A Note on the Infectivity to Mosquitos of Patients in the Asymptomatic, Symptomatic and Post-symptomatic Phases of Parasitic Relapses of Induced Infections with *Plasmodium vivax* (Madagascar Strain)

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In the seventh report of the WHO Expert Committee on Malaria reference is made to the limited knowledge available concerning low parasite and gametocyte density and their relation to subsequent infection of anopheles: [a]

" In malaria eradication it is important to know if subjects with a very low parasite and gametocyte density are capable of infecting mosquitos. Although various research projects have demonstrated that mosquitos may be infected by carriers with a low gametocytaemia, the number of oocysts which develop is small and the percentage of mosquitos infected is negligible. It appears highly probable from our present knowledge that only a small percentage of the anopheles that bite these ' hidden ' carriers become infected and that the chances of survival of such infected mosquitos and the danger of any considerable transmission are very small. Moreover, it is probable that mosquitos with only a few oocysts infect only a small proportion of the persons they bite. It would be valuable to secure more data bearing on this subject. "

In the present paper we propose to present some observations based on a study of a number of subjects with both high and low gametocyte densities who were tested for infectivity to mosquitos from the onset of a primary attack through the acute and into the chronic stage. Our studies are confined to *P. vivax*, since this is the only species of *Plasmodium* now used in this laboratory for malaria therapy.

The question of an asymptomatic patient being infective to mosquitos must be considered in relation to various phases in the course of the disease:

(1) during the early stage of the primary infection, before the onset of clinical symptoms;

(2) following the disappearance of clinical symptoms brought about by medication;

(3) during the period immediately preceding a clinical relapse;

(4) after a spontaneous clinical recovery, when there may be prolonged parasitaemia although the patient is able to lead a normally active life.

In two earlier papers [b, c] we described our findings regarding the infectivity of man to the mosquito (1) in the early stages of the primary attack and (2) in the asymptomatic phase of parasitic relapses in *P. vivax* infections.

In the second of these papers we described the results obtained when mosquitos were fed daily on 11 relapsing patients, beginning on the first day of parasitaemia, detected by thick film examination and continued until the onset of fever. Mosquitos were fed daily on the same 11 patients throughout the clinical relapse until the fever disappeared spontaneously or was interrupted by drug therapy. Batches of 50-100 laboratory-bred *Anopheles stephensi stephensi* were fed on each occasion, and after being kept at 27°C, 20 insects were dissected on the seventh day and the mid-guts examined for oocysts.

Case reports

Case reports of seven of these patients in whom the attack ended in spontaneous recovery are given below. In the first six of these the results seem to us to have some bearing on the problem of low parasite and gametocyte density and their relation to infection of mosquitos.

Case 1. During the pre-clinical phase of the first relapse parasitaemia was noted on six successive days. Mosquitos fed on the first two days failed to become infected and a prolonged search of blood films failed to reveal any gametocytes. [d] Infection

[a] World Health Organization, Expert Committee on Malaria (1959) *Wld Hlth Org. techn. Rep. Ser.*, **162**, *15*.

[b] Shute, P. G. & Maryon, M. (1957) *Trans. roy. Soc. trop. Med. Hyg.*, **51**, 403.

[c] Shute, P. G. & Maryon, M. (1959) *A study of the infectivity of patients to mosquitos in the asymptomatic phase of parasitic relapses of induced infections with* P. vivax (unpublished working document WHO/Mal/233).

[d] This also applied to five of the other six patients studied.

occurred in batches fed on each of the four succeeding days. On day 3, only macrogametocytes were found. On days 4, 5 and 6, microgametocyte densities of 10, 30 and 50 per mm^3 respectively were found. The clinical phase lasted three days, the fever being quotidian and ending in spontaneous recovery. Mosquitos fed on each of the three days became infected. On days 1, 2 and 3, counts of 50, 40 and 50 microgametocytes per mm^3 were made, but as one would expect, the number of oocysts per gut was greater than that encountered in the pre-clinical phase. In the post-clinical phase mosquitos became infected whenever a batch was fed over a period of 21 days. The gametocyte density can be illustrated by quoting days 9 and 21. On day 9 there were 50 microgametocytes per mm^3, and on day 21 none were found. It is interesting to note that of 20 mosquitos fed on the 21st day, only two became infected, in each case with only a single oocyst. In contrast, 95% of a batch of 20 fed on the ninth post-clinical day became infected, the number of oocysts per gut varying from nil to 100.

Case 2. During the pre-clinical phase of the first relapse, parasitaemia was noted on five successive days. Mosquitos fed on the first two days failed to become infected, but infection occurred in batches fed on each of the three succeeding days. On days 3 and 4 macrogametocytes only were found, but on day 5 there were 25 microgametocytes per mm^3. The clinical phase was classical tertian with only two paroxysms of fever followed by spontaneous recovery. Mosquitos fed on each day of the clinical phase were more heavily infected than during the pre-clinical period. On days 1, 2 and 3, counts of 30, 50 and 25 microgametocytes per mm^3 were made. This patient is of interest because during the post-clinical phase, when there were 50 microgametocytes per mm^3, he infected 85% of mosquitos on the first day. For the following five days he was practically non-infective, but when random feeding was carried out on the 15th post-clinical day, again 85% of mosquitos became infected (many with 30 oocysts), and microgametocytes were again 50 per mm^3.

Case 3. During the pre-clinical phase of the first relapse, parasitaemia was noted on four successive days. Mosquitos fed on the first two days failed to become infected, but infection occurred in batches fed on each of the two succeeding days, when there were 10 and 20 microgametocytes per mm^3. The clinical phase lasted for four days, the fever being low-grade quotidian (maximum peak 102.4°F, or 39.1°C), ending in spontaneous recovery. On days 1, 2, 3 and 4, microgametocytes were 20, 70, 20 and 10 per mm^3. There was very little difference in the percentage of mosquitos infected during the clinical and pre-clinical phases. During the post-clinical phase, batches of mosquitos became infected on only four occasions, the percentage of mosquitos infected was lower and the number of oocysts per gut fewer than in the other phases.

Case 4. During the pre-clinical phase of the first relapse, parasitaemia was noted on three successive days, but only the mosquitos fed on the third day became infected, and only macrogametocytes were seen. The clinical phase lasted five days and the fever was tertian, ending in spontaneous recovery. On days 1, 2, 3, 4 and 5, the microgametocyte density was 20, 50, 100, 150 and 100 per mm^3 respectively. A greater proportion of the mosquitos fed during this phase became infected than during the pre-clinical phase. During the post-clinical phase batches of mosquitos became infected on three occasions, but on the last of these infection was observed in only one insect, and only a single oocyst was seen and no gametocytes were found.

Case 5. This was a boy aged 16 years and the only individual in the series who gave a history of a previous malarial attack. He contracted malaria (species of parasite unknown) as a child, while living in Egypt where his parents were stationed. It is obvious that as the result of this infection he had acquired some degree of immunity.

When he was infected for therapeutic purposes the fever, although relatively severe, was of classical tertian type throughout the attack. He had 12 peaks of fever over a period of 24 days and was then given a course of quinine, 10 grains (650 mg) daily for seven days. Although thick blood films were examined daily from one week after the end of this course, no parasites were found until the 26th day. Mosquitos were fed on him during the first five days of parasitaemia, but none were infected. Batches fed on the sixth and seventh days became infected, 10% on the sixth and 15% on the seventh, with a maximum number of two oocysts per gut; there were 10 microgametocytes per mm^3 on each occasion. Those fed on the eighth and ninth days failed to become infected. The patient remained asymptomatic throughout the period of parasitaemia.

Case 6. During the pre-clinical phase of the first relapse, parasitaemia was noted on five successive

days. Mosquitos fed on the first two days failed to become infected, but infection occurred in batches fed on each of the three succeeding days. On days 3, 4 and 5, there were 20, 10 and 50 microgametocytes per mm^3 respectively. The clinical phase lasted for eight days, the fever being tertian, ending in a spontaneous recovery. On days 1-8 of this phase, there were 100, 250, 225, 1350, 350, 350, 150 and 100 microgametocytes per mm^3. During this phase mosquitos fed on him became so heavily infected that oocysts were too numerous to count (several hundreds per gut), the percentage infected being 95-100. During the first five post-clinical days mosquitos fed on each day became infected; on the last day infection occurred in every insect in the batch. On day 1 there were 20 microgametocytes per mm^3, and on day 5 there were 30. It was not possible to follow up this case beyond this period.

Case 7. This differed from the six cases reported above in that some batches of mosquitos fed on the patient during the clinical period failed to become infected. During the pre-clinical phase parasitaemia was noted on four successive days. Mosquitos fed on the first three days failed to become infected, but infection occurred in a batch fed on the fourth day, when there were 10 microgametocytes per mm^3. On the fourth day of the clinical period there were 450 microgametocytes per mm^3, and 95% of the mosquitos fed on that day became infected, some with as many as 50 oocysts per gut. On the fifth day of fever, however, although asexual parasites were numerous (four per 25 fields of a thin film), female gametocytes were scanty and none of the insects fed became infected. No infection occurred in batches fed during the post-clinical period.

Remaining cases. In the remaining four cases the fever was interrupted by drug therapy, and these are consequently of less interest. Batches of mosquitos were fed in each case from the first day on which parasitaemia was noted up to and including the day when drug treatment was begun.

All four cases followed the same pattern, i.e., during the first two pre-clinical days batches of mosquitos failed to become infected, while during the clinical phase mosquito infection occurred daily in each case until the first day of specific drug treatment. The percentage of mosquitos infected and the severity of infection in individual mosquitos was, as one would expect, proportionate to the intensity of parasitaemia.

Discussion

Although the series of cases here presented is a small one, we believe that the results probably indicate the general pattern of the infectivity of man to the mosquito in the pre-clinical, clinical and post-clinical phases of relapses in *P. vivax* malaria. It is evident that the patient may be ambulant, not being confined to bed when parasites are so scanty that prolonged search of a thick film would be required in order to find a parasite. On the other hand, parasites may be quite numerous in the absence of fever at a time when the subject may be leading a normal life in every respect. The explanation is undoubtedly related to the stage of the infection, i.e., whether this represents a primary attack or a subsequent relapse. It has to be remembered that under natural conditions in under-developed countries most of the infected subjects receive no anti-malarial drug, either prophylactically or therapeutically. It is under such conditions that the true natural history of malaria could best be studied, but it is unlikely that transmission experiments comparable with those in our series would be possible because of the difficulty in securing the co-operation of either adults or children in allowing batches of mosquitos to be fed on them daily over a period of weeks or even days. In our series the asymptomatic period was longer than the symptomatic in nearly all the patients, and because during the former period they were infectious to mosquitos as much as, and sometimes more than, in the latter their importance to the epidemiologist is considerable.

We are aware of the need for making investigations of a similar nature in *P. falciparum* infections but, as explained above, this is no longer possible at our laboratory. However, it is well known that *P. falciparum* infections differ from those with *P. vivax* in that gametocytes are seldom found until the eighth or ninth day of a primary attack and that, where trophozoites and gametocytes are detected in the peripheral blood at the same time, the patient must have been parasitized for at least a week before the film was taken. That a very low grade of gametogony can occur in *P. falciparum* infections has been demonstrated on numerous occasions, and quite recently we came across an instance of this.

A young Nigerian medical student who had been in England for about six months was accepted as a blood donor. The recipient developed a severe attack of *P. falciparum* malaria, but it was not until we had each spent about three hours searching thick films taken from the donor that a parasite was found.

This was a typical male " crescent " and it was not until several more films were examined that scanty trophozoites were detected. Whether or not this subject would have infected mosquitos we do not know, but we consider this to be doubtful.[e]

The observation that gametocytes of *P. falciparum* seldom, if ever, appear in the peripheral blood until about the eighth day of an attack is of considerable importance to the epidemiologist. During the Second World War, one of us (P. G. S.) was sent to Italy with instructions to infect a batch of mosquitos with *P. falciparum* by feeding them on volunteers in a hospital near Naples. The blood of many patients with fever was examined without a single film being found with numerous gametocytes. It was then decided to track down some patients who had been discharged from hospital up to a week previously and who had received a course of quinine therapy. This entailed visits to military units and convalescent camps and the results obtained were both interesting and enlightening. The blood of most of these recently discharged patients was found to contain numerous gametocytes, and mosquitos fed on them became very heavily infected. It was obvious that these symptom-free patients were being returned to their units at the very time when they were most highly infectious to mosquitos and so constituted an important link in maintaining the man-mosquito cycle. For at least one month, and possibly for a much longer period, these symptomless carriers were infectious to mosquitos and were probably much more dangerous than indigenous infections in which the attack has ended in spontaneous recovery.

[e] Grant, D. B., Perinpanayagam, M. S., Shute, P. G. & Zeitlin, R. A. (1960) *Lancet*, **2**, 469.

Cytological Aspects of the Taxonomy of Anophelines (Subgenus *Nyssorhynchus*)

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In the taxonomy of the anophelines, the researches of Frizzi [a, b] opened up a new path as regards the analysis of relationships between species belonging to the European and North American *maculipennis* complex. Comparison of the salivary bands made it possible to recognize a common structural plan and to analyse the differences between the species constituting the complex.

This method, which commenced with the chromosome analysis of *A. aquasalis*, by Frizzi & Ricciardi,[c] is at present being applied in this laboratory, in relation to Brazilian anophelines, to the study of the whole subgenus.[d, e, f] This note reports two cytological phenomena: firstly, the frequency of chromosome mutations (heterozygous inversions, asynaptic zones and translocations) among the different species of the subgenus, related to the formation of " variety complexes "; secondly, variation of the X chromosome, in which there is progressive loss of one of the arms, probably due to heterochromatin formation.

The subgenus *Nyssorhynchus* includes two "series": the *albimanus* series, with the species *aquasalis*, *albimanus* (not Brazilian), *strodei*, *noroestensis*, etc.; and the *argyritarsis* series, with the species *darlingi*, *argyritarsis*, *albitarsis*, etc.

The *albimanus* series includes some species which are well characterized and geographically distinct, as well as other species which are more difficult to characterize and consequently constitute an " *albimanus* complex " (species complex) similar to the *maculipennis* complex. We have not yet sufficient data on the salivary chromosome pattern to enable us to make any distinctions within this complex. The pattern of the right arm of the X, the only one so far studied, shows a certain homogeneity of the bands as between species, while the quantitative

[a] Frizzi, G. (1947) *Sci. genet. (Torino)*, **3**, 80.

[b] Frizzi, G. (1954) *Affinità genetiche fra* Anopheles *delle regioni paleartiche e neoartiche rilevate attraverso lo studio dei cromosomi salivari.* In: *Proceedings of the Ninth International Congress of Genetics (Caryologia (Torino)*, Suppl.), p. 671.

[c] Frizzi, G. & Ricciardi, I. (1954) *Symp. genet.*, **2**, 172.

[d] Guedes, A. S., Amorim, E. M. & Schreiber, G. (1957) *Rev. bras. Malar.*, **9**, 247.

[e] Schreiber, G. & Memória, J. M. P. (1957) *Rev. bras. Malar.*, **9**, 101.

[f] Schreiber, G. & Guedes, A. S. (1958) *Cytological and ecological researches on Brazilian Anophelids.* In: *Proceedings of the XVth International Congress of Zoology*, London, Section IX, paper 20.