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THE DIELMO PROJECT: A LONGITUDINAL STUDY OF NATURAL MALARIA INFECTION AND THE MECHANISMS OF PROTECTIVE IMMUNITY IN A COMMUNITY LIVING IN A HOLOENDEMIC AREA OF SENEGAL

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Abstract. The Dielmo project, initiated in 1990, consisted of long-term investigations on host-parasite relationships and the mechanisms of protective immunity in the 247 residents of a Senegalese village in which malaria is holoendemic. *Anopheles gambiae* s.l. and *An. funestus* constituted more than 98% of 11,685 anophelines collected and were present all year round. Inoculation rates of *Plasmodium falciparum, P. malariae,* and *P. ovale* averaged respectively 0.51, 0.10, and 0.04 infective bites per person per night. During a four-month period of intensive parasitologic and clinical monitoring, *Plasmodium falciparum, P. malariae,* and *P. ovale* were observed in 72.0%, 21.1% and 6.0%, respectively, of the 8,539 thick smears examined. Individual longitudinal data revealed that 98.6% of the villagers harbored trophozoites of *P. falciparum* at least once during the period of the study. Infections by *P. malariae* and *P. ovale* were both observed in individuals of all age groups and their cumulative prevalences reached 50.5% and 40.3%, respectively. Malaria was responsible for 162 (60.9%) of 266 febrile episodes; 159 of these attacks were due to *P. falciparum,* three to *P. ovale,* and none to *P. malariae.* The incidence of malaria attacks was 40 times higher in children 0–4 years of age than in adults more than 40 years old. Our findings suggest that sterile immunity and clinical protection are never fully achieved in humans continuously exposed since birth to intense transmission.

Despite uncertainty as to the accuracy and reliability of the methods used to measure malaria morbidity and mortality,[1–3] epidemiologic studies in regions of high malaria endemicity have consistently shown that the severity of the disease decreases considerably after the first years of life. Deaths from malaria occur mainly among infants and young children,[4,5] and a marked decrease in the incidence of clinical attacks is observed after five years of age.[6] In contrast, parasite prevalence and incidence remain high throughout adolescence and only decrease slowly in adults.[7,8] This nonsterilizing protective immunity has been progressively recognized since the historic studies of Christophers in the 1920s.[9] However, despite decades of research, knowledge of how parasitization results in disease is incomplete and no clear picture of the mechanisms of naturally acquired immunity to malaria has yet emerged.[10–12]

To understand the dynamics of the host-parasite balance, its relationship to transmission, and to identify those factors that contribute to the development of disease, it is essential to carry out studies of well-defined cohorts in whom malaria parameters are recorded longitudinally. With this objective, we began in 1990 a longitudinal study covering the whole population of the village of Dielmo in Senegal, in which malaria is holoendemic. By choosing a small and stable population in which numerous parameters could be studied over several years, our intention was to make an integrated analysis of individual data in persons of all ages with clearly defined epidemiologic, clinical, biological, and immunologic histories.

The present paper provides information on the study area, the methodology used to collect entomologic, parasitologic, and clinical data, and describes the main epidemiological characteristics of malaria in Dielmo.

MATERIALS AND METHODS

Choice of the study area. The village of Dielmo (13°45′N, 16°25′W) was chosen after preliminary surveys carried out from June to December 1989 in various villages of the Sine-saloum region of Senegal. In addition to the study of malaria endemicity, investigations were made during the preliminary surveys on the presence of chloroquine in urine samples, the presence of antimalarial drugs in the home, self-treatment practices, frequency of visits to health centers, the stability of the population, and the population's acceptance of the research protocol. Three criteria played a decisive role in the choice of Dielmo: a very high malaria prevalence (86% of the children's blood films were positive at the end of the dry season in June 1989), a total absence of chemoprophylaxis, and the low attendance at health centers (0.27 visits per inhabitant in 1989, which was four times less than the average for the district).

After reaching an agreement with the leaders and the population of the village and the national authorities, a field station was built in the village, including a laboratory building with a dispensary and seven huts to accommodate the project staff. The station was built between January and March 1990, and a major part of the construction work was carried out by the villagers themselves. The project protocol and objectives were carefully explained to the assembled village population at each stage of the study. Informed consent was obtained individually from all participants or their parents. Approval was obtained from the Ministere du Plan et de la Cooperation and the Ministere de la Sante Publique.

Area, climate, and population. Dielmo is in an area of Sudan-type savanna, 280 km southeast of Dakar and about 15 km north of the Gambian border, a 4-hr drive from Dakar (Figure 1). Rainfall occurs during a four-month period. The first rains usually start at the end of June and the last rains occur in mid-October. Annual rainfall, which averaged 1,000 mm before 1972, has decreased to 700 mm over the last 20 years. From our readings, it was 635, 611, and 583 mm in 1990, 1991, and 1992, respectively, when this study took place.

Dielmo is situated on the marshy bank of a small permanent stream (the Nema) that permits the persistence of anopheline breeding sites year round (Figure 2). Most of the houses are built in the traditional style with mud walls and thatched roofs. In 25 of the 87 houses (29%), corrugated iron replaces the thatching, but a space is left between the roof and the tops of the walls.

The inhabitants of Dielmo are settled agricultural workers. Millet and peanut crops are cultivated during the wet season; market gardening and rice growing are dry season agricultural activities made possible by the existence of the stream. Small herds of domestic animals, approximately four dozen head of beef cattle, 300 sheep and goats, and 20 donkeys, live in close contact with the houses. The ground is sandy and the original wooded savannah has been almost entirely cleared for cultivation.

In January 1990, the initial survey showed a population of 250 (sex ratio F:M = 0.98) of which 20.4% were children less than five years of age and 26.8% were children 5–14 years of age. The inhabitants were divided into 29 compounds: 20 in Dielmo itself (195 inhabitants) and nine in the small hamlet of Santhe-Mouride 300 meters away (55 inhabitants). The ethnic groups consisted of 78% Serere (Niominka: 58% and Sine/Baol: 20%), 13% Mandingue, and 9% miscellaneous. Persons who had attended school for at least a year represented 6% of the population more than six years of age (18% in the region in rural areas) and only one person could read and write.

Entomologic monitoring and typing of sporozoites. Entomologic studies began in April 1990. Night captures using human bait were conducted indoors and outdoors during the first week of each month, on two (April to June 1990) or three (July 1990 to June 1992) successive nights. The mosquitoes were captured from 9:00 PM until 7:00 AM by four groups of two collectors (two indoors and two outdoors), with each collector alternately working and resting for 1 hr. After identification, the anophelines were immediately dissected and examined for sporozoites. For rainy season captures, which yielded an abundant number of anophelines, only a representative sample with respect to hour and place was dissected.

Monoclonal antibodies (MAbs) directed to the circumsporozoite (CS) protein of *Plasmodium falciparum* (clone 2A10), *P. vivax* (clone NMRI 3/KA52.5), *P. ovale* (clone 110.54.3/JG1 5.5), and *P malariae* (clone 109.179.4/JCO9.5) were

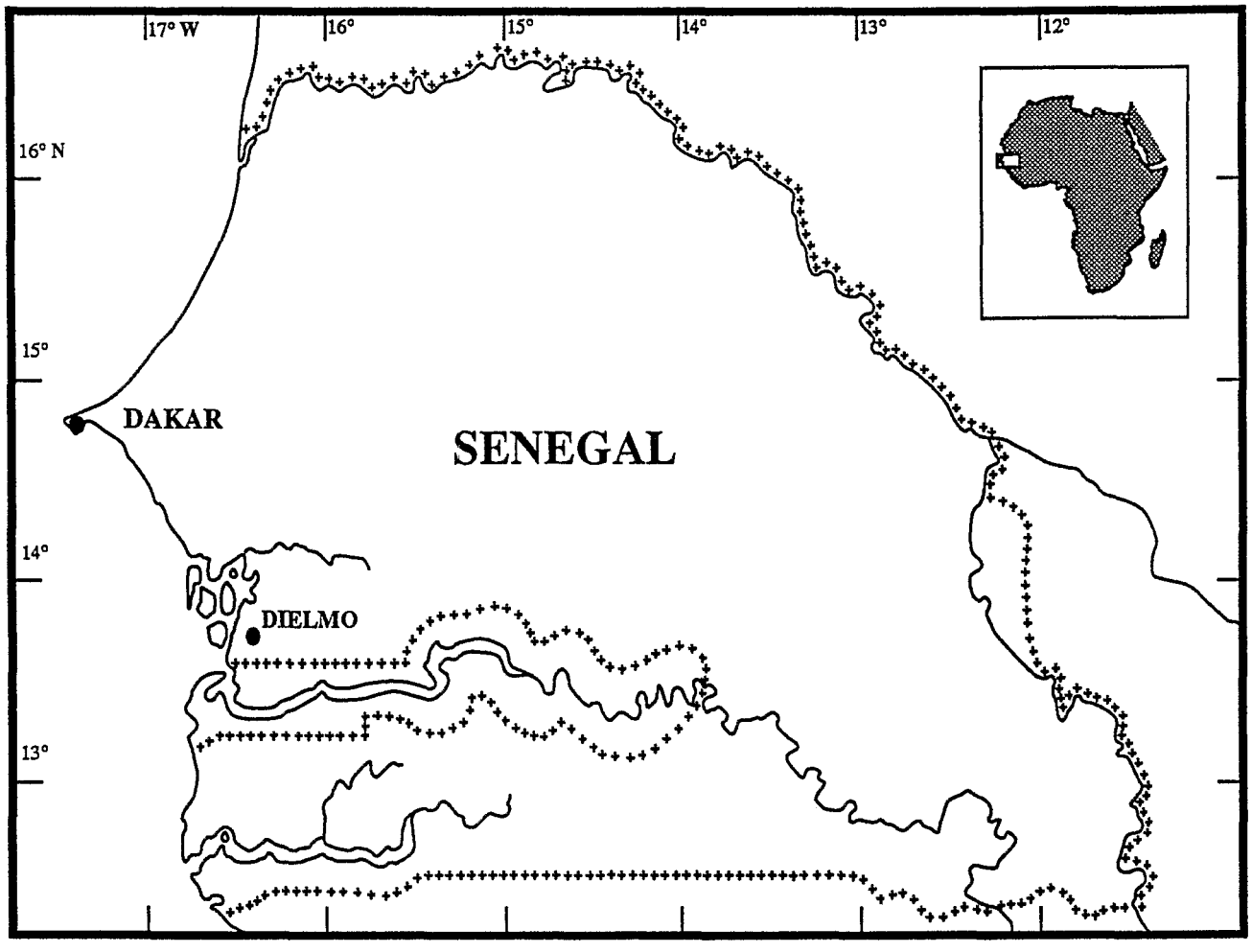

FIGURE 1. Map of Senegal showing the capital city, Dakar, and the study area, Dielmo.

reacted sequentially with sporozoites from each mosquito found infected. Acetone-fixed dissection slides were incubated with the first MAb at a concentration of 10 μg/ml in phosphate-buffered saline (PBS)-1% bovine serum albumin (BSA) buffer, washed, and reacted with fluorescein isothiocyanate–labeled anti-mouse IgG, IgA, and IgM at a 1:200 dilution. The number of labeled versus unlabeled sporozoites was determined by examination under epifluorescence combined with phase-contrast microscopy. Before proceeding with the next MAb, the slides were incubated in 0.2 M glycine buffer, pH 2.5, for 10 min to remove the antibodies and thereafter washed twice for 10 min in PBS-BSA buffer. Fluorescence was used to check that no residual labeling remained before incubating with the next MAb. *Plasmodium falciparum* and *P vivax* sporozoites raised by feeding *Anopheles stephensi* and *An. gambiae,* respectively, with the corresponding gametocytes were used as controls to check the specificity of this sequential procedure. No cross-reactivity between the four MAbs used was found.

Clinical and parasitologic monitoring. At the end of the initial census, each person was given a unique number for the project and a file was prepared that contained a photograph and details of genealogy and family ties, places of residence since birth, occupation, and other activities. Between May 28 and June 4, 1990, each villager was given a clinical examination and a series of biological tests that included basic hematology, glucose-6-phosphate dehydrogenase deficiency, stool and urine examinations for parasites, and urinalysis. Humoral and cellular responses to recombinant malarial antigens were also measured. Close follow-up examinations were also conducted and are reported here through September 1990.

Three times a week (Monday, Wednesday, and Friday or Tuesday, Thursday, and Saturday) each villager was visited at home to record his or her temperature and to fill out a questionnaire

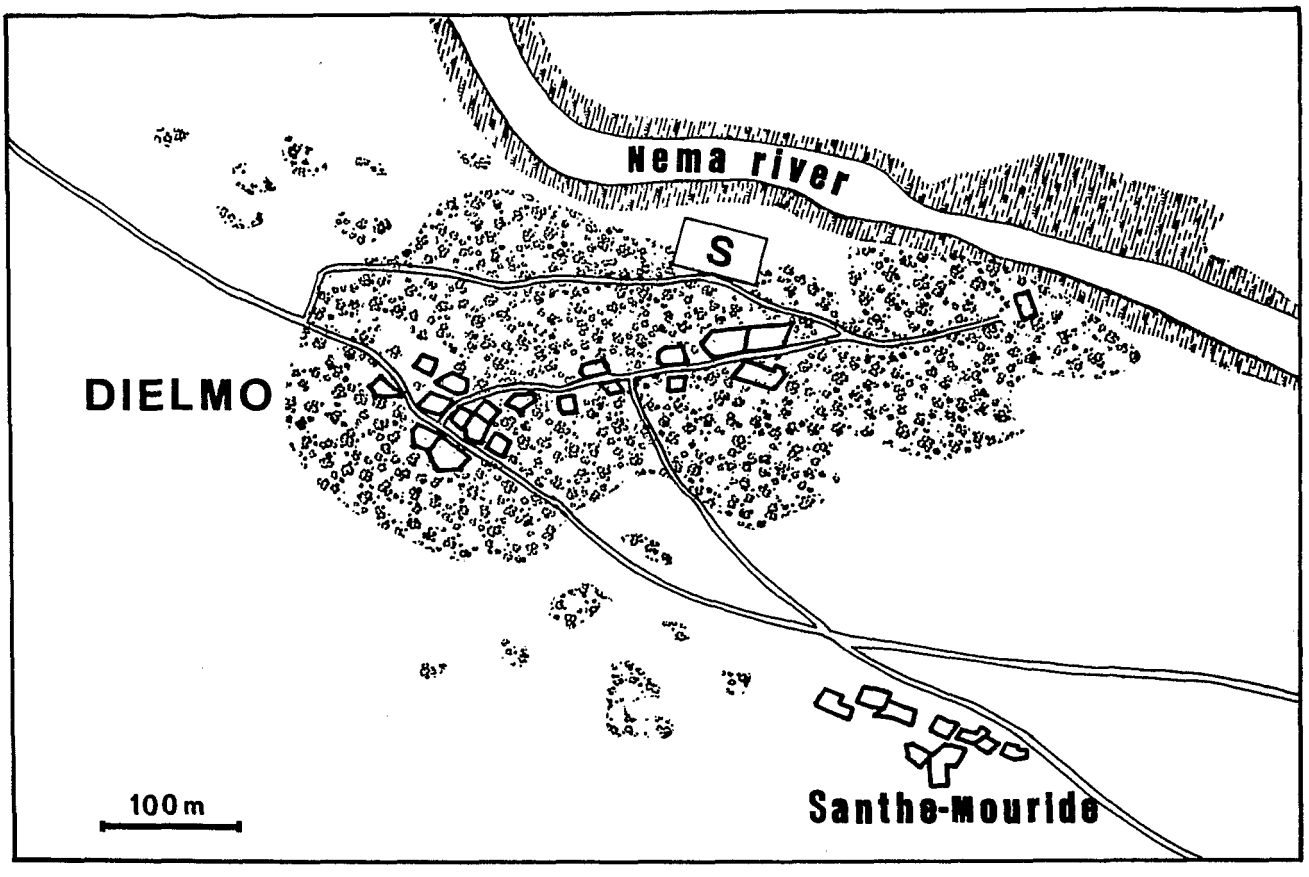

FIGURE 2. Map of Dielmo village and Santhe-Mouride hamlet showing the localization of the 29 compounds, the field station (S), and the Nema river.

about symptoms that had occurred during the previous 48 hr. Twice a week, during the first and last weekly visit, a thick blood film was made. Rectal temperature was read in children less than seven years of age, and axillary temperature was read in older persons. The questionnaires (in Serere and Wolof) were established with the assistance of linguists and doctors of the corresponding ethnic groups. They concerned nine categories of symptoms that were recognizable by the population; their time of onset as well as duration were also recorded.

In addition to the three systematic weekly visits, each compound was visited daily to rapidly detect new cases of fever. The dispensary at the station remained open day and night. Any patient reporting an episode of illness was examined by a doctor and information was recorded on standardized cards. In all patients with fever, an additional thick blood film was taken.

Patient management. Each episode of illness was immediately treated and patients with fever were visited at least twice a day until recovery. The only antimalarial drug used was quinine (Quinimax®; Sanofi-Labaz, Paris, France) at a dose of 25 mg/kg/day in three daily doses over three days.[13] It was administered only in the case of fever (axillary temperature $\geq$ 38.0°C or rectal temperature $\geq$ 38.5°C).

The criteria for malaria treatment, which were based mainly on age and parasite density in accordance with our own experience in a similar epidemiologic situation,[1] were established in advance for a trial period of one month, then continued for the following three months when it was confirmed that they were appropriate. Antimalarial treatment began immediately in the following cases: 1) fever with a parasite:leukocyte ratio $\geq$ 2 in children, 2) fever with a parasite:leukocyte ratio $\geq$ 0.5 in pregnant women, and 3) fever with a positive thick blood film in individuals with possible severe malaria (no case observed) or in individuals thought to have possibly lost protective immunity (villagers coming back from an area of low endemicity where they had lived for more than one year during the three years prior to the study).

For the remaining patients, symptomatic or specific treatment of nonmalarial diseases was

given according to the diagnosis. When fever persisted the next day, another thick blood film was taken. Criteria for antimalarial treatment remained unchanged, except for the following situations in which the requirement for antimalarial treatment was decided jointly by two doctors, taking into account all the patient's clinical, biological and epidemiologic data: 1) absence of clinical improvement with a parasite density close to the treatment threshold, 2) absence of clinical improvement with a positive thick blood film in infants and pregnant women, and 3) absence of clinical improvement with a parasite: leukocyte ratio ≥ 0.5 in adults.

During the first month of the study, only the first three doses of the treatment were administered by the medical staff, and the following two days' treatment was given to the patient for self administration. As a result of having observed several cases of incomplete treatment, we decided thereafter to control every dose of antimalarial drugs. The same applied for nonmalarial diseases requiring oral antibiotic treatment.

The population was asked not to use any drugs without informing the team, and urine tests were regularly carried out (on average once a month) to detect the presence of antimalarial drugs, using the modified Saker-Solomons test.[14] This test was carried out after any febrile episode. A total of 950 tests were done, of which only one positive result remained unexplained by our treatment. In this case, there had been a traditional treatment for diarrhea with an unknown plant root.

Thick blood film processing. All thick blood films taken from villagers were accompanied by the obtaining of a simultaneous temperature reading. In the absence of fever, two thick blood films were prepared (as a precautionary measure in case of detachment of blood or poor staining). Both were left to dry for 24–48 hr, then dehemoglobinized and stained with Giemsa. They were then stored, and apart from a few exceptions, were examined only after the study. In the case of fever, three thick blood films were prepared simultaneously. One of them was Giemsa-stained without previous dehemoglobinization and examined immediately so that a decision regarding treatment could be made (results were available 1–2 hr after blood sampling); the other two were dehemoglobinized, stained, and stored with the routine blood films to provide an independent control at the end of the study.

All thick blood film readings were standardized.[15] A total of 200 microscopic oil-immersion fields were examined on each slide (about 0.5 µl of blood). The ratio of trophozoites to leukocytes was established separately for each plasmodial species, either by counting the trophozoites until 200 leukocytes were observed (ratio ≥ 0.01) or from the total number of trophozoites observed on the 200 fields and an estimation of the average number of leukocytes per microscopic field (ratio < 0.01). The gametocytes were recorded separately and were not included in the asexual parasite count. For the blood films of febrile patients, a third reading was made in those cases where the parasite counts obtained with the rapid staining procedure differed significantly from those given by the independent control at the end of the study.

The mean number of leukocytes and its variation with age have been investigated in the study population (Rogier C and others, unpublished data). Average age-specific values were used for estimating the number of parasites per microliter of blood. Mean leukocyte counts ranged from 12,800/µl in infants to 5,400/µl in adults more than 60 years of age.

Staff and services. During the period from June to September 1990, clinical and biological data were collected by a field team of 16 persons consisting of three doctors, one biologist, two nurses, and 10 technicians. At least 12 of these persons, including two doctors, were present in the village 24 hr a day, six days a week. On Sunday, one doctor and three technicians were on duty. Entomologic data were collected by a scientist, two technicians and 18 villagers, who had been trained to capture mosquitoes.

RESULTS

Malaria transmission. *Vector density.* In 300 person-nights of captures over 26 months, 19,002 mosquitoes were caught, of which 11,685 (61.5%) were anophelines belonging to the following species: *An. gambiae* s.l., 7,227 (62.2%); *An. funestus,* 4,216 (36.1%); *An. pharoensis,* 152 (1.3 %); *An. rufipes,* 19 (0.2%); *An. ziemanni,* 15 (0.1%); and *An. squamosus,* 11 (0.1%). The number of *Anopheles* caught inside and outside of the dwellings was 6,385 and 5,300, respectively. The rate of endophagy was 52.7% for *An. gambiae* s.l. and 59.0% for *An. funestus.* Maximum aggressiveness for these two

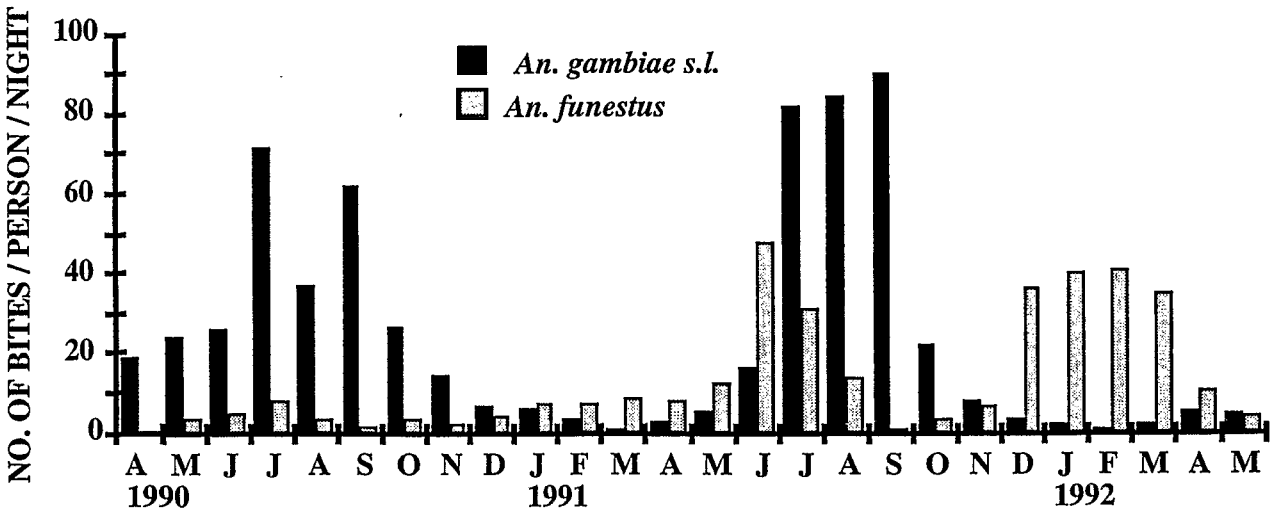

FIGURE 3. Monthly variations of the person-biting rate of *Anopheles gambiae* s.l. and *An. funestus* in Dielmo, April 1990–May 1992.

species was observed between 1:00 AM and 6:00 AM.

Both *An. gambiae* and *An. funestus* were captured every month over the 26-month study (Figure 3). For *An. gambiae,* the average annual density and seasonal variations differed only slightly from one year to another. A peak was invariably observed between July and September during the rainy season, with a maximum of 90.5 bites per person per night recorded in September 1991. In the dry season, the density of this vector was generally low (reaching a minimum of 0.9 bites per person per night in February 1991), but a distinct increase was noted each time the rainy season approached. For *An. funestus,* the average density was four times lower during the first year than during the second one. In the latter, two significant peaks were observed: the first just before the rainy season (48 bites per person per night in June 1991) and the second in the middle of the dry season (41 bites per person per night in February 1992).

Sporozoite rates and specific identification of sporozoites. The presence of sporozoites was determined by dissection of the salivary glands in 5,797 *An. gambiae* s.l. and 3,657 *An. funestus.* A total of 131 infections was observed: 83 in *An gambiae* s.l. (1.43%) and 48 in *An. funestus* (1.31%). Sporozoite rates were significantly higher in the second year than in the first year (1.57 versus 1.04; $P < 0.05$, by chi-square test), and in the rainy season than in the dry season (first year, 1.18 versus 0.83; $P < 0.05$; second year, 2.06 versus 1.14; $P < 0.05$). For each period, no significant difference was observed between *An. gambiae* s.1. and *An. funestus.*

The species of *Plasmodium* was determined on sporozoites samples using species-specific anti-CS MAbs in 110 of the 116 samples that were tested (presence of nonfluorescent sporozoites in three cases and loss of sporozoites from the slides in three other cases). Single *Plasmodium* species were present in 95 (86.4%) of 110 of the identified gland infections, and 15 (13.6%) of 110 contained two or more *Plasmodium* species (Table 1). It should be noted that the shape (length and thickness) of the sporozoites fluorescing with a given MAb made species identification possible. The relative proportions of each *Plasmodium* species in identified samples were 92.7% *P. falciparum,* 18.2% *P. malariae,* and 8.2% *P. ovale,* with no significant difference according to the season or the year (by Fisher's exact test or chi-square test). The proportions of *Plasmodium* species detected in *An. gambiae* s.l. and *An. funestus* were not significantly different (by Fisher's exact test or chi-square test).

Inoculation rates of P.falciparum, P. malariae, *and* P. ovale. Figure 4 shows monthly entomologic inoculation rates for each species of *Plasmodium* estimated from the following data: 1) mean number of *An. gambiae* and *An. funestus* bites per collector for the month in question, 2) mean sporozoite rate over three months (month of reference plus the month before and after), and 3) proportion of each species of *Plasmodi-*

TABLE 1

Species of Plasmodium *infections identified by epifluorescence in 110* Anopheles *mosquitoes with sporozoites in salivary glands detected by dissection*

Plasmodium species	No. (%) identified		
	An. gambiae	*An. funestus*	Total
P. falciparum	63 (84.0)	27 (77.1)	90 (81.8)
P. malariae	3 (4.0)	2 (5.7)	5 (4.5)
P. falciparum and *P. malariae*	5 (6.7)	1 (2.9)	6 (5.5)
P. malariae and *P. ovale*	1 (1.3)	2 (5.7)	3 (2.7)
P. falciparum, P. malariae, and *P. ovale*	3 (4.0)	3 (8.6)	6 (5.5)
Total	75	35	110

um in the 110 sporozoite samples identified by MAbs.

For the first year of clinical follow-up (June 1990–May 1991), the number of infective bites per person was estimated at 101.2, 19.9, and 8.9 for *P. falciparum, P. malariae,* and *P. ovale,* respectively, of which 74.3, 14.6, and 6.6, respectively, occurred between June and September 1990. Transmission increased considerably during the second year of the study with 272.5, 53.5, and 24.1 infective bites per person for the three species, respectively, despite no major variation in rainfall or other climatic parameter.

Spleen rate. At the beginning of June 1990 (dry season), the proportion of children 0–1, 2–9, and 10–14 years of age with an enlarged spleen was 61% (14 of 23), 87% (60 of 69), and 73% (19 of 26), respectively. The average enlarged spleen (AES) index was 2.57, 2.33, and 1.63, respectively. Seven (5.4%) of 129 adults had enlarged spleens. Three of them were between 15 and 17 years of age, the others were between 25 and 74 years of age, and all had lived for the major part of their life in Dielmo.

At the end of September (rainy season), the spleen rate and the AES index were 89% and 2.20, respectively, in children 2–9 years old and 72% and 1.61 in children 10–14 years old. No significant variation was observed between June and September (by Wilcoxon signed rank test). Table 2 shows individual variations in spleen size in children 2–14 years of age. For most children, the class of spleen remained unchanged.

Parasitology. *Monitoring rates.* Of the 247 inhabitants of Dielmo (118 children and 129 adults) present in the village at the beginning of June 1990, 222 (90%) could be followed-up over a four-month period with a compliance rate of 90–100% (at least 30 systematic thick blood films made two times each week on asymptomatic persons during the 17 weeks of study, and in case of absence, a maximum interruption of

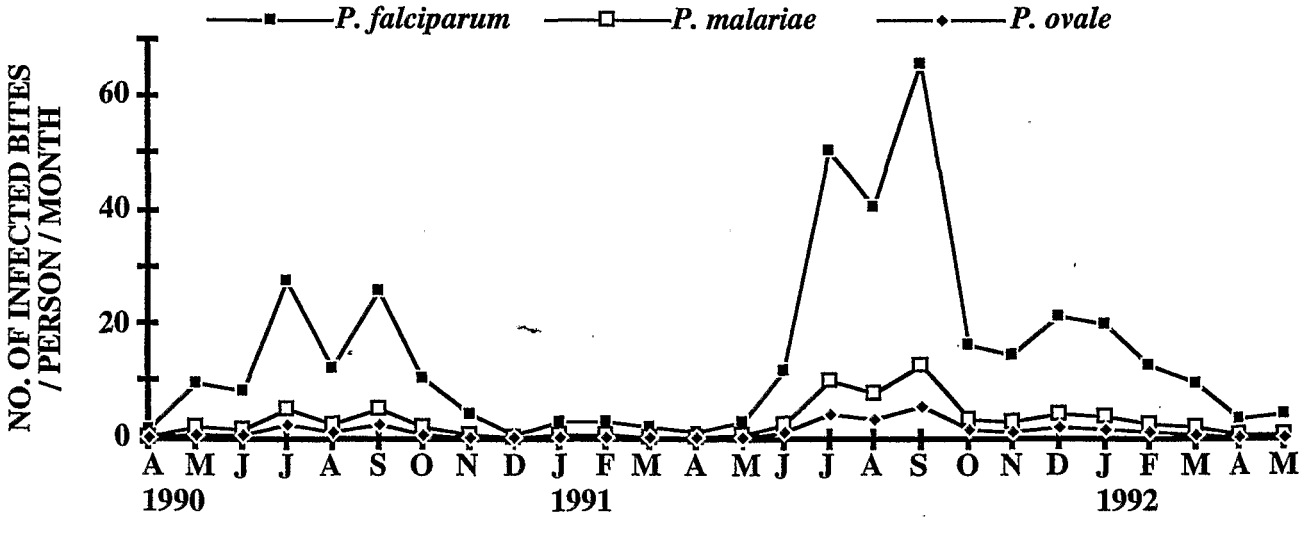

FIGURE 4. Monthly variations of the inoculation rates of *Plasmodium* species in Dielmo, April 1990–May 1992.

TABLE 2

Variations of spleen size between June and September 1990 in 88 children 2–14 years of age

Spleen size in September 1990*	Spleen size in June 1990					
	0	1	2	3	4	Total
0	8	4	1	0	0	13
1	3	10	8	0	0	21
2	3	7	10	13	0	33
3	0	0	7	9	2	18
4	0	0	0	1	2	3
Total	14	21	26	23	4	88

* Classification of spleen sizes according to Hackett's method.

10 days in the parasitologic and clinical follow-up). For these villagers, the total number of thick blood films carried out either systematically or during febrile episodes was 8,007, an average of 36 blood films per person.

For 25 inhabitants of Dielmo (12 children and 13 adults), the monitoring rate was less than 90%. Of these, three persons died after the beginning of the study (prematurity with microcephalism, bronchopneumonia, and hepatocarcinoma, respectively) and 22 were absent for a period of 11–30 days (for 10 persons) or for more than a month (12 persons). For these villagers, 532 thick blood films were made, an average of 21 films per person. No refusals were observed, either for blood tests or clinical monitoring.

Parasite rates. Of 8,539 thick blood films examined, 6,331 (74.1%) were parasite positive: *P. falciparum,* 4,254; *P. malariae,* 133; *P. ovale,* 50; *P. falciparum/P. malariae,* 1,435; *P. falciparum/P. ovale,* 227; *P. malariae/P. ovale,* 3; and *P. falciparum/P. malariae/P. ovale,* 229. Results by age groups for the whole study population are given in Table 3. Tables 4–6 show the slide positivity rate for each of the four months of the study in the 206 persons (101 children and 105 adults) who continued to live in Dielmo during the follow-up period (maximum absence ≤ 10 days) and met the following criteria: 1) at least 50% of their life since birth spent in Dielmo or an area of high malaria endemicity (95–100% of their life for most adults and nearly all children), 2) continued presence in the village or a maximum absence of 30 days during the six months preceding the study period, and 3) a return to Dielmo at least two years prior to the study for persons having lived for more than a year in areas of low malaria endemicity. In June, 99% of the 243 thick blood films taken from children 2–4 years of age showed the presence of *P. falciparum* trophozoites. This proportion was constantly more than 80% up to the age of 14 years, and gradually decreased to a minimum of 21% in adults more than 60 years old. For *P. malariae,* the slide positivity rate was also at a maximum in children 2–4 years of age (58%) and decreased by a factor of 10 in adults. *Plasmodium ovale* was observed in all age groups with a maximum of about 12% in children 1–9 years of age.

In general, no clear variation in positivity rates was observed between June, the last month of the dry season, and September. One significant exception concerned adults more than 40 years of age for whom *P. falciparum* prevalence doubled between June and August-September. In young children, the slight decrease observed

TABLE 3

Age distribution of the study population and crude slide positivity rate for Plasmodium *infection of 247 villagers in Dielmo in June–September 1990*

	Age groups (years)									
	0	1	2–4	5–9	10–14	15–19	20–39	40–59	≥60	Total
No. of subjects	11	12	31	38	26	22	61	28	18	247
No. of blood films	330	439	1,126	1,362	930	773	2,045	920	614	8,539
P. falciparum (% positive)	67.6	76.1	92.6	88.7	92.6	77.1	58.6	47.2	40.2	72.0
P. malariae (% positive)	13.3	21.2	49.6	35.7	38.5	6.9	5.8	5.4	6.3	21.1
P. ovale (% positive)	2.7	6.8	9.8	13.3	8.5	3.0	1.6	2.2	3.9	6.0
All species (% positive)	67.9	77.5	94.5	90.1	94.8	77.6	60.6	50.8	46.9	74.1

TABLE 4

Slide positivity rate for Plasmodium falciparum, P. malariae, *and* P. ovale *trophozoites according to age group in 206 permanent residents of Deilmo in June–September 1990**

Species and period	Age groups (years)†								
	0 (254)	1 (312)	2–4 (888)	5–9 (1,096)	10–14 (819)	15–19 (707)	20–39 (1,598)	40–59 (806)	≥60 (556)
P. falciparum									
June	54.9	82.8	98.8	83.6	83.1	66.0	37.1	22.0	20.8
July	71.2	84.2	96.6	85.3	91.2	61.2	51.7	33.7	27.9
Aug	68.4	80.6	81.8	91.3	91.9	69.1	50.4	47.7	46.6
Sept	56.7	64.9	92.0	93.2	91.4	68.1	50.8	44.2	39.0
Total	62.6	78.2	92.5	88.3	89.3	66.1	47.3	36.5	33.1
P. malariae									
June	8.5	27.6	58.4	35.0	27.9	11.4	4.0	4.2	8.1
July	15.2	25.0	54.4	27.1	38.4	6.2	4.2	6.8	1.4
Aug	0.0	20.8	50.5	33.5	34.3	2.3	5.2	1.5	4.6
Sept	11.7	20.8	45.8	45.2	35.4	6.7	7.0	5.3	12.5
Total	9.1	23.7	52.4	35.3	33.8	6.6	5.1	4.5	6.7
P. ovale									
June	0.0	14.9	8.6	14.0	3.7	2.7	1.7	2.3	4.0
July	0.0	14.5	15.1	13.2	10.3	5.6	0.5	2.0	3.6
Aug	0.0	5.6	13.1	13.7	8.1	4.5	1.0	0.5	5.3
Sept	0.0	0.0	9.8	16.4	13.1	0.0	1.8	2.6	3.7
Total	0.0	9.0	11.5	14.3	8.7	3.3	1.3	1.9	4.1

* Thick blood smears taken during malaria treatment or less than seven days after treatment are excluded.
† The total number of thick blood smears examined is shown in parentheses. Each monthly value (not shown) is approximately one quarter of this number.

for the three plasmodial species could be explained by the treatment given for clinical attacks due to *P. falciparum* (reinfection occurred generally 2–4 weeks after treatment, and the course of mixed infections from *P. malariae* and *P. ovale* was interrupted).

Parasite densities. Figure 5 shows variations with age of *P. falciparum* density in the 206 permanent residents of Dielmo. Parasitemia was at a maximum in the second year of life, and from then on steadily decreased. In children 12–23 months of age, 51% of the thick blood films showed more than 5,000 parasites/μl of blood; only 6% of the positive blood films showed less than 500 parasites/μl of blood. The prevalence of parasitemia greater than 5,000 parasites/μl decreased to less than 5% in those 10 years of age and older and to less than 1% in those 20 years of age and older. Whereas 81% of the thick blood films of children 2–4 years of age showed more than 500 trophozoites/μl, this proportion decreased to only 2% in adults more than 60 years of age.

For *P. malariae* (Figure 6), maximum parasitemia was estimated at 9,600 parasites/μl in a child two years of age. Up to four years of age, approximately 60% of the positive thick blood films showed parasitemias greater than 500 par-

TABLE 5

Slide positivity rate for Plasmodium falciparum, P. malariae, *and* P. ovale *gametocytes according to age group in 206 permanent residents of Dielmo in June–September 1990**

Species	Age groups (years)†								
	0 (254)	1 (312)	2–4 (888)	5–9 (1,096)	10–14 (819)	15–19 (707)	20–39 (1,598)	40–59 (806)	≥60 (556)
P. falciparum	49.6	42.3	48.1	38.4	37.1	38.3	16.5	15.5	11.3
P. malariae	1.2	13.8	28.7	4.5	2.9	0.3	0.3	0.0	1.1
P. ovale	0.0	2.2	1.9	1.7	0.5	0.7	0.1	0.2	0.4

* Thick blood smears taken during malaria treatment or less than seven days after treatment are excluded.
† The number of thick blood smears examined is shown in parentheses.

TABLE 6

Monthly variations of the slide positivity rate for Plasmodium falciparum, P. malariae, *and* P. ovale *gametocytes in 206 permanent residents of Dielmo in June–September 1990**

Species	Months† June (1,877)	July (1,745)	Aug (1,695)	Sept (1,719)	Total (7,036)
P. falciparum	26.2	29.1	31.3	35.0	30.3
P. malariae	6.0	6.7	4.7	4.5	5.5
P. ovale	0.9	0.9	0.9	0.6	0.8

* Thick blood smears taken during malaria treatment or less than seven days after treatment are excluded.
† The number of thick blood smears examined is shown in parentheses.

asites/μl. It decreased significantly in the 5–9-year-old age group (less than 50 parasites/μl for 65% of the positive thick blood films). For those 15 years of age and older, prevalence decreased to a very low level (less than 7%) and only 2% of the positive thick blood films showed more than 50 parasites/μl.

For *P. ovale* (Figure 7), parasitemia was generally very low at all ages. The proportion of thick blood films showing more than 50 parasites/μl was only 46% at one year of age, 26% from 2–4 years of age, and about 15% thereafter in children as well as in adults. We observed 67% of the cases with a parasitemia greater than 500 parasites/μl in children less than five years of age (maximum of 31,700 parasites/μl in a one-year-old child) and 13% in young adults.

Cumulative prevalence of P. falciparum, P. malariae, *and* P. ovale. Between June 1 and September 30, 1990, malaria parasites were observed in 243 of the 247 inhabitants of Dielmo (98.4%). The four persons whose blood films remained constantly negative were an infant who died of a congenital defect in the third month of follow-up, a child one year of age, and two adults 36 and 37 years of age. The presence of trophozoites of *P. falciparum, P. malariae,* and *P. ovale* was observed in 243 (98.4%), 123 (49.8%), and 98 (39.7%) persons, respectively. In August-September, 97.7% of the adults harbored trophozoites of *P. falciparum* at least once.

Table 7 shows the cumulative prevalence of the three *Plasmodium* species according to age in the 206 permanent residents of Dielmo. Whereas for *P. falciparum* infection occurred almost constantly regardless of age, the cumulative prevalence of *P. malariae* and *P. ovale* varied significantly with age ($P < 0.0001$ and $P < 0.01$, respectively, by chi-square test). For *P. malariae,* cumulative prevalence reached 50% at one year of age and continued to increase throughout childhood to a maximum of 91% in the 10–14-year-old age group. It decreased gradually in adults to a minimum of 25% in persons more than 60 years of age. For *P. ovale,* the variations according to age were different. The

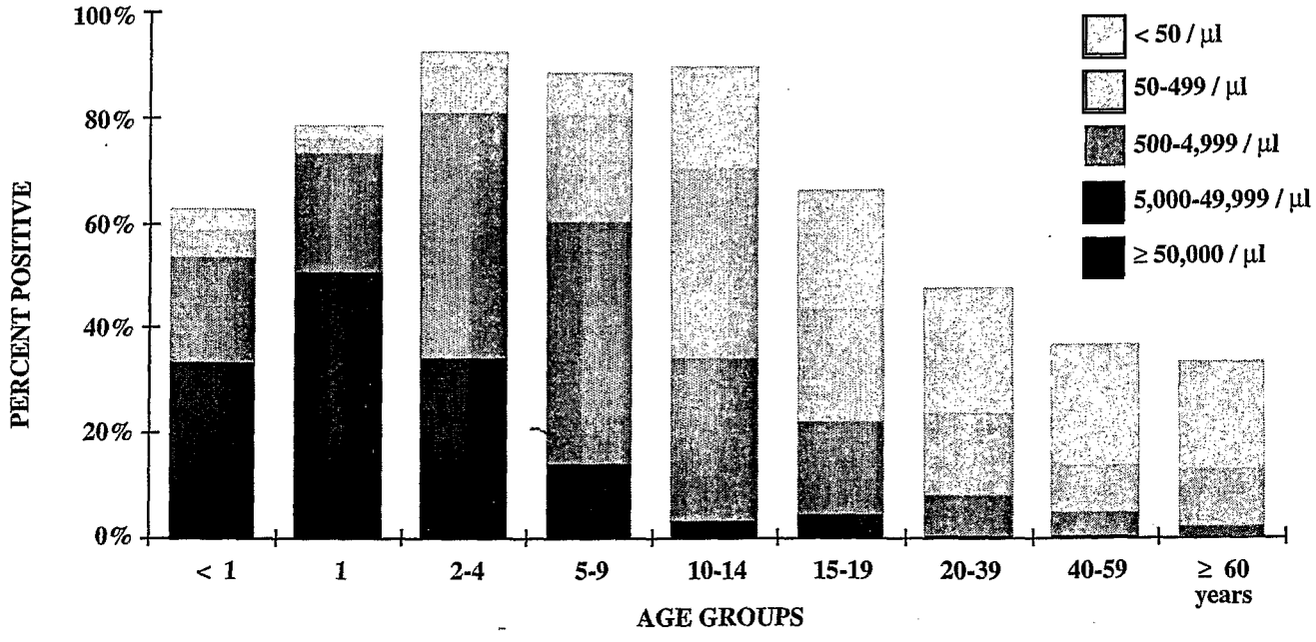

FIGURE 5. *Plasmodium falciparum* trophozoite density by age group in 206 Dielmo permanent residents (June to September 1990, 7,036 samples). The threshold of detection was 2 parasites/μl. Thick smears taken during malaria treatment or less than seven days after treatment are excluded.

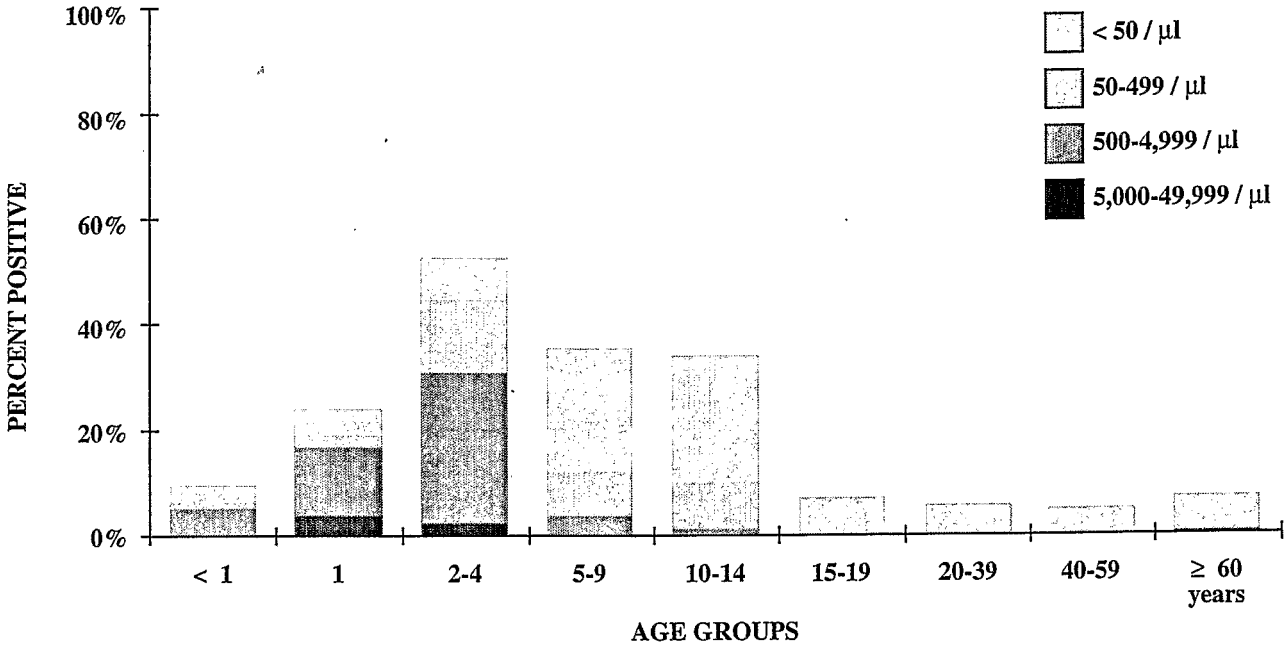

FIGURE 6. *Plasmodium malariae* trophozoite density by age group in 206 Dielmo permanent residents (June to September 1990, 7,036 samples). The threshold of detection was 2 parasites/µl. Thick smears taken during malaria treatment or less than seven days after treatment are excluded.

cumulative prevalence was a maximum of 62% in children 5–14 years of age; it decreased in young adults, but increased again in older adults and reached 50% after 60 years of age. Gametocytes were observed in 86% of the villagers for *P. falciparum,* 19% for *P. malariae,* and 9% for *P. ovale.* Table 7 shows that 90% of the persons presenting with *P. malariae* gametocytes were children, whereas this proportion was only 68% for *P. ovale* and 48% for *P. falciparum.*

Clinical attacks. During the four-month surveillance period, 366 days of fever (rectal temperature ≥ 38.5°C or axillary temperature ≥ 38.0°C) were observed in the 247 inhabitants of Dielmo: 339 in children 0–14 years of age and 27 in adults. The number of fever episodes was

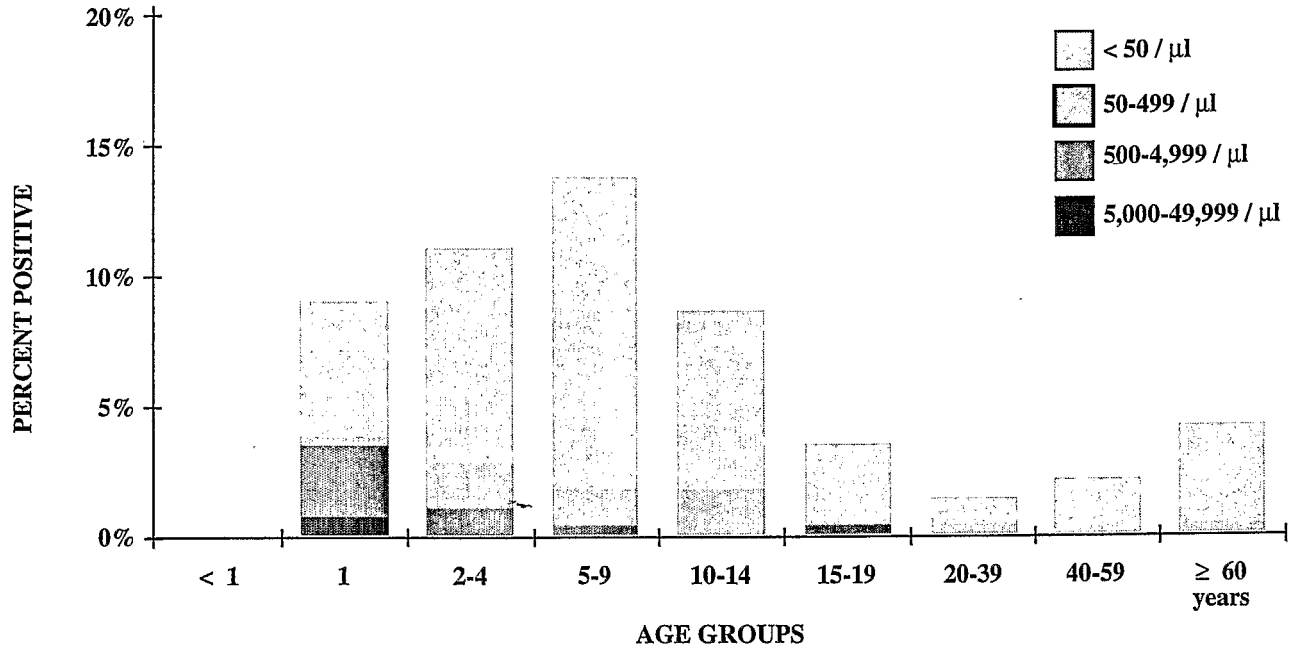

FIGURE 7. *Plasmodium ovale* trophozoite density by age group in 206 Dielmo permanent residents (June to September 1990, 7036 samples). The threshold of detection was 2 parasites/µl. Thick smears taken during malaria treatment or less than seven days after treatment are excluded.

TABLE 7

Cumulative prevalence of Plasmodium falciparum, P. malariae, *and* P. ovale *during a four-month period (June–September 1990) in 206 permanent residents of Dielmo**

Age groups, years (N)	*P. falciparum*		*P. malariae*		*P. ovale*	
	Trop (N)	Gamet (N)	Trop (N)	Gamet (N)	Trop (N)	Gamet (N)
<1	100	87.5	12.5	12.5	0.0	0.0
(8)	(8)	(7)	(1)	(1)	(0)	(0)
1	90.0	90.0	50.0	40.0	30.0	10.0
(10)	(9)	(9)	(5)	(4)	(3)	(1)
2–4	100	96.4	71.4	57.1	42.9	17.9
(28)	(28)	(27)	(20)	(16)	(12)	(5)
5–9	100	100	68.8	28.1	62.5	15.6
(32)	(32)	(32)	(22)	(9)	(20)	(5)
10–14	100	82.6	91.3	26.1	60.9	8.7
(23)	(23)	(19)	(21)	(6)	(14)	(2)
15–19	100	85.0	50.0	5.0	35.0	10.0
(20)	(20)	(17)	(10)	(1)	(7)	(2)
20–39	95.7	73.9	32.6	2.2	23.9	2.2
(46)	(44)	(34)	(15)	(1)	(11)	(1)
40–59	100	87.0	26.1	0.0	34.8	8.7
(23)	(23)	(20)	(6)	(0)	(8)	(2)
≥60	100	81.3	25.0	12.5	50.0	6.3
(16)	(16)	(13)	(4)	(2)	(8)	(1)
Total	98.6	86.4	50.5	19.4	40.3	9.2
(206)	(203)	(178)	(104)	(40)	(83)	(19)

* (N) = number of persons; Troph = trophozoites; Gamet = gametocytes.

TABLE 8

Age distribution of falciparum *malaria attacks in 206 permanent residents of Dielmo, June 1, 30–September 1990*

Age group	No. of persons	No. of attacks	Mean no. of attacks per person	No. of malaria patients	% malaria patients
1–11 months	8	16	2.00	7	87.5
12–23 months	10	19	1.90	8	80.0
2–4 years	28	55	1.96	18	64.3
5–9 years	32	31	0.97	19	59.4
10–14 years	23	2	0.09	2	8.7
15–19 years	20	2	0.10	2	10.0
20–39 years	46	5	0.11	5	10.9
40–59 years	23	2	0.09	2	8.7
≥60 years	16	0	0.00	0	0.0
Total	206	132	0.64	63	30.6

242 in children and 24 in adults. Malaria was clearly responsible for 130 fever episodes in children and 13 in adults (peaks of high parasitemia in persons with negative thick smears or low/medium-grade parasitemia the previous days, and no suspicion of any associated disease), and was the probable cause of 18 other fever episodes in children. Most clinical attacks were due to *P. falciparum; P. ovale* was responsible for only three attacks (in permanent residents of Dielmo one, four, and 18 years of age, respectively), and none was attributable to *P. malariae.*

Of the 206 permanent residents of Dielmo, 91 persons (73 children and 18 adults) presented with a total of 224 episodes of fever (children: 205 episodes, 282 days of fever; adults: 19 episodes, 22 days of fever). We attributed 135 (60.3%) of the episodes of fever to malaria: 125 (92.6%) in children and 10 (7.4%) in adults. At least one was seen in 54 children (maximum of six attacks) and 10 adults had one attack. Table 8 shows that the incidence of *P. falciparum* attacks was at a maximum in babies and young children (average: 2.0 attacks per person during the study period) and decreased considerably in older children and adults. The youngest person who had an attack was a two-month-old baby (parasitemia = 102,000/μl) and the oldest one was a 58-year-old woman (parasitemia = 25,000/μl).

DISCUSSION

Entomologic data support the idea that malaria transmission in Dielmo is intense and permanent, yet is subject to marked annual and seasonal fluctuations. Since *An. gambiae* s.l. is abundant only in the wet season, and *An. funestus* is dominant in the dry season, transmission is ensured alternatively by one or the other species; this is often observed in the Sudan-type savanna of West Africa when larval breeding sites can survive the dry season.[16] Although the quantity and distribution of rainfall was similar from one year to another, average vectorial density was significantly higher during the second year of the study than during the first year, mainly because of the abundance of *An. funestus.* We have no clear explanation for the two peaks of this species in June 1991 and from December 1991 to March 1992. For *An. gambiae* s.l., a distinct increase in the density of this vector always took place before the first rains, possibly in connection with the increase in temperature (which usually starts about April or May) and especially with the increase in humidity that occurs about a month before the first rainfall. The cytogenetic study of two samples of half-gravid females showed that the *An. gambiae* s.l. com-

plex is represented in Dielmo in the dry season as well as in the wet season by *An. arabiensis* and *An. gambiae* s.s.(Coluzzi M, Petrarca V, unpublished data).

Specific identification of the sporozoites shows that in all seasons *An. gambiae* s.l. and *An. funestus* are often simultaneously infected by two or three species of *Plasmodium.* Their identification by CS-specific MAbs correlated with differences in sporozoite morphology. Few entomologic studies in Africa have involved specific identification of sporozoites, and all used the enzyme-linked immunosorbent assay (ELISA).[17–19] The direct visualization of relative proportions of *P. falciparum, P. malariae,* and *P. ovale* sporozoites in mosquito samples from Dielmo confirms the ELISA results, and show data that closely resemble those found in a similar epidemiologic situation in Kenya.[17]

All the malariometric indices measured indicated that Dielmo is a typical holoendemic situation. The spleen rate in children 2–9 years of age is close to 90% and almost all the children's thick blood films are parasite positive. The measurement of parasitemia shows that a reduction in trophozoite density is observed for all *Plasmodium* species from the age of 2–4 years and older. In adults, the amplitude of seasonal variations of parasite rates increases with age. The trebling of transmission from July onwards results in a doubling of the apparent *P. falciparum* prevalence in adults more than 40 years of age. Study of selected blood samples by the polymerase chain reaction indicates that whatever the season, the true prevalence of *P. falciparum* in adults in Dielmo is, in fact, much higher than the one observed by standard examination of the thick blood smears because of the frequent occurrence of very low grade infections (Guanzirolli A and others, unpublished data). The variations in parasite density and detectability related to fluctuations in transmission may explain the considerable differences existing in the literature for the parasite rates of adults in holoendemic areas.

During the study, trophozoites of *P. falciparum* were found at least once in more than 98% of the villagers and in all adults more than 40 years of age. These findings confirm previous observations in holoendemic areas,[7, 20, 21] and clearly show that no individual reached sterile immunity against asexual forms of *P. falciparum* (and pre-erythrocytic stages), despite hundreds of inoculations every year during a lifetime. The observation of *P. falciparum* gametocytes in 84% of adults more than 60 years of age during a period of four months only suggests that the same is true for immunity against sexual forms of the parasite.

Plasmodium ovale is generally considered to be a rare species in adults in endemic areas. In the case of Dielmo, its prevalence is effectively very low in those more than 15 years of age. However, a very different picture is revealed by the longitudinal study, in which 32% of adults presented an infection during a period of four months. Moreover, the cumulative prevalence of this species reached 50% in adults more than 60 years of age, and four of the five oldest persons in the village, all more than 75 years of age, presented with an infection. The rarity of reports of *P. ovale* infection is in great part due to its low parasite density and the very short duration of patent infections, which rarely exceeds one week.

Although the average prevalence of *P. malariae* was almost four times higher than that of *P. ovale,* the cumulative prevalence of these two species was very similar, 50.5% and 40.3%, respectively, in permanent residents of the village. *Plasmodium malariae* infections in children generally had a long duration. In contrast, this species was relatively rare in adults and when observed, its parasite density was always less than 50/μl. Moreover, whereas gametocytes were observed in more than half of the positive blood films in children 1–4 years of age, the presence of gametocytes was rare in older children and exceptional in adults. In contrast to *P. falciparum,*[22, 23] the reservoir of *P malariae* seems almost entirely concentrated in young children.

Of the three plasmodial species, only *P. falciparum,* and to a much lesser extent, *P. ovale* were responsible for malaria attacks. For the latter species, parasitemia measured during the three clinical attacks observed always reached a level that for *P. falciparum* in the same age group is usually associated with a clinical attack. All other *P. ovale* infections were asymptomatic. For *P. malariae,* an equivalent level of parasitemia was never reached by any villager during the four-month study period and no fever was observed during peaks of medium-grade parasitemia. These observations suggest that under conditions of high *P. falciparum* endemicity, the

pyrogenic threshold of parasitemia in persons of a given age is similar for all plasmodial species.

The incidence of *P. falciparum* malaria attacks varied considerably depending on age. The mean number of attacks per person was highest in individuals 1–11 months old. However, no significant decrease was observed prior to five years of age. Despite a marked decrease after age nine, malaria attacks were observed at all ages. Thus, despite a very high and perennial transmission, complete protection was not achieved in adults, including those who had lived all their life in the village. With the exception of one case involving a pregnant woman, no associated pathologies or particular circumstances, such as other disease conditions, pregnancy, travel outside the area, or chemoprophylaxis, were observed in adults presenting with malaria attacks, which might be the cause of a decrease in acquired immunity and thereby contribute to the occurrence of the attack. Furthermore, it must be stressed that the present analysis only concerns episodes in which a definite fever (≥ 38.5°C) was observed. These typical malaria attacks represent in Dielmo only a part of the episodes directly due to malaria, particularly in older children and adults.

Entomologic, parasitologic, and clinical data collected systematically in Dielmo clearly show that the epidemiologic characteristics of malaria here are representative of holoendemic regions of tropical Africa. They provide baseline information on the natural history of malaria in a community exposed since birth to intense perennial transmission.

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