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Mosquito Infectivity and Parasitemia after Controlled Human Malaria Infection

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Abstract. Controlled Human Malaria Infection (CHMI) has become an increasingly important tool for the evaluation of drugs and vaccines. Controlled Human Malaria Infection has been demonstrated to be a reproducible model; however, there is some variability in time to onset of parasitemia between volunteers and studies. At our center, mosquitoes infected with *Plasmodium falciparum* by membrane feeding have variable and high salivary gland sporozoite load (mean 78,415; range 26,500–160,500). To determine whether this load influences parasitemia after CHMI, we analyzed data from 13 studies. We found no correlation between the sporozoite load of a mosquito batch and time to parasitemia or parasite density of first-wave parasitemia. These findings support the use of infected mosquito bite as a reproducible means of inducing *P. falciparum* infection and suggest that within this range, salivary gland sporozoite load does not influence the stringency of a CHMI.

Controlled human malaria infection (CHMI) by the bites of *Plasmodium falciparum*-infected, laboratory-reared *Anopheles* mosquitoes have been used to study the infection since the 1980s. In recent years, CHMI has become highly standardized and is used to assess the efficacy of antimalarial drugs and vaccines before large-scale field trials. This has been possible in part because of the high reproducibility of CHMI studies within and between centers.¹ Nevertheless, there is variability in time to detectable parasitemia between studies and between centers.^{2,3} As in any biological system, there is significant variability between batches of infected mosquitoes used for CHMI, most notably in the number of salivary gland sporozoites. Recently, CHMI centers have increasingly started using intravenous injection of cryopreserved *P. falciparum* sporozoites to initiate infection,^{4,5} in part, because it is easier to standardize dosage. However, using the natural route of infection via the mosquito still has advantages over intravenous injection. Most importantly, it includes the immune response in the skin, which likely plays a role in both vaccine-induced protection⁶ and the response to primary infection.⁷

To test whether differences in salivary gland sporozoite load between mosquito batches influenced the outcomes of CHMI studies at our center, we collected data from all past clinical trials since 2007 in which malaria-naive volunteers were challenged with bites from five NF54 strain *P. falciparum*-infected *Anopheles* mosquitoes, as described previously.³ In these studies, mosquito batch percent infectivity and sporozoite load were quantified 1 day before CHMI by dissection of salivary glands from a sample of 10 mosquitoes per batch. Dissected salivary glands were pooled and homogenized using a glass grinder; sporozoites were quantified in a counting chamber using a phase-contrast microscope. The mean salivary gland sporozoite number per mosquito was calculated. After the malaria infection, volunteers were followed up once to three times daily from day 6 after infection until 3 days after the treatment of parasitemia. Quantitative

real-time polymerase chain reaction (qPCR) was performed on blood samples either prospectively as the primary diagnostic test or retrospectively if thick blood smears were used as the primary diagnostic test.^{8,9}

We analyzed data from 13 CHMIs taking place between 2007 and 2016, involving 75 malaria-naive volunteers (Table 1). The mean sporozoite load of the mosquito batches was variable, between 26,500 and 160,500 (mean 78,415; 95% confidence interval: 59,627–97,204). We found no correlation between the mean sporozoite load of the batch used and the time to parasitemia detectable by qPCR (Spearman $r = 0.10$), Figure 1A. Day 7 parasitemia can be used as a reliable proxy measurement for liver parasite burden: parasite density on day 7, after the challenge, correlates strongly with the mean parasitemia of the first wave of parasites to emerge from the liver ($N = 50$; Spearman $r = 0.90$; $P < 0.0001$), when antimalarial treatment is initiated after the first peak. There was no correlation between mosquito sporozoite load and parasitemia on day 7 after CHMI (Spearman $r = 0.05$), Figure 1B. Forty-nine volunteers (65%) were exposed to exactly five mosquitoes, the rest required a second exposure because either a mosquito was unfed or a fed mosquito was uninfected. As unfed mosquitoes may still have probed, possibly transmitting sporozoites, we performed a second analysis adjusting the total infected mosquito exposure. Here, there was also no correlation with time to parasitemia (Spearman $r = 0.17$) or day 7 parasitemia (Spearman $r = 0.06$).

In CHMIs where the number of injected sporozoites is precisely controlled, as in intravenous injection studies, increasing the sporozoite number has an effect on prepatent period; injection of 50 or 3,200 sporozoites resulted in a prepatent of 13.3 and 11.2 days, respectively.⁴ However, this analysis shows that between our CHMI trials, there is no difference in infectivity to volunteers depending on salivary gland sporozoite load of the mosquito batch used. In contrast, a recent study found that the probability of malaria transmission to humans decreased when mosquitoes had sporozoite loads of less than 1,000.¹⁰ However, only mosquitoes with 1–10, 11–100, 101–1,000, and $> 1,000$ sporozoites were compared, a much lower load than the mosquitoes used in our analysis. Taken together, these

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TABLE 1
Controlled human malaria infection studies used in this analysis

| | Year | Trial registration number | Number of volunteers | Sporozoite prevalence (% mosquitoes infected) | Sporozoite intensity (mean number of sporozoites per mosquito) |
|----------|------|---------------------------|----------------------|---|--|
| Study 1 | 2007 | NCT00442377 | 5 | 80 | 31,500 |
| Study 2 | 2008 | NCT00509158 | 18 | 100 | 72,800 |
| Study 3 | 2009 | NCT00757887 | 5 | 96.5 | 88,000 |
| Study 4 | 2010 | NCT01002833 | 4 | 100 | 69,000 |
| Study 5 | 2011 | NCT01218893 | 5 | 100 | 79,500 |
| Study 6 | 2012 | NCT01422954 | 4 | 100 | 98,250 |
| Study 7 | 2012 | NCT01627951 | 5 | 100 | 101,250 |
| Study 8 | 2012 | NCT01728701 | 5 | 93.8 | 75,800 |
| Study 9 | 2012 | NCT01728701 | 4 | 100 | 98,000 |
| Study 10 | 2015 | NCT02080026 | 5 | 100 | 74,000 |
| Study 11 | 2015 | NCT02098590 | 2 | 90 | 26,500 |
| Study 12 | 2015 | NCT02098590 | 3 | 100 | 44,300 |
| Study 13 | 2016 | NCT02692963 | 10 | 100 | 160,500 |
| Total | | | 75 | | |

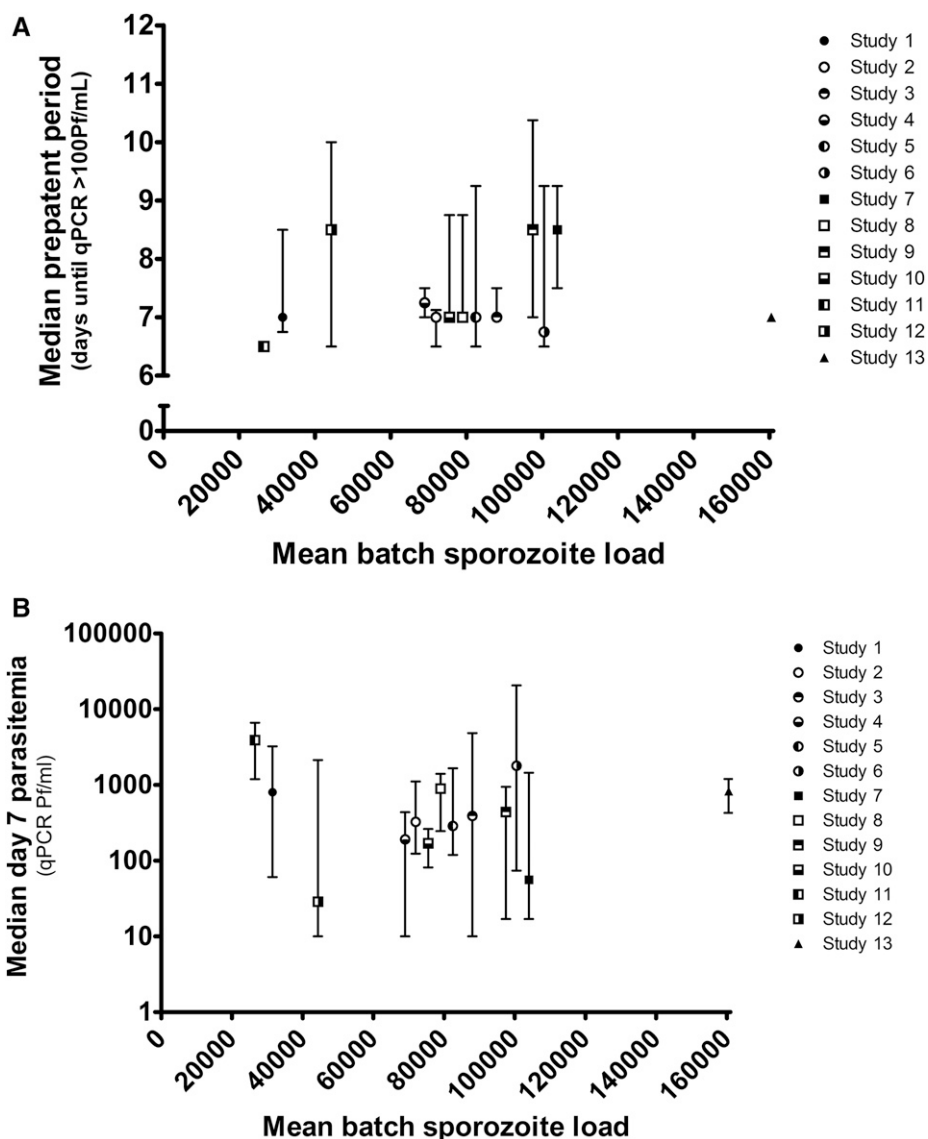


FIGURE 1. Correlation between *Plasmodium falciparum* mosquito infection and infectivity to humans. The mean salivary gland sporozoite load determined by the dissection of a sample of 10 mosquitoes compared with (A) the time to parasitemia detectable by PCR (> 100 parasites per milliliter) and (B) the height of parasitemia on day 7 postinfection. Points and error bars show the median and interquartile range.

observations suggest that above a certain threshold, more sporozoites in the salivary glands no longer increase the number of sporozoites transmitted.

In the past, findings on the association between the mosquito salivary gland load of *P. falciparum* and the number of sporozoites injected by a salivating mosquito have been contradictory, with some studies finding a correlation¹¹ and others not.^{12,13} In these studies, mosquitoes were typically induced to salivate onto glass cover slips or into capillary tubes. The number of sporozoites released during salivation was shown to be between 1 and nearly 1,000 sporozoites per mosquito, even when a much greater number was present in the salivary glands.^{13,14} More recently, studies with live imaging of fluorescent parasites have confirmed that only a small number of sporozoites are present in the mosquito salivary ducts and that each mosquito injects tens to hundreds of sporozoites during a feed.^{15,16} However, these types of studies have always been confined to rodent malaria models. Modeling of parasitemia after CHMI has calculated that an infected mosquito transmits an average of 21 sporozoites that successfully infect the liver,¹⁷ but studies directly linking the mosquito sporozoite load and liver parasite burden of *P. falciparum* are lacking.

The current data support the idea that the number of sporozoites injected by a feeding mosquito is independent of the number in the salivary glands, at high infection intensities. It is important to emphasize that all mosquito batches used in these studies had salivary gland sporozoite loads far exceeding that found in the field (usually less than 10,000).¹⁸ Other CHMI centers either have mosquito salivary gland sporozoite loads that are much lower or in the same range as the studies presented here.^{10,19,20} Irrespective of mosquito sporozoite load, most of these studies have prepatent periods similar to those at our center.^{9,19,20}

A weakness of this analysis is that it used only pooled mean mosquito batch sporozoite counts. In future studies, qPCR can be applied to analyze individual mosquito sporozoite loads in relation to volunteer prepatent periods. In such a study, it would also be interesting to generate mosquitoes with more variable sporozoite loads by titrating gametocyte concentration in their blood meal.

In conclusion, we demonstrate that the infectivity of mosquitoes to humans in CHMI studies at our center is independent of the salivary gland sporozoite load of the mosquito batch used. This finding supports the use of infected mosquito bite as a reproducible means of inducing *P. falciparum* infection and suggests that at high levels of infectivity, increased salivary gland sporozoite load does not increase the stringency of a mosquito challenge.

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