

## How does *Plasmodium vivax* infection modulate gene expression in *Anopheles aquasalis*?

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Malaria remains a major global health issue, with 263 million cases and 597,000 deaths reported worldwide. In the Americas, Brazil, Venezuela, and Colombia account for most cases, with the Amazon region representing 99.6% of infections. *Anopheles aquasalis*, a primary *Plasmodium vivax* vector in coastal regions, is a key laboratory model for malaria research, plays a critical role in the study of vector-parasite interaction. This study aimed to analyze changes in gene expression of *An. aquasalis* during *P. vivax* infection to identify potential molecular targets related to vector-parasite interactions. The female of *An. aquasalis* were fed via membrane with blood from adult volunteers ( $\geq 18$  years) infected with *P. vivax* (CAAE: 13653019.2.0000.0005) and inactivated blood was used for the control group. Fifteen days post-infection, a sub-sample of mosquitoes were dissected, to establish the infection rate, and afterwards, the RNA was extracted from pools of 10 females using TRIzol™ (Thermo Fisher Scientific, MA, USA). RNA-seq libraries were prepared and sequenced on the Illumina HiSeq2000 platform. Quality-checked was done by using FastQC v0.11.8 and downstream analyzes was performed using CLC Genomics Workbench v23 (Qiagen, Germany). RNA-seq libraries were prepared and sequenced on the Illumina HiSeq2000 platform. The *An. aquasalis* genome, available through VectorBase, was used to map the sequences, and the determination of the differentially expressed genes was selected by the following criteria; log2 fold change  $\geq 1.5$  and FDR  $p < 0.05$ . Comparative analyses revealed 279 differentially expressed genes, with 244 showing a fold change  $\geq 1.5$ . Of these, 209 (85.66%) were upregulated, and 35 (14.34%) were downregulated. Functional analysis identified genes involved in proteolysis ( $p = 0.006$ ), carbohydrate metabolism ( $p = 0.0008$ ), and cation binding ( $p = 0.012$ ). Genes associated with peptidase activity ( $p = 0.003$ ) and extracellular region localization ( $p = 0.006$ ) were also identified. In conclusion, *P. vivax* infection induces gene expression changes in *An. aquasalis*, activating genes related to biological processes, molecular functions, and cellular components, providing new insights into vector-parasite interactions and vector control.

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