Plasmodium Parasite-infection in the Malaria Vector Mosquito, Anopheles gambiae (Diptera: Culicidae)

Olayemi, I. K.* and Ande, A. T.

ABSTRACT

The need for a better understanding of the Plasmodium parasite-mosquito vector interactions in the ‘parasite-vector-host’ triad of malaria transmission, a pre-requisite for mosquito vector manipulation for malaria control, informed this study. To this end, the influence of Plasmodium berghei parasite-infected blood meal on reproductive and longevity fitness of Anopheles gambiae mosquito species was investigated under laboratory conditions. The results showed that with the exception of duration of embryogeny and egg-hatching rate, all fitness attributes investigated were significantly (P<0.05) affected in mosquitoes fed with parasite-infected blood. For example, the parasite-infected blood was much less attractive (47.08±8.40%) relative to the uninfected blood (68.27±5.18%); while post-blood meal pre-oviposition period was significantly extended by two days in the test mosquitoes (5.54±0.83 days). Also, Fecundity was significantly higher in the Control than test mosquitoes. The mosquitoes fed with uninfected blood lived almost twice longer (26.95±7.28 days), post-blood meal, than those offered infected-blood. These results show that though An. gambiae has been adjudged the most successful vector of malaria in sub-Saharan Africa, it does that at high fitness costs.

Key Words: Blood Meal, Duration Of Embryogeny, Egg Hatchability, Fecundity, Plasmodium berghei and Pre-oviposition period

1. Introduction

The Anopheles gambiae mosquito species is the most widespread and efficient vector of malaria in sub-Saharan Africa [1-2]. Where the disease accounts for about 10% of the Continent’s overall disease burden [3-5]. Malaria is holo-endemic in many localities in the region of distribution of An. gambiae and sometimes responsible for more than 50% Out-patient Hospital Attendance and 20% infant mortality [6-8]. According to World Health Organisation’s Statistics, malaria remains the bane of poverty and under-development in Africa, costing the Continent billions of dollars per annum, in terms of disease prevention and control. The intolerably high health and socio-economic burdens of malaria in sub-Saharan Africa, has been partly attributed to the efficiency with which the An. gambiae mosquitoes transmit the Plasmodium parasites in the region [9-10]. The high vectorial success of An. gambiae is, however, largely due to its superior reproductive capacity and biologic fitness, relative to the secondary anopheline mosquito vector species [9-11]. This development, therefore, necessitates a good understanding of the relationships between An. gambiae, as vector, and the other two components (namely, Plasmodium parasite and human host) of the tripod of malaria transmission.
However, while Literature is replete with information on interactions between An. gambiae and the human host of malaria [11-14], as well as, between the Plasmodium parasite and humans [15-22], there is a dearth of knowledge on the interactive influence of malaria Plasmodium parasite on vectorial success of the anopheline vectors. This bias results from the erroneous believe in some quarters that the life processes of anopheline vector mosquitoes are generally unaffected by the presence and activities of the Plasmodium parasites they transmit [23-28]. Yet, integrated malaria control programs that will be sustainably effective must incorporate strategies targeted, not only, at reducing human-mosquito vector contact (e.g., through the use of insecticide-treated bednets) and anti-parasite progression in the human host (i.e., chemotherapy) but also include those that will promote antagonism between the Plasmodium parasite its anopheline vector. This study was, therefore, carried out to elucidate the influence of Plasmodium-infected blood meal on reproductive and developmental fitness of An. gambiae mosquito species for malaria transmission.

2. Materials and Methods

2.1 Source and Laboratory Maintenance of Entomological and Parasitological Specimens

A cohort of 24 mice, of same age and approximately equal weight, were obtained from the National Veterinary Research Institute, Vom-Jos, Nigeria. Half of the mice were then infected with Plasmodium berghei, from an infectious mouse sourced from the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The An. gambiae mosquitoes used for the study came from a colony maintained in the Laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. The mice and mosquito specimens were maintained in the Laboratory at ambient conditions of 27.00±2.00°C, 72.00±3.29% RH and 12L: 12 D photoperiod; under which the experiments were subsequently carried out.

2.2 Adult Mosquito Rearing, Parasite infection and Determination of Reproductive Performance

Mosquito rearing and monitoring for reproductive performance followed standard procedures [26-27]. Two sets of 4 adult-holding mosquito cages (60x60x60cm) were set-up, to provide four replicates each for the Control (i.e., female mosquitoes fed with uninfected mice) and Test (i.e., mosquitoes fed with Plasmodium berghei-infected mice) experiments. Then, approximately, 100 one-day old Anopheles gambiae mosquitoes (approximately, 50 males and 50 females) were transferred to each cage, and fed with 10% sucrose solution, as source of sugar. On the third day of the experiment (i.e., when the mosquitoes were 4 days old), each of the Control and Test mosquito cages was randomly assigned two uninfected and infected mice, respectively, as source of blood meal for egg maturation. Two mice were assigned to each cage to take care of the influence of individual mouse differences on the mosquitoes. Also, the mice were introduced to the cages after thin blood smear from the infected individuals was confirmed to contain detectable gametocytes. To encourage blood feeding, the mosquitoes were deprived of sugar solution 24 hours prior to the introduction of mice in to the cages. Immediately after the retrieval of mice from the cages, Blood Feeding Rates of the female mosquitoes were determined, as the proportions that were engorged after presentation of source of blood meal. The male mosquitoes were not considered for any of the assessments, as they do not feed on blood and, hence, will not be affected by the presence or otherwise of parasites in the mice.

After blood-feeding, the female mosquitoes were retained in the holding-cages with the males for 24 hours to allow mating by individuals that, perhaps, did not do so prior to blood meal. Thereafter, 20 engorged female mosquitoes were randomly withdrawn from each cage and individually maintained in a 20ml plastic tube, half-filled with distilled water for oviposition. These mosquitoes were monitored for Pre-oviposition Period (i.e., number of days between blood meal and oviposition) and Fecundity (i.e., total number of eggs oviposited per female), after correspondingly matching each individual with its egg-cohort. The remaining female mosquitoes in the holding-cages were observed for post-blood meal Longevity (i.e., total number of days lived by the female mosquitoes after blood meal).

After oviposition, the eggs were carefully retrieved from the plastic tubes and each cohort independently monitored till eclosion, to determine Duration of Embryogeny (i.e., interval between oviposition and egg hatching) and Egg-hatching Rate (i.e., proportion of oviposited eggs that hatch in to larvae). The whole experiment was repeated within one month of the completion of the first.

2.3 Data Analysis

Data collected for the variables investigated were processed as mean±SD. The statistical significance of differences in mean values obtained for uninfected and infected blood meals were determined using the student’s t-test.

3. Results

Figure 1 shows the feeding responses of An. gambiae mosquitoes to presence or absence of Plasmodium berghei parasites in mice hosts. The infected mice were significantly (P<0.05) less attractive to the mosquitoes (47.02±8.40%) than their uninfected counterparts (68.27±5.18%), as reflected by the proportion of individuals that, perhaps, did not do so prior to blood meal.

Fig 1: Blood-feeding rate of Anopheles gambiae mosquitoes fed with uninfected and Plasmodium berghei-infected blood meal. The influence of Plasmodium berghei-infected blood meal on reproductive attributes of the mosquitoes is presented in Table 1. While, Pre-oviposition Period and Fecundity of the mosquitoes were significantly (P<0.05) influenced by the presence of parasites in the blood meal of the mosquitoes, the reverse was the case for Duration of Embryogeny (DE) and Egg-hatching Rate (ER). Both DE (range = 1.57±0.20 to 1.61±0.26 hrs) and ER (67.38±1.50 to 69.16±2.68%) varied within narrow limits between the mosquitoes fed with uninfected and parasite-infected blood. The Control mosquitoes oviposited significantly (P<0.05) higher number of eggs (72.89±2.44 eggs/female) than those fed with infected blood (56.52±6.29 eggs/female). Post blood meal Pre-oviposition Period was also significantly extended by almost two days in the group of mosquitoes fed with infected blood (5.54±0.83 days).
Fig 1: Blood-feeding rate of Anopheles gambiae mosquitoes fed with uninfected and Plasmodium berghei-infected blood meal.

Table 1: Reproductive performance of Anopheles gambiae mosquitoes fed with Uninfected and Plasmodium berghei-infected blood meal

<table>
<thead>
<tr>
<th>Reproductive Attributes</th>
<th>Blood Meal Uninfected</th>
<th>Infected</th>
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<tbody>
<tr>
<td>Pre-oviposition Period (days)</td>
<td>3.82±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.54±0.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fecundity (eggs/female)</td>
<td>72.89±2.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.42±6.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Duration of Embryogeny (hours)</td>
<td>1.61±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.59±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg-hatching Rate (%)</td>
<td>67.38±1.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.16±2.68&lt;sup&gt;a&lt;/sup&gt;</td>
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*Values followed by same superscript alphabets, in a row, are not significantly different at P = 0.05.

On the whole, mosquitoes given uninfected blood meal lived Almost twice longer, post-blood meal, than their counterparts that fed on parasite-infected blood, i.e., 26.95±7.28 and 14.30±3.37 days, respectively (Figure 2).

Fig 2: Longevity of Anopheles gambiae mosquitoes fed with uninfected and Plasmodium berghei-infected blood meal

4. Discussion

Important reproductive and longevity variables of An. gambiae mosquitoes, investigated in this study, were significantly influenced by the ingestion of Plasmodium berghei-infected blood. This finding runs contrary to the reports of certain similar studies on the subject<sup>[23-25]</sup> that concluded that vectors including mosquitoes are generally unaffected by the activities of the pathogens that require them for dispersal. The subtractive influence of parasite-infected blood meal on most of the variables observed in this study, however, agrees with the results of more recent studies<sup>[26-34]</sup>. The insignificant influence of parasite-infected blood meal on the Duration of Embryogeny, though relatively new in Literature, may be due to the fact that embryo development in insects is more dependent on environmental temperature, in terms of day-degree accumulation of heat<sup>[35]</sup>, than oogenetic factors that could have been adversely affected by Plasmodium-antigen and/or the elicited mosquito-antibody<sup>[13,36-37]</sup>

The parasite-infected blood was significantly less attractive to the mosquitoes. This observation indicates that An. gambiae, perhaps, possesses the ability to perceive presence or secretions of Plasmodium parasites in mammalian hosts, either prior to
prospecting for blood meal or during pre-blood ingestion activities, such as the pumping of anti-coagulant in to site of proboscis insertion. Studies have shown that the chemo-octatory sense organs of mosquitoes are well developed; and such are used reliably to detect oviposition attractants or repellents before selecting egg deposition sites that will provide adequate developmental rates and survival advantages to the immature stages [38-40]. Thus, if parasite-infected blood meal exerts subtractive influence on reproductive and longevity fitness of An. gambiae, as evident from the results of this study, the female mosquitoes would be inclined to discriminate against such a blood meal, especially, if they are equipped with the necessary sense organs. However, unlike this study, in field experiments involving the use of human subjects, individuals manifesting clinical conditions of Plasmodium falciparum gametocytes (the stage transmissible to mosquitoes) were found to be significantly more attractive to An. gambiae mosquitoes, as source of blood meal [41]. This extreme disparity in attractiveness of Plasmodium parasite-infected humans and mice may be due to the mosquito’s tolerance of different Plasmodium species (i.e., P. falciparum or P. berghei), or additional factors in the mammalian hosts, that either attract or repel mosquitoes prospecting for blood meal. Studies have shown that principal attractants of mosquitoes to humans include excretory wastes, such as CO₂, NH₃ and L-lactic acid, [42-43] which humans produce in relatively high amounts.

Post-blood meal Pre-oviposition Period of the mosquitoes was significantly extended under the influence of parasite-infected blood meal. Usually, the post-blood meal pre-oviposition period in mosquitoes provides time to digest and utilize the vital components (especially, erythrocytic blood cells) for egg maturation. Since Plasmodium parasites are known to destroy erythrocytes in their mammalian hosts [44] thus reducing the protein content of their blood; a factor critical for egg maturation in engorged mosquitoes, then female mosquitoes feeding on such parasite-infected, erythrocyte-depleted, blood may take a longer time to aggregate sufficient protein for oogenesis and, probably, require multiple blood meal to mature a batch of eggs. This reasoning, probably, explains the significant extension of post-blood meal Pre-oviposition Period observed in the mosquitoes fed with parasite-infected blood in this study.

The Control mosquitoes recorded significantly higher fecundity than those fed with parasite-infected blood. This result, in a way, re-enforces the explanation given earlier for the extended Pre-oviposition Period among the Test experiment mosquitoes; as due to probable low protein content of the infected blood. If the explanation holds, then, it could be that as a result of insufficient protein in the infected blood, the mosquitoes were unable to mature a complete batch of eggs but rather had to resorp the incompletely formed eggs. Egg-resorption during or immediately after oogenesis [45], due to factors ranging from inadequate teneral reserve [46-47], or denial of suitable oviposition site [48] is common in mosquitoes. It may mean that the adverse effects of the low protein content of infected blood on Pre-oviposition Period and Fecundity became pronounced because the female mosquitoes in this study were allowed only one blood meal while, in the wild, mosquitoes make-up for nutritional protein deficiency by taking multiple blood meal in the course of maturing a single batch of eggs [49]. Earlier, it was found that infection with the malaria parasite, P. falciparum, increased the frequency of multiple feeding by An. gambiae mosquitoes [29].

Plasmodium parasite-infected blood meal had no significant effect on Egg-hatching rate of the mosquitoes. This finding is not surprising, as evidence abound of egg-hatchability in mosquitoes determined principally by mating success and fertility of the male mosquitoes [50]. These two reproductive attributes (i.e., mating competitiveness and fertility) of male mosquitoes wouldn’t have been affected by the parasite-infected blood meal in this study, as male mosquitoes generally do not feed on blood (due to their lack of piercing mouthparts) but instead depend solely on plant nectar for sustenance [51].

The Test mosquitoes lived for only about half the life-span of their counterparts that fed on parasite-free blood. This result indicates that Plasmodium though been a successful parasite, as it does not cause the death of its mosquito host before it completes sporogony and is, perhaps, transmitted [52-53], it never-the-less reduces the vector’s life-span considerably. However, the significant reduction in life-span of An. gambiae to about two weeks, observed in this study, is not sufficient to reduce the vectorial capacity of the mosquito below the threshold required to sustain malaria transmission cycle. Anopheles mosquitoes require only 9 - 11 days to support the completion of sporogony and make the first infective bite while, every additional extension of life-span by two days, i.e., the gonotrophic cycle of An. gambiae ranging from 2 - 4 days depending on environmental temperature [54], allows for probable successive multiple infective bites [55-56]. The significant reduction in longevity of mosquitoes fed with infected blood may be due to adverse effects of Plasmodium parasites on mosquitoes including, damage to gut wall by oocysts, which interferes with food absorption [55]; depletion of blood erythrocyte-protein content, needed not only for oogenesis but also for replacement of damaged or worn-out tissues [Mons, 1986] [55,56]; and costly production of antibodies against parasite antigens [57-58]. Any one or combinations of these adverse effects will quickly wear-out the mosquitoes, with a consequent reduction in life-span, as observed in this study.

5. Conclusion

Ingestion of parasite-infected blood meal by the An. gambiae mosquitoes resulted in significant subtractive influence on critical vectorial fitness attributes of the species, thus, underscoring the veritable potentials of parasite manipulation in the anopheline vectors, as a strategy for malaria vector control. Though, An. gambiae has been adjudged the most successful vector of malaria in sub-Saharan Africa, it does that at high reproductive and longevity fitness costs that, in turn, should exert serious limiting effects on the species’ ecological adaptability. The findings of this study have shed more light on the parasite-vector interactions in the malaria-transmission requirements tripod. However, serious issues have been raised for further studies, especially, those related to post-ingestion nutritional and toxicity status of Plasmodium parasite-infected blood meal in mosquitoes, as well as, the mechanisms of the subtractive costs of such blood meal.

6. References


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