Annals of Tropical Medicine & Parasitology, Vol. 92, No. 8, 889–890 (1998)

# Estimation of *Plasmodium falciparum* oocyst survival in *Anopheles arabiensis*



Oocysts begin to develop in the vectors of malarial parasites 24–72 h after the vectors have ingested the infective bloodmeal. Within 7–15 days of the bloodmeal, the exact time depending on the species of parasite and the temperature (Garnham, 1988), the oocysts have matured and release thousands of sporozoites into the haemocoel. The numbers of *Plasmodium falciparum* oocysts found, 3 and 8 days post-bloodmeal (pbm), in *Anopheles arabiensis*, a major malaria vector in Africa and part of the *An. gambiae* complex, were investigated in the present study.

Carriers of *P. falciparum* gametocytes were identified among naturally infected patients reporting at dispensaries in Dakar, Senegal, in September–December 1997, and a sample of venous blood was collected (from each of those who consented) into a heparinized Vacutainer® (Becton Dickinson) and immediately offered to hungry female *An. arabiensis*, 3–4 days after the mosquitoes had emerged from pupae. The mosquitoes used were raised from larvae collected from market-garden wells in Dakar, and reared in an insectary at 27–29°C and 70%–90% relative humidity. The females were placed in cups covered by mosquito netting, and blood from gametocyte carriers was offered to them, while held at 37°C, using a membrane-feeder system. The mosquitoes, kept in the dark, were allowed to feed for 15 min and then any that were not fully engorged were removed. The engorged mosquitoes were maintained without any further bloodmeals. About one third of those then surviving were examined on day 3 pbm for oocysts. Dissected midguts were stained with an anti-Pfs25 monoclonal antibody conjugated to fluoroscein isothiocyanate, so that any young oocysts could be identified and counted under a fluorescence microscope (Gouagna *et al.*, 1998). All the surviving mosquitoes were dissected on day 8 pbm and any oocysts were then counted using mercurochrome stain.

The experiment was repeated until there were results for 19 replicates in each of which at least nine midguts had been examined on each of days 3 and 8 pbm and at least one oocyst had been observed. The results for these 19 replicates are given below, mostly as means and (S.D.). The blood for each replicate came from a different gametocyte carrier (mean age = 21.8 years; range = 9–45 years). The mean density of gametocytes in the 19 donors of blood used to produce the results analysed was 197 (235)/$\mu$l blood (range = 6–963/$\mu$l). The mean number of fully fed mosquitoes per replicate was 41 (10) and the mean numbers of midguts examined in each replicate were 11.7 (3.6) on day 3 pbm and 17.5 (5.2) on day 8. The maximum numbers of oocysts found in single mosquitoes were 13 on day 3 and eight on day 8. Overall, 127 oocysts were counted in the 222 midguts examined on day 3, and 71 in the 333 checked on day 8.

The mean proportions (%) of dissected mosquitoes found to have at least one oocyst were 26.7 (15.1) and 8.9 (14.6) on days 3 and 8, respectively, representing a reduction of 67% in 5 days ($P = 0.02$, by Wilcoxon's test on paired groups). There was a poor correlation between the proportions of mosquitoes found positive for oocysts on days 3 and 8 ($r = 0.30$; $P = 0.21$).

There were similar trends in the numbers of oocysts observed. The mean numbers of oocysts per mosquito were 0.61 (0.79) on day 3 and 0.21 (0.39) on day 8, representing a reduction of 66% in 5 days ($P = 0.02$; by Wilcoxon's test on paired groups). Although the numbers of oocysts observed per mosquito dissected on days 3 and 8 were poorly correlated ($r = 0.30$; $P = 0.22$), the numbers of oocysts observed per infected mosquito on

0003-4983/98/080889-02 $9.00
Carfax Publishing Ltd

days 3 and 8, with means of 1.86 (1.61) and 0.98 (1.34), respectively, were strongly linked ($r = 0.53$; $P = 0.018$).

Oocyst survival from day 3 to day 8 pbm can be estimated as [(proportion of mosquitoes with oocysts on day 8) × (mean number of oocysts/mosquito on day 8)]/[(proportion of mosquitoes with oocysts on day 3) × (mean number of oocysts/mosquito on day 3)] = 0.115 (i.e. about one oocyst out of nine survived from day 3 to day 8).

In comparable experiments carried out in Cameroon, Gouagna *et al.* (1998) observed a much higher parasite survival, of 0.78 between days 2 and 7 pbm, using *P. falciparum* in *An. gambiae* s.s. The apparently better survival of *P. falciparum* in *An. gambiae* s.s. than in *An. arabiensis* is of interest for two reasons. Firstly, it may help explain why the sporozoite indices observed in *An. gambiae* s.s. in Senegalese villages are higher than those in sympatric *An. arabiensis*, even when the two species present similar anthropophilic and parous rates (Lemasson *et al.*, 1997; Diatta *et al.*, 1998). Secondly, it implies that young oocysts are potentially a better indicator of infectivity, compared with mature oocysts, when transmission-blocking immunity is to be determined.

ACKNOWLEDGEMENTS. We would like to thank R. Sauerwein for providing the fluorescent antibody used, and Y. Dutheil for his partnership. This study was financially supported by ORSTOM and by SmithKline Beecham Africa International.

H. P. AWONO-AMBENE
Laboratoire de Paludologie,
ORSTOM, B.P. 1386,
Dakar, Senegal, and
Université Cheikh Anta Diop,
Faculté des Sciences et Techniques,
Département de Biologie Animale,
Dakar, Senegal

V. ROBERT*
Laboratoire de Paludologie,
ORSTOM,
B.P. 1386,
Dakar, Senegal

*Received 20 May 1998, Revised and accepted 8 September 1998*

* Author to whom correspondence should be addressed. E-mail: vincent.robert@orstom.sn; fax: +221 832 16 75.

## REFERENCES

DIATTA, M., SPIEGEL, A., LOCHOUARN, L. & FONTENILLE, D. (1998). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92**, 270–272.

GARNHAM, P. C. C. (1988). In *Malaria. Principles and Practice of Malariology*, Vol. 1, eds Wernsdorfer, W. H. & McGregor, I. pp. 61–96. Edinburgh: Churchill Livingstone.

GOUAGNA, L. C., MULDER, B., NOUBISSI, E., TCHUINKAM, T., VERHAVE, J. P. & BOUDIN, C. (1998). *Tropical Medicine and International Health*, **3**, 21–28.

LEMASSON, J. J., FONTENILLE, D., LOCHOUARN, L., DIA, I., SIMARD, F., BA, K., DIOP, A., DIATTA, M. & MOLEZ, J. F. (1997). *Journal of Medical Entomology*, **34**, 396–403.