

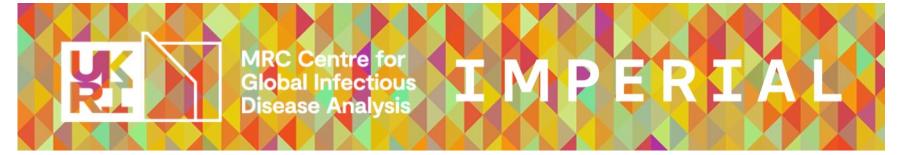
# Malaria Molecular Surveillance Study Design Workshop

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*MRC Centre for Global Infectious Disease Analysis*

*WHO Collaborating Centre for Infectious Disease modelling*

*School of Public Health  
Imperial College London*



# Welcome!

- No fire alarms planned
- Restrooms
- WIFI: Network **Aloft**, no password
- Check in and breakfast vouchers
- Teas, Coffees, refreshments

## Acknowledgements



MRC Centre for  
Global Infectious  
Disease Analysis

IMPERIAL

BILL & MELINDA  
GATES *foundation*

**Demonstrators:** Dr. Gina Cuomo-Dannenburg and Dr. Sequoia Leuba

**Guest lecture:** Hinda Doucoure

# 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

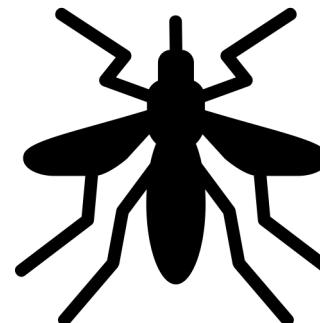
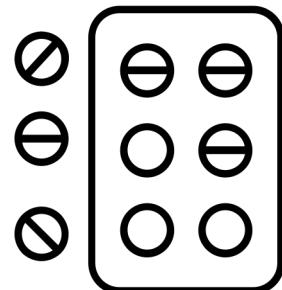
1. Monitoring the prevalence of established molecular markers of drug resistance (*crt*, *dhfr*, *dhps*, *mdr1*).
2. Detecting the emergence of rare variants of concern and tracking their spread in space and time (e.g. *k13*).
3. Measuring the prevalence of *hrp2/3* gene deletions as part of decision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



# 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

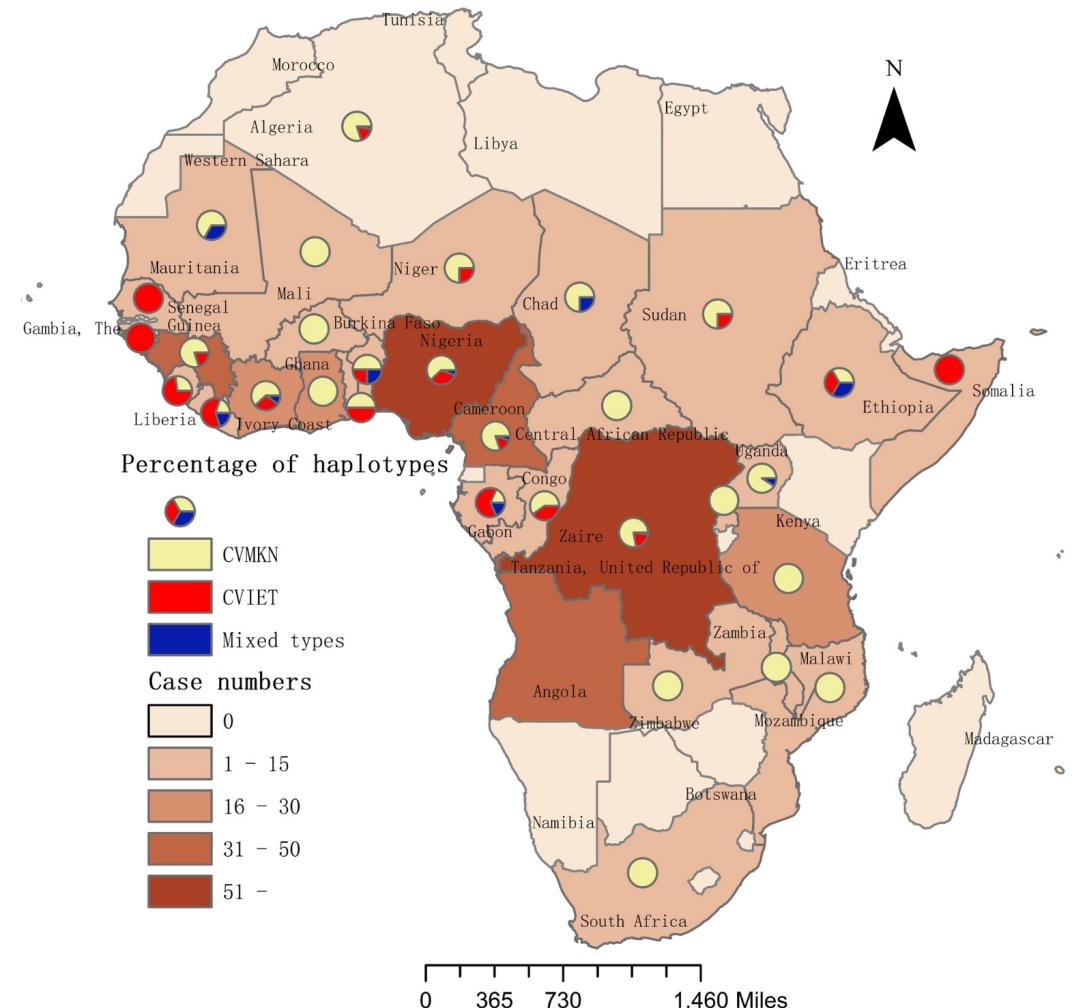
*pfmdr1*

*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

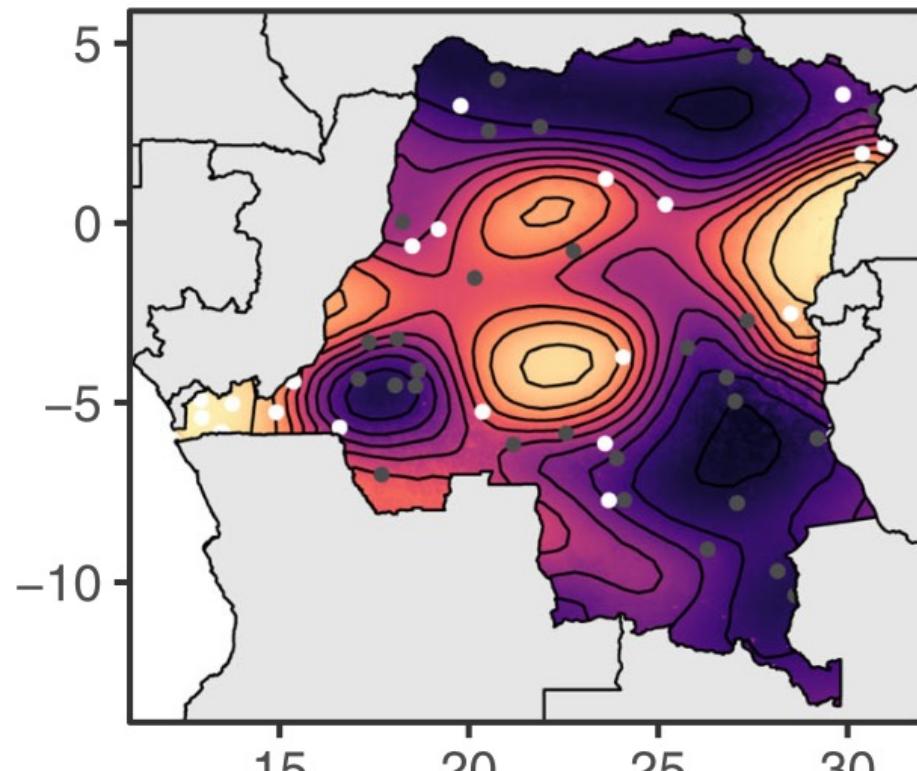


**Fig. 1** The number of imported *P. falciparum* cases from Africa and percentage of haplotypes of *PfCRT*

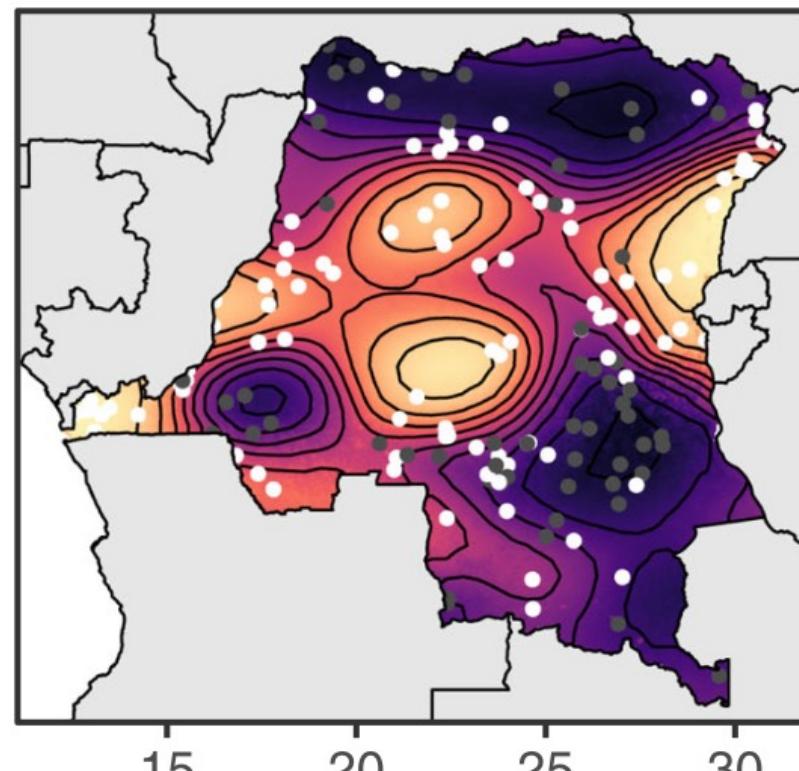
*pfCRT*

## Democratic Republic of the Congo

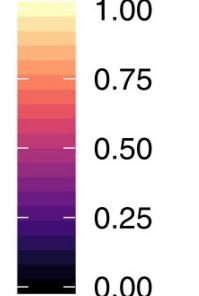
2007



2013



estimated  
allele  
frequency



1.00
0.75
0.50
0.25
0.00

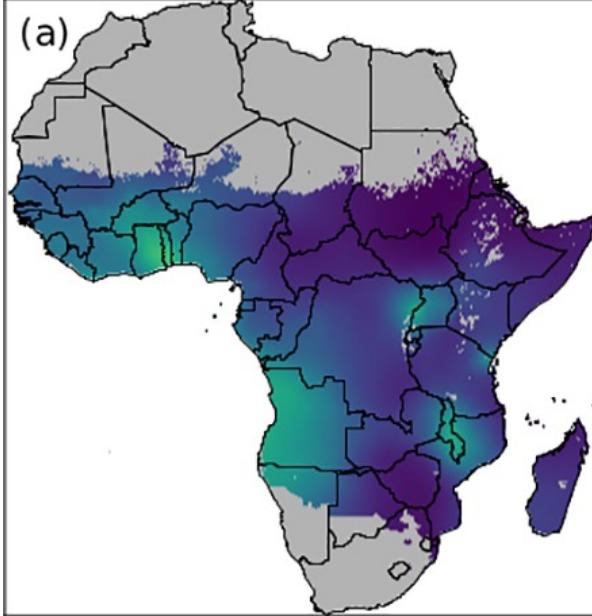
CIET

longitude

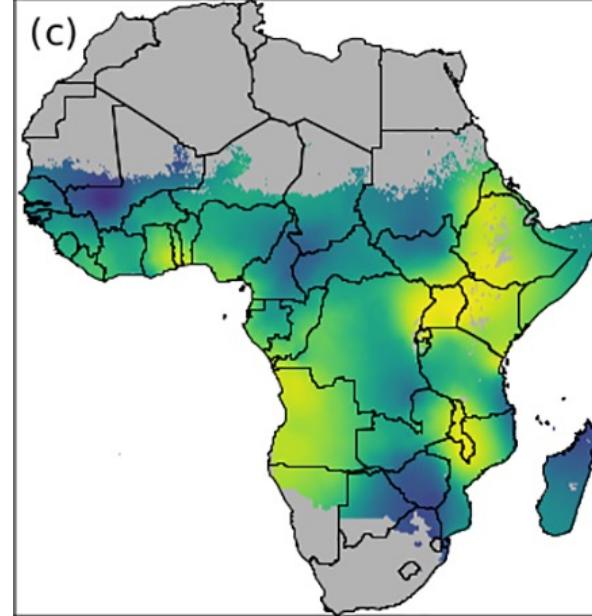
# Monitoring markers of drug resistance

## *pfdhps A437G*

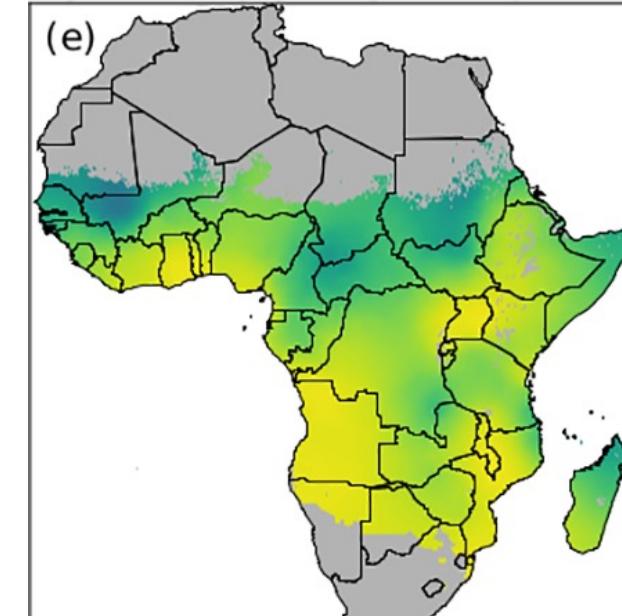
Median map: dhps437, 1990



Median map: dhps437, 2005

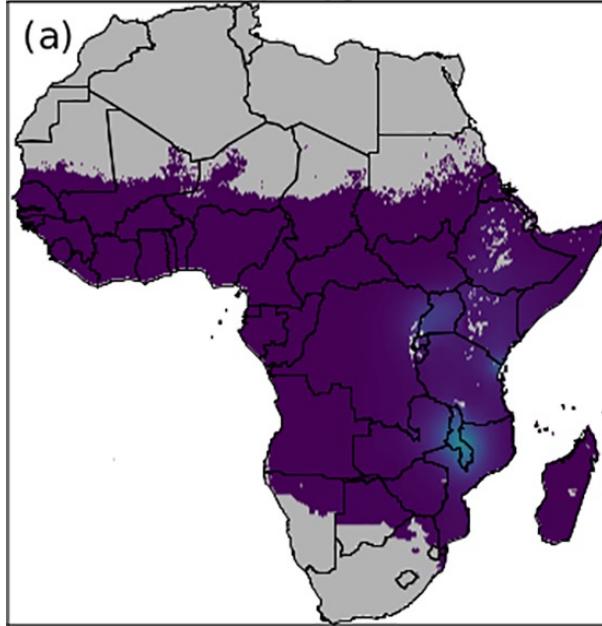


Median map: dhps437, 2020

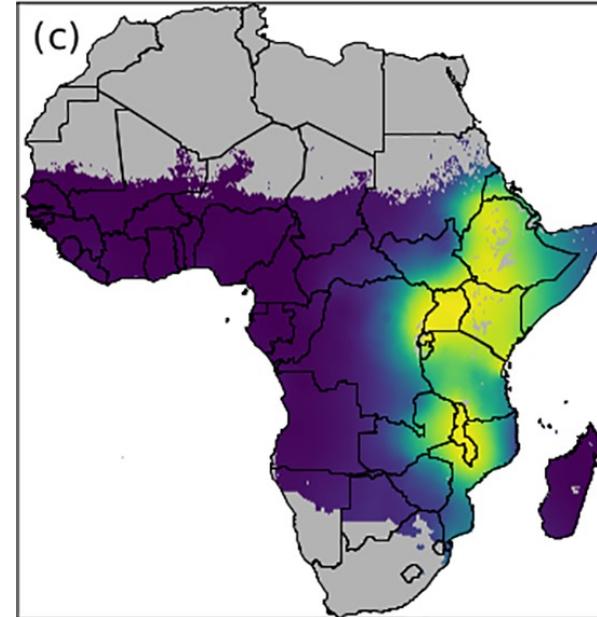


## *pfdhps* K540E

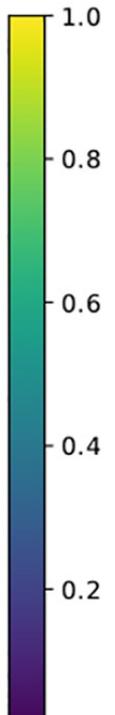
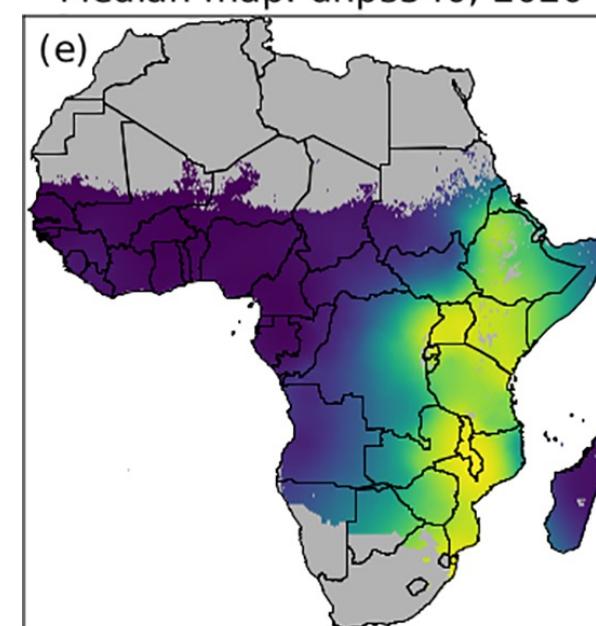
Median map: dhps540, 1990



Median map: dhps540, 2005



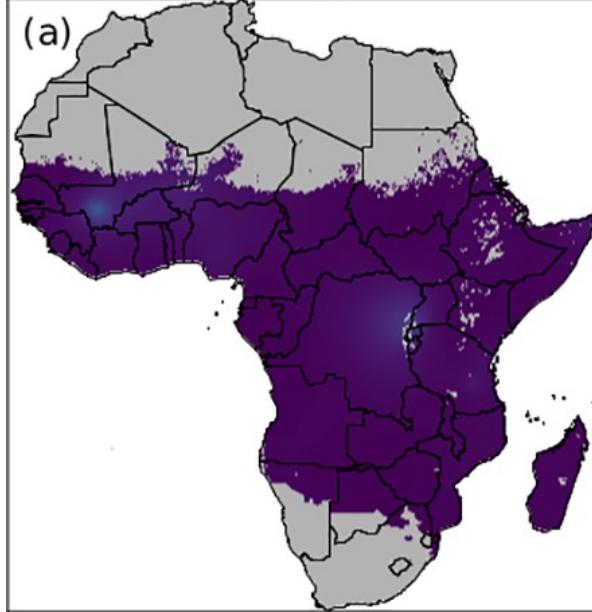
Median map: dhps540, 2020



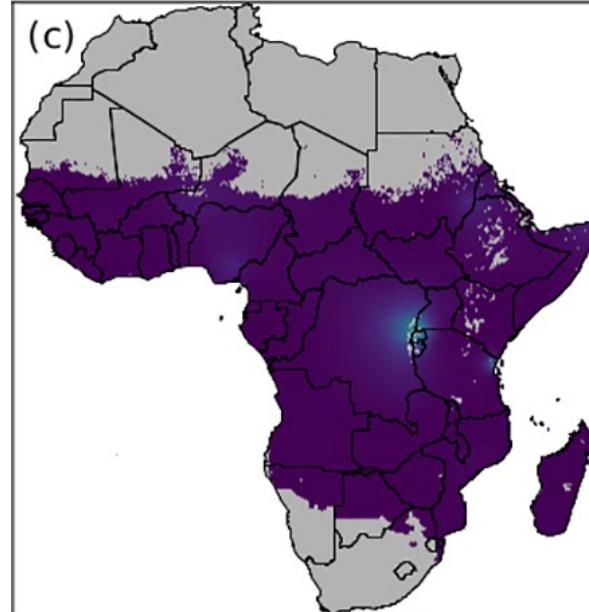
# Monitoring markers of drug resistance

## *pfdhps A581G*

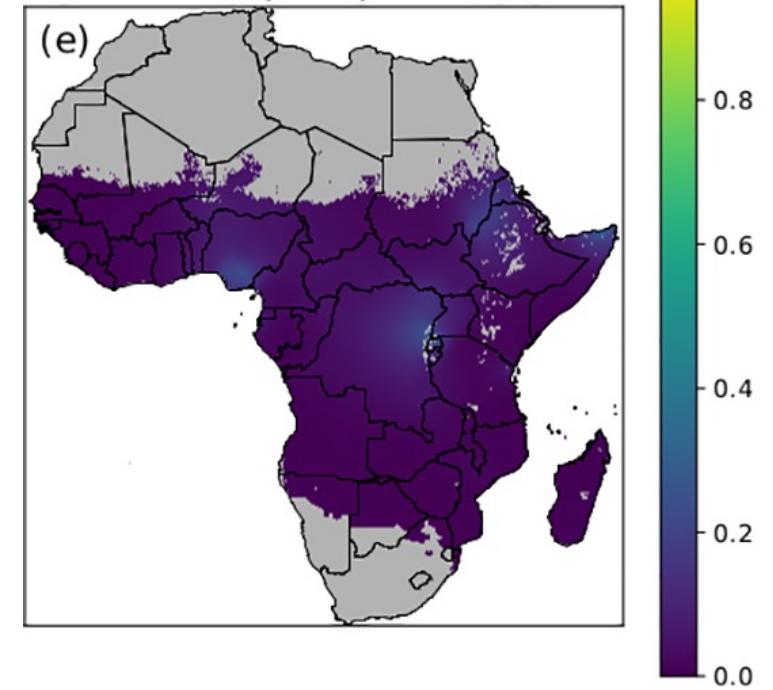
Median map: dhps581, 1990



Median map: dhps581, 2005

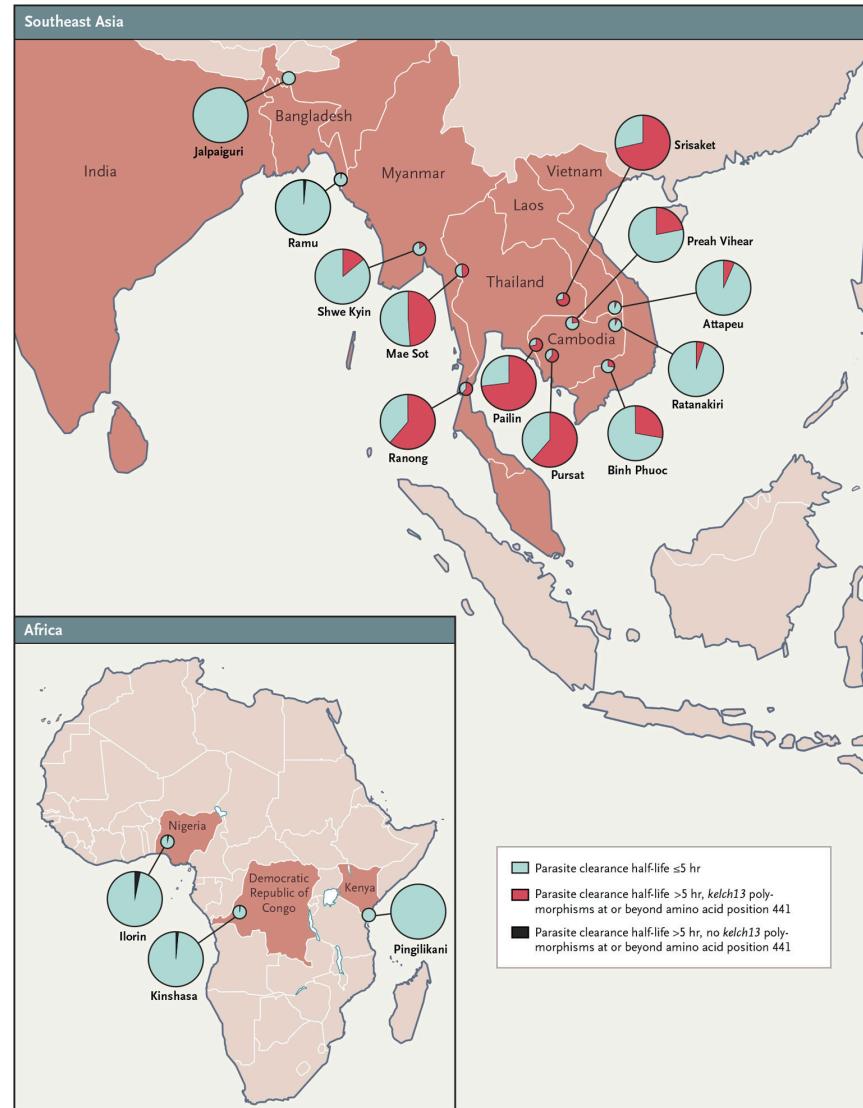


Median map: dhps581, 2020



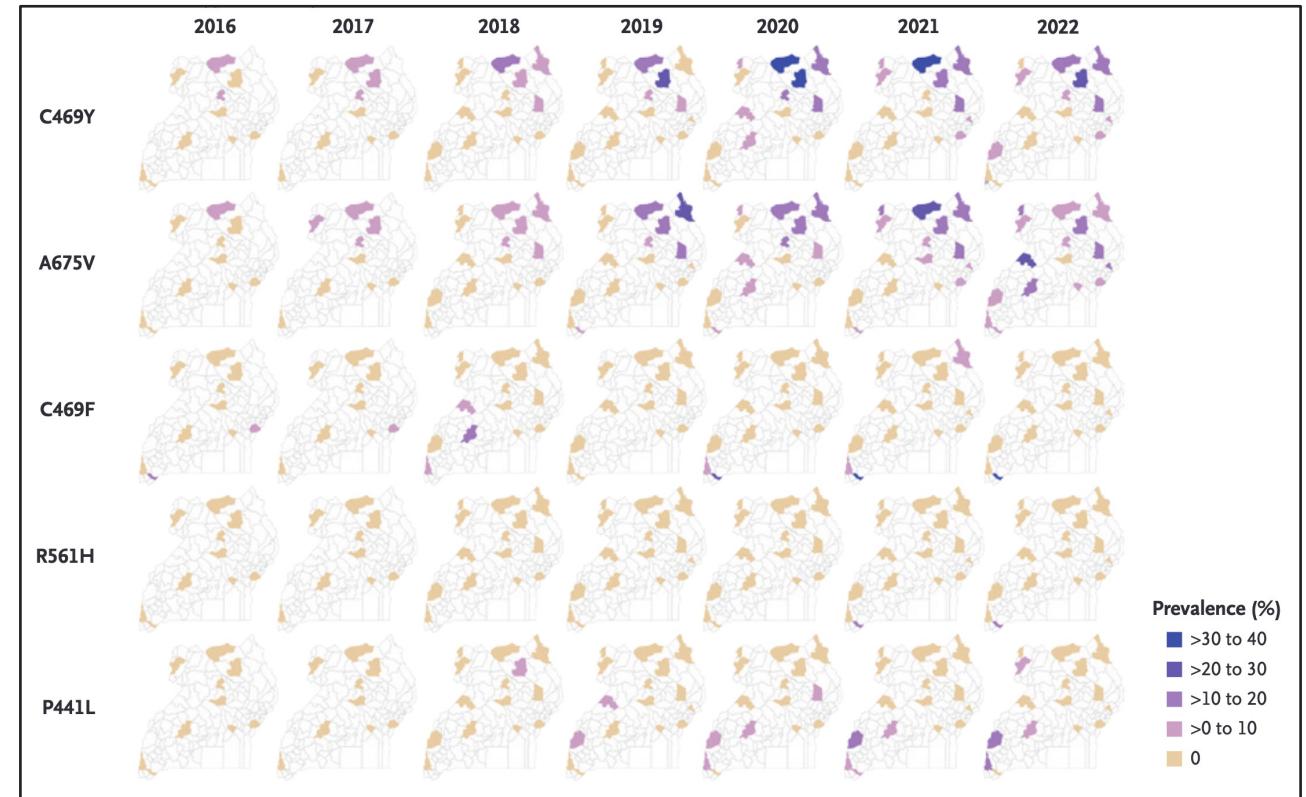
# Detecting pfk13 variants

- 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)

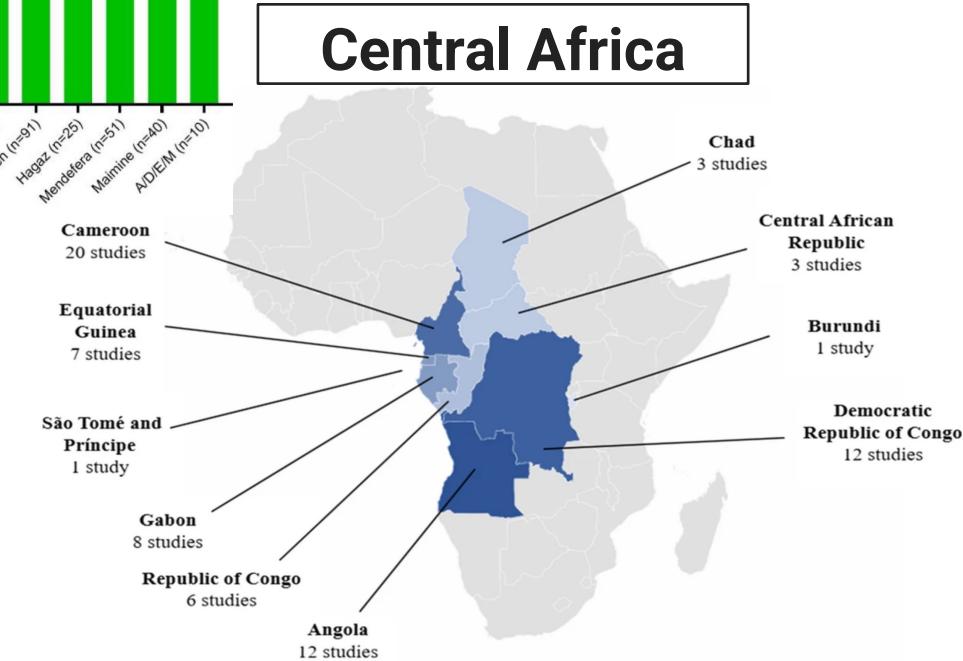
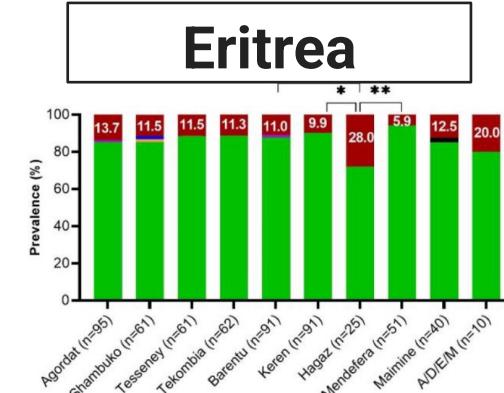
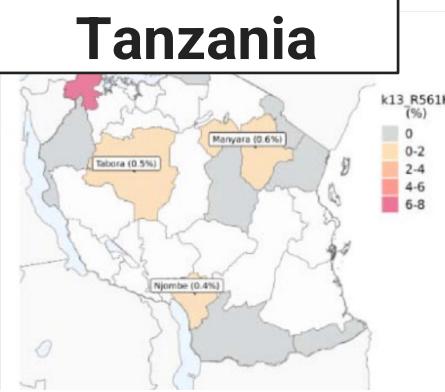
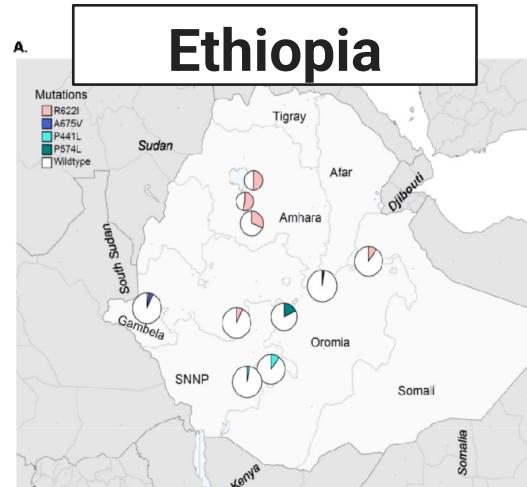
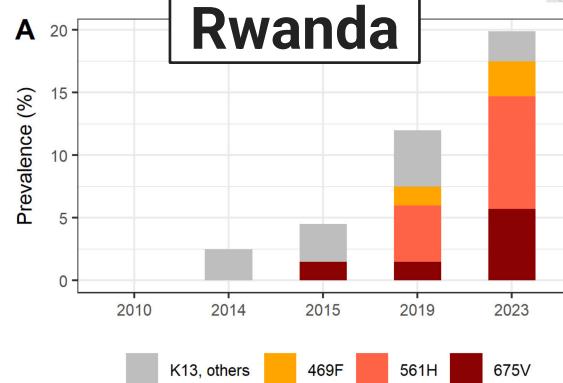
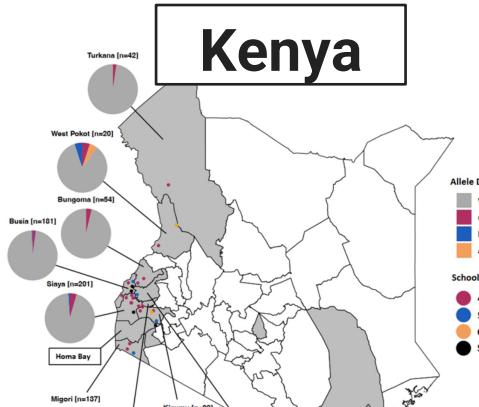


# Detecting pfk13 variants

- 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)

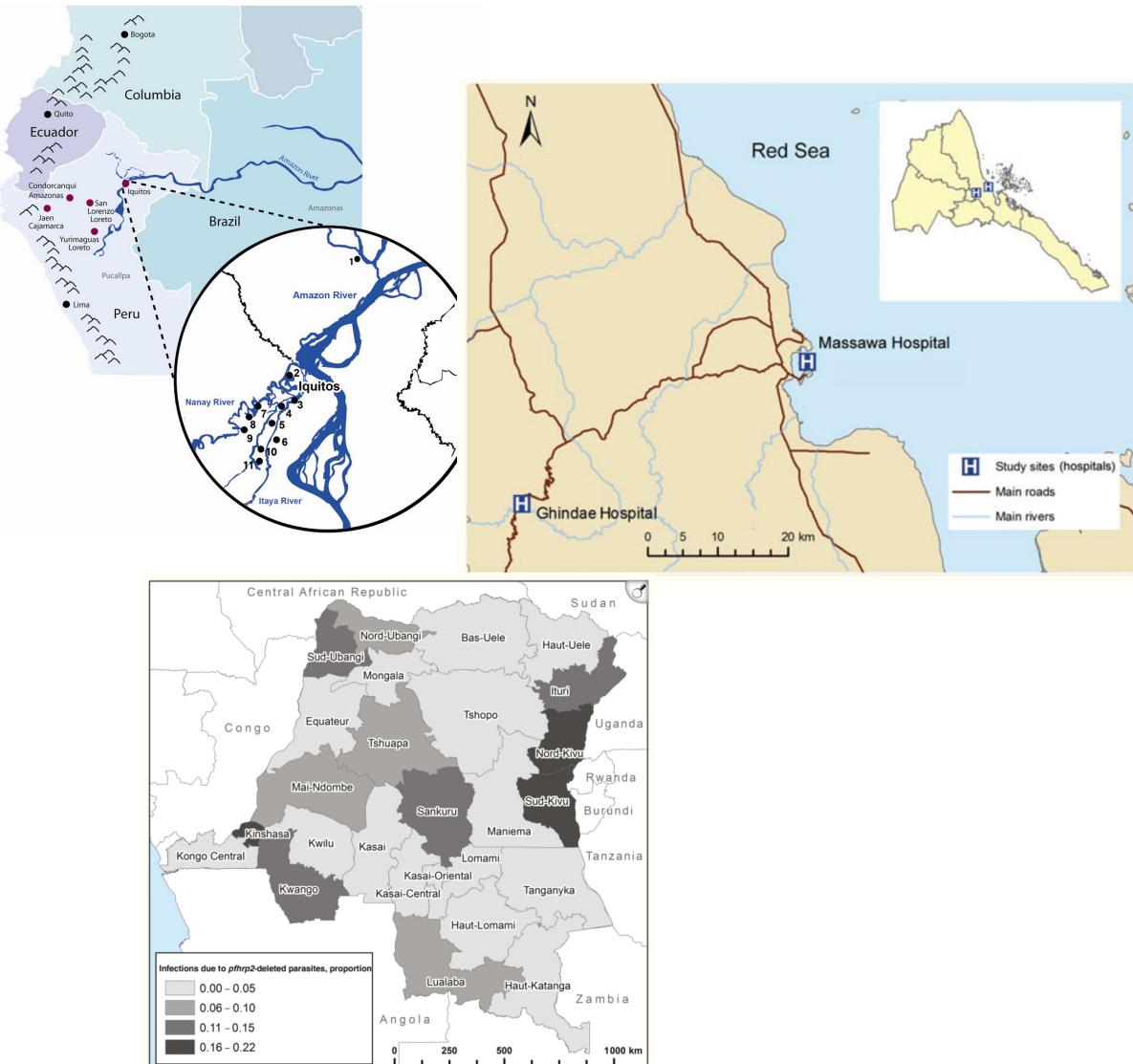


## *pfk13* mutations now found throughout Sub-Saharan Africa

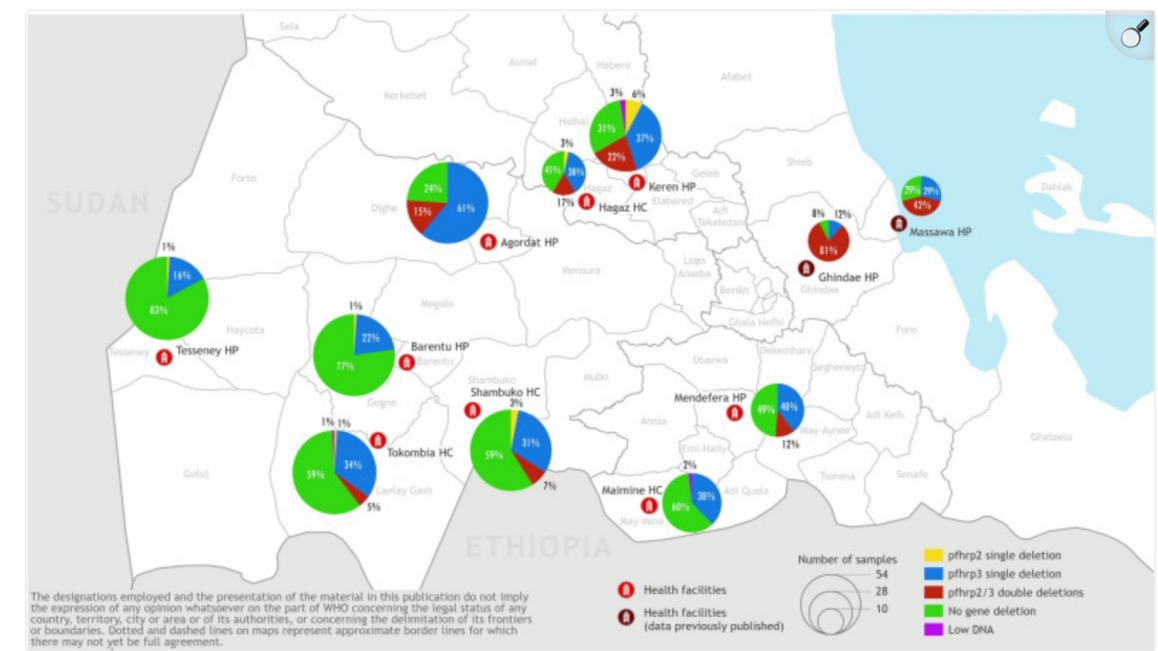
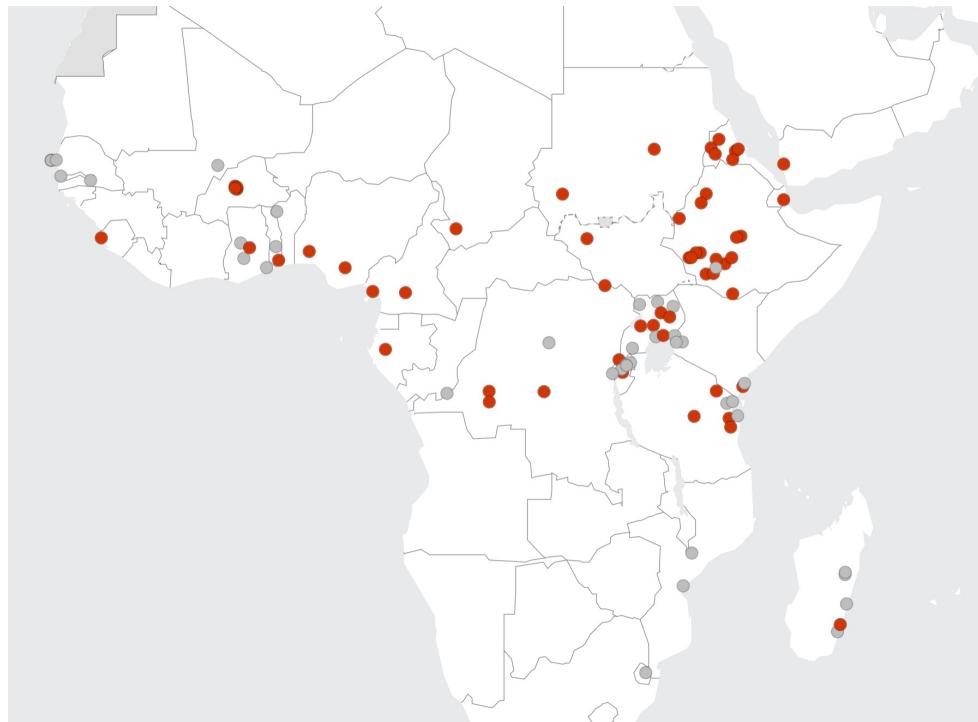


# Identifying and quantifying *pfhrp2/3* deletions

- 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



## 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

## How does study design come into this

### Major changes in MMS...

- Scale-up in number of sites and samples
- Deeper and wider sequencing
- Changes in distribution of genomic infrastructure

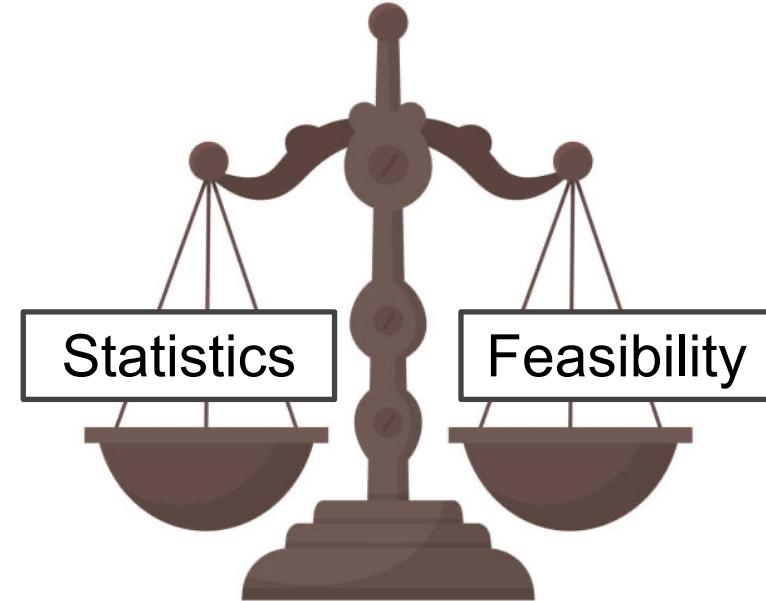
### Few general guidelines on...

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



## 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

## Monday

- M1: Sampling from a population
- M2: Sample size based on precision
- Structured discussion: experiences and challenges

*Dinner at Antoines*



## Tuesday

- Guest lecture: Hinda Doucoure
- **M3:** Hypothesis testing and power
- **M4:** Multi-cluster studies
- **Lunch**
- **M5:** The DRpower tool
- **M6:** Designing studies for multiple endpoints
- Structured discussion: Future steps

## We want to hear from you!

- Name
- Affiliation
- Your reasons for attending this workshop