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MALARIA MORBIDITY AMONG CHILDREN EXPOSED TO LOW SEASONAL TRANSMISSION IN DAKAR, SENEGAL AND ITS IMPLICATIONS FOR MALARIA CONTROL IN TROPICAL AFRICA

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Abstract. To measure morbidity due to malaria and to study its relationship with transmission and parasitemia in children living in an area of low malaria endemicity, a cohort study of 343 schoolchildren was undertaken during a one-year period in Dakar, Senegal. From parallel investigations on transmission and the frequency of malaria as a cause for outpatient visits, three different seasons were chosen for close monitoring of different clinical, parasitologic, and sero-immunologic parameters. The daily incidence rates of malaria parasitemia and primary attacks were at a maximum level during the high transmission season (0.00198 and 0.00185 new cases/person/day, respectively) and decreased considerably during the season of low transmission. For each given period, the values of these two rates were close to each other, suggesting that each new infection was followed by a clinical attack. During the period of maximum transmission, clinical malaria prevalence was 1.36% and malaria was responsible for 36% of school absences due to medical reasons. At the end of the period of minimum transmission, clinical malaria prevalence was 0.15% and malaria was responsible for 3% of school absences due to medical reasons. In contrast, parasite prevalence hardly varied with the season (minimum 3.6%, maximum 7.5%). In a one-year period, the total number of new malarial infections was estimated between 173 and 230. Because of the existence of a vector density gradient in the area concerned, the annual malaria incidence varied considerably according to the children's place of residence. Although this rate reached one infection per year in children living near a marsh where *Anopheles* breeding sites were localized, we did not observe a higher clinical tolerance in these children than in those less exposed to malaria. These findings show that schoolchildren in Dakar have no protective immunity and that for them, malaria is a major cause of morbidity despite low endemicity. The implications for malaria control strategies based on the reduction of human-vector contact are discussed.

The level of malaria endemicity in tropical Africa is generally much lower in urban areas than in rural ones.[1,2] The urbanization process tends to reduce transmission by eliminating *Anopheles* breeding sites, by spacing out the persistent anopheline populations within a denser human population, and by limiting their dispersion from breeding places.[3–5] This phenomenon and its consequences have been well-documented by recent studies in regard to entomologic, parasitologic, and sero-immunologic features. In contrast, except for impact on severe malaria,[6] the clinical consequences of urbanization have not been studied.

The relationship between malaria morbidity and urbanization in tropical Africa occurs within the general framework of relationships among the entomologic inoculation rate, incidence and recovery rates of malaria parasitemia, and the incidence of malaria attacks in stable endemic areas. Transmission levels in tropical Africa vary considerably, according to ecologic conditions, from approximately 10^{-2} to 10^{3} infective bites per person per year. At a given age, the degree of acquired protective immunity differs according to the level of transmission and has marked consequences on the absolute and relative importance of malaria morbidity (Trape JF and others, unpublished data). Thus, whereas in areas of high transmission the incidence of malaria attacks is approximately 40 times higher in children less than five years of age than in adults, the differences among the age groups tend to decrease in areas of low transmission, and in older

children and adults, the incidence of clinical attacks is liable to be higher in areas of low transmission than in those of high transmission (Trape JF and others, unpublished data). In fact, despite their important implications for malaria control, the effects of transmission level on the incidence of malaria attacks in persons of a given age are not well known. This knowledge is necessary, however, to appreciate the global impact of malaria pathology, to decide on the advisability of a campaign to reduce transmission, and to assess the short-, medium-, and long-term effectiveness of all forms of malaria control.

The first objective of this study, carried out in Dakar, Senegal, was to accurately measure malaria morbidity in older children living in a town in tropical Africa where malaria is hypoendemic. The second was to use the phenomenon of vector density gradients observed in urban areas[5] to carry out simultaneously at different transmission levels a quantitative analysis of the relationships among transmission, parasitemia, and morbidity in these children.

MATERIALS AND METHODS

Background

This study is part of a research program in epidemiology and malaria control in Dakar that has been underway since 1987. After a systematic survey of the main *Anopheles* breeding sites, one district of the town near a vast marsh (Pikine Ancien) was chosen. The study area, which extends to a distance of 910 meters from the marsh, has been presented in a previous report.[5] Malaria transmission, as estimated from the results of an entomologic study (indoor-resting pyrethrum spray collections), varied from 0.382 to 0.014 infecting bites per person per year according to the distance from the marsh.[5]

Average parasite prevalence in the community was 3.8%. In children less than 15 years of age, malaria accounted for between 4% and 40% of fever cases among outpatients at local clinics depending on the time of year. These prevalence figures were 4%, 33%, and 8% in June 1987, November 1987, and February 1988 (date of the present study), respectively.[7] These observations were made before the first cases of chloroquine-resistant malaria appeared in Dakar.[8]

Surveys

From June 1987 to June 1988, four surveys were carried out among a group of schoolchildren between seven and 11 years of age in the 7A-Badiane-Gangue school, situated in the center of the study area. At the preparatory stage, six classrooms were selected and an individual health card was filled out for each child, showing the exact address of the child's home, which was then marked on a map of the district. The number of children followed longitudinally was originally limited to 313 for operational reasons (only children having their home in a given number of blocks of each sector of the study area were included), but was increased to 419 children for the second survey (the low rate of school absences during the first survey led us to include all blocks of the study area). We were able to add a certain number of the children who had initially been excluded based on residence, but who had nevertheless undergone systematic parasitologic tests during the preparatory stage (all children from three of the six classrooms) so that the final analysis of the results covered a group of 343 children. Since the children missing from the final analysis (18%) were almost exclusively determined by initial classroom composition and changes between the two academic years covered by the study, the representative nature of the study population was probably not affected. Informed consent of the schoolchildren was obtained and their parents or guardians. Approval for the study was obtained from the Ministere de l'Education Nationale, the Ministere du Plan et de la Cooperation, and the Ministere de la Sante Publique.

The first three surveys lasted 13 days (June 1–13, 1987), 15 days (November 2–16, 1987), and 14 days (February 2–16, 1988). These specific dates were fixed according to data collected on monthly variations in malaria transmission and the frequency of malaria as a cause for children's outpatient attendance in this district of Dakar (February: beginning of the minimum transmission period, June: end of the minimum transmission period, November: period of maximum clinical incidence).

Each of the first three surveys consisted of the following: 1) initially interviewing each child to determine whether any febrile syndromes had occurred during the previous month and any malarial chemoprophylaxis or treatment was used,

2) daily axillary temperature readings during the survey period (except Wednesdays and Sundays, when school was closed), and 3) daily questioning of children about any new symptoms that might be related to malaria (except on Wednesdays and Sundays), 4) taking two systematic thick blood smears 10 days apart (day D0/D9, D1/D10, or D2/D11), 5) taking a sample of capillary blood by fingerprick for sero-immunologic studies (except during the survey of June 1987), 6) taking supplementary thick blood smears and capillary blood when a child presented with fever or malaria-related symptoms during or in between daily visits, and 7) making a house visit on the same day to clarify absence from school. If this absence was for medical reasons, a thick blood smear was immediately taken.

The fourth survey took place on June 6 and 7, 1988. To complement the previous surveys, its purpose was to help establish for a period of one year the children who had at least one malaria infection. On these two days, a thick blood smear and a sample of capillary blood for sero-immunologic tests were taken from all the children included in the previous surveys.

When any pathologic symptoms were observed, a note was given to the child to visit a neighboring medical center, the usual procedure in the school where the study took place. The thick blood smears were only examined after the last survey, with 200 microscopic oil immersion fields being systematically examined (approximately 0.5 μl of blood). Parasite density was estimated from the parasite:leukocyte ratio using a previously described method.[9] Plasma from the samples of capillary blood was examined by indirect immunofluorescence (IFA) using *Plasmodium falciparum* as antigen (dilutions used: 1:200, 1:600, 1:1,200, 1:1,800, 1:5,400, and 1:16,200). Since samples were collected during the last three surveys, serologic profiles were only available for the period November 1987–June 1988.

During the year of the study, all the children's homes were visited and each head of the household was questioned. The following parameters were investigated: 1) presence of chloroquine in the home, 2) whether the child resided with his or her parents, 3) level of education and the professional categories of the parents, 4) ethnic group, 5) presence of running water and electricity in the dwelling, 6) type of dwelling, and 7) presence of animals or vegetation in or around the house.

An entomologic survey was also carried out in the homes of 216 children. This consisted of indoor-resting pyrethrum spray collections, and all mosquitoes caught were identified by species. As a general rule, five bedrooms (only four bedrooms were present in five homes) were sampled in each house.

The model of Bekessy and others[10] was applied to the results of blood samples taken 10 days apart for estimating the incidence rate h and the recovery rate r of patent malaria parasitemia. These were calculated using the formulas:

$$h = -\alpha \log (1 - \alpha - \beta)/(\alpha + \beta)t$$
$$r = -\beta \log (1 - \alpha - \beta)/(\alpha + \beta)t$$

where α is the proportion of children positive at the second survey who were negative at the first survey ($\alpha = N_{-+}/N_{-+} + N_{--}$), β is the proportion of children negative at the second survey who were positive at the first survey ($\beta = N_{+-}/N_{++} + N_{+-}$) and t is the time interval (in days) between surveys.

RESULTS

June 1987 survey

Observations were made on nine days for a period of 13 days (D0, D1, D3, D4, D5, D8, D10, D11, and D12) and covered 313 children. The total number of days children were present at school was 2,628 (93.3% of 2,817 school days) and there were 189 absences (6.7%). Of the absences, 149 were for nonmedical reasons and 40 were for medical ones. Of the 2,709 temperature readings taken at school or at home, 39 (1.4%) showed a temperature greater than 37.9°C and six (0.2%) showed a temperature greater than 38.4°C.

The first series of blood tests showed that 16 (5.1%) of the 313 children had positive thick blood smears. These were exclusively *P. falciparum* infections. The second set of tests 10 days later also showed that 16 (5.1%) of 313 children had positive thick blood smears. These were also *P. falciparum* infections. In all, 15 children were positive for both tests, one child was positive for the first test only, and another child was positive for the second test only. Parasite densities were generally less than 500 parasites/μl (Table 1). During the 13 followup days, 62 supplementary thick blood smears were taken from children experiencing fever or other symptoms. No other

TABLE 1

*Prevalence and density of malaria parasites in children surveyed in the study (systematic samples only)**

Surveys	Slide positivity rate	Parasite density classes†				
		1	2	3	4	5
June 1987	32/626 (5.1)	11 (34.4)	15 (46.9)	5 (15.6)	1 (3.1)	0 (0)
November 1987	46/794 (5.8)	10 (21.7)	13 (28.3)	15 (32.6)	6 (13.0)	2 (4.4)
February 1988	52/802 (6.5)	25 (48.1)	12 (23.1)	8 (15.4)	7 (13.4)	0 (0)
June 1988	15/419 (3.6)	6 (40.0)	7 (46.7)	2 (13.3)	0 (0)	0 (0)

* Values are the no. positive/no. tested (%) or no. (%) positive.
† 1 = <50 parasites/μl; 2 = 50–<500; 3 = 500–<5,000; 4 = 5,000–<50,000; 5 = ≥50,000.

malarial infection, other than those detected during the systematic tests, was observed.

The model of Bekessy and others[10] was applied to the results of blood samples taken 10 days apart. The daily incidence rate of malaria parasitemia (h) was 0.00035 ± 0.00035 (mean ± SD) and the daily recovery rate (r) was 0.00647 ± 0.00647. The average duration of episodes of patent parasitemia ($1/r$) and period without parasitemia ($1/h$) were 155 and 2,857 days, respectively.

Of the 17 children who had a positive thick blood smear at least once during the study, 14 presented with no clinical symptoms. One child had a primary attack that began during the followup period. Two other children presented with mild symptoms (headache without fever) for one day, but their association with a positive thick blood film was probably fortuitous (low-grade stable parasitemia). During 4,069 followup days (313 children, 13 days), malaria caused 6–8 days of illness. The daily incidence of primary attacks was 0.00025 (95% confidence interval [CI] 0.00006–0.00137), or one new malaria attack per 4,000 children. The clinical malaria prevalence (primary attacks and relapses) was between 0.15% and 0.20%. During 2,817 days of school (313 children, nine days), only one day of absence (0.04%) was due to malaria, while 40 days of absence (1.42%) were due to illness.

November 1987 survey

Observations were made on 10 days during a period of 15 days (D0, D1, D4, D5, D7, D8, D10, D11, D12, and D14) and covered 397 children. The number of days children were present at school was 3,851 (97.0% of 3,970 school days) and there were 119 absences (3.0%). Of these absences, 61 were for nonmedical reasons and 58 were for medical ones. Of the 3,891 temperature readings taken at school or at home, 61 (1.6%) showed a temperature greater than 37.9°C and 18 (0.5%) a temperature greater than 38.4°C.

The first series of blood tests showed that 24 of the 397 children (6.0%) had positive thick blood smears. These were *P. falciparum* infections in 23 cases and *P. malariae* in one case. The second set of tests, conducted 10 days later, showed that 22 of 397 children (5.5%) had positive thick blood smears. These were *P. falciparum* infections in 21 cases and *P. malariae* in one case. In all, 16 children were positive for both tests (*P. falciparum*: 15, *P. malariae*: 1) eight children were positive for the first test only, and six children were positive for the second test only. Parasite densities are shown in Table 1. During the 15 followup days, 152 supplementary thick blood smears were taken for children experiencing fever or other symptoms. Four malarial infections other than those detected during the systematic tests were observed, three of which occurred during the 10-day period between the tests.

The model of Bekessy and others[10] was applied to the results of blood samples taken 10 days apart. The daily incidence rate of malaria parasitemia (h) was 0.00198 ± 0.00082 and the daily recovery rate (r) was 0.04101 ± 0.01466. The average duration of episodes of patent parasitemia ($1/r$) and period without parasitemia ($1/h$) were 24 and 505 days, respectively.

Of the 34 children who had a positive thick blood smear at least once during the tests, 13 presented no clinical symptoms during followup and 21 had a malaria attack. There were 19 primary attacks (eight of which had begun before D0) and two probable clinical relapses (first attacks 10 days and three weeks before follow-up). During 5,955 followup days, (397 children, 15 days) malaria caused 81 days of illness. The daily incidence of primary attacks was 0.00185 (95% CI 0.00092–0.00330), or one new malaria attack per 541 children. The clinical malaria prevalence

(primary attacks and relapses) was 1.36%. During 3,970 days of school (397 children, 10 days), 21 days of absence (0.53%) were due to malaria, while 58 days of absence (1.46%) were due to illness.

February 1988 survey

Observations were made on 11 days during a period of 15 days (D0, D2, D3, D4, D6, D7, D9, D10, D11, D13, and D14) and covered 401 children. The number of days children were present at school was 4,309 (97.7% of 4,411 school days) and there were 102 absences (2.3%). Of these absences, 53 were for nonmedical reasons and 49 were for medical ones. Of the 4,372 temperature readings taken at school or at home, 35 (0.8%) showed a temperature greater than 37.9°C and 10 (0.2%) a temperature greater than 38.4°C.

The first series of blood tests showed that 30 (7.5%) of the 401 children had positive thick blood smears. The second set of tests, conducted 10 days later, showed that 22 (5.5%) of 401 children had positive thick blood smears. In both series of tests, infections were exclusively *P. falciparum*. In all, 21 children were positive for both tests, nine children were positive for the first test only, and one child was positive for the second test only. Parasite densities are shown in Table 1. During the 15 followup days, 168 supplementary thick blood smears were taken in the case of fever or other symptoms. No other malarial infection, other than those detected during the systematic tests, was observed.

According to the model of Bekessy and others,[10] the daily incidence rate of malaria parasitemia (h) was 0.00032 ± 0.00032 and the daily recovery rate (r) was 0.03573 ± 0.01198. The average durations of episodes of patent parasitemia ($1/r$) and periods without parasitemia ($1/h$) were 28 and 3,125 days, respectively.

Of the 31 children who had a positive thick blood smear at least once during the tests, 22 presented no clinical symptoms during followup and nine had a malaria attack. These were two primary attacks that had begun before D0 and seven clinical relapses (primary attacks in December or January). During 6,015 followup days (401 children, 15 days) malaria caused 30 days of illness. The daily incidence of primary attacks was nil (95% CI 0–0.00061). The clinical malaria prevalence (primary attacks and relapses) was 0.50%. During 4,411 days of school (401 children, 11 days), three days of absence (0.07%) were due to malaria, while 49 days of absence (1.11%) were due to illness.

June 1988 survey

This survey covered 419 children who were included in at least one of the previous surveys. Thick blood smears with positive results were observed in 15 (3.6%) of these children, with 13 being *P. falciparum* infections and two *P. malariae* infections. Only three cases (two of *P. malariae* and one of *P falciparum*) were new infections since the previous February.

Annual malaria incidence

The annual malaria incidence was studied in 343 children whose age at enrollment ranged from seven to 11 years (22, 119, 132, 57, and 13 children per year of age, respectively). A total of 261 children took part in all four surveys, with 82 children taking part only in the three most recent surveys. In 142 (41.4%) children, parasitologic and serologic data enabled us to rule out any malarial infections during the year of the study (the results of IFA and thick blood smears were consistently negative). In 156 (45.5%) children, parasitologic and/or serologic data showed without a doubt that one or several malarial infections occurred. These included 1) 42 children whose thick blood smear was negative in June 1987 and later became positive, 2) 46 children whose IFA result was negative in November 1987 and later became positive, 3) 44 children whose IFA result was highly positive in November 1987 (titer ≥ 1:1,800), whereas the thick blood smear in June 1987 was negative, 4) eight children who were only enrolled in November 1987 but whose serologic profile was identical to that of the previous group, and 5) 16 children in whom the IFA titers from November 1987 to June 1988 indicated a malarial infection (increase in antibody titers by at least two dilutions). In 45 children (13.1%), the available parasitologic and serologic data were insufficient to determine whether or not a malarial infection occurred during the year of the study (34 children who participated in all four surveys and 11 children who were enrolled in November 1987).

Including the multiple infections detected parasitologically or suspected serologically in 28 children (two infections in 27 children and three

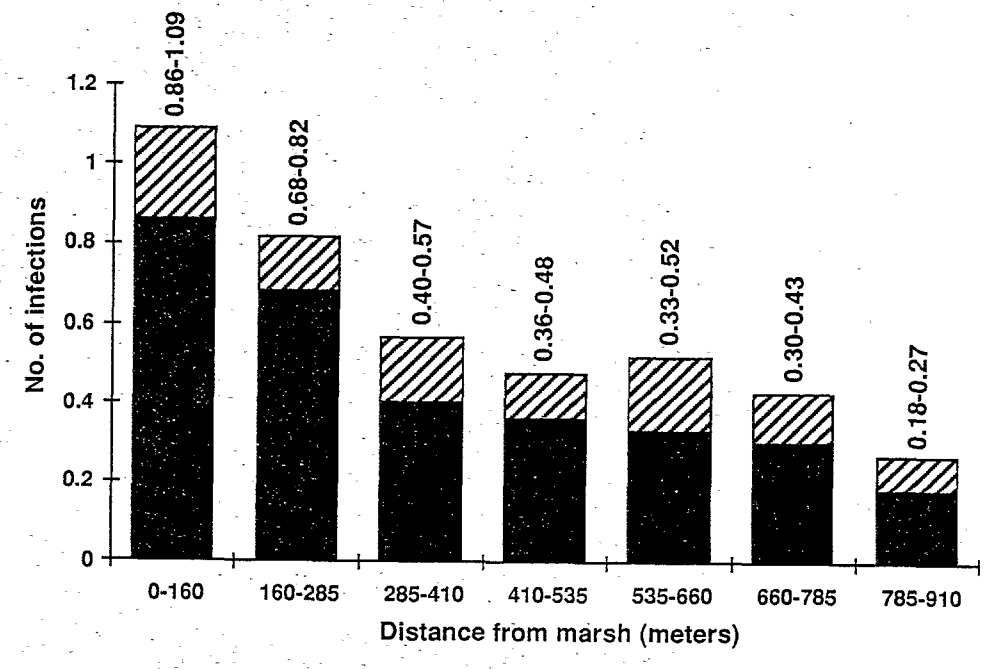

FIGURE 1. Annual malaria incidence rate (maximum and minimum estimates) according to the distance separating the children's homes from the marsh bordering the study area.

infections in one child), the total number of malarial infections that occurred during the study year was between 173 and 230, or an annual incidence rate between 0.50 and 0.67. The minimum and the maximum incidence rates were 0.45–0.61, 0.54–0.70, and 0.56–0.74 in children 7–8, 9, and 10–11 years of age, respectively.

Epidemiologic analysis

The relationships between the occurrence of malarial infection and different epidemiologic parameters were studied in the 343 children who were followed for one year. No significant differences (by chi-square test, analysis of variance, or Spearman's rank test) were detected with respect to the following variables: 1) ethnic group, 2) level of education of the father and/or the mother, 3) professional category of the father, 4) whether or not the child resided with his or her parents, 5) type of dwelling, 6) presence of animals and or vegetation in or around the house, 7) presence of running water and/or electricity in the dwelling, 8) presence of chloroquine in the home, 9) the child's statement as to the use of antimalarial chemoprophylaxis and or a mosquito net, and 10) the number of non-anopheline mosquitoes captured in the child's home.

The number of malarial infections varied greatly in relation to two parameters: 1) the number of female *Anopheles arabiensis* captured in the child's home ($P < 0.0001$, by Spearman's rank test) and 2) the distance separating the child's home from the marsh bordering on the study area ($P < 0.0001$, by analysis of variance). Analysis by logistic regression showed that these two parameters were linked ($P < 0.02$).

The variations in malaria incidence according to the distance separating the child's home from the marsh bordering the study area are shown in Figure 1. It can be seen that this incidence sharply decreases with distance from the marsh by approximately one infection per year in children residing at a distance of 0–160 meters (maximum estimation 1.09 ± 0.06, minimum estimation 0.86 ± 0.07) to one infection every four or five years in those residing at a distance of 785–910 meters.

To find out if the infections occurring in children residing near the marsh were more often

TABLE 2
Main findings of the three longitudinal surveys

	June 1987	November 1987	February 1988
No. of children	313	397	401
No. of followup days	4,069	5,955	6,015
Parasite prevalence (%)*	5.1/5.1	6.0/5.5	7.5/5.5
Parasite incidence†	0.00035	0.00198	0.00032
Parasite recovery†	0.00647	0.04101	0.03573
Primary attacks incidence†	0.00025	0.00185	<0.00061
Clinical malaria prevalence (%)	0.15	1.36	0.50
School absences, all diseases (%)	1.42	1.46	1.11
School absences, malaria (%)	0.04	0.53	0.07
School absences, malaria: all diseases (%)	3	36	6

* Two samples taken at a 10-day interval.
† Daily rate.

asymptomatic or of less clinical severity than those occurring in the other children (acquisition of protective immunity in children residing near the marsh), we compared clinical data collected during the daily followup sessions. Of 43 episodes of parasitemia found in children living at a distance of 0-285 meters from the marsh, 19 (44.2%) had clinical signs during one of the daily followup periods (11 of 15 for the November session). On average, these clinical attacks lasted 3.9 ± 0.9 days, of which 0.6 ± 0.2 days of school absence were observed (4.2 ± 1.0 days and 0.8 ± 0.2 days, respectively, for the 14 primary attacks). Of the 39 episodes of parasitemia observed in children residing at a distance of 285–910 meters from the marsh, 14 (35.9%) had clinical signs during one of the daily followup periods (10 of 19 for the November session). The average duration of these 14 clinical attacks was 3.2 ± 0.5 days, with 1.0 ± 0.4 days of absence from school (3.8 ± 0.8 days and 1.5 ± 0.7 days, respectively, for the primary attacks). None of the differences between the infections in the two groups of children were significant (by chi-square or Mann-Whitney tests).

DISCUSSION

The results of the present study show that the proportion of schoolchildren harboring malaria parasites has always been low and relatively stable (between 3.6% and 7.5%). In contrast, seasonal variations were considerable for all measures assessing malaria morbidity and the dynamics of malaria parasites. Table 2 summarizes the main findings. Between June (end of the low transmission season) and November (maximum transmission season), we observed an increase reaching a factor of six for parasite incidence and recovery, a factor of seven for primary attack incidence, a factor of nine for clinical malaria prevalence, and a factor of 13 for school absence due to malaria. In February (beginning of the low transmission season), all of these rates decreased approximately the same proportion, except the parasite recovery rate, which was still very high, and, to a lesser extent, the prevalence of clinical malaria (because of clinical relapses). The fact that for a given period the daily incidence rates of malaria parasitemia and primary attacks were very close to each other suggests that each new infection was rapidly followed by a clinical attack. Their seasonal fluctuations conformed to fluctuations in transmission[5, 11] and symptomatic infections were mainly recently acquired ones. It is interesting to note that malaria was responsible for 36% of school absences for medical reasons in November, but for only 6% and 3% in February and June, respectively. These figures closely correspond with those obtained in the outpatient clinic of the study area, which concern the frequency of visits to the center for malaria.[7]

Of all the risk factors studied, only those implying high exposure to vectors appeared to be epidemiologically significant: the number of anophelines captured in the child's home and the proximity of the child's residence to a neighboring marsh harboring the main *Anopheles* breeding sites. The incidence of malarial infections sharply decreased with distance from this marsh from approximately one infection per year in children living at a distance of less 160 meters to one infection every four or five years in those residing at a distance of 825–910 meters. This is a consequence of the vector density gradient observed in this area.[5] However, when measured by entomologic methods, the differences in exposure to malaria as a function of distance of the home from the marsh were considerably greater (factor of 27).[5] This suggests that the proportion of malarial infections not contracted at home increases rapidly with distance from the marsh.

In nonimmune persons, it is generally assumed that each infective bite is followed by an episode of parasitemia, and that this always causes a clinical attack. After repeated infections, acquired immunity rapidly results in a decrease of clinical incidence,[12, 13] whereas its effect on parasitemia (decrease in parasite incidence and increase in recovery) occurs later and is more progressive.[10, 14] For the schoolchildren in this study, the incidence rates of malaria parasitemia and primary attacks were nearly the same, including in those children presumed to have higher immunity because of the proximity of their homes to the marsh (84% of the children of the area bordering on the marsh have lived in the same house since birth). In addition, the duration of the disease and its presumed severity were similar whether the child lived near or far from the marsh. This suggests that at a rate of transmission of one infective bite per person per year, acquired immunity at the age 10–11 years is still insufficient to reduce the clinical incidence of malaria.

All entomologic, parasitologic, and sero-immunologic data collected in Dakar show that the level of malaria endemicity is much lower than in most other regions of tropical Africa. For older children in this area, malaria is nevertheless a cause of morbidity the incidence of which almost reaches that of areas of high malaria endemicity. In a village in the Congo that we recently studied using a similar methodology,[15] the number of malaria attacks was 2.4, 1.9, and 1.0 per year in schoolchildren 7–8, 9–10, and 11–13 years of age, whereas transmission reached 250 infective bites per person per year (a level of transmission 250–1,000 times higher than for the Dakar schoolchildren), and the parasite rate was 84%. In Dielmo village, Senegal, where malaria is holoendemic, only eight clinical attacks were observed in a cohort of 38 children with an age range of 7–14 years who were visited daily over a four-month period.[12] The average transmission during that study period was 75 infective bites per person. Thus, the comparison of the results of these different studies suggests that in regions of high transmission, i.e., most rural areas of tropical Africa, malaria control strategies based on the reduction of human-vector contact (impregnated bed nets or curtains, insecticidal control operations) can only have a low long-term impact on the overall incidence of malaria attacks in the community, since the decrease in the number of attacks will be limited to the youngest age groups, and an increase will probably occur in the other age groups.

Hypoendemic malaria in Dakar ranks first as a cause of outpatient visits, accounting for more than 25% of fever cases in older children and adults, i.e., the most active part of the population (Lefebvre-Zante E and others, unpublished data). Furthermore, since severe cases requiring efficient treatment occur at all ages, the economic impact of malaria is probably much higher in area such as Dakar than in highly endemic areas of tropical Africa. On the other hand, in urban areas such as Dakar where transmission is already naturally limited by ecologic conditions, vector control could result in a significant decrease in the general incidence of malaria attacks in the community. For younger and older children, this decrease would be directly proportional to the reduction in human-vector contact for all transmission levels of less than one infective bite per person per year.

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VERTICAL TRANSMISSION OF WEST NILE VIRUS BY *CULEX* AND *AEDES* SPECIES MOSQUITOES

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Abstract. Experiments were conducted to determine whether West Nile (WN) virus was transmitted vertically by colonized strains of *Aedes albopictus, Ae. aegypti,* and *Culex tritaeniorhynchus.* Female mosquitoes were infected by intrathoracic inoculation with WN virus, and the F_1 progeny were tested for virus by the fluorescence antibody technique and the newborn mouse assay. Each of the three mosquito species transmitted WN virus to F_1 adults derived from immature forms reared at 26°C. The minimal filial infection rate (MFIR) ranged from 1:124 to 1:138 for *Ae. albopictus,* from 1:62 to 1:172 for *Ae. aegypti,* and from 1:325 to 1:859 for *Cx. tritaeniorhynchus.* The MFIR for *Cx. tritaeniorhynchus* reared at 20°C was 1:213 for larvae and 1:390 for pupae, and 1:208 for larvae and 1:554 for pupae reared at 26°C. These data are the first reported evidence of vertical transmission of WN virus by mosquitoes, and therefore warrant further studies to determine whether vertical transmission occurs among WN viral-infected mosquitoes in nature.

West Nile (WN) virus is an important human pathogen that has caused large epidemics of febrile disease in Africa, Asia, and Europe.[1] These epidemics have coincided with an increase in the population density of mosquitoes, especially *Culex* species, during the summer in temperate regions and during the rainy season in the tropics. West Nile virus has been isolated from several species of *Culex,* and to a lesser extent from *Aedes* and *Anopheles* mosquitoes, and argasid and ixodid ticks.[1] However, most isolates have been obtained from *Cx. univittatus* in Egypt,[2] Israel,[3] and the Republic of South Africa,[4,5] *Cx. molestus* in France,[6] and the *Cx. vishnui* complex, including *Cx. pseudovishnui, Cx. tritaeniorhynchus,* and *Cx. vishnui* in India[7] and Pakistan.[8] Experimental studies have shown that several of these *Culex* species were readily infected with WN virus and capable of transmitting the virus to laboratory animals.[9–13] Evidence of WN viral infection has been demonstrated in several species of domestic and wild vertebrates, but only wild birds have been incriminated as viral-amplifying hosts.[14–20] These ecologic and epidemiologic observations indicated that the primary maintenance and transmission cycle of WN virus during the summer season involved *Culex* species mosquitoes and wild birds.

The maintenance mechanism(s) for WN virus during periods unfavorable for adult mosquito activity, especially during the winter in temperate regions is unknown. Initial experimental attempts to demonstrate vertical transmission of the virus by *Cx. tritaeniorhynchus* and *Ae. aegypti* as a possible overwintering mechanism were unsuccessful.[13,21] Since vertical transmission by mosquitoes of other flaviviruses has been conclusively demonstrated,[22–27] this study was conducted to retest the hypothesis that WN virus could be transmitted vertically by *Culex* and *Aedes* species mosquitoes.

MATERIALS AND METHODS

Mosquitoes

Culex tritaeniorhynchus, Ae. albopictus, and *Ae. aegypti* used in these experiments during 1979 and 1980 were obtained from colonies established during 1968 and 1969 from adults and immature forms collected in Pakistan. Immature mosquitoes were reared at 26°C for sustaining colonies, and at 20°C and 26°C for use in exper-