# *Plasmodium falciparum* sporozoites increase feeding-associated mortality of their mosquito hosts *Anopheles gambiae* s.l.

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## SUMMARY

There is some evidence that pathology induced by heavy malaria infections (many oocysts) increases mortality of infected mosquitoes. However, there is little or no published evidence that documented changes in feeding behaviour associated with malaria infection also contribute to higher mortality of infected mosquitoes relative to uninfected individuals. We show here for the first time that, in a natural situation, infection by the sporozoites of the malaria parasite *Plasmodium falciparum* significantly reduced survival of blood-feeding *Anopheles gambiae*, the major vector of malaria in sub-Saharan Africa. To estimate feeding-associated mortality of infected mosquitoes, we compared the percentage of sporozoite infection in host-seeking mosquitoes caught before and after feeding. The infection rate was 12% for mosquitoes caught during the night as they were entering a tent to feed; however, only 7·5% of the surviving members of the same cohort caught after they had had the opportunity to feed were infected. Thus, *Plasmodium falciparum* sporozoites increased the probability of dying during the night-time feeding period by 37·5%. The increase in mortality was probably due to decreased efficiency in obtaining blood and by increased feeding activity of the sporozoite-infected mosquitoes that elicited a greater degree of defensive behaviour of hosts under attack.

Key words: malaria, virulence, *Plasmodium falciparum*, *Anopheles gambiae*.

## INTRODUCTION

It generally has been suggested that malaria-infected mosquitoes survive at the same rate as uninfected cohorts (Macdonald, 1957). Some experimental data from laboratory studies support this contention (Chege & Beier, 1990; Robert, Verhave & Carnevale, 1990), although data from other studies support the opposite conclusion (Klein *et al*. 1982, 1986). These studies examined only direct mortality as a result of the physical presence of parasites in mosquito tissues. Similarly, in a study by Lyimo & Koella (1992) of a natural population of mosquitoes, the authors interpreted their data to mean that high oocyst burdens of large mosquitoes induce mortality in a direct way, perhaps as a result of metabolic burden. In nature, mosquitoes are likely to be subject to other sources of mortality, such as that associated with attacking a defensive host, and such mortality may be exacerbated by malaria infection.

Mosquitoes must bite at least 1 host each reproductive cycle to obtain blood. Studies of defensive behaviour of vertebrate hosts under attack by mosquitoes have demonstrated that mosquitoes are killed while trying to feed (Day & Edman, 1984; Edman, Day & Walker, 1984). A number of studies (Rossignol, Ribeiro & Spielman, 1984, 1986; Wekesa, Copeland & Mwangi, 1992) have shown that sporozoite-infected mosquitoes probe more often and spend more time probing than their uninfected counterparts because sporozoite-infected salivary glands produce less apyrase than uninfected glands (Rossignol *et al*. 1984). Mosquitoes positive for *Plasmodium falciparum* circumsporozoite protein also bite more people per night than uninfected mosquitoes (Koella, Sørensen & Anderson, 1998). Sporozoite-infected mosquitoes have also been demonstrated to be more persistent in returning to bite after thwarted feeding attempts (Anderson, Hurd & Koella, 1999). These changes may benefit malaria because transmission increases with the biting rate of mosquito hosts; however, they also stand to increase the mortality costs of the mosquitoes because of the risk associated with each extra feeding attempt.

To our knowledge, survival of malaria-infected mosquitoes relative to uninfected individuals during blood feeding has never been evaluated. We present experimental evidence from a field study that

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*Anopheles gambiae* s.l. infected with *P. falciparum* are more likely to die during feeding than are uninfected cohorts.

## MATERIALS AND METHODS

Our field study site is located in the village of Njage, 80 km west of Ifakara in the Kilombero Valley of Tanzania. This area is hyperendemic for malaria and has been described by Tanner *et al*. (1987). We designed a human-baited mosquito trap as follows. A site with an open-walled house of bamboo frame and a grass-thatch roof was chosen. Under the roof, 2 light-weight canvas tents (dimensions: 1 m × 1 m × 2 m, i.e. approximately the sleeping area available in average mud and wattle houses used by local residents) were suspended. Each tent had 4 entrances for mosquitoes, 2 on each long side near the top. The entrances narrowed from slots 90 cm long by 10 cm high to a circular port 3 cm in diameter. These baffle entrances reduced the probability that mosquitoes would escape once inside (Service, 1976). Two of the slots on each tent opened into mesh-bag entrance traps (diagonal from each other) that prevented access to sleeping humans in the tent. The other 2 slots (also diagonal from each other, so that mosquitoes approaching a given side of the tent had equal opportunity to end up in an entrance trap or with access to hosts) gave access to the human hosts in the tent. The slots allowing ingress to either the entrance traps or the inside of the tent were covered with the same density and thickness of mesh to equalize the airflow, and thus quality of odour plumes coming through each. Thus, mosquitoes were segregated into 2 samples: (*a*) those that were unable to make contact with hosts, and (*b*) those that had been in contact with human hosts overnight, the normal feeding period for *Anopheles*.

The tents were occupied by local residents from dusk until dawn. All volunteers were provided with chloroquine prophylaxis during and after the study. All mosquitoes in the entrance traps and in the sleeping areas were collected between 06.30 and 07.30 hours. The head and prothorax of each mosquito were tested for evidence of malaria infection by ELISA based on a monoclonal antibody to the $\text{NANP}_{40}$ repeat region of *Plasmodium falciparum* circumsporozoite protein (Burkot, Williams & Schneider, 1984). To make comparisons among plates more reliable, we standardized ELISA values for each plate by dividing them with the mean of the positive controls and multiplying them by 100. Transformed values higher than the mean of all negative controls plus 3 times their standard deviation were considered positive. The positives were confirmed by re-analysis. To control for mosquito size as a possible confounder of our analysis, we measured the wings to the nearest 0·05 mm from the tip, excluding the fringe, to the distal end of the allula with a dissecting microscope equipped with a micrometer.

If malaria-infected mosquitoes are subject to disproportionate mortality because they feed in a more risky manner, we expected that the proportion of infected mosquitoes remaining after blood feeding should be less than the proportion of infected mosquitoes entering the trap. The proportion of infected mosquitoes in entrance traps and sleeping areas was compared by logistic analysis blocked by date and tent. As there may be a non-linear association between wing length and infection (Lyimo & Koella, 1992), wing length and its square were included in the model as confounders. The number of infected mosquitoes was too low to investigate the interactions between wing length and collection area with this analysis. Therefore, we did an additional analysis, in which we categorized the mosquitoes according to 2 size classes.

An association between malaria infection and the probablity of successful blood-feeding was investigated with Fisher's exact test of the mosquitoes caught in the sleeping area. For this test, bloodmeal size was split into 2 categories: less than half-fed and at least half-fed.

## RESULTS

In total 1565 *A. gambiae* Giles s.l. mosquitoes were collected over a 4 day collection period. Of these 218 mosquitoes were collected in the entrance traps and 1347 were collected from the sleeping areas. Wing lengths ranged from 1·70 to 2·65 mm with a mean of 2·15 mm, with no difference between the mosquitoes from the entrance traps and the sleeping areas ($t = 1{\cdot}404$, D.F. $= 1563$, $P = 0{\cdot}161$). Of all mosquitoes tested 8% were positive for sporozoite infection. Twenty-six (12%) of the mosquitoes in the entry traps, but only 103 (7·5%) of the mosquitoes in the post-feeding group were infected (Table 1, Fig. 1). Thus, the proportion of infected mosquitoes decreased by 37·5% during the feeding period. Before and after feeding, the proportion of infected mosquitoes was highest in the intermediate sized mosquitoes and lowest in the largest mosquitoes (Table 1, Fig. 2). The pattern of the size-dependence changed during the feeding period, though not quite at a statistically significant level (Table 2). While in the smallest mosquitoes there was almost no difference in infection between mosquitoes caught in the entrance traps and the ones allowed to feed, the infection level dropped during the feeding period by more than half in the largest ones (Fig. 2).

With regard to blood-feeding success of the surviving mosquitoes in the sleeping areas, there was a greater tendency for infected mosquitoes to obtain

Table 1. Logistic analysis of the proportion of *Anopheles gambiae* infected with sporozoites of *Plasmodium falciparum* as a function of collection location (entrance trap or sleeping area), blocked by the date and the tent where the mosquitoes were caught and corrected for wing length and its square as confounding factors

| Source | D.F. | $\chi^2$ | $P$ |
|---|---|---|---|
| Date collected (random) | 3 | 3·309 | 0·346 |
| Tent (random) | 1 | 0·040 | 0·842 |
| Wing length | 1 | 5·479 | 0·019 |
| (Wing length)$^2$ | 1 | 5·919 | 0·015 |
| Trap | 1 | 5·688 | 0·017 |
| No. of observations | 1565 | | |

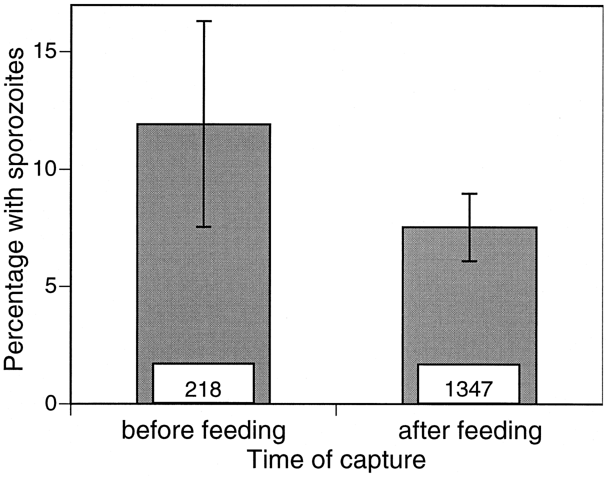

Fig. 1. Proportion of mosquitoes containing sporozoites caught in entry traps before they had an opportunity to feed, compared with those in the sleeping area after they had fed or attempted to feed during the night. The bars represent the percentages, the vertical lines the 95 % confidence intervals of the percentages, and the numbers in the bars the sample sizes.

at least half of a bloodmeal (87·5 %) in comparison with uninfected individuals (81·5 %) (Fisher's exact one-tailed test: $P = 0·08$).

## DISCUSSION

That the proportion of *Anopheles* harbouring malaria sporozoites decreased during their feeding period suggests that the infection increased the risk of biting-induced mortality. This suggestion is supported by previous studies on the blood-feeding behaviour of infected and uninfected mosquitoes. First, infection by malaria sporozoites decreases the efficiency with which mosquitoes can find blood, so that infected mosquitoes must probe more often to obtain their bloodmeal (Rossignol *et al.* 1984, 1986; Wekesa *et al.* 1992). Second, infected mosquitoes are more likely than uninfected ones to bite more than once to obtain a full bloodmeal (Koella *et al.* 1998) and sporozoite-infected mosquitoes are more likely than uninfected mosquitoes to return after being thwarted from successful feeding (Anderson *et al.* 1999). Both of these effects – clumsy and risky feeding behaviour – are likely to increase the risk of being killed during feeding. In our study, the suggestion that infected mosquitoes are more persistent is supported by the blood-feeding data which indicate a greater degree of engorgement for infected mosquitoes.

The suggestion is further supported by details of the interaction. As the largest mosquitoes are most likely to obtain heavy infections (Lyimo & Koella, 1992) and their biting rate is increased most by the parasite (Koella *et al.* 1998), it is the largest ones that should suffer most from parasite-induced mortality. This is consistent with the size-specific pattern observed in Fig. 2 and with an earlier study that suggested the highest level of parasite-induced mortality in the largest mosquitoes (Lyimo & Koella, 1992).

Alternative interpretations of the data are possible, but unlikely. First, it is possible that the ingress ports leading to the entrance traps are more attractive than the ports leading to the sleeping area for the infected mosquitoes, but not for uninfected mosquitoes. However, it is difficult to imagine a plausible mechanism for such a difference in behaviour, as the routes by which mosquitoes ended up in either entrance traps or the sleeping areas were identical. Furthermore, the tents were designed to avoid the possible bias of infected mosquitoes approaching from a direction different from uninfected mosquitoes. One of the 2 ingress ports on each long side of the tents opened into the sleeping area and the other ingress port opened into an entrance trap. Therefore, mosquitoes approaching from either side had an equal chance of ending up in either the sleeping area or an entrance trap. The disparity in total numbers of mosquitoes recovered from the entrance traps and the sleeping areas is somewhat puzzling due to the balanced nature of the respective entrance slots. Previous studies have shown that unfed mosquitoes are more active and thus more likely to leave baffled areas than are fed mosquitoes (Smith, 1965). Thus, mosquitoes that had entered the traps but could not access the sleeping humans may have found their way out at a greater rate than those mosquitoes in the sleeping area with access to a host. This explanation, however, would affect our conclusions only if, in addition, uninfected mosquitoes were more active than infected ones, contrary to a previous study in which infected mosquitoes were the most persistent in trying to obtain a bloodmeal (Anderson *et al.* 1999).

Second, as the development of the sporozoites

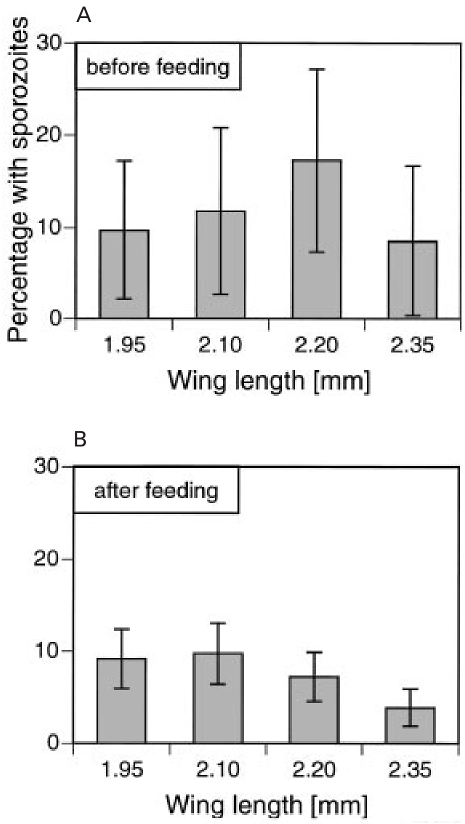

Fig. 2. Effect of wing length on the proportion of mosquitoes containing sporozoites in (A) entry traps and (B) in sleeping areas. In each graph, mosquitoes are grouped into 4 size classes with similar number of mosquitoes, and the wing length shows the midpoint within each class. The bars represent the percentages and the vertical lines the 95% confidence intervals of the percentages. Note that the analysis shown in Table 2 is based on only 2 size groups.

Table 2. Logistic analysis to test for an interaction between mosquito size (wing length) and collection location (entrance trap or sleeping area) with regard to the proportion of infected mosquitoes

(Mosquitoes are grouped into 2 size classes so that a sufficient number of infected mosquitoes is obtained within each group. The analysis is blocked by the date and the tent where the mosquitoes were caught.)

| Source | D.F. | $\chi^2$ | *P* |
|---|---|---|---|
| Date collected (random) | 3 | 3·217 | 0·359 |
| Tent (random) | 1 | 0·033 | 0·856 |
| Trap | 1 | 6·492 | 0·011 |
| Size class | 1 | 0·475 | 0·491 |
| Trap × size class | 1 | 2·993 | 0·084 |
| No. of observations | 1565 | | |

takes several days, mosquitoes generally harbour sporozoites only after their 4th or 5th gonotrophic cycle (Gillies & Wilkes, 1965). Therefore, infected mosquitoes are, on average, older than uninfected ones, so that the apparent mortality of infected mosquitoes could be a reflection of increased mortality rates in old mosquitoes. In fact, Clements & Patterson (1981) have suggested that mortality increases proportionately with age in *Anopheles gambiae*, based on reanalysis of Gillies & Wilkes (1965) data. However, the daily increase in mortality suggested by them for this species of mosquito is much less than the 37·5% jump in mortality indicated by our data and they admit that a linear relationship may hold for the first 6 gonotrophic cycles. Additionally, 2 other major studies of survivorship in Afro-tropical *Anopheles* species have found that mortality rate is independent of age at least up to and including the 6th or 7th gonotrophic cycle (Gillies & Wilkes, 1965; Lines, Wilkes & Lyimo, 1991), after which the low number of survivors reduces the accuracy of analysis. This suggests that at least some of the mortality observed in our study is due to the presence of the parasite.

It is theoretically possible that certain mosquitoes are intrinsically more persistent than others (due to genetic or other factors) and thus, most likely to become infected because of larger bloodmeal size. Consequently, the observed higher mortality of infected mosquitoes may be due to this intrinsic variability in persistence, rather than the effect of the parasite. However, these more persistent individuals would also be more likely to die from blood feeding-associated mortality at one of the intervening feeding bouts before sporozoites become available for transmission. Therefore, an intrinsically variable biting rate among mosquitoes is unlikely to explain the observed pattern.

In accordance with the laboratory-based studies of Klein *et al.* (1982, 1986) and the field data presented by Lyimo & Koella (1992), we provide strong evidence from a natural situation that mosquitoes infected with human malaria have a higher risk of mortality than uninfected mosquitoes, and that a portion of this mortality is associated with the feeding activity of the mosquitoes. As the mosquito's mortality is one of the principal entomological factors determining the rate of transmission (Macdonald, 1957), parasite-induced mortality will have important implications for the epidemiology of malaria. In particular, control programs, which are generally designed to decrease the intensity of infection, may have surprising results by decreasing mortality of lightly infected individuals which are still able to transmit the parasite.

From the perspective of the relationship between malaria and its mosquito host, our demonstration of feeding-associated mortality as a cost to the mosquito of malaria infection and evidence from studies of fecundity reduction (Hogg & Hurd, 1996) associated with malaria infection are evidence that malaria dominates this evolutionary interaction.

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## REFERENCES

ANDERSON, R. A., HURD, H. & KOELLA, J. C. (1999). The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle. *Proceedings of the Royal Society of London, B* **266**, 1729–1733.

BURKOT, T. R., WILLIAMS, J. L. & SCHNEIDER, I. (1984). Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene* **33**, 783–788.

CHEGE, G. M. M. & BEIER, J. C. (1990). Effect of *Plasmodium falciparum* on the survival of naturally infected Afrotropical *Anopheles* (Diptera: Culicidae). *Journal of Medical Entomology* **27**, 454–458.

CLEMENTS, A. N. & PATTERSON, G. D. (1981). The analysis of mortality and survival rates in wild populations of mosquitoes. *Journal of Applied Ecology* **18**, 373–389.

DAY, J. F. & EDMAN, J. D. (1984). Mosquito engorgement on normally defensive hosts depends on host activity patterns. *Journal of Medical Entomology* **21**, 732–740.

EDMAN, J. D., DAY, J. F. & WALKER, E. D. (1984). Field confirmation of laboratory observations on the differential antimosquito behaviour of herons. *Condor* **86**, 91–92.

GILLIES, M. T. & WILKES, T. J. (1965). A study of the age composition of populations of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in North-Eastern Tanzania. *Bulletin of Entomological Research* **56**, 237–262.

HOGG, J. C. & HURD, H. (1996). The effects of natural *Plasmodium falciparum* infection on the fecundity and mortality of *Anopheles gambiae* s.l. in north east Tanzania. *Parasitology* **114**, 325–331.

KLEIN, T. A., HARRISON, B. A., ANDRE, R. G., WHITMIRE, R. E. & INLAO, I. (1982). Detrimental effects of *Plasmodium cynomolgi* infections on the longevity of *Anopheles dirus*. *Mosquito News* **42**, 265–271.

KLEIN, T. A., HARRISON, B. A., GROVE, J. S., DIXON, S. V. & ANDRE, R. G. (1986). Correlation of survival rates of *Anopheles dirus* A (Diptera: Culicidae) with different infection densities of *Plasmodium cynomolgi*. *Bulletin of the World Health Organization* **64**, 901–907.

KOELLA, J. C, SØRENSEN, F. L. & ANDERSON, R. A. (1998). The malaria parasite *Plasmodium falciparum* increases the frequency of multiple feeding of its mosquito vector *Anopheles gambiae*. *Proceedings of the Royal Society of London, B* **265**, 763–768.

LINES, J. D., WILKES, T. J. & LYIMO, E. O. (1991). Human malaria infectiousness measured by age-specific sporozoite rates in *Anopheles gambiae* in Tanzania. *Parasitology* **102**, 167–177.

LYIMO, E. O. & KOELLA, J. C. (1992). Relationship between body size of adult *Anopheles gambiae* s.l. and infection with the malaria parasite *Plasmodium falciparum*. *Parasitology* **104**, 233–237.

MACDONALD, G. (1957). *The Epidemiology and Control of Malaria*. Oxford University Press, London.

ROBERT, V., VERHAVE, J. P. & CARNEVALE, P. (1990). *Plasmodium falciparum* infection does not increase the precocious mortality rate of *Anopheles gambiae*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 346–347.

ROSSIGNOL, P. A., RIBEIRO, J. M. C. & SPIELMAN, A. (1984). Increased intradermal probing time in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene* **33**, 17–20.

ROSSIGNOL, P. A., RIBEIRO, J. M. C. & SPIELMAN, A. (1986). Increased biting rate and reduced fertility in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene* **35**, 277–279.

SERVICE, M. W. (1976). *Mosquito Ecology: Field Sampling Methods*. Applied Science Publishers, London.

SMITH, A. (1965). A verandah-trap hut for studying the house-frequenting habits of mosquitoes and for assessing insecticides. I. A description of the verandah-trap hut and of studies on the egress of *Anopheles gambiae* Giles and *Mansonia uniformis* (Theo.) from an untreated hut. *Bulletin of Entomological Research* **56**, 161–167.

TANNER, M., DEGRÉMONT, A. D., DE SAVIGNY, D., FREYVOGEL, T.A., MAYOMBANA, C. & TAYARI, S. (1987). Longitudinal study on the health status of children in a rural Tanzanian community: study area and design. *Acta Tropica* **44**, 119–136.

WEKESA, J. W., COPELAND, R. S. & MWANGI, R. W. (1992). Effect of *Plasmodium falciparum* on blood feeding behavior of naturally infected *Anopheles* mosquitoes in Western Kenya. *American Journal of Tropical Medicine and Hygiene* **47**, 484–488.