Clinical malaria reduces human attractiveness to mosquitoes

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Evolutionary fitness concepts dictate that blood parasites should regulate their transmission success by enhancing the responsiveness of arthropod vectors to infectious hosts. We observed that the presence of trophozoite stages of *Plasmodium falciparum* in peripheral blood, combined with clinical malaria symptoms, actually reduced the attractiveness to *Anopheles gambiae* mosquitoes of one Kenyan male, relative to another. Their innate levels of attractiveness were restored within days, prior to the onset of gametocytaemia. These findings support the theory that a parasite-modulated change in host attractiveness occurs, but not at the stage when transmission from the human host to mosquito vector can be effected.

Keywords: *Anopheles gambiae*, mosquito, *Plasmodium falciparum*, parasite, clinical malaria, human attractiveness

Some humans are more attractive to host-seeking mosquitoes than others, contributing substantially to heterogeneity of exposure to malaria and stability of endemicity in afflicted populations (Smith *et al.* 2005). For instance, various species of *Anopheles* exhibit preferences for men rather than women, pregnant rather than non-pregnant women, and individuals with blood group O rather than those of other blood groups (Hurd 2003). Furthermore, key vector species of malaria in Africa are attracted to humans in direct proportion to their body weight, with the result that most malaria transmission originates from adults and older children, rather that the more infectious children (Ross *et al.* 2006). Disease control programmes typically focus on protecting the most vulnerable

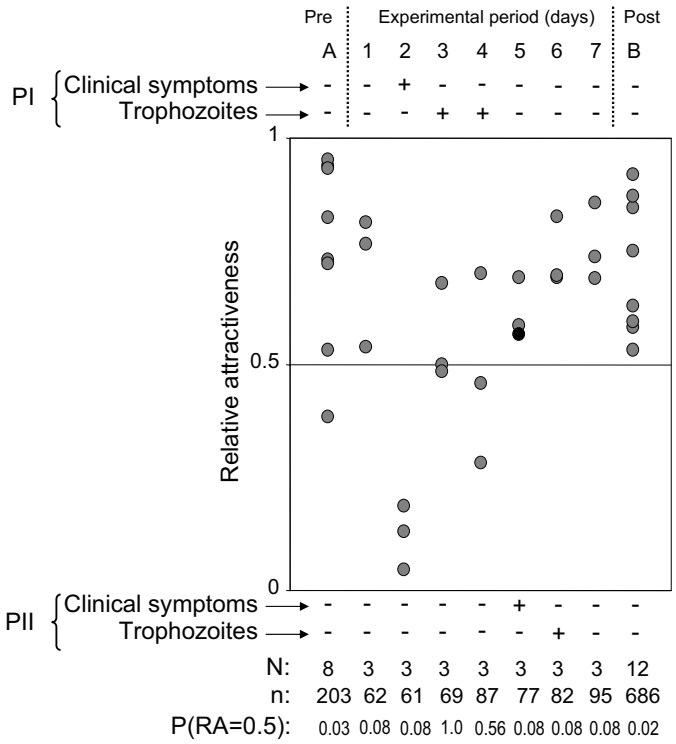
but could achieve greater community-level transmission control by targeting those groups contributing most to transmission (Smith *et al.* 2005, Ross *et al.* 2006). More specifically, the success of control programmes can be improved by determining whether susceptible mosquitoes prefer to feed on infective rather than non-infective humans or *vice versa* (Lacroix *et al.* 2005). Although there is evidence that malaria parasites manipulate their vectors, this mainly derives from rodent malaria models and no consensus view has emerged from studies of *Plasmodium falciparum* in humans (Lacroix *et al.* 2005, Bousema & Sauerwein 2005).

MATERIAL AND METHODS

Here we describe how a rare window of opportunity enabled us to measure the effect of clinical symptoms of *Plasmodium falciparum* infection on the relative attractiveness of two male Kenyans (designated as Person 1 and Person 2, 19 and 22 years of age, respectively) to the African malaria mosquito *Anopheles gambiae* Giles. Relative attractiveness of the two persons was measured on seven consecutive days during which the individuals were both uninfected or alternately displayed parasitaemia and clinical symptoms of falciparum malaria. Malaria infection status was tested daily by microscopically examining thin and thick smears of finger-pricked blood stained with Giemsa. When positive, the men were treated with a single complete dose of sulfadoxine-pyrimethamine (FANSIDAR®). Three half-hour dual-choice experiments were carried out every evening and relative attractiveness of the subjects' total body odour was calculated by dividing the number of mosquitoes trapped by the emanations of Person I by the sum of those trapped by Person I and Person II. The significance of differences in attractiveness between the two persons was assessed by Kendall's W test for related samples, and the significance of changes in relative attractiveness between experiments was assessed by the Kruskal-Wallis H test for independent samples.

RESULTS

Person I always attracted more mosquitoes than Person II, when both were uninfected, but fever and profuse sweating associated with falciparum malaria suppressed his relative attractiveness and reversed the usual ranking of the pair to near significance (p=0.08). Furthermore, the relative attractiveness of Person I, when suffering these clinical symptoms (day 2), differed significantly from all other nights before (p=0.01), during (p=0.050) and after (p=0.009) the seven-day window. The relative attractiveness of the two individuals returned to baseline levels over the following two days, during which no symptoms were observed but trophozoites were readily detected in the blood of Person I. Immediately after Person I no longer exhibited clinical symptoms or patent parasitemia,



$P(RA_1 = RA_2)$:

RA_1 \ RA_2	1	2	3	4	5	6	7	B
A	0.68	**0.01**	0.10	0.07	0.22	0.54	0.84	0.22
1		**0.05**	0.13	0.13	0.51	0.83	0.83	0.67
2			**0.05**	**0.05**	**0.05**	**0.05**	**0.05**	**0.009**
3				0.51	0.28	**0.05**	**0.05**	0.31
4					0.51	0.28	0.13	0.31
5						0.08	0.13	0.67
6							0.83	0.31
7								0.31

Figure 1. The relative attractiveness of persons I and II to *Anopheles gambiae* when both subjects were uninfected (periods A and B; days 1 and 7) or alternately displaying clinical symptoms (day 2, person 1; day 5, person 2) or trophozoites (day 3 and 4, person 1; day 6, person 2) of falciparum malaria. Plus (+) and minus (-) signs depict the presence and absence of trophozoites/clinical symptoms, respectively. The black dot represents the only record when person II exhibited clinical symptoms. N represents the number of replicates and n the total number of mosquitoes used in the experiments. A and B represent results of experiments conducted 2-5 days before (A) and 770-773 days after (B) the seven consecutive experimental days. Points above the 0.5 mark represent higher attractiveness of person I. P (RA=0.5) represents the significance of differences in relative attractiveness (RA) between person I and person II in the same day (1-7) or period (A and B). P (RA_1=RA_2) represents the change in relative attractiveness between experiments *e.g.* the relative attractiveness of the test subjects between day 2 and period B was significantly different (p=0.009).

Person II developed clinical malaria symptoms (chills) and then patent *P. falciparum* trophozoite infection but these did not cause an obvious effect in the relative attractiveness of the two subjects (Fig. 1), probably because of the lower baseline relative attractiveness of Person II under natural conditions (Mukabana *et al.* 2004). While neither of the participants exhibited gametocyte-stage parasites, these commonly infect mosquitoes at densities below the limit of microscopic detection (Schneider *et al.* 2006).

DISCUSSION

We have shown that the world's most important malaria vector avoids symptomatic carriers of malaria, an aspect consistent with evidence that malaria infection exerts selection pressure on mosquitoes, resulting in complementary, heritable but non-behavioral traits that reduce the transmission of *P. falciparum* to *An. gambiae* (Niare *et al.* 2002). Since no observations were made when any of the participants was positive for gametocyte stages, which are infectious to susceptible mosquito vectors and generally occur after peaks of parasitaemia, we cannot directly extrapolate our results in terms of disease transmission. Moreover, as treatment with Fansidar leads to poor parasite clearance (Bousema *et al.* 2003) and the majority of gametocyte carriers harbour gametocytes beyond the microscopic detection limit (Nassir *et al.* 2005), our results only peg on the effects of malaria-associated clinical symptoms on human attractiveness to mosquitoes. The extent to which mosquitoes avoided the symptomatic subject underscores the need to understand the true relationship between malaria infection status and exposure to mosquitoes so that its contribution to heterogeneity of transmission can be quantified and appropriate control measures can achieve maximum impact in terms of preventing malaria transmission within afflicted communities.

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