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Early Sporogonic Development in Local Vectors of *Plasmodium falciparum* in Rural Cameroon

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In ongoing studies on experimental transmission of Plasmodium falciparum *in the city of Yaounde gametocyte carriers are daily being identified among dispensary patients with malaria-like complaints. This species comprises 93% of all parasitemias and because of the selection criteria most patients have it as a recent infection. 17% of all* P. falciparum*-positives carry detectable gametocytes with little difference between youngsters and adults. Blood of adult carriers is taken and infection of* Anopheles gambiae *mosquitoes is attempted by membrane feeding; the establishment of infection is judged by the presence of oocysts.*

Key words: early sporogony - local vectors - *Plasmodium falciparum* - Cameroon

Out of 140 gametocyte carriers 62% turns out to be infective to mosquitoes (Tchuinkam et al. 1993) and the commitment in the present EEC-project is to search for factors in human blood that interfere with the development of parasites in mosquitoes.

We found one factor that determines the chance of mosquitoes to become infected: the gametocyte density (Table I).

At the time we used oocysts as a parameter of infection, but the early sporogonic or pre-oocyst stages were thought to be the vulnerable part of the development in the mosquito. Chances of infection rise when the plasma is replaced by non-malarious plasma (Mulder et al. 1993, 1994), suggesting the presence of blood-factors that reduce efficiency of transmission. Competition ELISA's are being developed to identify transmission-reducing antibodies against surface antigens of sexual stages (Roeffen et al. 1993).

It was further hypothesized that early phagocytosis of activated gametes by monocytes and neutrophils in the bloodmeal would take away a considerable quantity of gametes. Phagocytosis does occur, and so far the results suggest that this amounts to about 10% of macrogametes, but this is not the major cause for loss of sexual material (Tchuinkam 1994).

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We have tried out the detection of pre-oocyst stages in individually ground midguts of at least 20 laboratory-reared *An. gambiae* mosquitoes per experiment at 3 and 21 hours post-feeding, using a fluorescent microscope and FITC-labelled monoclonal antibody against the neo-antigen 25 kD. This surface antigen is formed after activation of gametocytes in female gametes, zygotes (both called round forms), retort forms and ookinetes and is a serious candidate for a sexual-form vaccine (Lensen et al. 1992).

The method was subsequently applied in the village of Mbebe, situated in the rain forest at the border of the Sanaga river. There is continuous transmission and people receive about 200 infective bites per year from *An. nili*, *An. gambiae* and *An. funestus*.

The field-studies were done from January to July 1993 i.e. in the dry season when *An. gambiae* is prevalent, due to the many breeding sites in the drying river bed. Initially mosquitoes were caught weekly, during the early mornings in bedrooms of four houses following pyrethroid spraying. Mosquitoes were transported in an isotherm box to the OCEAC-laboratory in Yaounde and examined upon arrival, after 2-3 h. Later, the fluorescent microscope and centrifuge were carried to the field and sampling was done during 10 subsequent days in an open bednet, under which a gametocyte carrier was invited to sleep. Dissection was performed on intervals between 0-24 h after the bloodmeal; guts

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TABLE I
Association gametocytaemia and mean percentage positive mosquitoes

Gametocity density	0-50	51-100	101-200	201-450	>450
Ooocyst positive n= 91	3	7	10	13	17
Pre-oocyst positive n= 10	0	34	57	63	89

were homogenised, mixed with the labelled Pfs25 mab and completely screened.

In the first part of the field study (January-April) 87 midguts were checked for the origin of the blood and 100% of these mosquitoes appeared to have fed on humans. Most of the mosquitoes were in the half-gravid stage, meaning that the degree of digestion had progressed for 2-12 hours. 94% *An. gambiae* and 71% *An. funestus* were half-gravid, indicating that *An. gambiae* was significantly less endophilic. Pre-oocyst stages were found in 12% of 534 mosquitoes, with a mean of 2 per positive mosquito. This could be split up in 77% round forms, 10% retort forms and 13% ookinetes. Retorts are short-lived, transient stages that were always found in combination with either or both other forms. Retorts and ookines together were present in 3.4% of the midguts, which is an expression of the natural reduction of parasites inside the midgut. Ookinetes began to appear between 12-15 h post feeding. No pre-oocyst forms were found after more than 26 h, i.e. at the gravid stage. No difference was found in the two anopheline species.

The 35 inhabitants of the 4 houses were screened for parasites and parasitaemias appeared rather constant over two months; plasmodial indices were typically decreasing with age. Gametocytes were observed in 38% of the inhabitants at a geometric mean of 1.1 / µl (range 0.5-7). The house with the highest gametocyte densities also yielded highest percentage of anophelines with pre-oocyst stages and number of pre-oocyst stages per positive mosquito. The results underline the importance of low gametocytaemias in natural transmission and it should be stressed that the baseline of one gametocyte per µl is far below the detection level that is routinely applied in epidemiological studies (1 in 0.2 µl).

In the second part of this field study (April-July) six gametocyte carriers were identified and asked to sleep for one night under a bednet that was not tucked in. At regular times mosquitoes that had entered and fed were collected and screened for species and pre-oocyst forms. One of them was chosen to sleep under a bednet for the following 10 days because virtually all mosquitoes proved to have at least one pre-oocyst stage. His gametocytaemia was 8/µl with a mean of 10 male and 31 female gametocytes per 5 µl and this remained fairly constant during the experimental period.

Out of 172 fed mosquitoes fed on him 74% showed at least one pre-oocyst stage. 41% was *An. gambiae* , 16% *An. funestus* and 74% *An. nili* (Table II).

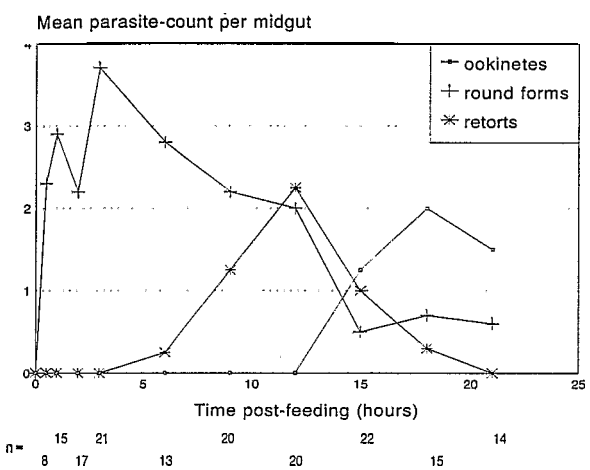
TABLE II
Mbebe-mosquitoes and their mean parasite content

Species	*Anopheles gambiae*	*Anopheles funestus*	*Anopheles nili*
Total number of mosquitoes	70	28	74
Positive mosquitoes	58	21	48
% sp.	83%	75%	65%
% total	46%	17%	38%
Round forms	2.4	2.5	1.9
Retorts	1.6	1.6	0.9
Ookinets	2.2	0.8	1.4

Towards the rainy season *An. funestus* appeared less frequent and *An. nili* had increased. The percentage of positive *An. nili* is significantly lower than that of *An. gambiae* (chi-square test, $\alpha = 0.05$, 1 df), which may be explained by their smaller size and hence smaller bloodmeal.

SPOROGONIC POPULATION DYNAMICS

The figure shows the development in time of the three pre-oocyst stages. In general terms all three anopheline species show comparable figures, thus we have combined all data. Round forms peak at 3 hours post-feeding, retorts at 12 h and ookinetes at 18 h. The mean pre-oocyst count of all mosquitoes remains fairly constant for 12 hours and then starts to decrease. Between 12 and 21 h we found a 2.25-fold decrease in parasite numbers. Apparently the transient retorts represent a vulnerable stage.



Development of sporogonic stages in bloodmeals of gorged anophelines in Mbébé

These data differ from those found in Nijmegen using cultured parasites in *An. gambiae*: round forms at 3 h, retorts at 15 h and ookinetes at 20-22 h post-feeding. This may be explained by several differences between field and laboratory: temperature, humidity, parasite load and uniformity of mosquitoes. Particularly the numbers of ookinetes per mosquito may be a factor 100 different! Developmental times in the lab can be reduced and efficiency improved when gametocyte-blood is diluted (Ponnudurai et al. 1987). A major difference in statistical significance between results in the lab and the field is to be expected (Medley et al. 1993); numbers of examined mosquitoes in the field need to be largely increased as parasite densities are very low.

Vaughan et al. (1992) recently reported even longer periods of development for retorts and ookinetes in the laboratory (24-31 h), whilst we never saw a retort at 24 h! Stepwise decreases from round forms to ookinetes found in laboratory-based studies with enormous numbers of gametocytes in the bloodmeal are 10 to 40 x, suggesting a more optimal situation for the parasites in low numbers under field conditions (maximally 3 ookinetes in our study and an average of 2 in that of Beier et al. 1992).

Our next goal will be to expand the field data and study human bloodfactors that may influence the success of parasitic development in the mosquito, i.e. antibodies against sexual stages, cytokines and complement.

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