

# Malaria Molecular Surveillance Study Design Workshop

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*School of Public Health  
Imperial College London*

# Welcome to Toronto!



- Representation from 12 countries
- Range of levels from early career researchers to group leads

*You are not expected to be an expert in statistics!*

# Instructors



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## Acknowledgements



MRC Centre for  
Global Infectious  
Disease Analysis

IMPERIAL

Gates Foundation

# Goals of Malaria Molecular Surveillance

# What is Malaria Molecular Surveillance (MMS)?



**Genomic epidemiology:** the study of the genetic characteristics of pathogens to **understand** their transmission, distribution, and evolution. Combines genetic data with epidemiological information to **improve our understanding** of disease.

**Genomic surveillance:** the systematic, ongoing collection and analysis of pathogen genetic data to **monitor** for genetic changes that could impact public health. Focuses on **actionable information** and impacts on control.

## High priority areas for surveillance

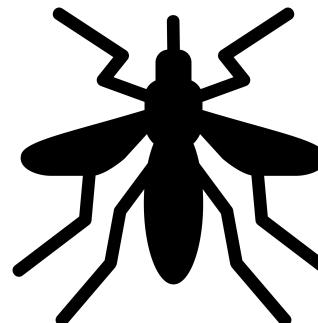
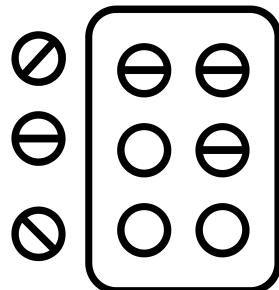
1. Monitoring the prevalence of established molecular markers of drug resistance (*crt*, *dhfr*, *dhps*, *mdr1*).
2. Detecting the emergence of rare variants of concern and tracking their spread in space and time (e.g. *k13*).
3. Measuring the prevalence of *hrp2/3* gene deletions as part of decision frameworks that directly impact control strategies.

## Other applications of MMS

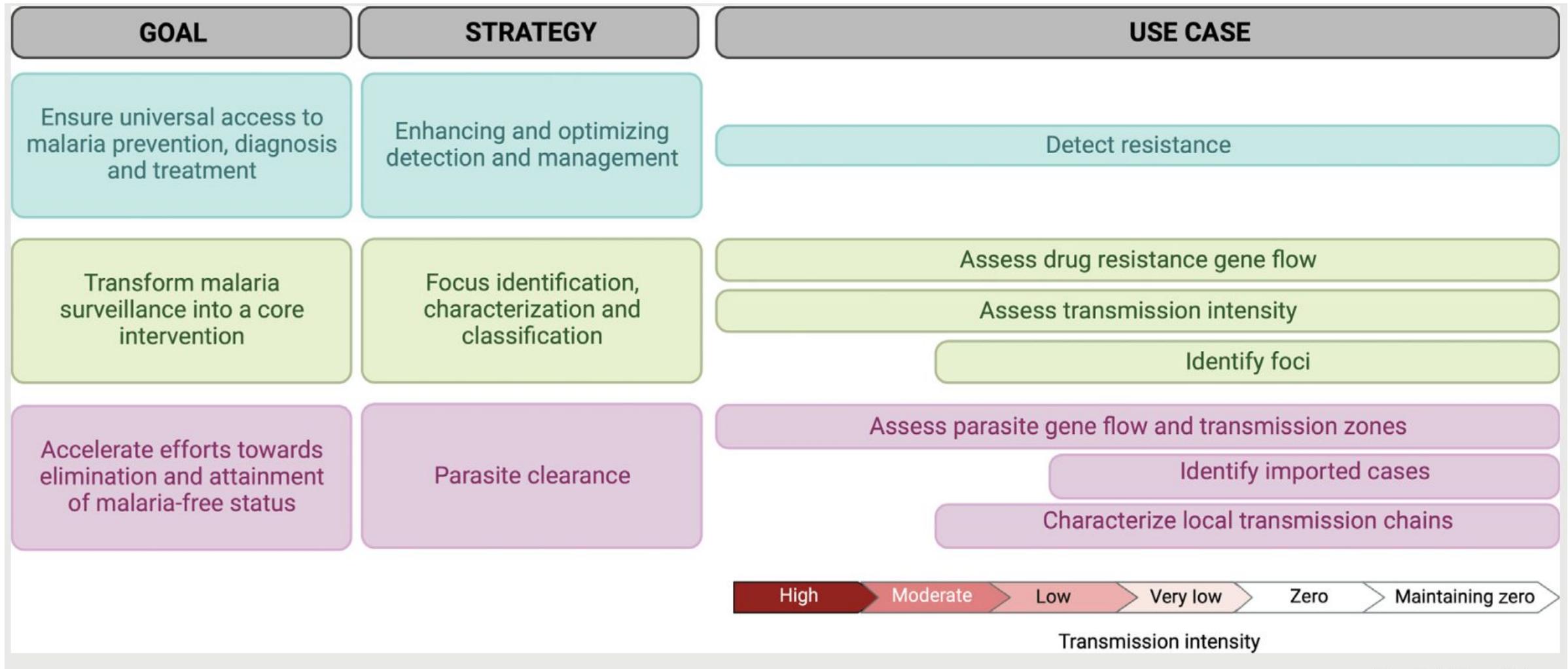
1. Detect imported vs. locally acquired cases
2. Measure migration and connectivity between populations
3. Estimate transmission chains and networks
4. Measure changes in transmission (e.g. impact of interventions)
5. Classifying infections as reinfection, recrudescence, and relapse (vivax)

## Other things we will NOT cover here

1. Therapeutic Efficacy Studies (TES)
2. Vector surveillance/genomics
3. Study designs for measuring interventions, e.g. clinical trials



# Considerations depending on local context



# Considerations depending on local context

1 Use case	2 Goal	3 Target population	4 Sampling approach	5 Frequency	6 Parameters for sample size
	Early warning of biological threats	Detect emerging artemisinin resistance variants	Malaria clinical cases among children	Multi-cluster sampling	Cross-Sectional
	Detection of transmission sources	Case classification (local/imported)	Whole population	Dense (all malaria cases in elimination settings)	Continuous
	Inform transmission dynamics	Changes in transmission	Whole or sentinel population	All malaria cases in a representative area	Longitudinal

# Current state of play

## Chloroquine

*pfcrt*

*CVIET* haplotype, K76T

*SVMNT* haplotype, A220S

## Sulfadoxine-Pyrimethamine (SP)

*pfdhps*

**A437G, K540E, A581G**

*pfdhfr*

**N561I, C59R, S108N, I164L**

## Mefloquine and Lumifantrine

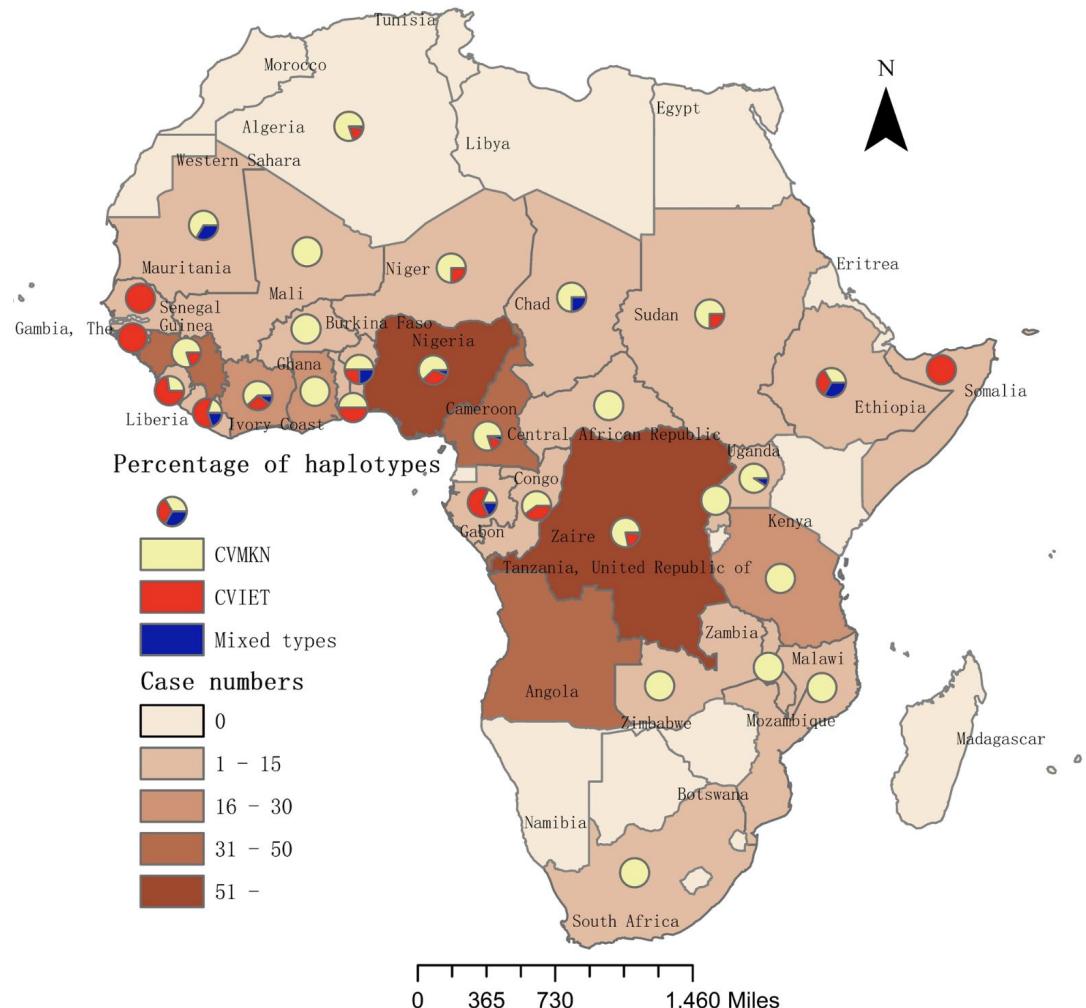
*pfmdr1*

*N86Y, Y184F, D1246Y*

# Monitoring markers of drug resistance

## *pfCRT*

- Historically (pre-2000) at high prevalence, following intense use of chloroquine
- Decline in some places following switch to ACTs
- Current distribution is patchy

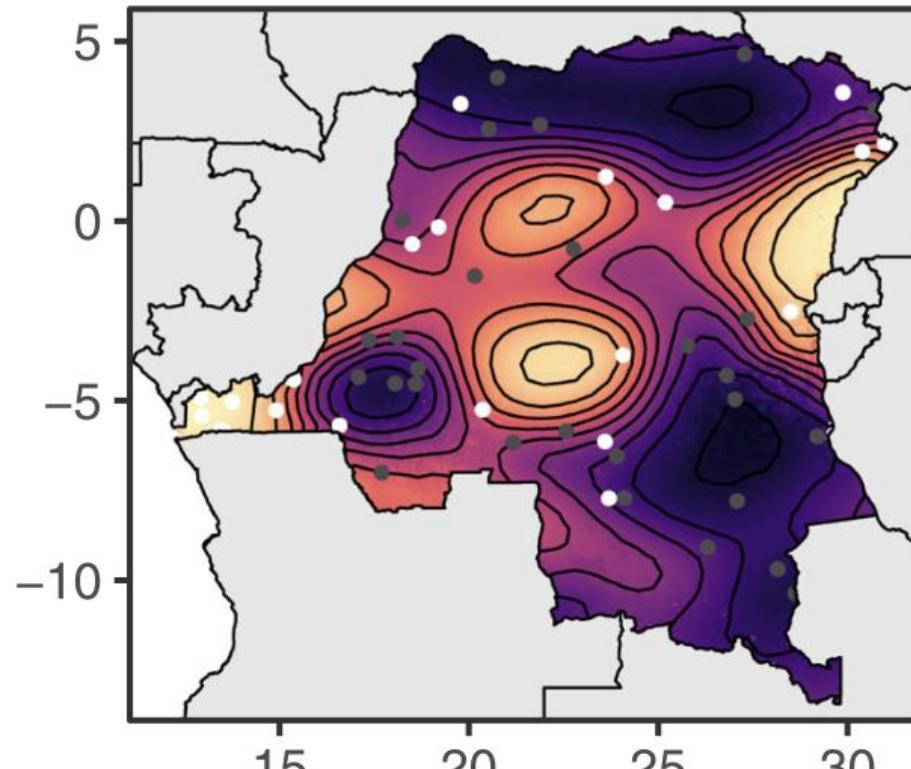


**Fig. 1** The number of imported *P. falciparum* cases from Africa and percentage of haplotypes of *PfCRT*

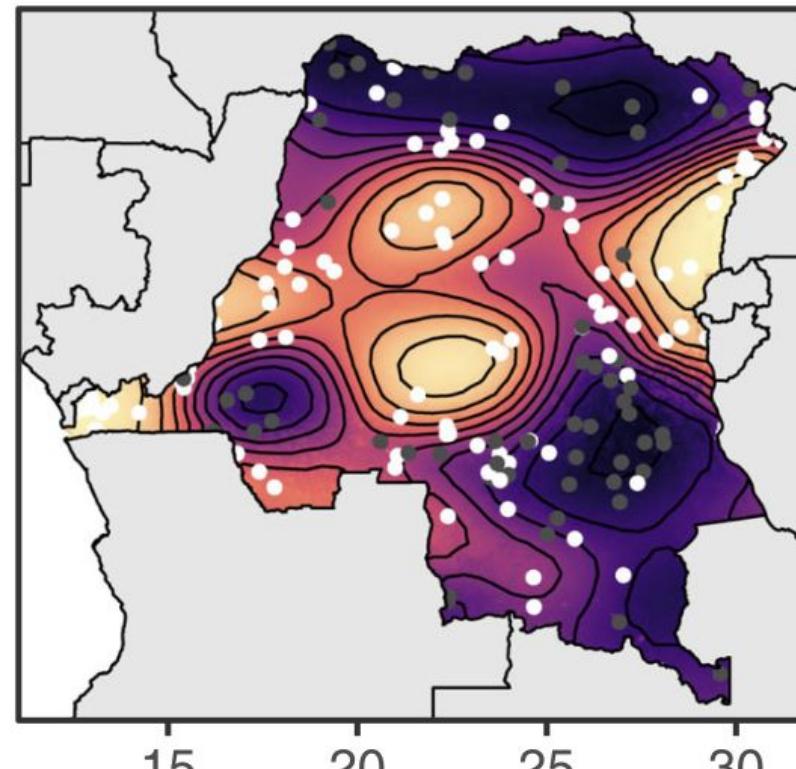
*pfCRT*

## Democratic Republic of the Congo

2007



2013



CIET

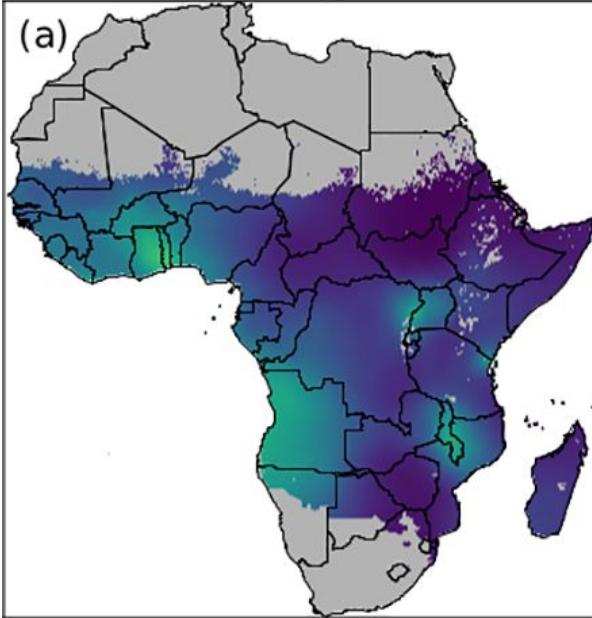
estimated  
allele  
frequency

1.00
0.75
0.50
0.25
0.00

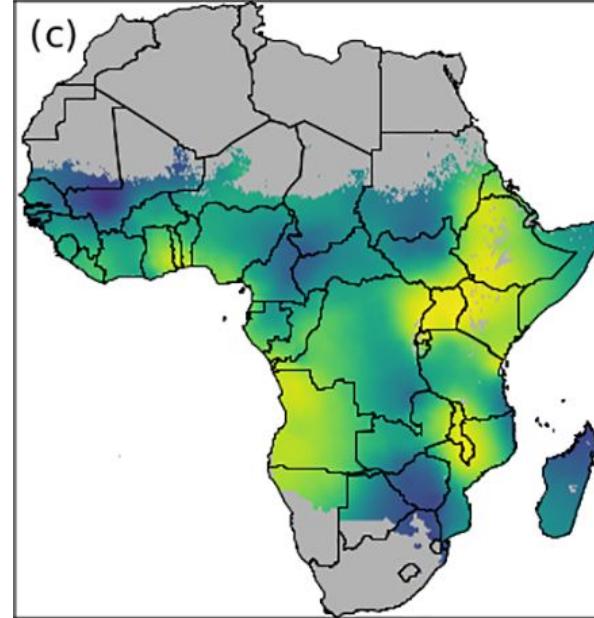
longitude

## *pfdhps A437G*

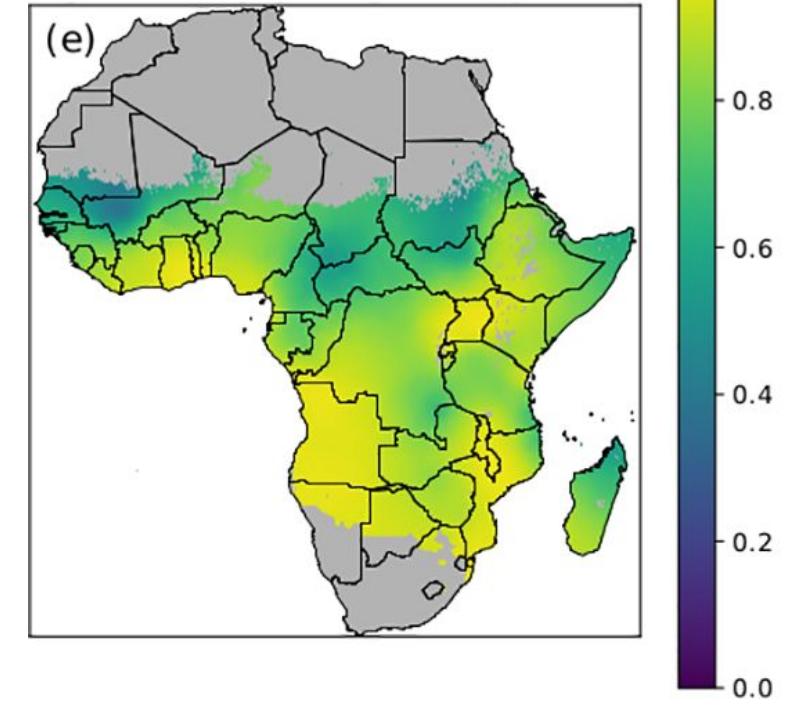
Median map: dhps437, 1990



Median map: dhps437, 2005

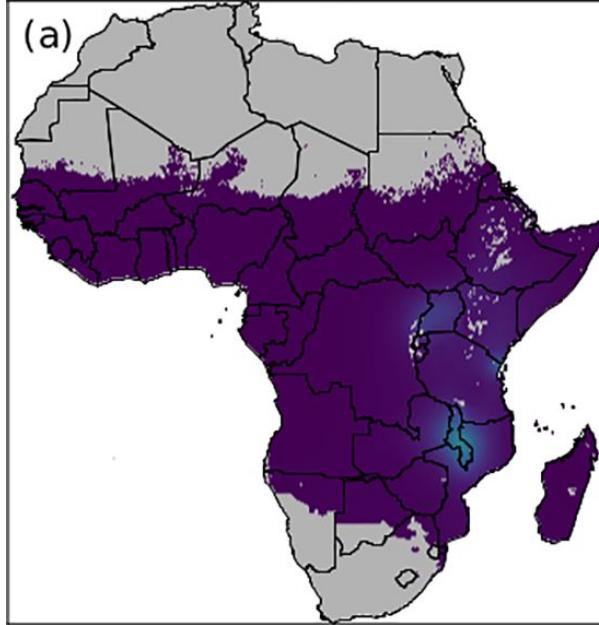


Median map: dhps437, 2020

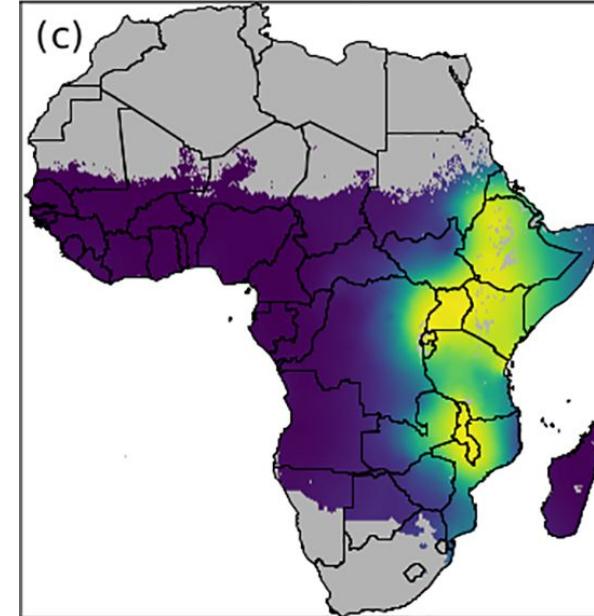


## *pfdhps K540E*

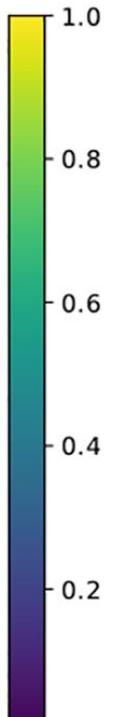
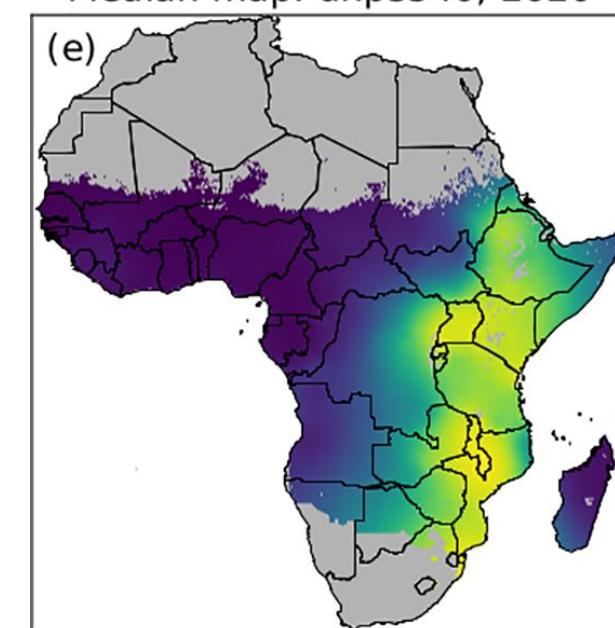
Median map: dhps540, 1990



Median map: dhps540, 2005



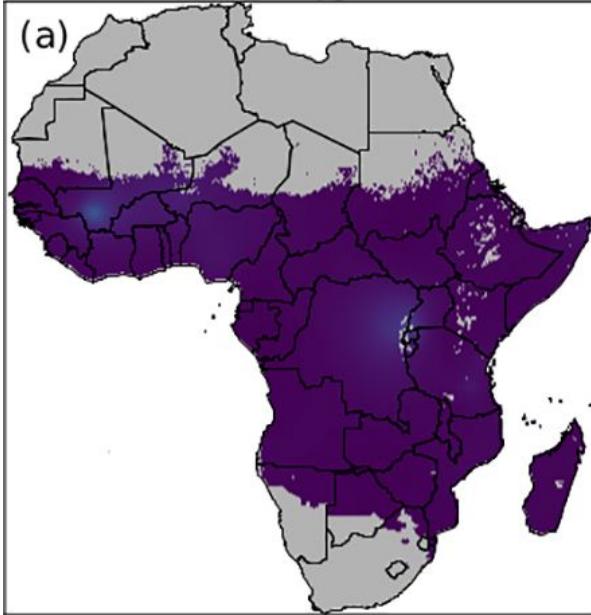
Median map: dhps540, 2020



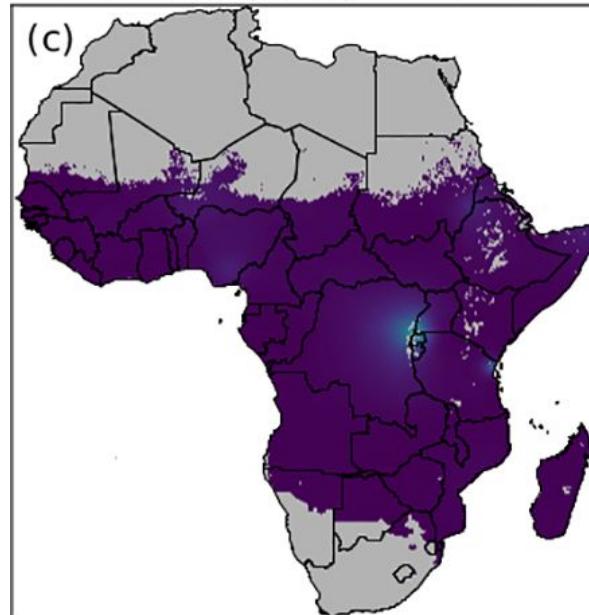
# Monitoring markers of drug resistance

## *pfdhps A581G*

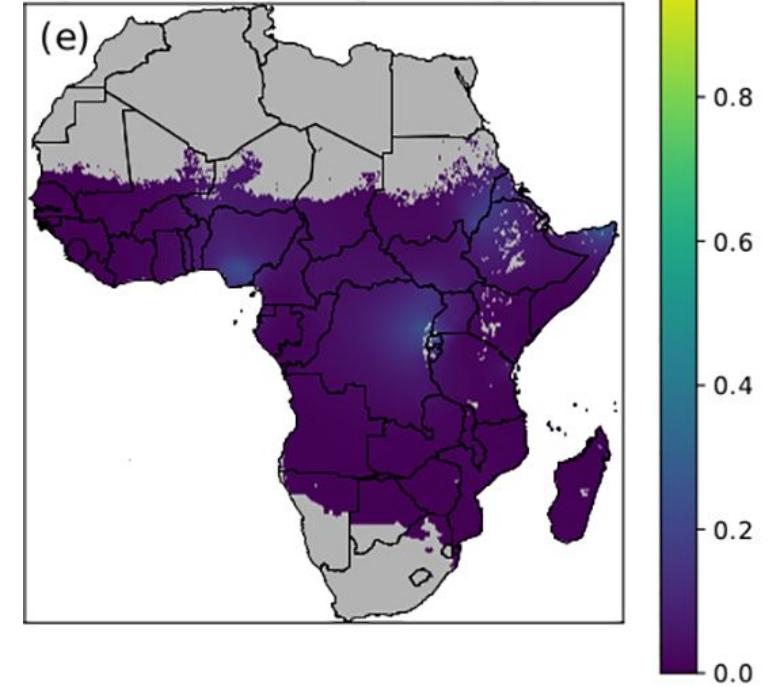
Median map: dhps581, 1990



Median map: dhps581, 2005

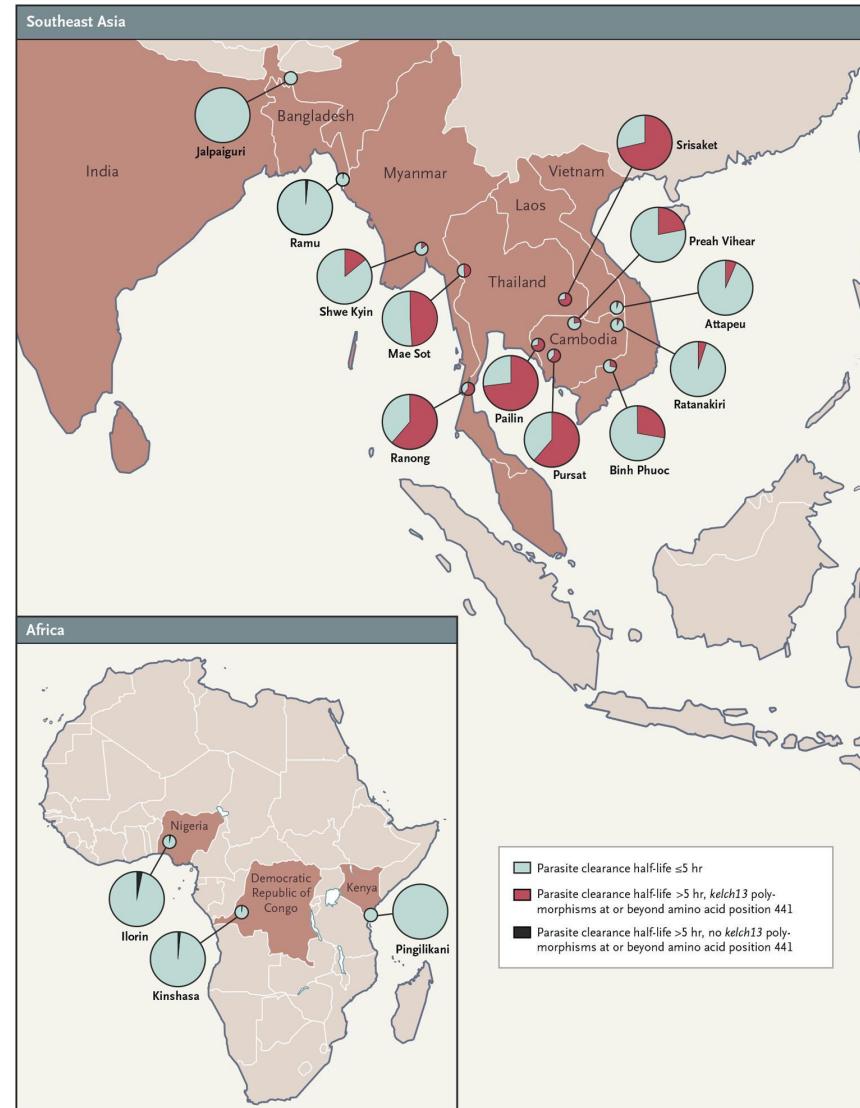


Median map: dhps581, 2020



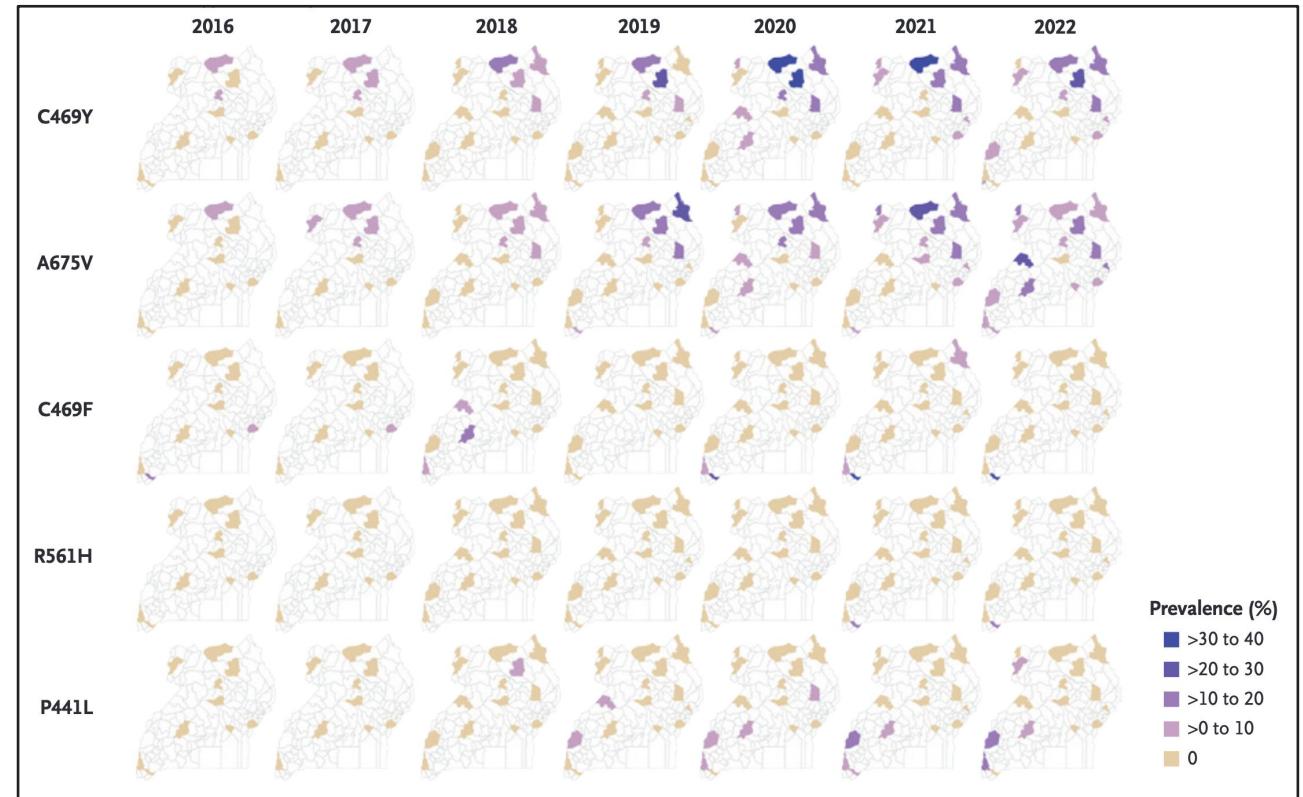
# Detecting pfk13 variants

- Delayed parasite clearance following artemisinin treatment, Western Cambodia (2000s)
- Identification of *kelch* 13 domain (2013)
- High prevalence of delayed clearance, and strong association with *pfk13* (2014)

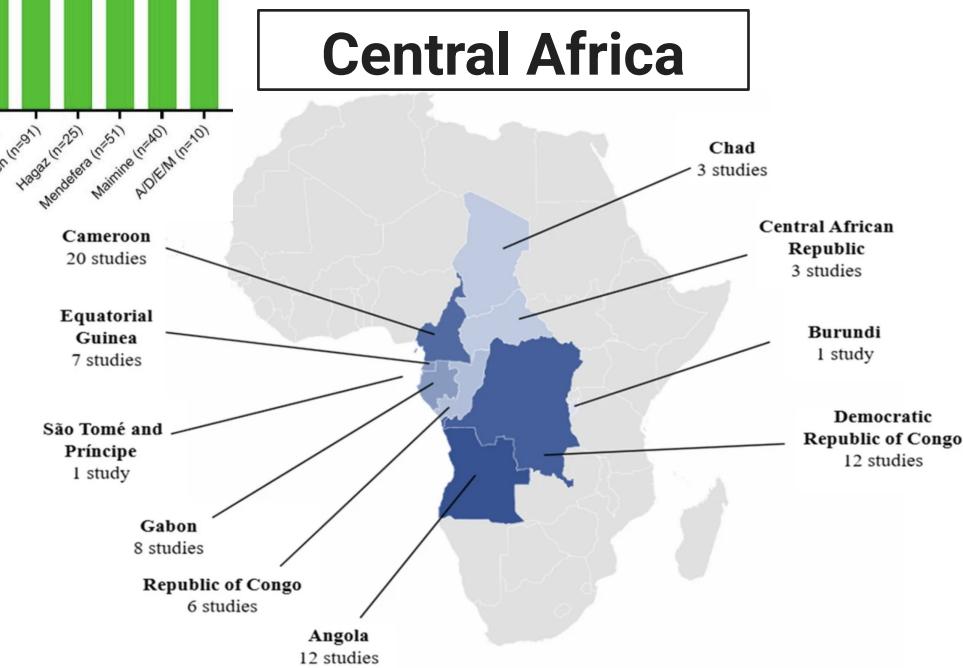
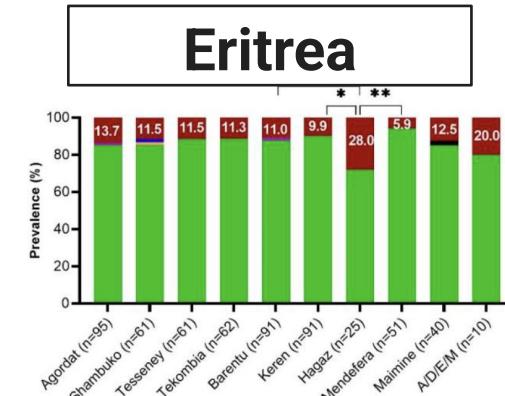
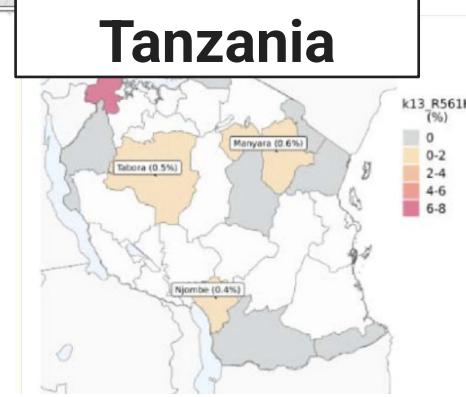
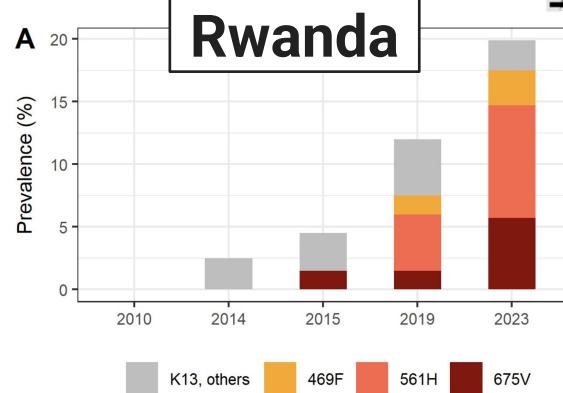
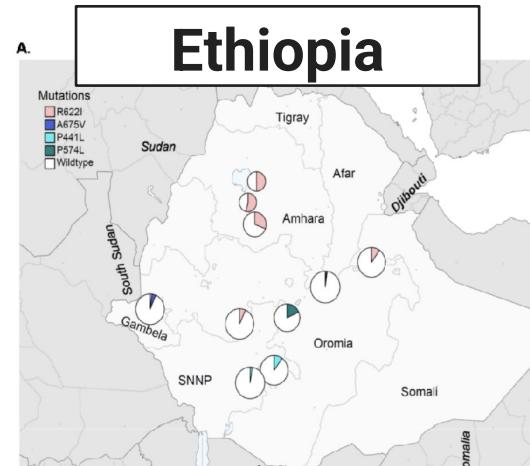
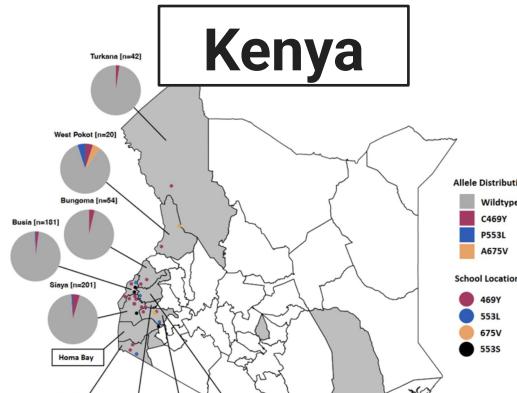


# Detecting pfk13 variants

- Enhanced survival of parasites after *in vitro* artemisinin exposure in Northern Uganda (2018)
- In Rwanda, *pfk13* mutations found to have increased between 2015 and 2018
- Spread in space and time from Northern Uganda (2023)

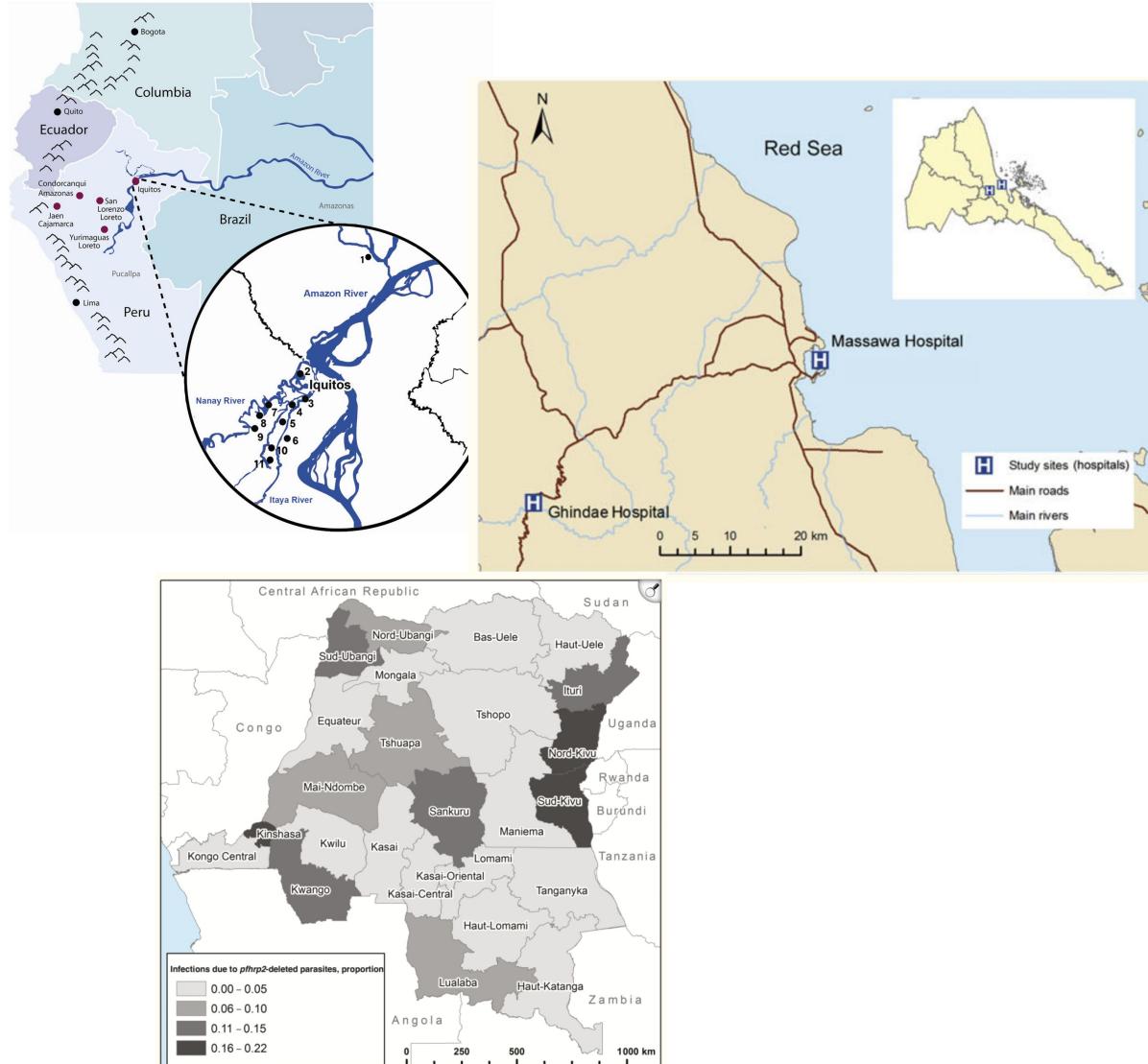


## *pfk13* mutations now found throughout sub-Saharan Africa



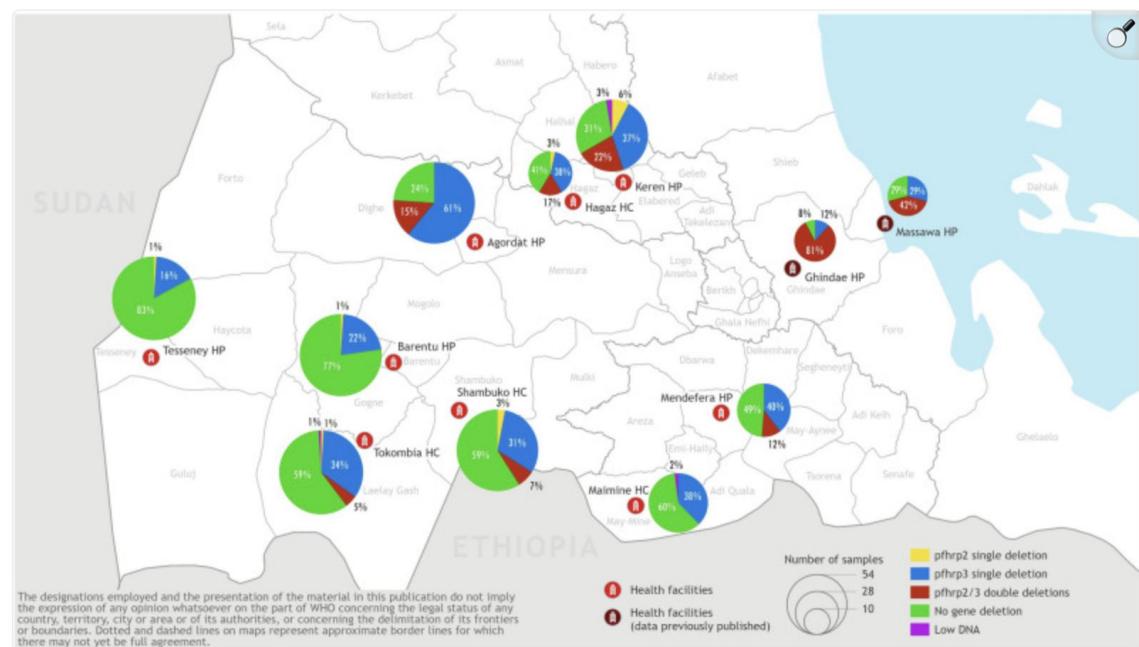
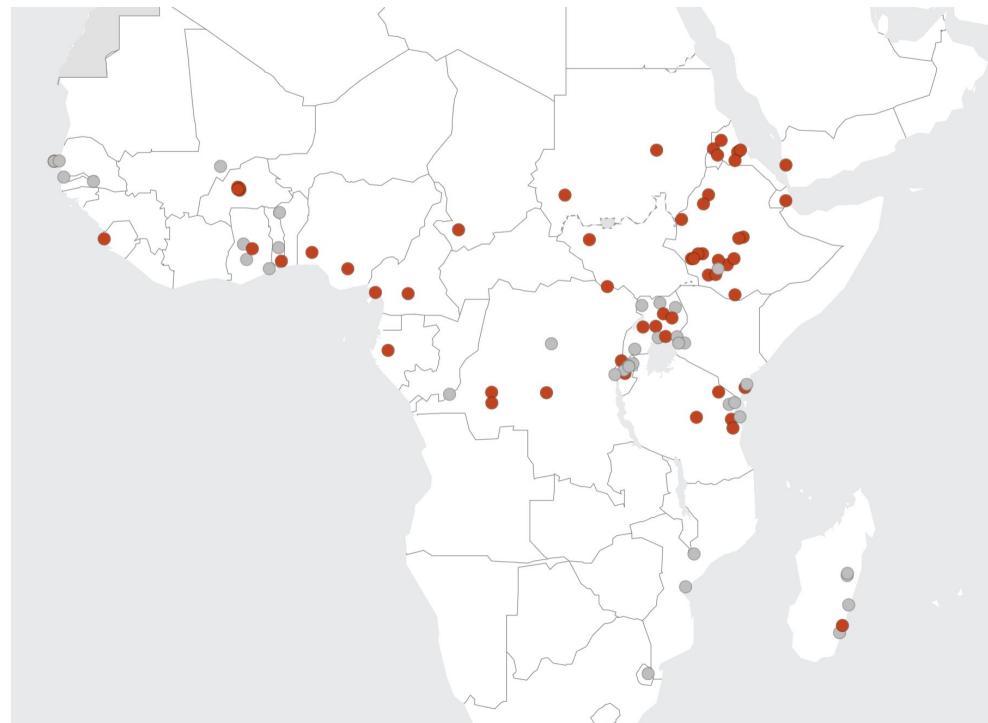
# Identifying and quantifying *pfhrp2/3* deletions

- First reports in Peru in 2010
- Turning point in 2016, identification in Eritrea and India
- Similar time (2017) identification in DRC from large cross-sectional surveys
- Moderate prevalence in Kenya, scattered prevalence in Mozambique and Tanzania



# Identifying and quantifying *pfhrp2/3* deletions

***Pfhrp2/3* deletions now found throughout sub-Saharan Africa, and at high prevalence in the Horn of Africa**



## Partner drug resistance

Patchy distribution throughout SSA. Some markers close to fixation, others spreading or receding

## Artemisinin resistance

Distinct epicenters in Northern Uganda and the Horn of Africa

## *pfhrp2/3* deletions

High prevalence in the Horn of Africa, identified throughout SSA

# Back to study design

## Major changes in MMS...

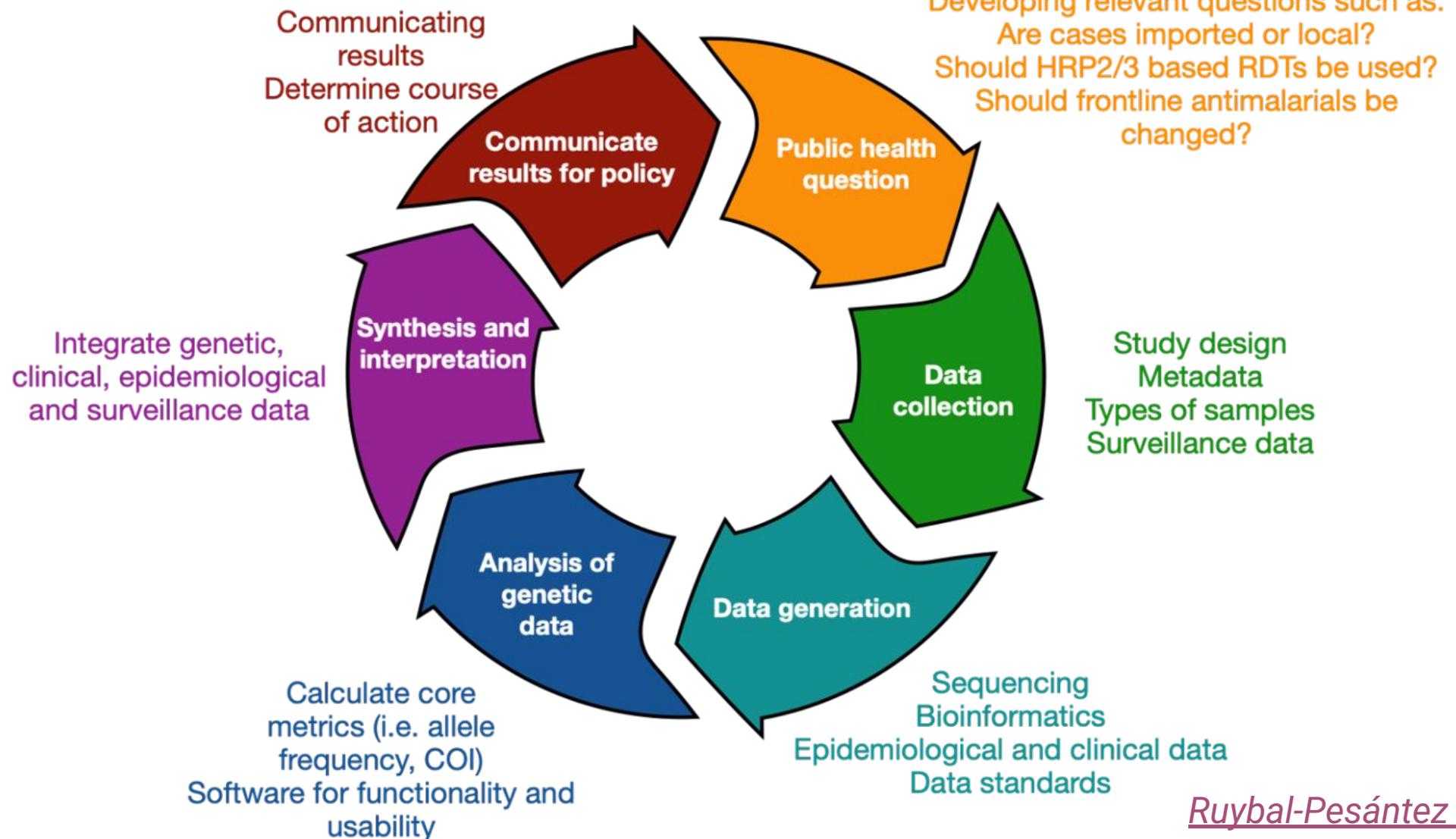
- Scale-up in number of sites and samples
- Deeper and wider sequencing
- Panels include multiple molecular markers (drug resistance, *pfhpr2/3* genes, diversity markers)
- Changes in distribution of genomic infrastructure

## Few general guidelines on...

- Study structure
- Minimum sample size
- Type of sequencing technology
- Which analysis tools to use

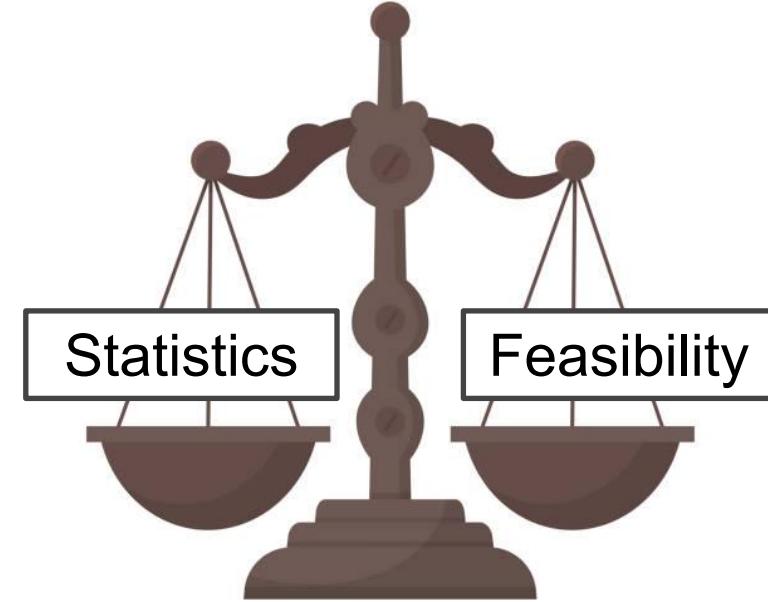


# Ideal framework



## Strengthen our statistical plans

- Precision and confidence intervals
- Power analysis
- Sample size calculation
- More advanced tools



## Put this in real world context

- Combine statistics with logistics, feasibility, budget etc.
- Discuss challenges and share solutions
- Identify areas for future development

# Plan for the workshop

## Housekeeping

- Breakfast and lunch served outside of the Rainbow room
- Bathrooms located outside the room to the left
- Prayer room is Windsong room directly across exit down the hallway past the kitchen area
- Dinners:
  - Thursday dinner, **18:00 at Mercatto Taverna**
  - Friday, **17:15 boxed dinner at hotel**
  - Saturday dinner, **17:15 at 360 CN Tower**



### Thursday

- **M1:** Sampling from a population
- **M2:** Sample size based on margin of error
- Flash talks: Getting to know each other
- Structured discussion: in-country experiences and challenges in MMS

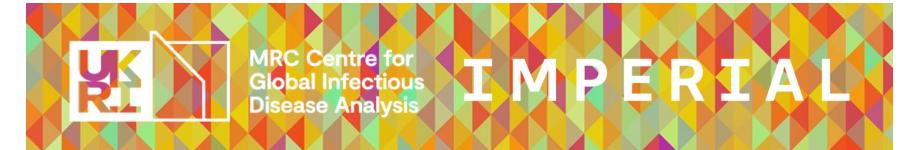
## Friday

- **M3:** Hypothesis testing
- **M4:** Statistical power
- **M5:** Multi-cluster studies
- Structured discussion: outlook and future of MMS

## Saturday

- **M6:** The DRpower tool
- **M7:** *Group project* - Designing studies for multiple endpoints

## Workshop materials



- All workshop materials will be available at our dedicated website:  
[mrc-ide.github.io/MMS-SD\\_workshop](https://mrc-ide.github.io/MMS-SD_workshop)
- Modules consist of lecture slides and practical (these are in a LearnR interactive format, available online)

