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Epidemiological assessment of malaria transmission in an endemic area of East Pakistan and the significance of congenital immunity *

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A longitudinal epidemiological study was carried out in a hyperendemic malarious area of East Pakistan. Transmission of malaria was observed with two peaks, one in the pre-monsoon period, the other in the pre-winter period. New infections occurred in 51.7% of the infants surveyed who were 2 months old or under when first examined: there were frequent superinfections. The maternal immunity passively transferred to the infants did not play a significant role in restricting the development of the asexual erythrocytic stage or that of gametocytes, as indicated by the fact that the average parasite density and the gametocyte counts were highest in the 0–2-month age group and declined with increasing age. Mosquitos were collected for identification and for dissection to determine parity, the sporozoite rate, the vectorial capacity, and other basic indices. An. minimus *was the principal vector, but* An. leucosphyrous *was also found to be positive.*

Chittagong Hill Tracts in East Pakistan is considered as a hyperendemic malarious district: Kuraishy & Talibi (1962) recorded a spleen rate of 91% in children 2–9 years of age in this area. The natural transmission of malaria, in particular mosquito bionomics, and the infectivity of the anophelines occurring in the area have not been studied very thoroughly.

A longitudinal epidemiological study was therefore carried out to investigate the seasonal transmission of malaria and the significance of congenital immunity in an endemic area of East Pakistan and to determine the various indices for epidemiological assessment.

THE STUDY AREA

The study area was at Khagrachberi, in a large fertile valley bounded on both sides by chains of hills in the northern part of Chittagong Hill Tracts (Fig. 1). Several drains and natural streams from this valley run into the Chengi river. The area is not flooded during the rainy season and the flat land and most of the hills that have been cleared of forest are under cultivation. Rice is the main crop but some sugar cane is also grown.

The houses are made of split bamboo poles and consist of a central raised platform. The people usually stay indoors at night. The men and children wear very little clothing. The animals are tethered outside, usually in a field near the house. Shelters are sometimes erected for the animals but these shelters are usually without side walls.

The area has a tropical monsoon climate, the average annual rainfall being about 250 cm. The maximum and minimum temperatures recorded during the study were 32°C and 10°C, respectively: during the period from February to November, the mean temperature was about 27°C and during the winter months of December and January the mean temperature fell to 18°C. The relative humidity ranged from 70.3% to 93.4% at 07.00 hours and from 41.7% to 85% at 16.00 hours.

MATERIALS AND METHODS

Villages with high spleen and parasite rates were selected on the basis of a rapid malariometric survey. The basic observations included determinations of the infant parasite rate, and the parasite species, estimates of parasite density, gametocyte counts, and determination of vector bionomics, sporozoite and

* Subsequent to the completion of this work the name of East Pakistan was changed to Bangladesh.

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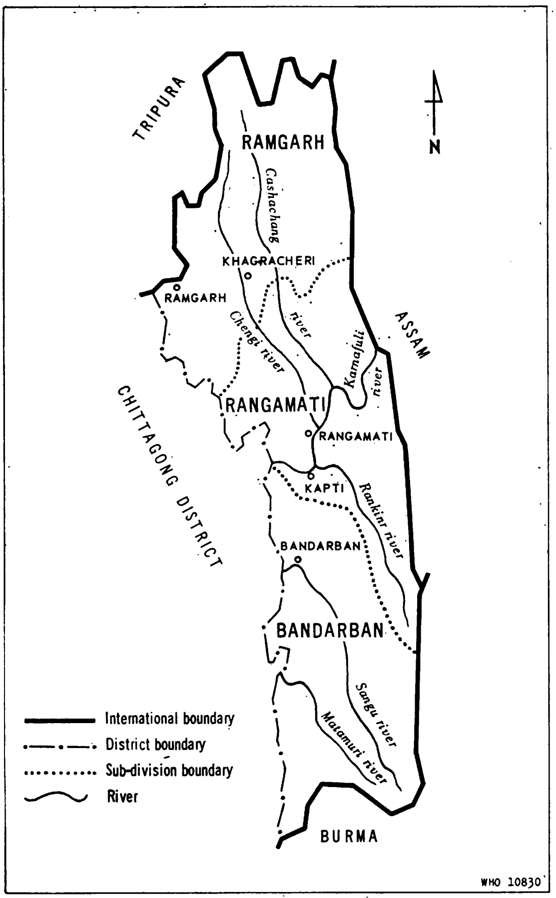

Fig. 1. Chittagong Hill Tracts District.

oocyst rates, and abdominal categories. The expectation of life, the vectorial capacity, and the basic reproduction rate of the vector species and the inoculation rate were also considered.

Twelve villages were chosen for routine monthly observations of the occurrence of new infections in infants and for the collection of anopheline mosquitos for a full year from July 1966 to June 1967. A complete census of houses, population, and particularly infants was made and the census was kept up to date by means of the monthly visits. Infants less than 8–10 days old were usually not available for examination.

Parasitological observations

Monthly parasitological observations were made on all available infants (0–11 months) in the study area. The blood slides were dehaemoglobinized and fixed and were then stained with 3% Giemsa stain for 35 min. At least 200 fields were examined before a slide was considered negative. The parasite count was made against 400 white blood cells (WBC), 12 000 WBC per cubic millimetre of blood being taken as the standard for the purposes of this study.

Entomological observations

Adult mosquitos and larvae were collected by several different methods. Adult mosquitos were caught in the early morning from four rooms in each village, using an aspirator for 15 min in each room: after these catches, adults were caught with a spray sheet in one sleeping-room per village. In addition, adult mosquitos were caught in different biotopes in the village and the surrounding areas, using an aspirator: these catches were made inside houses other than those selected for the regular catches, underneath the platforms of houses and in cattle sheds, goat and chicken houses, holes in trees, holes in earth banks, the bases of banana trees, and artificial shelters.

At night, mosquitos were caught off human bait in one village. A team of four insect collectors (two indoors and two outdoors) exposed their arms and legs to mosquito bites starting 15 minutes before sunset and continuing throughout the night until 15 minutes after sunrise: collections were made for 30 minutes each hour. Larvae were collected from suitable breeding places, such as streams, springs, ponds, and rice fields.

The work of identifying the mosquitos, abdominal grading, dissection for parity—by observing the stretching of the tracheoles of the ovaries (Detinova, 1962)—and the preparation of precipitin papers was carried out in the field laboratory immediately following the collections. The salivary glands and gut were usually dissected on the same day. As far as possible the same insect collectors were retained throughout the study and the usual procedure of rotating the duties of insect collectors for indoor and outdoor night collection was maintained.

RESULTS

Parasitological observations

The monthly incidence of new parasite infections in infants has been found to be a sensitive indication of the transmission season. All infants positive for parasites were followed up for determination of the other indices, such as parasite density and gametocyte count.

The 147 infants available for examination in the locality were of different ages when first examined, but routine monthly surveillance for infection was possible on 41 infants from the age of 1 month and on 19 from the age of 2 months.

Table 1 shows the results of the monthly collections of blood slides from the 60 infants by age group. A total of 31 infants were found to be posi-

Table 1. Parasite incidence determined at the monthly examinations of blood slides from 60 infants who were 2 months old or under when first examined

Infant slides examined		Age group (months)				
		0–2	3–5	6–8	9–11	Total
August	no.	7	0	0	0	7
	no. positive	0	0	0	0	0
September	no.	10	3	0	0	13
	no. positive	1	0	0	0	1
October	no.	11	6	0	0	17
	no. positive	1	1	0	0	2
November	no.	8	17	0	0	25
	no. positive	0	6	0	0	6
December	no.	10	14	4	0	28
	no. positive [a]	0	3 (2)	0	0	3 (2)
January	no.	11	14	6	0	31
	no. positive	2	0	0	0	2
February	no.	14	13	14	0	41
	no. positive [a]	0	1	(1)	0	1 (1)
March	no.	12	14	14	4	44
	no. positive [a]	3	2	1 (1)	0	6 (1)
April	no.	6	15	14	6	41
	no. positive [a]	1 (1)	3 (1)	2 (1)	0	6 (3)
May	no.	2	10	12	11	35
	no. positive [a]	0	0	2	1 (1)	3 (1)
June	no.	0	8	11	13	32
	no. positive [a]	0	0	(1)	1 (2)	1 (3)
total (August–June)	no.	91	114	75	34	314
	no. positive [a]	8 (1)	16 (3)	5 (4)	2 (3)	31 (11)

[a] Figures in parentheses refer to superinfection.

Table 2. Results of monthly examinations of blood slides from 87 infants who were more than 2 months old when first examined

Age group (months)	Distribution by age group (%)	Total no. of blood slides examined	No. positive	Average parasite density index	Average gametocyte counts/mm³			
					P. vivax	*P. falciparum*	*P. malariae*	All species
3–5	33.3	50	9	VI	500	190	0	397
6–8	46.0	104	24	VI	615	223	0	404
9–11	20.7	138	40	VII	636	301	510	400
total	—	292	73	—	—	—	—	—

tive, 11 of them showing superinfections. The term superinfection, for the purposes of this study, denotes the detection of a subsequent infection with a heterologous species during the course of the study and this has been considered as evidence of fresh transmission. The incidence of new infections for the year under study was 51.7%.

The results of the parasitological observations on the 87 infants who were more than 2 months old at the time of the first examination are shown in Table 2.

Fig. 2 shows the monthly incidence of new infections in the 60 infants. The data in Table 1 show that the incidence of new infections was highest in the period from March to June and in November and December, reflecting two principal transmission seasons, which probably have peaks during the period from February to May (pre-monsoon) and in October and November (pre-winter).

The parasite density of a population gives an indication of the collective load of infection and is related to immunity. Among the 31 infants found to be positive, the parasite density was highest in the 0–2-month age group (Class 8) and decreased to Class 5 in the 9–11-month age group. The definition of parasite density used in this study is similar to that used in West Africa by Bruce-Chwatt (1958).

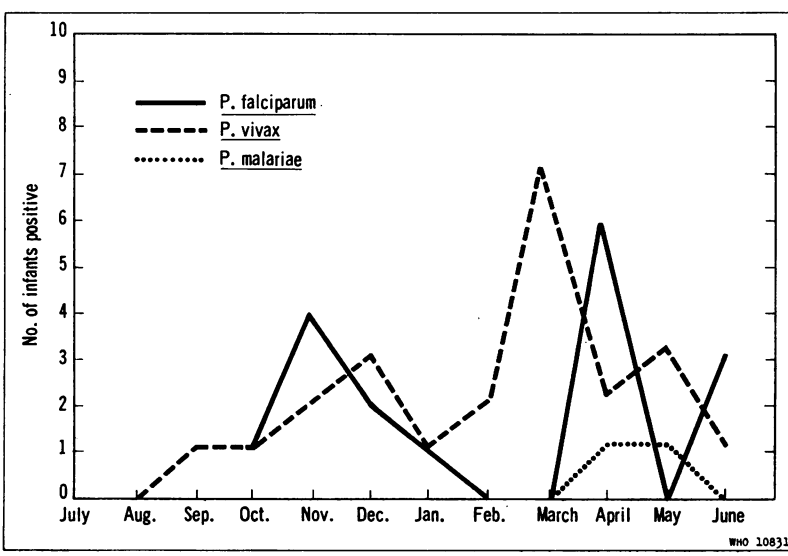

Fig. 2. Occurrence of new infections among 60 infants who were 2 months old or under when first examined.

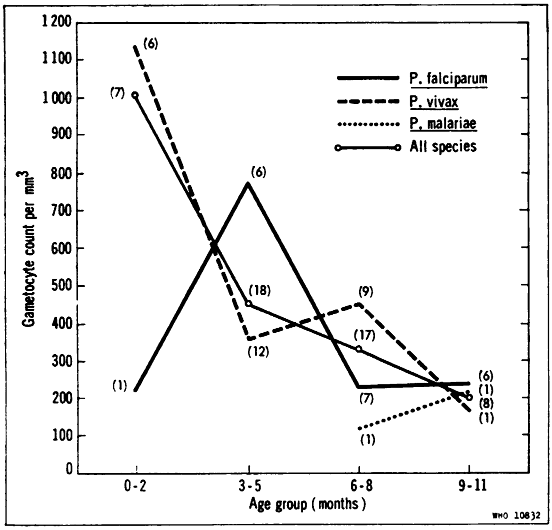

Fig. 3. Average gametocyte counts, by age group, for the 31 infants found positive out of the 60 infants examined who were 2 months old or less when first examined. The figures in parentheses give the numbers of gametocyte-positive slides in each category.

Fig. 3 shows, by age group, the average gametocyte count per positive examination of 31 infants. For the purposes of these calculations of the average for all species, a slide showing gametocytes of more than one species was considered as two or more separate entities. The highest count was found in the 0–2-month age group for *P. vivax*. The highest counts of *P. falciparum* were observed in the 3–5-months age group. The average gametocyte count for all species was highest in the 0–2-month age group and declined with increasing age.

The prevalence of parasitaemia in infants of different ages is shown in Table 3. If the proportions of infected infants in the different age groups are compared with the curves given by Macdonald (1957) for the estimation of inoculation rate, which is the daily number of bites inflicted on one individual by mosquitos infected with sporozoites which are actually infective, a daily inoculation rate of 0.0025 is obtained.

Table 3. Prevalence of parasites in infants who were 2 months old or under when first examined, by age group

Age group (months)	Median age (days)	No. examined	No. positive	Proportion
0–2	20	91	9	0.099
3–5	127	114	31	0.272
6–8	218	75	23	0.307
9–11	310	34	11	0.324

The continuation of the highly malarious situation in the area during the study period was confirmed by means of two malariometric surveys among children 2–9 years of age. These were conducted in May 1966, immediately prior to the initiation of this study, and in November 1966 during the expected peak of the malaria season. The results are summarized in Table 4.

Entomological observations

Vector species. Twenty-one species of *Anopheles* mosquito were collected and identified from the area. Three of the species, *An. vagus*, *An. minimus*, and *An. aconitus*, were common throughout the year, and two species, *An. culicifacies* and *An. jeyporiensis*, were less common. The remaining species occurred in very small numbers.

An. minimus was usually found inside houses. One specimen was found in a cattle shed and another in an artificial shelter. Other species found resting inside houses were, in order of prevalence, *An. vagus*, *An. aconitus*, *An. jeyporiensis*, *An. varuna*, *An. culicifacies*, *An. fluviatilis*, *An. kochi*, and *An. subpictus*.

Table 4. Results of malariometric surveys in children 2–9 years of age

Date of survey	No. of children examined [a]	Spleen rate (%)	Parasite rate (%)	Relative prevalence (%)			Gametocyte rate (*P. falciparum*)
				P. falciparum	*P. vivax*	*P. malariae*	
May 1966	344 (283)	58.8	69.6	50.1	39.0	10.8	33.3
Nov. 1966	188 (140)	54.0	56.4	59.3	31.5	9.3	32.1

[a] Figures in parentheses relate to the parasite survey.

Man–vector contact. A total of 363 anopheline mosquitos were caught biting at night off human bait and these belonged to 14 species. Of these, 248 (68%) were collected biting indoors and 115 (32%) biting outdoors. Of the total night catch, 57.3% was *An. minimus.* The average man-biting rate for *An. minimus* was found to be 5.7 for the year and man-biting activity was most pronounced in the months of October and November. Of the total of 208 *An. minimus* collected, 182 (87.5%) were caught biting indoors and 26 (12.5%) were caught biting outdoors.

The period of man-biting activity for *An. minimus* is shown in Fig. 4 and it may be observed that the maximum biting activity occurred 7 hours after sunset, i.e., at midnight.

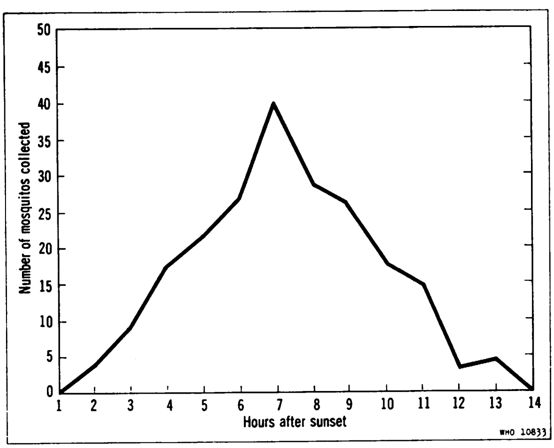

Fig. 4. Night-biting activity of *An. minimus.*

Abdominal categories. During abdominal grading, gravid *An. vagus, An. minimus, An. aconitus, An. culicifacies, An. jeyporiensis,* and *An. varuna* were found in the day catches from human dwellings.

The proportions of the different abdominal categories for *An. minimus* were as follows: unfed, 5.3%; freshly fed, 49.0%; half-gravid, 34.6%; and gravid, 11.1%.

The low proportion of gravid females in the day catch suggests some degree of exophily and the proportions of fully fed females that were half-gravid and gravid, 0.52 and 0.48, respectively, indicate a 2-day gonotrophic cycle. To avoid any tendency for specimens between late-fed stage and subgravid to be classified as half-gravid the two-stage classification of fed and gravid was adopted.[1]

Serological identification of the blood meal. A total of 1 235 blood smears were made from *Anopheles* mosquitos collected from resting places indoors and outdoors. Precipitin tests were carried out at the Lister Institute of Preventive Medicine, London, and showed that 93% of the *An. minimus* fed on human blood.

Dissection for determination of parity and sporozoite rate. All the freshly fed and unfed mosquitos with ovaries in Christopher's stage II that were collected biting at night, or by other collection methods, were dissected to determine parity and the probability of survival. The probability of survival was highest (90%) for *An. minimus*; that for *An. vagus* was 86%, for *An. jeyporiensis* 81%, and for *An. philippinensis* 72%.

A total of 2 432 mosquitos of 16 species were dissected to detect salivary gland infections. Of the total of 2 060 *An. minimus* dissected, 27 were infected, giving a sporozoite rate of 1.31%. Only 4 *An. leucosphyrus* were collected throughout the year and 1, from a night catch, was infected with sporozoites. *An. minimus* is considered to be the principal vector in the area.

Estimation of vectorial capacity and other indices. Using the table of Oganov-Rayevski, as reproduced by Pampana (1963), the average duration of sporogony for *P. vivax* at the temperatures found in this area is 10 days. For *P. falciparum* the duration of sporogony is 12 days. Using the probability of survival based on age grading, read with the table given by Macdonald (1957), the average expectation of infective life (longevity factor) of female *An. minimus* was determined for *P. vivax* as 3.3 days and for *P. falciparum* as 2.7 days.

The expected rate of malaria inoculations in man per infective case per day, i.e., the vectorial capacity (Garrett-Jones, 1964), which is the product of the man-biting rate, the longevity factor, and the index of man-biting, for *An. minimus* was 4.008 for *P. vivax* and 3.24 for *P. falciparum.* The number of new infections that would be expected in the surrounding population from a totally nonimmune and untreated case, if that population had been untouched by malaria until the case arrived in their midst, i.e., the basic reproduction rate (Macdonald, 1957), was calculated, and was 320.7 for *P. vivax* and 259.1 for

[1] This classification was proposed in: *Practical entomology in malaria eradication,* Part I, p. 54 (unpublished WHO document MHO/PA/62.63)

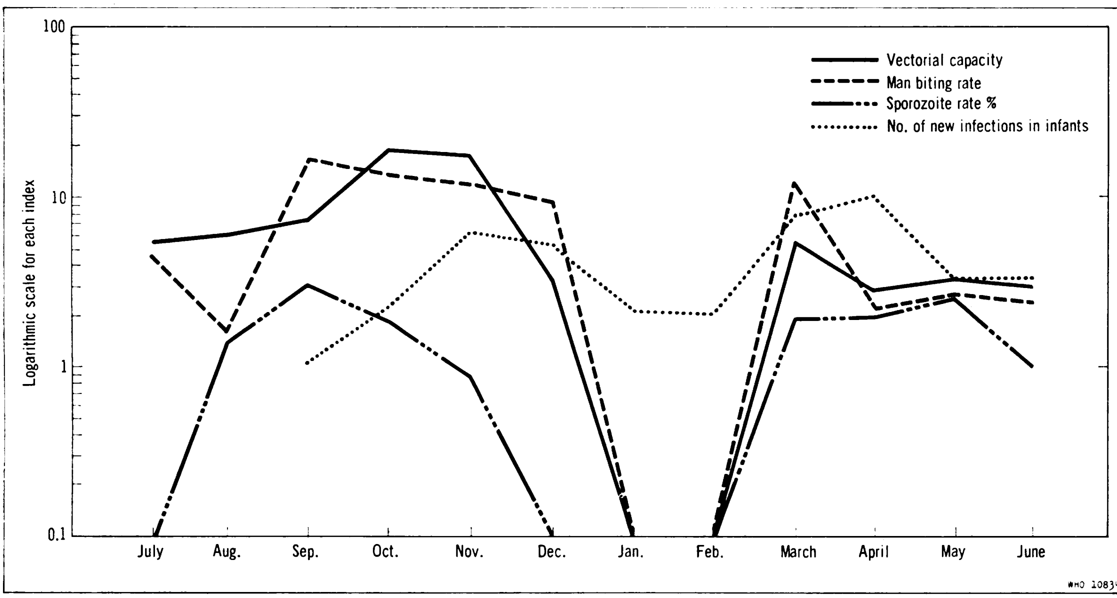

Fig. 5. Trends of vectorial capacity, man-biting rate, sporozoite rate, and the occurrence of new infections in infants.

P. falciparum. The actual reproduction rate as determined for the study area was 1.03 for *P. vivax* and 1.05 for *P. falciparum*. The stability index of the study area was 4.4. The mean daily number of bites inflicted on one individual by mosquitos infected with sporozoites that were actually infective, or the inoculation rate (Macdonald, 1957), was determined as 0.034.

The malaria transmission trend as derived from various indices is presented in Fig. 5.

DISCUSSION

Congenital transmission of malaria is an exceedingly rare phenomenon (Covel, 1950). Bruce-Chwatt (1952) found not a single case of congenital malaria in 332 African newborn babies in Lagos. Similar negative results have been quoted by Cannon (1958) and Spitz (1959) from Nigeria and by Jilly [1] from Ghana. A study by Jelliffe [2] in Kampala, Uganda, showed that although 5.6% of 570 pregnant women had infected peripheral blood and 16.1% of them had a malaria-infected placenta, only 1 case of congenital malaria was seen among the neonates. Thus the incidence of new infections in infants can be regarded as a sensitive index of transmission.

In this study, transmission of malaria occurred throughout the year with the exception of the months of July and August. It was possible to continue routine monthly surveillance of 41 infants from the age of 1 month and of a further 19 from the age of 2 months. Out of the total of 42 episodes of transmission that occurred in 31 infants, 24 (57%) were detected during the period from March to June and 11 (26%) in November and December, reflecting two main transmission seasons; one in the pre-monsoon period in February–May and one in the pre-winter period in October and November. The pre-winter transmission period was shorter than the pre-monsoon period, possibly because of the onset of winter. The infrequent occurrence of new infections in infants for the first 2 months of study might have been the result of the small number of infants examined, rather than of the absence of transmission.

The occurrence of new infections in 51.7% of the infants examined and the occurrence of 11 super-infections with heterologous species in 10 positive infants (out of a total of 31 positives) indicate a fairly high level of transmission in the area. *P. vivax*

[1] Jilly, P. (1966) *Anaemia in parturient women with special reference to malarial infection of the placenta and sickle-cell trait* (Unpublished document WHO/Mal/66.563).

[2] Jelliffe, E. F. (1966) *Low birth weight and malaria infection of the placenta* (Unpublished document WHO/Mal/66.558).

cases were well distributed throughout the months of the study except July and August, but *P. falciparum* cases were found only in November, December, and January and again in April and June. The largest number of new *P. vivax* infections was observed in March whereas the largest number of new *P. falciparum* infections was in April.

Congenital transmission of immunity in malaria was the subject of a comprehensive review by Bruce-Chwatt (1963). Davidson & Draper (1953), commenting on the discrepancy between the potential and actual malaria inoculation rates in Tanganyika, state that " The cause appears to be within the infant, taking the form of an immunity which suppresses the majority of infections but as we have seen exerting little restraint on those which break through." Gilles (1957) and McGregor et al. (1956) have reported low incidence and low parasite densities in very young infants. Four possible causes for this have been considered by McGregor (1960) and by Bruce-Chwatt (1963): (1) the relative aversion of *Anopheles* to feeding on infants, (2) the deficiency of *p*-aminobenzoic acid in the exclusive milk diet during early infancy, (3) the inability of the fetal and other haemoglobins to support plasmodial development, and (4) passive immunity inherited from the mother.

In a hyperendemic situation, such as that in the study area, where transmission is continuous and intense, the adult population, including the expectant mothers, enjoy a reasonably high degree of acquired immunity, which may be passively transferred to the infants. If this passively transferred immunity is of some significance, and if it lasts for the first 3 months, the highest average parasite density and the highest average gametocyte count should not occur in the 0–2-month age group.

In the present study the average parasite density and the average gametocyte count were highest in the 0–2-month age group and decreased with increasing age: there were 7 positives in the 0–2-month age group (one of whom was 1 month old) out of the total of 31 positive infants. Thus it seems that in hyperendemic areas, where transmission is continuous and intense, the maternal immunity passively transferred to the infants may not play a significant role in restricting the development of the asexual erythrocytic stage and of the gametocytes.

Macdonald (1957) has said that " the earliest immune response is a reduction of gametocyte output, and in intensely malarious places it is visible by three months of age in the form of greatly reduced densities, without at first marked reduction of rate ". Thus, the gradual decline of the average parasite density indices and that of the average gametocyte counts with increasing age in infants that were observed in this study are indicative of the building up of active immunity as a result of acute infection rather than the occurrence of infection in an already immune population.

The large number of anopheline species collected from the area points to the richness of the mosquito fauna. Only two species, however, *An. minimus* and *An. leucosphyrus*, were infected with sporozoites. *An. minimus* was the main vector in the area and from the estimates of the basic reproduction rates (320.7 for *P. vivax* and 259.1 for *P. falciparum*), the actual reproduction rates (1.03 for *P. vivax* and 1.05 for *P. falciparum*), and the stability index (4.4), it is clear that this species was responsible for maintaining highly stable malaria in the area.

The occurrence of new infections in infants followed broadly the seasonal prevalence of *An. minimus*. The two peak months of new infections were preceded by increased densities of *An. minimus* and a fall in density was followed by a decrease in the occurrence of new infections. As regards other possible vectors of malaria in the area, the density of *An. jeyporiensis* in relation to man was 12.2% and its human blood index was 34.5%; thus it appears that this species could be a vector of some importance.

Although *An. philippinensis* does not seem to play an important part in malaria transmission at present, its presence in night catches (2 indoors and 20 outdoors) is indicative of exophilic and exophagic behaviour in the area. No specimens were found in day collections from indoor resting places. If this species were to become a vector in the area, the spraying of insecticides inside houses might not be effective.

The vectorial capacity of *An. minimus* was as high as 12.2–16.2 during March, September, October, and November. During the remaining months it varied from 2.0 to 4.2 except in January and February. During these cold months it was not possible to estimate the vectorial capacity as no mosquitos were caught at night. This may be explained by the fact that the mosquito collectors were located in cold sites. It is, however, probable that mosquitos were biting the local inhabitants in the warmer indoor rooms.

The inoculation rate for this area calculated from entomological data is 0.034, and this value was 14 times that calculated on the basis of the proportion of infants infected, which was 0.0025. In areas of high endemicity, a discrepancy of this kind is quite common. Discussing possible reasons for these discrepancies, Macdonald (1957) stated that the entomological approach estimates the total number of inoculations whereas the parasitological approach estimates only the successful inoculations. Elaborating further, Pampana (1963) stated that many infections are distributed in infants who are already infected and who have already developed some immunity; in addition, the inoculations probably contain so few sporozoites that only a few subjects develop parasitaemia.

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RÉSUMÉ

ÉVALUATION ÉPIDÉMIOLOGIQUE DE LA TRANSMISSION DU PALUDISME DANS UNE RÉGION D'ENDÉMICITÉ DU PAKISTAN ORIENTAL; RÔLE DE L'IMMUNITÉ CONGÉNITALE

On a mené une enquête dans une région d'endémicité paludéenne du Pakistan oriental afin d'étudier les aspects saisonniers de la transmission du paludisme, d'apprécier l'influence de l'immunité congénitale et d'établir les indices indispensables à une évaluation épidémiologique.

Après une rapide enquête paludométrique, on a choisi des villages où les indices plasmodiques et spléniques étaient élevés. L'examen de tous les enfants de 0 à 11 mois a été répété mensuellement. Des captures de jour et de nuit ont permis de récolter des moustiques adultes; on les a identifiés, évalué le stade de la digestion sanguine, différencié les femelles pares des nullipares et recueilli des échantillons de repas de sang pour les épreuves de séroprécipitation.

Sur un total de 147 enfants étudiés, 60 (41 âgés de 1 mois et 19 âgés de 2 mois) ont fait l'objet d'examens parasitologiques mensuels; 31 d'entre eux ont été trouvés positifs, ce qui correspond à une incidence parasitaire annuelle chez les nourrissons de 51,7%. On a décelé 11 cas de surinfection par une espèce hétérologue, indice d'une transmission récente. La fréquence des nouvelles infections a été particulièrement élevée de mars à juin et en novembre-décembre, avec deux clochers de transmission maximale de février à mai (avant la mousson) et en octobre-novembre (avant l'hiver). Le nombre quotidien de piqûres infectantes, par individu, a été estimé à 0,0025. L'indice de densité parasitaire et l'indice gamétocytaire, très élevés chez les enfants de 0 à 2 mois, s'abaissaient chez les enfants plus âgés. On en conclut que l'immunité néonatale ne joue aucun rôle déterminant.

Deux espèces d'anophèles, *Anopheles minimus* et *A. leucosphyrus*, ont été trouvées infectées par des sporozoïtes, la première étant considérée comme le principal vecteur dans la région. Le taux d'agressivité d'*A. minimus* est maximal vers minuit, 7 heures après le coucher du soleil. La durée de son cycle gonotrophique est de 2 jours. Les épreuves de séroprécipitation indiquent qu'il se nourrit essentiellement sur l'homme (93%). Sur 2060 femelles d'*A. minimus* disséquées, 27 étaient infectieuses (indice sporozoïtique: 1,31%). La durée probable de vie infectante est de 3,3 jours pour *Plasmodium vivax* et de 2,7 jours pour *P. falciparum*. L'aptitude vectorielle d'*A. minimus* est estimée à 4,008 pour *P. vivax* et à 3,24 pour *P. falciparum*.

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