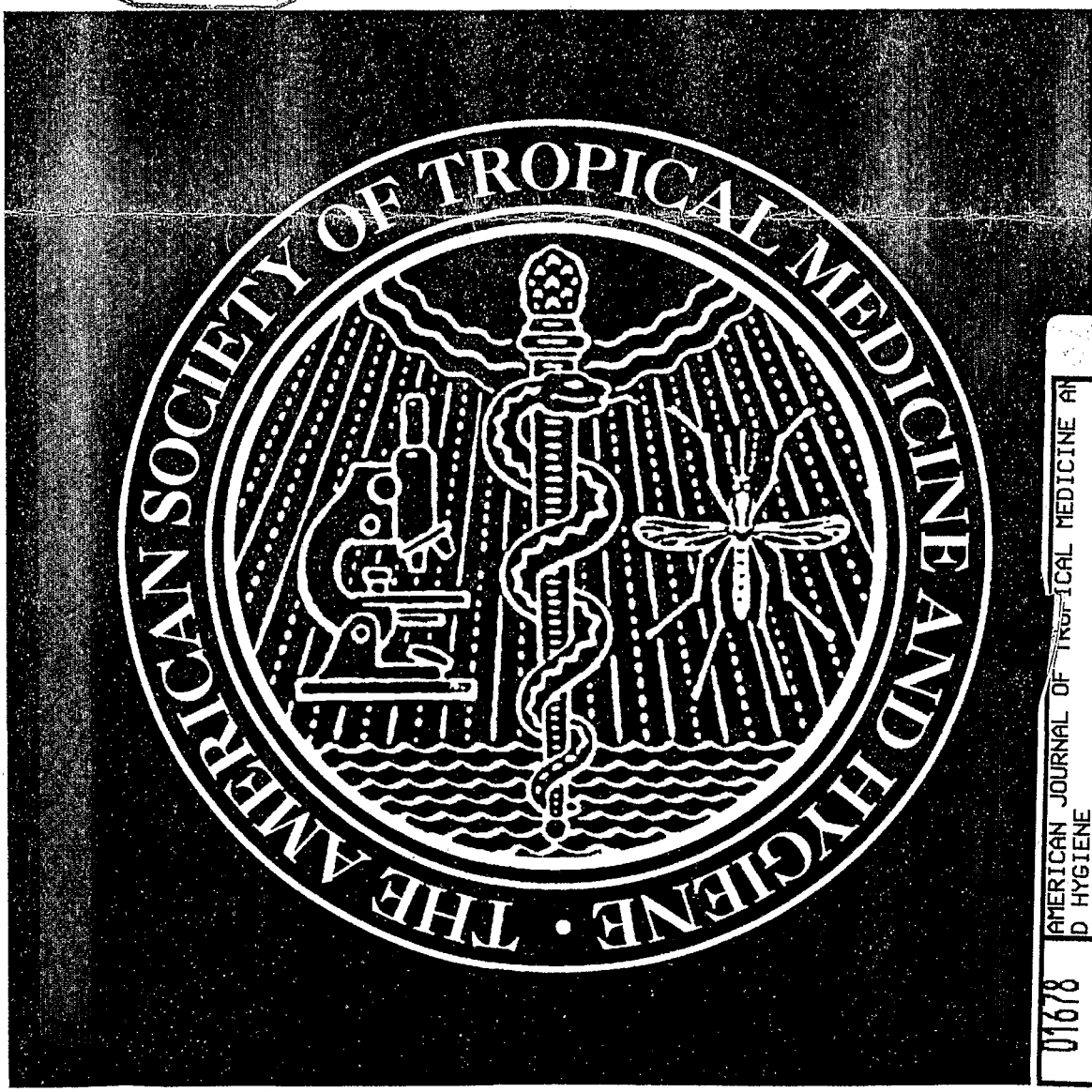

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