Insights into the early physiological responses of the *Anopheles darlingi* midgut to *Plasmodium vivax* infection

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The *Anopheles-Plasmodium* interaction in the midgut depends on a dynamic interplay between peritrophic matrix (MP), microbial community, and innate immunity. Understanding how *Plasmodium vivax* modulates these interdependent physiological layers is essential for identifying targets to disrupt transmission. Here, we investigated the impact of *P. vivax* infection on the immune response, MP homeostasis, and bacterial microbiota load and composition in *Anopheles darlingi*. Females (3-5 days old) were fed on *P. vivax*-infected blood, while the control group received heat-inactivated blood (42°C for 30 minutes) from the same donors. At 24 and 48 hours post-blood meal (hpb), midguts were pooled (n = 10/pool) for RT-qPCR analysis of immune (*gambicin*, *defensin* and c*ecropin*) and PM-related gene (*peritrophin*), normalized to *RPL32*. Expression was analyzed via linear models, including 'donor' as a covariate. Additionally, total 16S rRNA load was quantified by qPCR and microbiota composition was assessed at 24 hpb through 16S rRNA (V4) metabarcoding. Beta-diversity was evaluated using Unifrac metrics (PERMANOVA) and differential abundance was determined via ANCOM-BC2. Preliminary results suggest that *P. vivax* does not induce additional stimulation of AMP genes ($p > 0.05$) at 24 or 48 hpb. However, infection triggers a transcriptional response related to PM homeostasis, as evidenced by a consistent 30% (1.3 fold) increase in *peritrophin* expression at 24 hpb ($p = 0.002$, $R^2 = 0.74$). This modulation is likely driven by parasite invasion, as microbiota load and composition – known to influence PM formation – remained unchanged by the infection ($p > 0.05$). Notably, the genus *Asaia* exhibited 100% prevalence and high mean abundance (>70%) across all samples at 24 hpb. The consistent dominance of *Asaia*, regardless *P. vivax* infection, identifies it as a core component of the *An. darlingi* microbiota and a key candidate for future investigations about its role in vector competence.

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