

# **Proposal title:**

Molecular Epidemiology  
of Mixed Malaria Infections in  
Tanzania

**Author:** Vito Baraka

**Coauthors:**

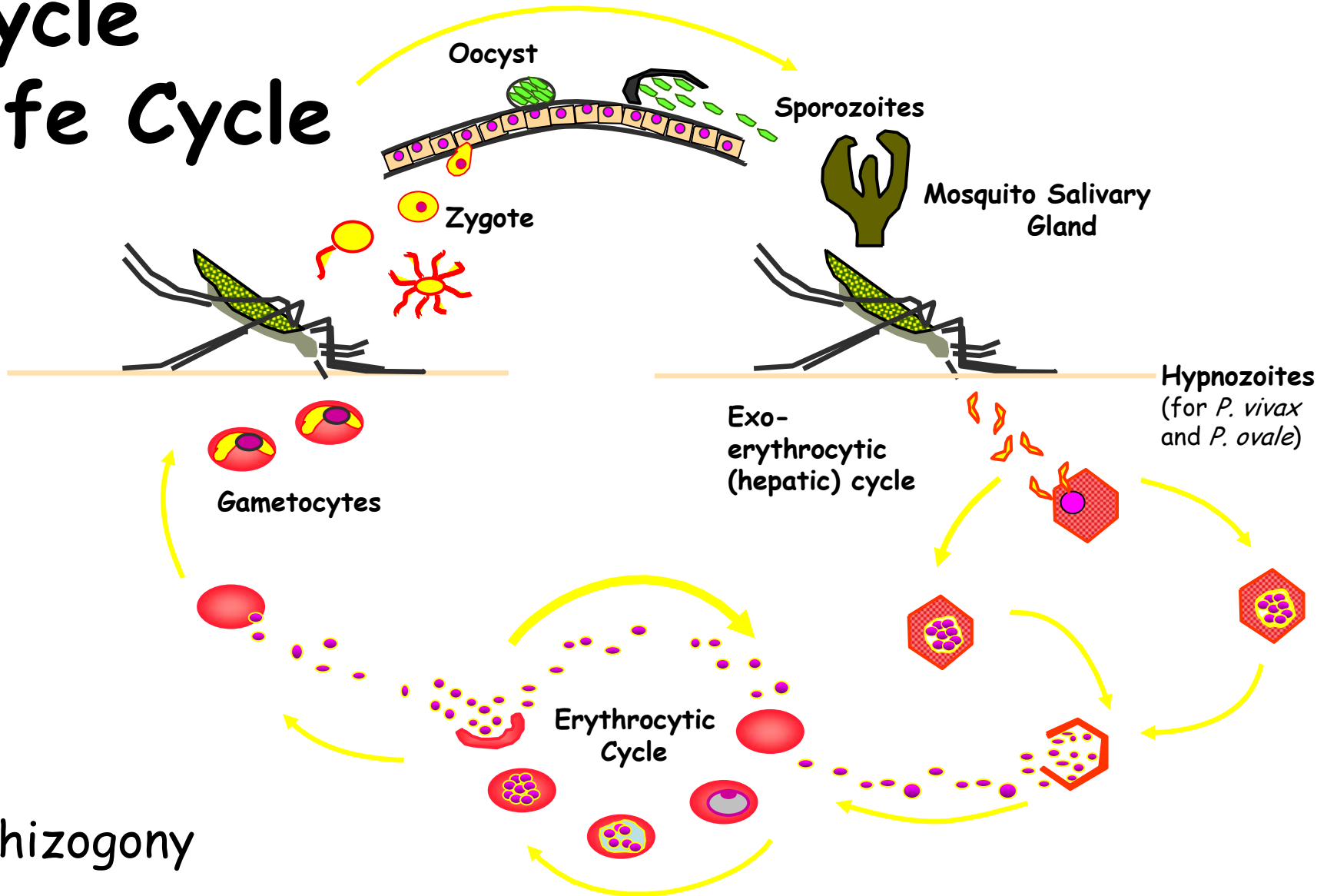
**Institution :** NIMR-Tanga Medical  
Research Centre

# Introduction

- Malaria is a vector-borne infectious disease caused by protozoan parasites.
- The disease is caused by protozoan parasites of the genus *Plasmodium*.
- Four types of the plasmodium parasite can infect humans, *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*.
- Most serious spp are *P. f* and *P. v* but other species *P. ovale*, *P. malariae* also affect humans and their role in the burden of the disease can never be underestimated.
- Mixed malaria infection is the concurrent infection of malaria species >> *P. falciparum*, *Plasmodium vivax*, *P. ovale* and *P. malariae*.
- Double-species mixed infections are more common than triple- and quadruple-species mixed infections (Mayfong et al, 2004)

# Malaria Life Cycle

## Sporogony



# Introduction cont...

## Types of Malaria Infections

- Recrudescence
  - persisting parasitemia due to survival of erythrocytic forms despite chemotherapy (*P.f.*, *P.m.*)
- Relapse
  - reactivation of hypnozoites forms of parasite in liver, separate from previous infection with same species (*P.v.* and *P.o.*)
- Recurrence or reinfection
  - exo-erythrocytic forms infect erythrocytes, separate from previous infection (all species)

# How do malaria parasites interact?

- **P.vivax and P.falciparum**

Several different studies have now reported that *P. vivax* infections help to reduce the severity of *P. falciparum* malaria by maintaining the stable *P.f* parasitaemia (Luxemburger *et al*,1997)

- **P.falciparum and P.malariae**

Mathematical model suggest *P.m* can reduce peak parasitaemia of subsequent infection by 50%(Mason,2000).

Mixed infection *P.f+P.m* protects against clinical diseases by *P.f* .

Children with *P.f+P.m* had fewer or no symptoms compared with those with *P.f* infection only (Alifrangis *et al*,1999).

*P.f* gametocytes increases when there is concurrent infection with *P.m* (Mackenzie *et al*,2002,Teun *et al* 2006).Also *P.f+P.m* increase the antibody titre compared to single infection???

- **P.falciparum and P.ovale**

Mixed *P.f+P.o* protects against protects against clinical diseases by *P.f*.

- **P.falciparum+ P.malariae+ P.ovale**

Triple infection associated with severe anaemia compared with that of single infection. (Mayxay *et al*,2001,May *et al*,2000,Krudson *et al*,1999).

# Why do we need studies on mixed malaria infections??

- **Impact on malaria transmission**

Increased Gametocyte production is enhanced in *P. falciparum* when there is concurrent infection with *P. malariae* (Mackenzie et al, 2002, Teun Beusema et al 2006)

- **Impact of Mixed malaria infection on Disease interventions**

Species interactions reduce the severity of malaria illness, therefore **vaccine** or **drug development programs** targeting one species disrupt an important balance in human infection and increase the risk of severe disease in endemic populations eg Spf66, RTS/AS.

- **No specific Treatment recommended**

All primary treatment of *P. f.* kill asexual stage of all other human Plasmodium spp (ACT). However these will not kill hypnozoites of *P. ovale*, treatment should include hypnozoicidal drug eg Primaquine.

- **Mixed infection has been underestimated**

The overall proportion of mixed infection has been underestimated. Mixed spp infections are not uncommon but they may often be overlooked because there is a tendency for *P. falciparum* to predominate

# Malaria Diagnosis

- Diagnosis of malaria involves identification of malaria parasite or its antigens/products in the blood of the patient.
- The efficacy of the diagnosis is subject to many factors.
  - The different forms of the four malaria species; the different stages of erythrocytic schizogony;
  - symptoms;
  - Problems of recurrent malaria, drug resistance, persisting viable or non-viable parasitemia,
  - sequestration of the parasites in the deeper tissues;
  - use of chemoprophylaxis or even presumptive treatment

# Malaria Diagnostic tools

- **Microscopic Tests**

Direct microscopic visualization of the parasite on the thick and/or thin blood smears has been the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys. Currently remains the "gold standard" for malaria diagnosis.

- **RDTs**

The immunochromatographic tests for the detection of malaria antigens, HRP2, pLDH, P Aldolase. Parachek, OptiMal, Parasight,

- **ELISA**

Rely on identification of specific antibodies.

- **Flowcytometry:**

Flowcytometry and hematological analyzer can detect abnormal cell clusters and small particles with DNA fluorescence, free malarial parasites, seen on automated hematology analyzers and malaria parasites can be detected on the scatter plots produced on the analyzer.

# PCR Based techniques

- Several PCR assays for malaria diagnosis have been developed, most often based on species-specific sequences of the parasites' 18S subunit rRNA gene.
- **Advantages PCR Based techniques**
  - ✓ highly sensitive and specific for detecting all 4 species of malaria, particularly in cases of low level parasitemia and mixed infections.
  - ✓ 10-fold more sensitive than microscopy, with one study reporting a sensitivity to detect 1.35 to 0.38 parasites/ $\mu\text{L}$  for *P. falciparum* and 0.12 parasites/ $\mu\text{L}$  for *P. vivax*.
  - ✓ The PCR test has also been found useful in unraveling the diagnosis of malaria in cases of undiagnosed fever.
  - ✓ Good for epidemiological studies

## Disadvantages

- Do not produce quantification (exception Real time PCR)
  - PCR can fail: - Contamination & false positives
  - Expensive
  - Cumbersome, requires multiple PCR assays.
- Although these factors could limit the role that PCR diagnosis can play in clinical settings in most regions it is clear that PCR diagnosis of malaria will play an increasing role in epidemiology of malaria

# Mixed infection as detected by PCR (Mayfong *et al*,2004)

Table 1. Mixed infections not detected by microscopy of admission blood slide, but detected by PCR techniques<sup>a</sup>

Mixed infections tested positive by microscopy (%)	Mixed infections tested positive by PCR (%)	Cryptic species	Countries	Refs
0/23 (0)	4/23 (17)	Pv	Australia	[51]
1/137 (0.5)	9/173 (5)	Pv	Thailand	- <sup>b</sup>
0/189 (0)	52/189 (27.5)	Pm	Guinea Bisseau	[52]
1/196 (0.5)	25/196 (13)	Pv; Pm	Thailand	- <sup>c</sup>
0/48 (0)	6/48 (12.5)	Pf; Pv	Thailand	[53]
18/475 (4)	356/548 (65)	Pf; Pv; Pm; Po	Thailand	[9]
0/100 (0)	17/100 (17)	Pf; Pv	Venezuela	- <sup>b</sup>
11/159 (7)	44/159 (28)	Pf; Pm; Po	Equatorial Guinea	[54]
2/192 (1)	9/192 (5)	Pv; Pm; Po	Spain	- <sup>d</sup>
80/1470 (0.005)	113/173 (65)	Pf; Pv; Pm; Po	Papua New Guinea	[55]
2/58 (3.4)	27/117 (23.1)	Pf; Pv; Pm; Po	Laos	[28]
7/300 (2.3)	26/151 (17)	Pf; Pv; Pm; Po	Thailand	[56]
0/160 (0)	21/160 (13)	Pf	Thailand	[34]

<sup>a</sup>At the presentation of the patients, mixed-species malaria infection is usually underestimated when detected microscopically, but this is more common when detected by the more sensitive technique – PCR. The overall median (range) ratio of PCR to microscopy diagnosed mixed infection in this review was 6% (0–25%). Please note that the results from different papers might not be directly comparable. Abbreviations: Pf, *Plasmodium falciparum*; Pm, *Plasmodium malariae*; Po, *Plasmodium ovale*; Pv, *Plasmodium vivax*.

<sup>b</sup>Data obtained from Postigo *et al.* (1998) and Brown *et al.* (1992) as cited in Ref. [40].

<sup>c</sup>Data obtained from Snounou *et al.* (1993) as cited in Ref. [39].

<sup>d</sup>Data obtained from Rubio *et al.* (1999) as cited in Ref. [54].

# Mixed infection as detected by PCR

Ivo Mueller *et al*, PNG

Table 1. Studies comparing LM and PCR diagnosis of *Plasmodium malariae* and *Plasmodium ovale* infections

Region and country	Population	<i>P. malariae</i>			<i>P. ovale</i>			Refs
		LM (%)	PCR (%)	Mixed <sup>a</sup> (%)	LM (%)	PCR (%)	Mixed <sup>a</sup> (%)	
<b>Pacific</b>								
PNG (Drikikir)	CS <sup>b</sup> , all ages	14.4	37.0	91.8	0.0	15.6	100.0	[31]
PNG (Liksul)	CS, all ages	1.1	10.0	75.8	0.0	5.2	88.2	[32]
PNG (Wosera)	CS, all ages	2.0	21.8	72.9	0.1	6.2	80.0	[32]
PNG (Wosera)	CS, all ages	4.0	12.4	69.3	0.3	5.5	77.3	[29]
<b>Africa</b>								
Cameron	CS, pregnant women	1.1	7.6	100.0	0.0	2.5	100.0	[77]
Equatorial Guinea	CS, under six years old	18.7	39.2	92.9	2.8	9.3	60.0	[59]
Guinea-Bissau	CS, all ages	0.0	23.3	97.7	0.0	6.9	100.0	[58]
<b>Americas</b>								
Brazil	CS, all ages	1.2	11.9	69.5	- <sup>c</sup>	-	-	[14]
Brazil	Malaria patients	0.0	9.4	88.9	-	-	-	[63]
<b>Asia</b>								
Thai-Myanmar border	Patients	2.2	24.3	99.2	0.4	3.8	100.0	[46]

<sup>a</sup>Proportion of *P. malariae* or *P. ovale* infections diagnosed by PCR that are coinfections with *P. falciparum* and/or *P. vivax*.

<sup>b</sup>Cross-sectional population survey.

<sup>c</sup>- indicates not assayed.

# Rationale of the study

- **Statement of the research problem**

- The overall proportion of mixed infection has been underestimated in Tanzania. Mixed malarial infections of *P. falciparum* and *P. malariae/P. ovale* are often overlooked because there is a tendency for *P. falciparum* to predominate and also due reliance on microscopy technique for epidemiological data and clinical diagnosis.
- Therefore this study will conduct molecular epidemiology of the mixed malaria and provide epidemiological data of mixed malaria that will be important for designing appropriate intervention to reduce malaria burden.

# Hypotheses

- i) The prevalence of mixed malaria infection is greater than previously projected using microscopy
- ii) The distribution of mixed malaria infection is lower in highland than lowland
- iii) There is a relationship between the prevalence of mixed malaria infection with age group and genotypes of the individual
- iv) There is a high degree of false negativity using malaria microscopy as compared to PCR based technique

# Objectives

## Broad Objective

To determine the prevalence of mixed malaria infection using PCR based technique.

## Specific objectives

- ii) To compare the effectiveness of PCR-Ligase Detection Reaction Assay and microscopy in detection of the mixed infection burden
- iii) To determine the relationship between the age, Hemoglobin status and  $\alpha$  thalassemia and G6PD of the individuals in relation to mixed malaria infection.
- iv) To establish the degree of false negativity for malaria microscopy using PCR as a standard technique
- v) To compare between pattern and distribution of the mixed malaria infection in highland and lowland.

# Significance of the study

- This study is will produce epidemiological data of mixed malaria infections both in endemic and non endemic areas.
- Molecular diagnosis will reveal patterns of mixed malaria risk that are significant different from those obtained by Microscopy techn..
- These data will be useful to design interventions by to reduce mixed malaria burden in Tanzania.

# Study area and Ethical clearance

- Ethical clearance

Will be obtained for NIMR-MRCC

- Study area, sample size and inclusion and exclusion criteria will be decided later

# METHODS

- Diagnosis of blood smears and collection of filter paper will be done as described previously.
- DNA will be extracted from filter paper by Chelex 100 method as describe previously(Pearce *et al*,2003)
- PCR amplification and Probe hybridization for spp differentiation will be done(Mcanamara *et al*,2004).
- Human SNPdetection will also be done(Anders *et al*,2005).

# Study Methodology

- **Data Management and Analysis**
- **Achievement of the study and dissemination of results**
- **Budget:**
- **Budget summary**

# References

1. Howard, S.C. et al. (2001) Methods for estimation of association between multiple species parasite infections. *Parasitology* 122, 233–251
2. McKenzie, F.E. et al. (2002) *Plasmodium malariae* infection boosts *Plasmodium falciparum* gametocyte production. *Am. J. Trop. Med. Hyg.* 67, 411–414
3. Peter A. Zimmerman, Rajeev K. Mehlotra, Laurin J. Kasehagen and James W. Kazura Why do we need to know more about mixed *Plasmodium* species infections in humans? *TRENDS in Parasitology* Vol.20 No.9 September 2004
4. Alexandre Lorenzetti et al Mixed *Plasmodium falciparum* infections and its clinical implications in four areas of the Brazilian Amazon region
5. DAVID T. MCNAMARA, LAURIN J. KASEHAGEN, BRIAN T. GRIMBERG, JENNIFER COLE-TOBIAN, WILLIAM E. COLLINS, AND PETER A. ZIMMERMAN\* DIAGNOSING INFECTION LEVELS OF FOUR HUMAN MALARIA PARASITE SPECIES BY A POLYMERASE CHAIN REACTION/LIGASE DETECTION REACTION FLUORESCENT MICROSPHERE-BASED ASSAY. *Am. J. Trop. Med. Hyg.*, 74(3), 2006, pp. 413–421
6. F. ELLIS MCKENZIE, GEOFFREY M. JEFFERY, and WILLIAM E. COLLINS *PLASMODIUM MALARIAE* INFECTION BOOSTS *PLASMODIUM FALCIPARUM* GAMETOCYTE PRODUCTION *Am J Trop Med Hyg.* 2002 October ; 67(4): 411–414.

A photograph of two soccer players in action on a green field. The player on the left is wearing a blue jersey with the number 13 and is falling towards the ground. The player on the right is wearing a white jersey with the number 15 and is also falling, reaching for a yellow and green soccer ball. The background is a blurred green field.

**We cannot afford to lose the battle against  
Malaria,mbona Zanzibar wameweza???**

**:Thank you for listening**