

Production of *Plasmodium falciparum* sporozoites by *Anopheles plumbeus*

W. Eling¹, G-J van Gemert¹, O. Akinpelu², J. Curtis² and C.F. Curtis^{2,3}

¹ University of Nijmegen, Netherlands ² London School of Hygiene & Tropical Medicine, London WC1E 7HT, U.K. ³ author for correspondence, email: chris.curtis@lshtm.ac.uk

Abstract

The progeny of two *Anopheles plumbeus*, collected biting a human in London, were fed on *Plasmodium falciparum* gametocytes grown *in vitro*. 18-21 days after the blood feed, six out of ten of the mosquitoes had visible sporozoites in their salivary glands and these glands showed strong positivity in an ELISA test for *P. falciparum* circumsporozoite protein. This confirms earlier suggestions that, unlike *An. atroparvus*, *An. plumbeus* can nurture completion of sporogony of tropical *P. falciparum*.

Following a case of *P. falciparum* malaria in the north of England more than 70 years ago in a person who had not travelled to the tropics (Blacklock, 1921), and two much more recent such cases in Germany (Krüger *et al.*, 2001), it was suggested that *An. plumbeus* could have been the vector. If so, this tree-hole breeding species (Blacklock & Carter, 1920b) must differ in parasite susceptibility from the marshland breeding *An. atroparvus*, which is accepted as having been the vector of *P. vivax* in northern Europe (Hackett, 1937), but not being susceptible to tropical *P. falciparum* (Shute, 1940; Ramsdale & Coluzzi, 1975; Dashkova & Rasnicyn, 1982). There is evidence that *P. vivax* can develop as far as the sporozoite stage in *An. plumbeus* (Blacklock & Carter, 1920a) and Shute & Maryon (1974) suggest that it may have been the vector of two autochthonous cases of *P. vivax* recorded in London in 1953. *Plasmodium falciparum* can develop at least as far as the oocyst stage after feeding *An. plumbeus* on gametocytes of this malaria parasite (Blacklock & Carter, 1920c; Marchant *et al.*, 1998). It remains to check whether sporogony of *P. falciparum* could be completed in these mosquitoes.

An opportunity to acquire material with which to test this arose when two *An. plumbeus* came to bite one of us at about 1100h and 1600h during August 2002, near Primrose Hill, not far from central London, indoors in an apartment near old trees in which there may well be suitable tree holes for *An. plumbeus* breeding. Biting indoors in daylight is a known habit of this species (Blacklock & Carter, 1920b). We caught the two biting mosquitoes and, with difficulty, fed them both on human blood. Eggs were subsequently laid and 39 adults were reared in the insectaries of the London School of Hygiene & Tropical Medicine and transported by a sea-traveller to the Netherlands who delivered them to the University of Nijmegen. There, 11 of the 19 females were successfully fed through a membrane on gametocytes of the NF54 strain of *P. falciparum* produced by methods described by Ponnudurai *et al.* (1988). *Anopheles stephensi* were simultaneously fed as controls and, as expected, developed numerous oocysts.

After the blood feed the mosquitoes were kept at 26°C and 80% RH. One *An. plumbeus* was dissected after a few days to check that oocysts were developing. Table 1 shows the results for the other 10 *An. plumbeus* dissected 18-21 days. All but one had oocysts, either empty or full and the oocyst density was of the same order of magnitude as in the *An. stephensi* controls. Six of the *An. plumbeus* had visible sporozoites in the salivary glands. The glands were returned to London and the ELISA for *P. falciparum* (Burkot *et al.*, 1984) was applied. All except the glands from the mosquito with no visible oocysts proved positive for *P. falciparum* circumsporozoite protein (CSP) by ELISA. Readings made without knowledge of the dissection results showed that all six which had had visible sporozoites gave strong coloration in the ELISA. The three with oocysts but no visible sporozoites gave faint ELISA coloration, suggesting the presence of CSP not bound to sporozoites.

Thus *P. falciparum* can complete sporogony in *An. plumbeus*. Even if global warming raises the summer temperatures in northern Europe to levels at which sporogony could readily go to completion out of doors, we do not expect any more than very rare autochthonous cases of *P. falciparum* to occur. This is because the considerable number of travellers who arrive in, or return to, northern Europe with *P. falciparum* infection have their illness well looked after by the existing medical systems. They therefor seldom remain infected long enough for gametocytes to develop which could infect an *An. plumbeus* if it happened to bite them.

Table 1. Results of dissection and ELISA for *P. falciparum* on 10 *Anopheles plumbeus* that had been fed on *Plasmodium falciparum* gametocytes grown *in vitro*.

Mosquito no.	Number of full oocysts	Number of empty oocysts	Visible sporozoites in salivary glands?	Coloration in ELISA on salivary glands
1	27	13	Yes	Strong
2	2	10	No	Faint
3	0	8	Yes	Strong
4	1	0	No	Faint
5	4	3	Yes	Strong
6	0	0	No	None
7	12	19	Yes	Strong
8	1	6	No	Faint
9	0	2	Yes	Strong
10	6	40	Yes	Strong

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