STUDIES ON THE INFECTIVITY OF TROPICAL AFRICAN STRAINS
OF PLASMODIUM FALCIPARUM TO SOME SOUTHERN EUROPEAN VECTORS OF MALARIA

by

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The distribution of Plasmodium falciparum extends from the tropics into the temperate zones. Until its disappearance as a result of malaria eradication programmes, it included southern Europe, where it was endemic to some areas, and in others was responsible for epidemics, sometimes of devastating severity. The possibility of the re-introduction of falciparum malaria into the now non-immune communities of southern Europe is, therefore, of concern to the health authorities.

P. falciparum is able to infect a great number of anophelines, including all the European vector species (Garnham, 1966), but its infectivity can be dependant on the geographical origin of either the parasite or the mosquito (James et al., 1932; Boyd & Kitchen, 1936; Boyd et al., 1936; Boyd et al., 1938; Shute, 1940; Jeffery et al., 1950; Collins, 1962; Collins et al., 1963). Anopheles atroparvus of English origin, a good vector of European strains of P. falciparum, has been shown to be completely refractory to strains of the same parasite coming from Malaya, India, and East Africa (James et al., 1932; Shute, 1940) and from West Africa (Shute & Maryon, personal communication, 1947).

In view of the ever increasing movement of people between Europe and the tropics, the degree of susceptibility to infection with tropical strains of P. falciparum of the European mosquitoes, and particularly of the southern European vector species, is of obvious interest. This paper presents data obtained in studies of the susceptibility to infection with tropical African P. falciparum of two southern European malaria vectors. These studies, with A. atroparvus and A. labranchiae from the Italian peninsula, were conducted in two stages, a short preliminary investigation at Garki, near Kano, Nigeria, and a more extended study at Kisumu, Kenya. A summary of these studies has been reported elsewhere (de Zulueta et al., 1975).

METHODS

1. Garki, Nigeria

Newly emerged females, first generation offspring of wild A. atroparvus collected from the upper Voltturno Valley, north of Naples, were taken by air to Garki, Nigeria, where two suitable gametocyte carriers, six and four years old, were available. An on-the-spot examination had shown these children to have densities of 32 and 34 P. falciparum gametocytes per mm³ of peripheral blood.

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The mosquitoes were all fed on the same day by applying cages to the limbs of the two gametocyte carriers. It proved difficult for the children to keep still after the mosquitoes started to probe and, of some 60 female *A. atroparvus* available, only 32 fed. Of these, nine were fully and 23 partially gorged. The two groups of fed mosquitoes were transferred to clean cages and held in the laboratory inside an insulated box to protect them from extreme climatic fluctuations. During the incubation period relative humidity in this box never fell below 70%, while temperatures, recorded twice daily, were between 24°C and 28°C. The mosquitoes were not offered additional blood meals, but were supplied with a sugar solution.

2. Kisumu, Kenya

After the preliminary investigation at Gariki laboratory colonies of *A. labranchiae* from the Tyrrenian coast near the town of Tarquinia, in the province of Viterbo, and of two strains of *A. atroparvus*, one from the valley of the River Orcia, in the province of Siena, and the other from the upper Voltarno Valley, were established in Rome. Adults of the second cage generation of each of these colonies were then taken to Kisumu, Kenya, to compare their susceptibility to infection with the local strain of *P. falciparum* with that of an indigenous vector species. All the indigenous mosquitoes used in this study were the laboratory reared offspring of wild *A. gambiae* collected from houses at Ahero, in the Kano Plain, near Kisumu, where regular chromosomal examinations over many months have shown the population to be composed exclusively of species B.

Of 317 schoolchildren from the Kisumu area to be examined during the week preceding this investigation, only 30 were positive for *P. falciparum* gametocytes, almost all with very low densities. Examination, at the commencement of the study, of further blood slides taken from these children permitted the selection of seven carriers, all with counts of 20 or more gametocytes per mm².

Feeding on these gametocyte carriers took place over a period of three weeks, each mosquito receiving only one infected blood meal. On each occasion pairs of cages, one containing one of the European strains, and the other containing *A. gambiae*, were applied to the limbs or body of a gametocyte carrier. As at Gariki, it was difficult to keep the carriers still, and not all mosquitoes, *A. gambiae* as well as the European strains, imbibed the same amount of blood, and on each occasion some did not feed. Others had to be discarded after sustaining damage to the mouthparts when feeding through the netting of the cages. Fed mosquitoes were transferred to clean cages and were only offered sugar during the incubation period.

Insectary conditions were similar to those occurring out-of-doors, but with less marked fluctuations. Temperatures varied between 22°C and 30°C, and relative humidity rose from 30% in the afternoon to as high as 75% in the early morning. The mosquitoes were held in cages made from paper cups, and were placed under frames covered by damp towelling so as to eliminate extremes of temperature and aridity. Consequently, micro-climatic conditions in the holding cages, though ideal for mosquito survival, were cooler and more humid than the insectary conditions. Sporogony takes 9 days at 30°C, 10 days at 25°C, 11 days at 24°C, and 23 days at 20°C (Garnham, 1966). During this study, sporozoites appeared in the salivary glands of *A. gambiae* 12-14 days after the infective blood meal, indicating that the mean temperature in the holding cages fluctuated around 23°C.

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1 In view of the rarity of detectable densities of gametocytes in the peripheral blood of adults inhabiting the extremely malarious areas of Gariki and Kisumu, recourse had to be made to the employment of children as gametocyte carriers. In each case, permission was obtained from the parents after careful explanation of the objectives of the studies and of the methods to be employed. The parents were informed that the experiments involved exclusively laboratory-reared mosquitoes the harmlessness of the bites of which had been demonstrated in many trials.
RESULTS

1. Garki, Nigeria

All surviving *A. atroparvus*, 8 of the fully and 12 of the partially gorged, were dissected and examined nine days after their infected blood meal. In none of these specimens was there evidence of oocysts, normal or degenerated, or of sporozoites.

2. Kisumu, Kenya

Results of examinations of the guts of the three strains of European mosquitoes and of *A. gambiae* which fed simultaneously on the same gametocyte carriers are set out in Table 1. In each comparative series, gut infections readily developed in *A. gambiae*, with oocyst rates of 76%, 70% and 82% respectively. On the other hand, among the 17 *A. labranchiae*, and 117 *A. atroparvus* (48 of the Orcia, and 69 of the Volturino strains), only three infected guts were found, all in the Volturino strain. Two of these mosquitoes, dissected on the ninth and tenth days after feeding, had single oocysts only. The third specimen was not dissected until 19 days after its infected blood meal. Three oocysts were observed on the gut, but they had not completed their development and contained no sporozoites.

Some dissections were delayed until after the time necessary for completion of the extrinsic cycle of *P. falciparum*, when the salivary glands were also examined (see Table 2). With some specimens of *A. gambiae* sporozoites reached the glands 12 days after feeding, and all were sporozoite positive by the 14th day. None of the European mosquitoes, including the oocyst positive specimen dissected 19 days after feeding, had sporozoites in the glands.

DISCUSSION

The relatively slow development of *P. falciparum* in the mosquito, which does not take place or is very much retarded at mean temperatures below 20°C, has facilitated its eradication from many areas where *P. vivax* persists. Nevertheless, all temperatures within the range 20°C-30°C are suitable for completion of its extrinsic cycle (Garnham, 1966), and studies on the comparative susceptibility to infection with *P. falciparum* of North American and Caribbean mosquitoes were conducted at a mean temperature of 20°C (Boyd et al., 1938).

Temperatures at which mosquitoes were incubated in the present study at Kisumu could not, therefore, have been a factor inhibiting development of the parasite in the European mosquitoes, especially since sporogony developed normally in *A. gambiae*.

Not all *A. gambiae* at Kisumu developed infections, and the number of oocysts per gut varied, even between mosquitoes feeding at the same time on the same gametocyte carrier. Obviously, gametocyte density was only one of the factors influencing infectivity. Work with bird material has shown that susceptibility to infection of the mosquito hosts is genetically determined, and can vary between species, strains and individuals (Huff, 1931; Kilama & Craig, 1969). On the other hand, due to the difficulty of keeping the gametocyte carriers still while the mosquitoes were feeding, and to the fact that all mosquitoes had access to sugar prior to their blood meal, the amount of blood imbibed must have been subject to considerable variation. However, a sufficiently high rate of infection was obtained with *A. gambiae* to make the results with the European mosquitoes entirely convincing; particularly so, since due to their much larger size as compared to *A. gambiae*, they tended to ingest a greater volume of blood.

The results show that refractoriness to infection with the Kisumu strain of *P. falciparum* appears to be virtually complete in all three strains of European mosquitoes studied, and that, with the Volturino strain of *A. atroparvus* at least, this refractoriness extends also to the West African Garki strain of the parasite. Refractoriness does not seem to be dependant on one factor only. The gut wall plainly forms an almost impenetrable barrier, but the present study indicates that in the rare instance of successful penetration the parasite still finds itself in a hostile environment, and is unable to complete sporogony.
It must be noted here that gametocyte densities were rather low, a reflection of the high levels of endemicity both at Garki and at Kisumu, and that higher densities could be expected in an epidemic situation. However, existing evidence indicates that refractoriness of European mosquitoes to infection with exotic strains of *P. falciparum* is not affected by high gametocyte densities. In many years of work with non-immune subjects, Shute (1940) has repeatedly challenged *A. atroparvus* with multiple feeds on carriers with massive gametocyte densities, and has failed to produce a single gut infection. Other attempts, involving hundreds of mosquitoes, to infect *A. claviger* with Indian and African *P. falciparum* were entirely unsuccessful (P. G. Shute, personal communication). Shute & Maryon (personal communication, 1947) observed a further example of refractoriness to infection with tropical African *P. falciparum* when they were unable to infect *A. atroparvus* in a trial in Lagos, Nigeria, though *A. gambiae* feeding on the same gametocyte carriers readily took up the infection.

Infecivity studies with European mosquitoes have now been made with laboratory adapted and with wild populations. Experiments with English *A. atroparvus* involved the "Horton" laboratory strain. The infectivity experiments with *A. claviger* were made with females reared from fourth instar larvae collected from natural breeding places in the south of England. The Italian *A. atroparvus* used in the present work at Garki were the laboratory reared offspring of wild females. The two strains of *A. atroparvus* and the strain of *A. labranchia* studied at Kisumu were, in each case, adults of the second cage generations, little adapted to laboratory conditions. Refractoriness to infection with tropical *P. falciparum* is not, therefore, a condition existing in a particular laboratory strain of *A. atroparvus*, but is a characteristic of the species.

From the present work and from all previous studies it must be concluded that *A. atroparvus* and probably *A. labranchia* are not suitable hosts of African *P. falciparum*. It follows that human carriers of *P. falciparum* from tropical Africa seem unlikely to cause fresh outbreaks of malaria within the distribution areas of *A. atroparvus* and *A. labranchia*, which include considerable parts of the previously malarious regions of Europe. It is also possible that elsewhere in Europe, where other members of the *A. maculipennis* complex, including *A. sacharovi*, were the vectors, importations of African *P. falciparum* may be similarly unimportant epidemiologically, but the matter requires further investigation. On the other hand, it seems probable that the strains of *P. falciparum* previously prevalent in Europe must have been genetically different from those in tropical Africa in view of their adaptation to the local vector species. This points to a high degree of genetical adaptability of the malaria parasite, which also follows from the experiments of Corradetti et al. (1970) who was able to adapt, by selection, *P. gallinaceum* to *A. stephensi*, a species which had previously shown almost complete refractoriness.

**ACKNOWLEDGEMENTS**

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**SUMMARY**

Preliminary experiments in Garki, Nigeria, with *A. atroparvus* from Italy have shown a refractoriness to infection with the local strain of *P. falciparum*. Comparative studies involving *A. labranchia* and two strains of *A. atroparvus*, all from Italy, and one local strain of *A. gambiae* species B, were carried out at Kisumu, Kenya. While oocysts developed in 77% of *A. gambiae* and, from day 14 onwards, 100% of the oocyst-positive mosquitoes had sporozoites in the salivary glands, oocysts were seen in only 4% of one of the *A. atroparvus* strains; the development of these oocysts appeared to be arrested at an early stage. *A. labranchia* and the other strain of *A. atroparvus* showed no oocysts. None of the Italian mosquito strains was positive for sporozoites.
It is concluded that the importation of falciparum malaria cases from tropical Africa is unlikely to produce epidemics in those southern European areas where A. labranchiae and A. atroparvus used to be malaria vectors.

RESUME

Certains rapports ayant indiqué que le Plasmodium falciparum d'Afrique tropicale, de Malaisie et de l'Inde ne se développait pas chez les espèces européennes des vecteurs du paludisme, il est apparu souhaitable d'éprouver l'infectiosité des souches de P. falciparum d'Afrique tropicale pour certains Anopheles maculipennis spp d'Europe méridionale qui, auparavant, étaient des vecteurs de P. falciparum local.

Les expériences préliminaires faites à Garki (Nigéria) avec des A. atroparvus venant d'Italie ont montré que ces insectes étaient réfractaires à l'infection par la souche locale de P. falciparum. Des études comparatives utilisant A. labranchiae et deux souches d'A. atroparvus provenant toutes d'Italie, et une souche locale d'A. gambiae espèce B, ont été effectuées à Kisumu (Kenya). Si des oocystes se sont développés dans 77% des A. gambiae et si, dès le 14ème jour, 100% des moustiques porteurs d'oocystes avaient des sporozoïtes dans les glandes salivaires, 4% seulement des insectes appartenant à l'une des souches d'A. atroparvus étaient porteurs d'oocystes. Le développement de ces oocystes semblait être arrêté à un stade précoce. Aucun oocyste n'a été observé chez A. labranchiae ni dans l'autre souche d'A. atroparvus. Aucune des souches de moustiques italiens n'était porteuse de sporozoïtes.

On en conclut que l'importation de cas de paludisme à falciparum en provenance d'Afrique tropicale a très peu de chance de déclencher des épidémies dans les régions d'Europe méridionale où A. labranchiae et A. atroparvus ont jadis été des vecteurs du paludisme.

REFERENCES

Boyd, M. F., Kitchen, S. F. & Mulrennan, J. A. (1936) Ibid., 16, 159-161
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<th>Serial number of carrier</th>
<th>Gametocyte density (per mm$^3$)</th>
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<th>A. gambiae species B. (Ahero strain)</th>
<th>Oocyst density</th>
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**Table 2. Numbers of Mosquitoes with Sporozoite Positive Salivary Glands in Dissections Made 12 or More Days After the Infected Blood-Meal. (Numbers of Mosquitoes Dissected Shown in Brackets.)**

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<tr>
<th>Serial number of carrier</th>
<th>A. labranchiae (Tarquinia) incubation period in days</th>
<th>A. atroparvus (Orcia) incubation period in days</th>
<th>A. atroparvus (Volturino) incubation period in days</th>
<th>A. gambiae sp B* (Ahero) incubation period in days</th>
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* Mosquitoes with oocyst positive guts. Of 22 oocyst negative A. gambiae none had sporozoites in the glands.
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