Development of a new molecular tool to study the transmission of *Plasmodium falciparum* parasite inside its main vector, the mosquito *Anopheles coluzzii*

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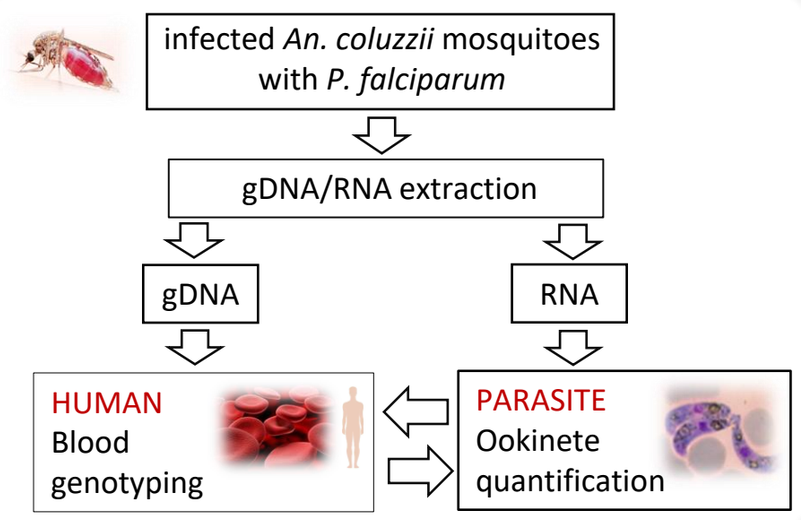
INTRODUCTION AND AIM OF THE WORK

- Malaria transmission from human to mosquito strictly depends on the presence of *P. falciparum* gametocytes in the peripheral human blood.
- Gametocytemia is affected by the human genetic background.
- The accurate detection and quantification of specific parasite stages in human and mosquito are crucial to study the role of human genetic variation on the dynamics of malaria transmission.

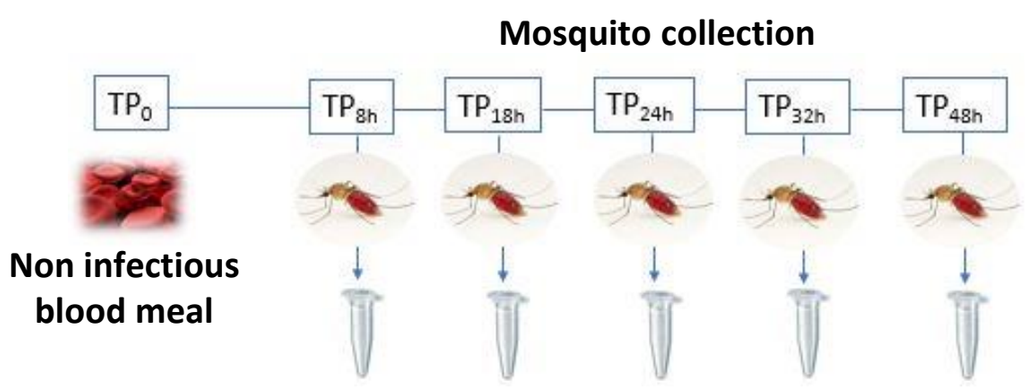
Objective: Developing a new molecular method based on RTqPCR analyses of nucleic acids to quantify *P. falciparum* ookinetes (parasite RNA) and to identify human polymorphisms (gDNA) inside single infected *An. coluzzii* mosquitoes. The quantification of ookinete markers will be correlated with the human genetic background.

PROJECT WORKFLOW



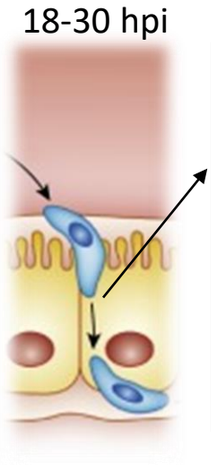
MATERIAL AND METHODS

1. Identification of human genetic backgrounds



- Conservation in RNA Later Solution at +4°C
- **gDNA extraction (evaluation of human gDNA persistance up to 48 pbf)**
- Human genotype identification (SNPs assay)

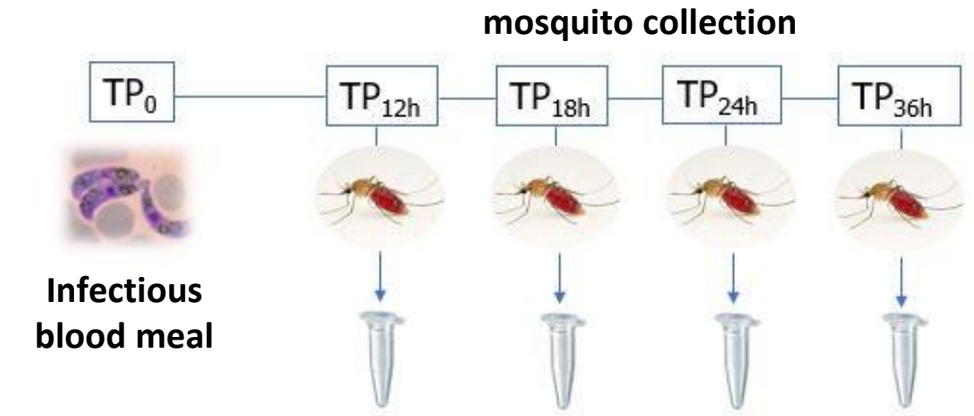
2. Selection of ookinete specific markers



Selection of 4 micronemal associated genes specifically expressed during ookinete development and mosquito midgut invasion:

- CTRP (Circumsporozoite and TRAP Related Protein)
- WARP (von-Willebrand factor A domain-related protein)
- SOAP (Secreted Ookinete Adhesive Protein)
- CHT1 (Chitinase 1)

3. Identification and quantification of *P. falciparum* ookinete

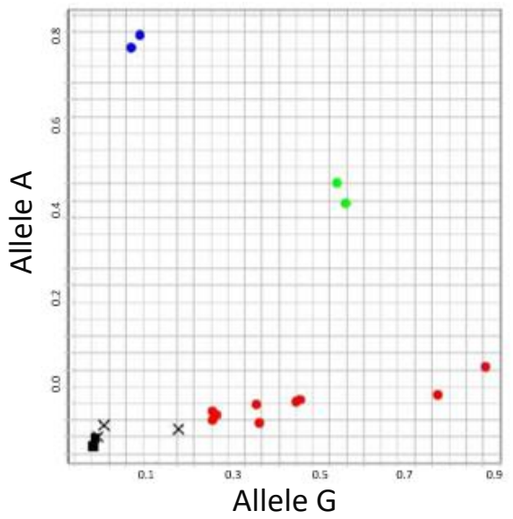


- Conservation in RNA Later Solution at +4°C
- **RNA extraction (evaluation of the best timepoint for ookinete quantification)**
- Trascriptional analysis of ookinete marker genes (Ook stage identification)
- Absolute quantification of ookinete marker genes

RESULTS

1. Detection of human G6PDA- polymorphism

Allellic Descrimination Plot



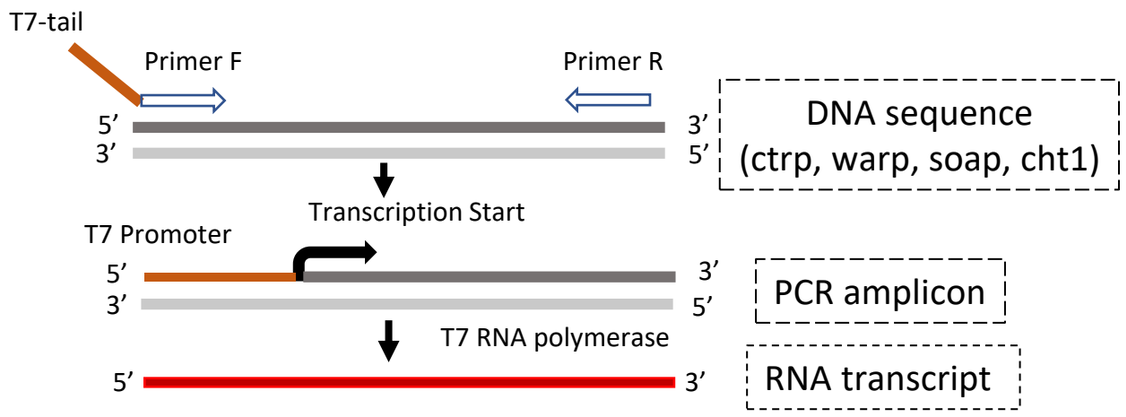
Legend
Homozygous G/G
Heterozygous G/A
Homozygous A/A
X Undetermined

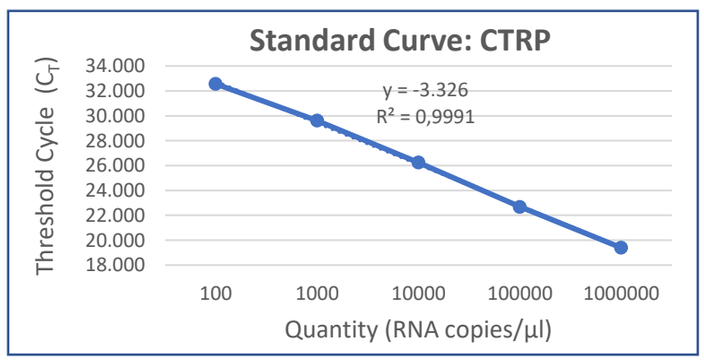
Human gDNA extracted from single fed mosquitoes at 8, 18 and 24 hpbf.

G6PDA- TaqMan specific assay on gDNA from single blood fed mosquitoes correctly identifies human polymorphisms.

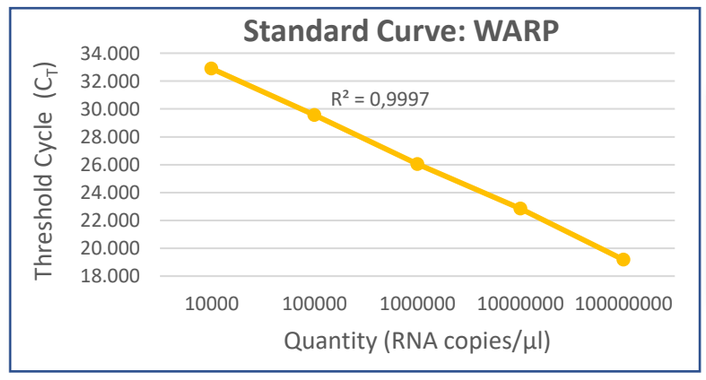
2. Absolute quantification of ookinete marker genes

Scheme of RNA *in vitro* synthesis needed for absolute quantification





CTRP and WARP standard curves. These curves will allow absolute quantification of ookinete markers in infected mosquitoes



We are currently analysing a first set of infected mosquitoes to quantify the ookinete markers *in vivo*.

RESULTS, PERSPECTIVES AND FUTURE APPLICATION

- Our approach focuses on the evaluation of malaria transmission dynamics through a RTqPCR analysis of single fed mosquitoes
- The successful implementation of this assay will enable developing a novel and potentially high-throughput epidemiological approach for malaria transmission studies in endemic countries bypassing issues related to human blood collection in the field and laboratory work.

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