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MALARIA PFHRP2/3 GENE DELETION – WHO GUIDELINES AND EXPERIENCE SHARING FROM UGANDA AND ETHIOPIA.

Navigating Malaria Testing in the Era of Gene Deletion.

11 April 2024

*Lateral Flow Lipoarabinomannan

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Introduction and Welcome

Mr. Bokretsion Gidey
(B. Sc, M. Sc, PhD in progress)
Team Leader,
Malaria and NTDs research team
Ethiopian Public Health Institute (EPHI)

Dr. Agaba Bosco
(PhD, MSc, MPH, Post-Doct)
Post-Doc Scientist- LSHTM, Senior Research Fellow- MUST, Coordinator- Malaria Genomic surveillance/Emerging Pathogens
National Malaria Program Uganda

Dr. Xavier Ding
(PhD)
Global Clinical Strategy Manager
Abbott Rapid Diagnostics

Agenda

Time	Topic	Presenter
5’	Opening and welcome	Dr. Evans Mathebula Medical and Scientific Affairs Manager, Africa, Abbott
10’	WHO guidelines on Malaria pfhrp2/3 Gene Deletion Surveillance	Dr. Xavier Ding (PhD) Global Clinical Strategy Manager
15’	The emergence of pfhrp2/3 gene deletion for the escape of plasmodium falciparum detection in Ethiopia	Mr. Bokretsion Gidey (B. Sc, M. Sc, PhD in progress) Team Leader, Malaria and NTDs research team Ethiopian Public Health Institute (EPHI)
15’	Threat of HRP2 Deletion, Surveys and Implementation Experience from Uganda	Dr. Agaba Bosco (PhD, MSc, MPH, Post-Doct) Post-Doc Scientist- LSHTM, Senior Research Fellow-MUST, Coordinator- Malaria Genomic surveillance/Emerging Pathogens National Malaria Program Uganda
10’	Q&A Session	Facilitated by Dr Evans Mathebula Medical and Scientific Affairs Manager, Africa, Abbott
5’	Closing Remarks	Dr. Evans Mathebula Medical and Scientific Affairs Manager, Africa, Abbott

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DR. XAVIER DING

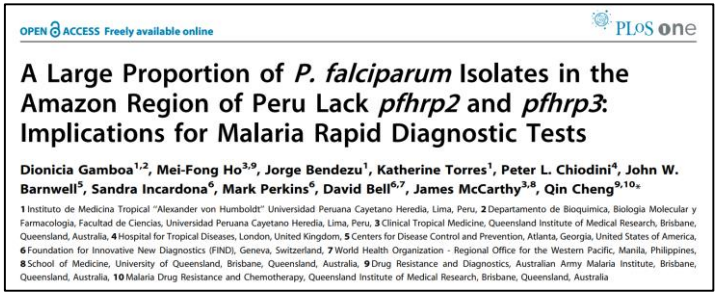
WHO guidelines on Malaria pfhrp2/3 Gene Deletion Surveillance

INTRODUCTION

Background

- First report of *hrp2* and *hrp3* deletion in **January 2010** by Dionicia Gamboa, Qin Cheng, and colleagues
- Study calling for **surveys to identify and monitor the presence and spread of parasites with deletion**
- **Largely ignored** and considered a peculiarity of the Amazon basin **until 2018**, when an alarming high rate of false negative RDT in Eritrea led to the identification of a similar issue in Eastern Africa
- **WHO issued a response plan in 2019** covering:
  - Selection criteria for malaria RDTs
  - Surveillance recommendation and protocols
  - Recommendation on when and how to switch to malaria RDTs recommended for use in area of high deletion prevalence

https://mesamalaria.org/resource-hub/resource-compilation-responding-threat-pfhrp23-deletions/



OPEN ACCESS Freely available online PLoS one

A Large Proportion of *P. falciparum* Isolates in the Amazon Region of Peru Lack *pfhrp2* and *pfhrp3*: Implications for Malaria Rapid Diagnostic Tests

Dionicia Gamboa[1,2], Mei-Fong Ho[3,9], Jorge Bendezu[1], Katherine Torres[1], Peter L. Chiodini[4], John W. Barnwell[5], Sandra Incardona[6], Mark Perkins[6], David Bell[6,7], James McCarthy[3,8], Qin Cheng[9,10]*

1 Instituto de Medicina Tropical "Alexander von Humboldt" Universidad Peruana Cayetano Heredia, Lima, Peru, **2** Departamento de Bioquímica, Biología Molecular y Farmacología, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru, **3** Clinical Tropical Medicine, Queensland Institute of Medical Research, Brisbane, Queensland, Australia, **4** Hospital for Tropical Diseases, London, United Kingdom, **5** Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **6** Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland, **7** World Health Organization - Regional Office for the Western Pacific, Manila, Philippines, **8** School of Medicine, University of Queensland, Brisbane, Queensland, Australia, **9** Drug Resistance and Diagnostics, Australian Army Malaria Institute, Brisbane, Queensland, Australia, **10** Malaria Drug Resistance and Chemotherapy, Queensland Institute of Medical Research, Brisbane, Queensland, Australia

"As Peru borders on several countries that share the Amazon River basin and where malaria transmission occurs without respect for national borders, it is unlikely that parasites lacking pfhrp2 and pfhrp3 are confined to Peru."

"It is therefore important that investigations be performed in other areas in South America urgently where P. falciparum is endemic to determine the presence and geographical spread of parasites lacking the pfhrp2 and pfhrp3 genes before the large scale implementation of malaria RDTs in this area."

"Investigations should also be carried out to monitor the presence and spread of parasites with gene deletions in areas outside of South America to ensure the best performance of malaria RDTs globally."

https://www.who.int/publications/i/item/9789240002036

WHO Surveillance template protocol

- Recommended template protocol has been developed by WHO **to guide surveillance for *pfhrp2/3* gene deletions in malaria endemic countries**
- "This surveillance activity is intended to determine whether the local prevalence of mutations in the P. falciparum hrp2/3 genes causing false negative RDTs has reached a threshold that might require a local or national change in diagnostic strategy."

Target population	Individuals seeking care for febrile illness at health facilities
Survey type	Cross-sectional, multi-site
Primary output measures	1. Prevalence of suspected false-negative HRP2 RDT results among symptomatic patients with *P. falciparum* malaria. 2. Prevalence of *pfhrp2/3* gene deletions among symptomatic falciparum patients with a false-negative HRP2 RDT result 3. Prevalence of *pfhrp2/3* gene deletions causing false-negative HRP2 RDTs amongst all symptomatic *P. falciparum* confirmed cases.
Secondary output measures (optional)	1. Parasite density, as measured by quantitative PCR and/or microscopy, in patients with suspected false-negative HRP2 RDT results.
Sample size	A sample size of 600 confirmed *P. falciparum* cases per sampling domain (60 per health facility) is recommended to quantify whether or not the prevalence of *pfhrp2* deletion is above 5%. Once the sample of 600 *P. falciparum* cases have been enrolled then molecular confirmation of *pfhrp2* deletions amongst suspected false-negative *P. falciparum* cases should ensue.
Sampling method	In at least 10 pre-selected health facilities per sampling domain e.g. province at risk, a cross-sectional survey will measure the suspected and confirmed prevalence of *pfhrp2/3* gene deletions causing false-negative HRP2 RDT results. 60 *P. falciparum* confirmed cases should be included in each health facility.

Data collection

1. Identify provinces to be included in the study.
2. Select at least 10 health facilities per province for testing (facility sample size may vary depending on logistical and budgetary constraints). Any facility where RDTs are being used is eligible; however, microscopy services are not a requirement.
3. Test all individuals presenting with suspected malaria using both a WHO-recommended HRP2 RDT and a non-HRP2 method (e.g., pf-pLDH RDT (separate single or multiple test line RDT) or quality – assured microscopy in the health facility and collect minimum two dried blood spots (DBS).
4. Record demographic and clinical history details and all test results
5. Administer antimalarial therapy based on results from (either) RDT and/or microscopy and according to national guidelines.
6. Send minimum of two DBS from all Pf patients with negative HRP2 RDT and positive pf-pLDH RDT or microscopy for molecular +/-- serological analysis.[1]
7. Surveillance activity can stop once 600 individuals with confirmed *P. falciparum* malaria (ideally ~60/site across the 10 sites in the province) have been recorded as having *P. falciparum* in step 4.
8. Supplemental data collection options are described in Appendix 1.
9. Discard all RDTs, microscopy slides and DBS after survey results finalized and reported

https://www.who.int/publications/i/item/9789240002036

Outcomes and actions

Outcome 1:

Repeat in 2 years

Outcome 1: The estimated proportion is lower than 5% and the upper limit of the 95% CI is below 5%. In this case there is a high statistical confidence that the proportion of parasites with pfhrp2/3 deletion causing false-negative RDT results within symptomatic patients is below 5%.

Outcome 2: The estimated proportion is higher than 5% and the lower limit of the 95% CI is above 5%. This result means that there is a high statistical confidence that the proportion of pfhrp2/3 deletion causing false-negative RDT results in symptomatic Pf patients is greater than 5%.

Outcome 3: The statistical analysis shows that it is inconclusive (5% contained within the 95% CI) as to whether or not the prevalence of pfhrp2/3 deletion causing false negative RDT results in symptomatic Pf patients is greater than or less than 5%

Outcome 3:

Repeat in 1 or 2 years or keeping screening for a larger sample size

Outcome 2:

"If outcome 2 is obtained, *pfhrp2* deletions are found to be prevalent (lower 95% CI is > 5%) in any province, the country programmes should **make a nationwide switch to RDTs** that do not rely exclusively on HRP2 for detecting *P. falciparum*, prioritized on the basis of the prevalence of *pfhrp2* deletions across provinces"

"**A threshold of 5% was selected because it is somewhere around this point that the proportion of cases missed by HRP2 RDTs due to non-hrp2 expression may be greater than the proportion of cases that would be missed by less-sensitive pLDH based RDTs**"

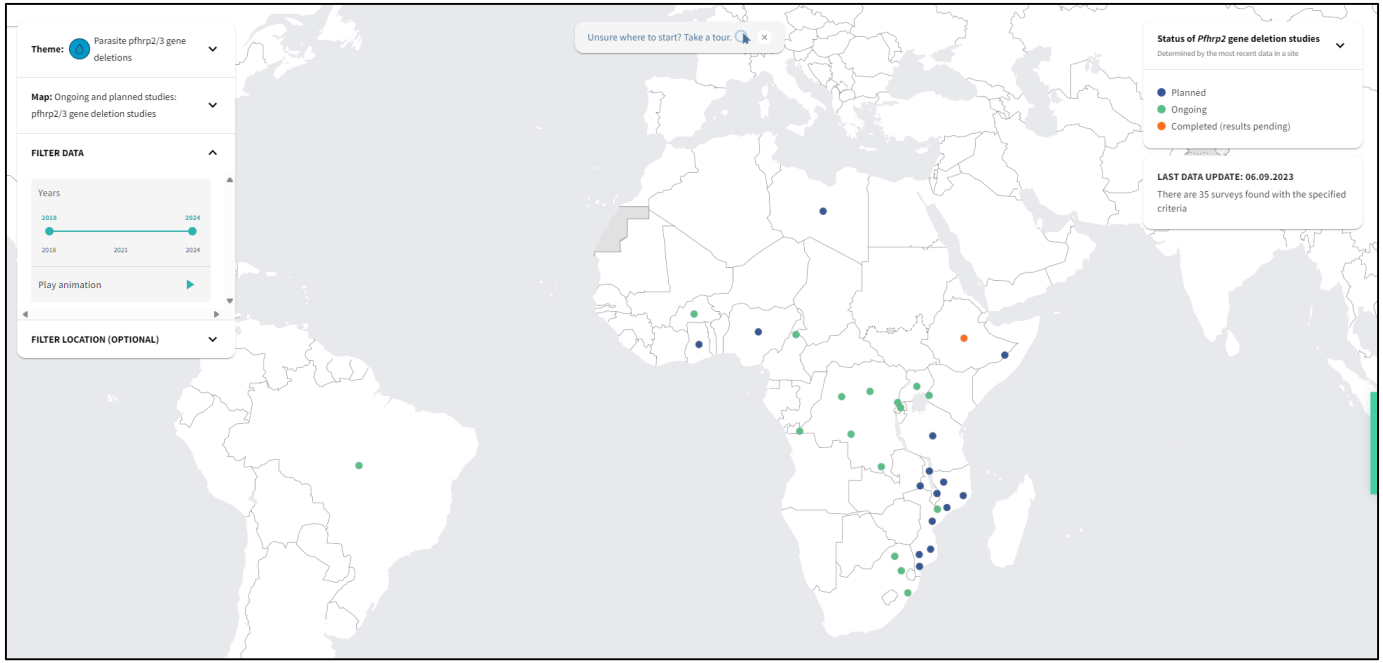
"A nationwide change is suggested because mathematical models show parasites lacking pfhrp2 genes will spread […]"

HRP2 DELETION SURVEILLANCE

https://apps.who.int/malaria/maps/threats

WHO threat maps

Online map to track planned, ongoing and completed surveys



https://apps.who.int/malaria/maps/threats
https://www.medrxiv.org/content/10.1101/2023.10.21.23297352v3.full.pdf

WHO threat maps

Online map to track reported presence or absence of deletions

- "In the thirteen years since the first report of pfhrp2-deleted parasites in 2010, the World Health Organization (WHO) has found that 40 of 47 countries surveyed worldwide have reported pfhrp2/3 gene deletions."
- In the last five years, Eritrea, Djibouti and Ethiopia have switched or started switching to using alternative RDTs, that target pan-specific-pLDH or P. falciparum specific-pLDH alone of in combination with HRP2

https://www.medrxiv.org/content/10.1101/2023.10.21.23297352v3.full.pdf

Global risk of deletion selection and spread

- "In the thirteen years since the first report of pfhrp2-deleted parasites in 2010, the World Health Organization (WHO) has found that 40 of 47 countries surveyed worldwide have reported pfhrp2/3 gene deletions."
- In the last five years, Eritrea, Djibouti and Ethiopia have switched or started switching to using alternative RDTs, that target pan-specific-pLDH or P. falciparum specific-pLDH alone of in combination with HRP2

Global risk of selection and spread of *Plasmodium falciparum* histidine-rich protein 2 and 3 gene deletions

Oliver J. Watson[1], Thu Nguyen-Anh Tran[2], Robert J Zupko[2], Tasmin Symons[3], Rebecca Thomson[4], Theodoor Visser[5], Susan Rumisha[3], Paulina A Dzianach[3], Nicholas Hathaway[6], Isaac Kim[7,8], Jonathan J. Juliano[9,10,11], Jeffrey A. Bailey[7,8,12], Hannah Slater[13], Lucy Okell[1], Peter Gething[3,14], Azra Ghani[1], Maciej F Boni[2,15], Jonathan B. Parr[10,11], Jane Cunningham[16]

Conclusions

- Since its first identification in 2010, *hrp2/3* deletions have been found to **occur in the majority of malaria endemic countries**
- **It is a pernicious issue, that can spread undetected for years** and that can severely impact the effectiveness of HRP2-based RDTs and ultimately prevent adequate clinical management of patients
- **Well-planned surveys are essential** to monitor the occurrence and spread of this issue. WHO has developed recommend protocol to facilitate best-practices and data comparison
- **The 5% threshold is based on the historical more limited sensitivity of LDH-based RDTs compared to HRP2-based RDTs**
- **If equally sensitive LDH-based RDTs can be made available, the switch could be made at a lower prevalence percentage or even preemptively**, rendering the need for repeated survey less acute

MR. BOKRETSION GIDEY

The emergence of pfhrp2/3 gene deletion for the escape of Plasmodium falciparum detection in Ethiopia

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Ethiopian Public Health Institute

The emergence of pfhrp2/3 gene deletion for the escape of plasmodium falciparum detection in Ethiopia

Bokretsion Gidey

Ethiopian Public Health Institute (EPHI)

April 2024

Content outlines

- Malaria Overview in Ethiopia
- pfHRP2/3 gene deleted parasite in Ethiopia: phase one
- Nationwide distribution of pfhrp2/3 gene deletion in Ethiopia: Phase two

Malaria Overview in Ethiopia

- In Ethiopia, *P. falciparum* and *P. vivax* are the most common malaria with a ratio of 60 vs 40%
- 2nd populous country in Africa and 75% of the population is at risk
- In 2022, more than 5 million episodes are reported
- Ethiopia launched elimination program since 2017, a step wise malaria elimination to align with vision of malaria free Ethiopia by 2030

Diagnosis, Malaria Elimination Challenges:

➢ Biological challenges

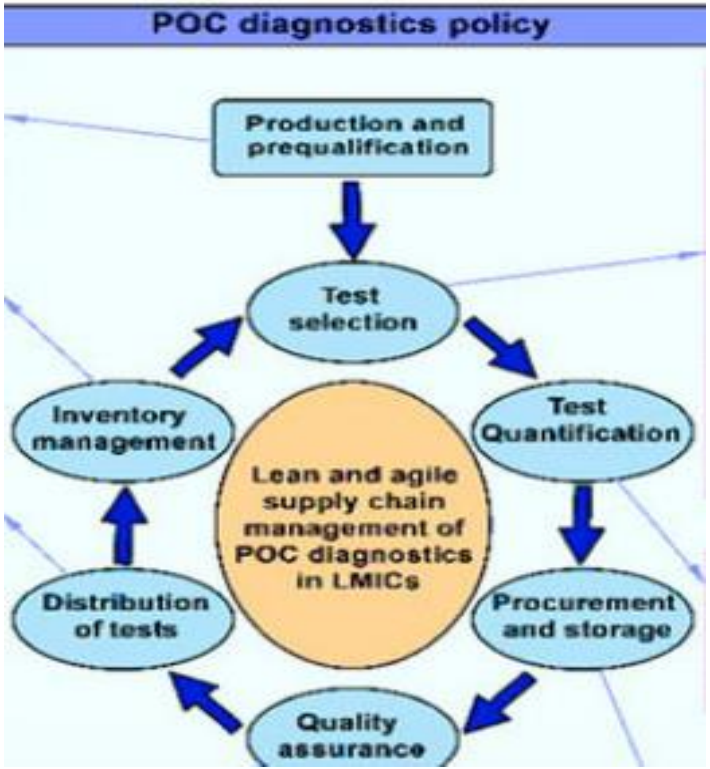
1. Parasite Biology (**Diagnostic resistant parasite (HRP2/3) gene deletion,** and drug resistance parasite)
2. Host Biology (G6PD deficiency , Duffy receptor etc)

Cont…

- RDTs offers community level diagnosis
- Allowed test and treat strategy
- As of 2020, more than 70% of malaria test in Ethiopia done by RDTs

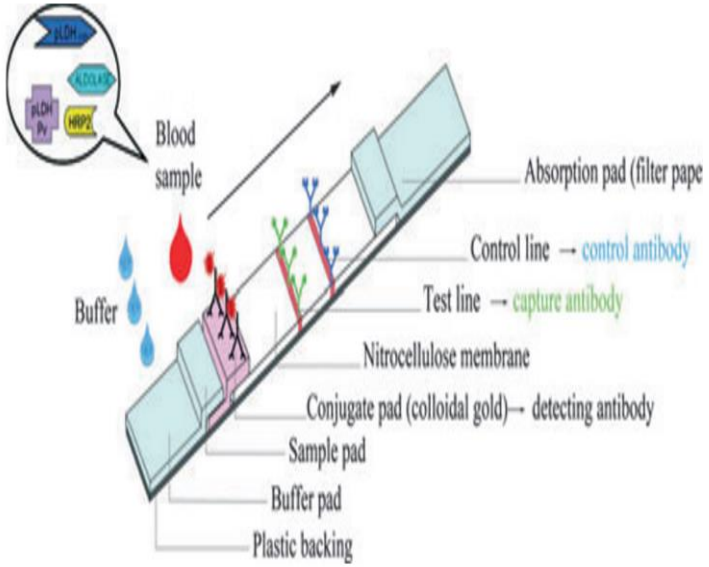
❖ **Challenges with RDTs**

- ➢ Manufacturing (Lot & product quality)
- ➢ Storage and transportation
- ➢ Operator error
- ➢ **Parasite genetic change (Diagnostic target gene deletion)**



Conti…

- Three reliable antigens are known targets for RDTs:
  - HRP2
  - PLDH
  - Aldolase
- HRP2 antigen is most preferred RDT target antigen due to its:
  - High abundance in the blood stream
  - Repetitive binding epitopes increased sensitivity
  - P. falciparum-specificity
  - Thus, most malarious countries including **Ethiopia** uses pf*HRP2* detecting RDTs



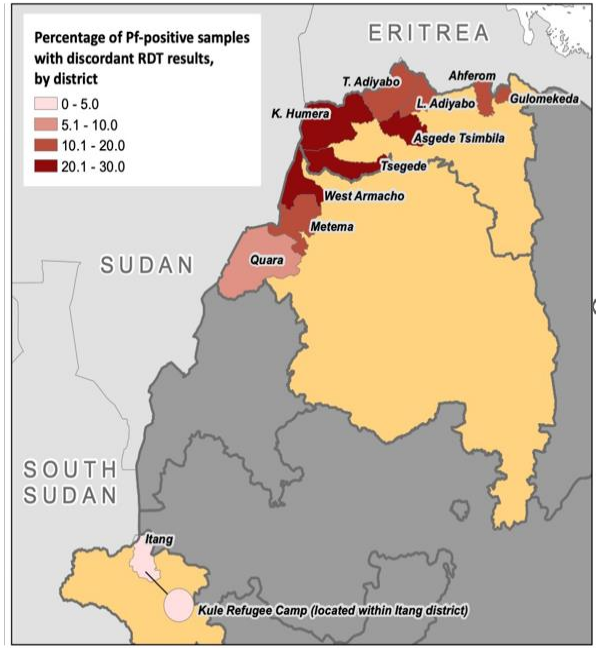
pfHRP2 and HRP3 gene deletion parasite in Ethiopia

- Many countries in Africa, Asia and Latin America have reported *pfHRP2/3* gene deletion, where the first was from Peru.
- **In 2016,** High prevalence of *HRP2/3* gene deletion reported in Eritrea
- By 2017/18, therefore, EPHI conceived the problem, proposed to study the *pfHRP2/3* gene deletion status in districts neighboring to Eritrea, Sudan and South Sudan
- Initial study, to assess the emergence of *pfHRP2/3* gene deletion in Ethiopia

Study Methods

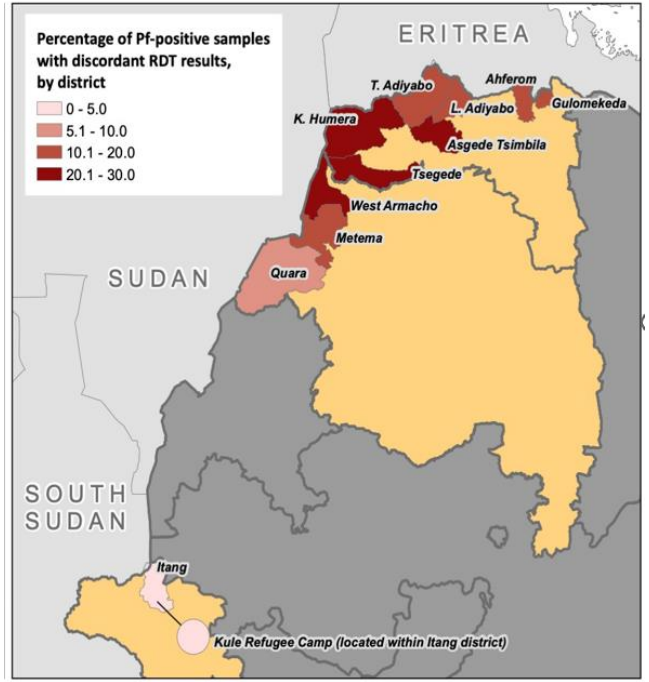
- **Study site:** 108 health facilities in **11 districts**: Tigray (6), Amhara (4) and Gambella (1)
- **Study period: 2017/18**
- **Malaria suspected self presented patients screened**
    1. Carestart *PfHRP2/PvPLDH* and
    2. SD-Bioline Pf/Pf (HRP2/PLDH)RDTs
- **Discordant and 20% of concordant Pf samples tested with:**
    1. PCR assays and Luminex-based serological assay,
    2. whole-genome sequencing,
    3. Molecular inversion probe (MIP) deep sequencing

Study sites in Tigray, Amhara and Gambella Regions



Result…

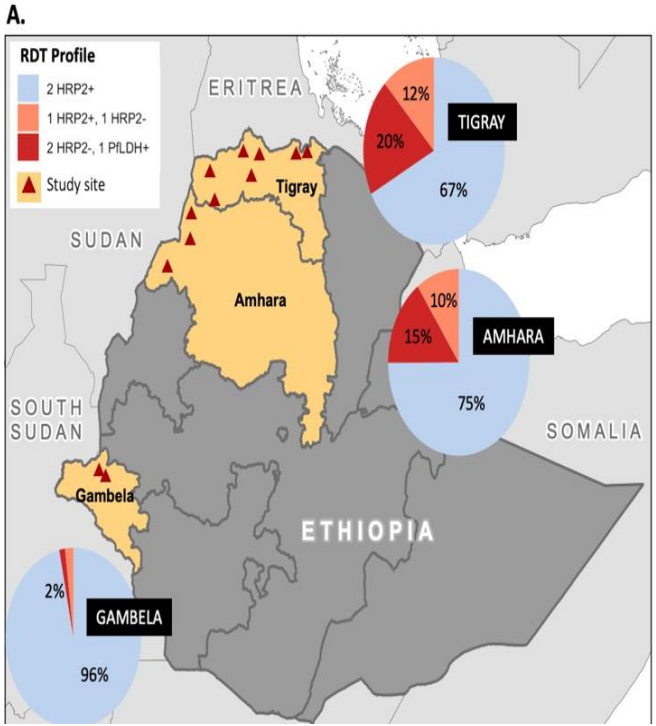
- A subset of 824 samples from Amhara (n = 529), Tigray (n = 224), and Gambella (n = 71), were sent to UNC for molecular analysis (for species and pfhrp2/3 qpcr assay)
- 613 samples were confirmed for *P. falciparum* infection by qPCR and were eligible for qpcr pfhrp2/3 assay



Result…

qPCR result revealed

- 135 samples (22%) were pfhrp2-/3-,
- 193 (32%) pfhrp3- only and ,
- 28 (4.6%) pfhrp2- only

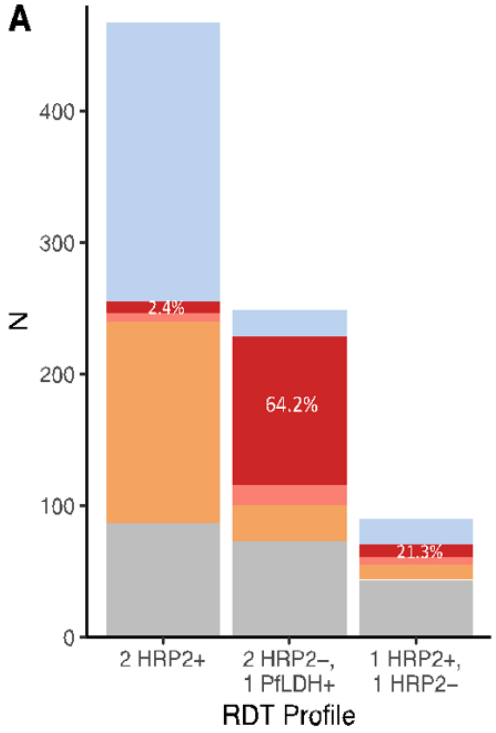


Cont…

Among RDT discordant profile

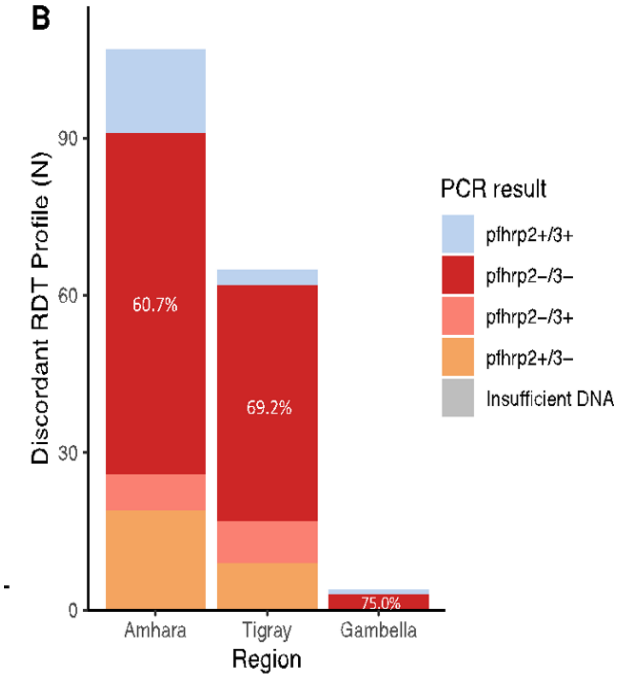
- **64.2% were PCR *pfhrp2-/3-* and,**
- **8.5% (5-14) *pfhrp2-/3+ and***
- **15.9% (11-22) *pfhrp2+/3-***

- A high proportion of samples HRP2-positive by RDT were negative for *pfhrp3* by PCR (42%; 95% CI 37- 48)



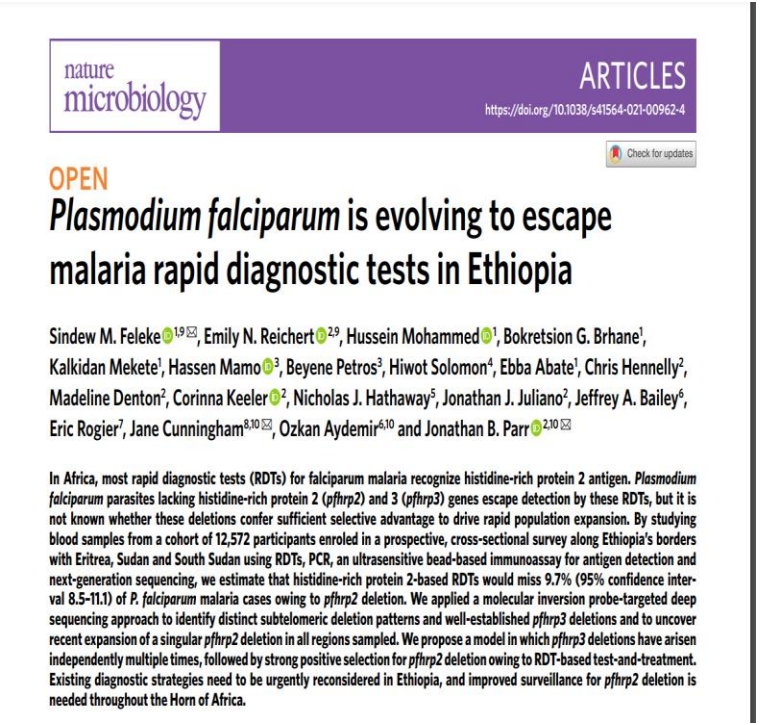
Results…

- The population level estimate of *P. falciparum* infections with *pfhrp2* deletions was 9.7% among all study sites
- The regional pf*hrp2* deletion prevalence estimates:
  - Tigray-15%; 95% CI: 11-19)
  - Amhara- 11%; 95% CI: 9-14 and,
  - Gambella -1.2%; 95% CI: 0.6-2.4



Study Conclusion and recommendation

- Large scale surveillance in Ethiopian districts bordering with Eritrea, South Sudan confirmed the presence of *pfhrp2/3*-deleted parasites in all surveyed regions
- Therefore, policy consideration was recommended



nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-021-00962-4

Check for updates

OPEN

Plasmodium falciparum is evolving to escape malaria rapid diagnostic tests in Ethiopia

Sindew M. Feleke[1,9], Emily N. Reichert[2,9], Hussein Mohammed[1], Bokretsion G. Brhane[1], Kalkidan Mekete[1], Hassen Mamo[3], Beyene Petros[3], Hiwot Solomon[4], Ebba Abate[1], Chris Hennelly[2], Madeline Denton[2], Corinna Keeler[2], Nicholas J. Hathaway[5], Jonathan J. Juliano[2], Jeffrey A. Bailey[6], Eric Rogier[7], Jane Cunningham[8,10], Ozkan Aydemir[6,10] and Jonathan B. Parr[2,10]

In Africa, most rapid diagnostic tests (RDTs) for falciparum malaria recognize histidine-rich protein 2 antigen. *Plasmodium falciparum* parasites lacking histidine-rich protein 2 (*pfhrp2*) and 3 (*pfhrp3*) genes escape detection by these RDTs, but it is not known whether these deletions confer sufficient selective advantage to drive rapid population expansion. By studying blood samples from a cohort of 12,572 participants enroled in a prospective, cross-sectional survey along Ethiopia's borders with Eritrea, Sudan and South Sudan using RDTs, PCR, an ultrasensitive bead-based immunoassay for antigen detection and next-generation sequencing, we estimate that histidine-rich protein 2-based RDTs would miss 9.7% (95% confidence interval 8.5–11.1) of *P. falciparum* malaria cases owing to *pfhrp2* deletion. We applied a molecular inversion probe-targeted deep sequencing approach to identify distinct subtelomeric deletion patterns and well-established *pfhrp3* deletions and to uncover recent expansion of a singular *pfhrp2* deletion in all regions sampled. We propose a model in which *pfhrp3* deletions have arisen independently multiple times, followed by strong positive selection for *pfhrp2* deletion owing to RDT-based test-and-treatment. Existing diagnostic strategies need to be urgently reconsidered in Ethiopia, and improved surveillance for *pfhrp2* deletion is needed throughout the Horn of Africa.

Decision made by FMoH

- The FMoH have reviewed the results and acknowledged the study findings
  - Result was not conclusive for the national level decision making as study site were not representative
- Therefore, decision was made to conduct nationwide *pfhrp2/3* gene deletion survey

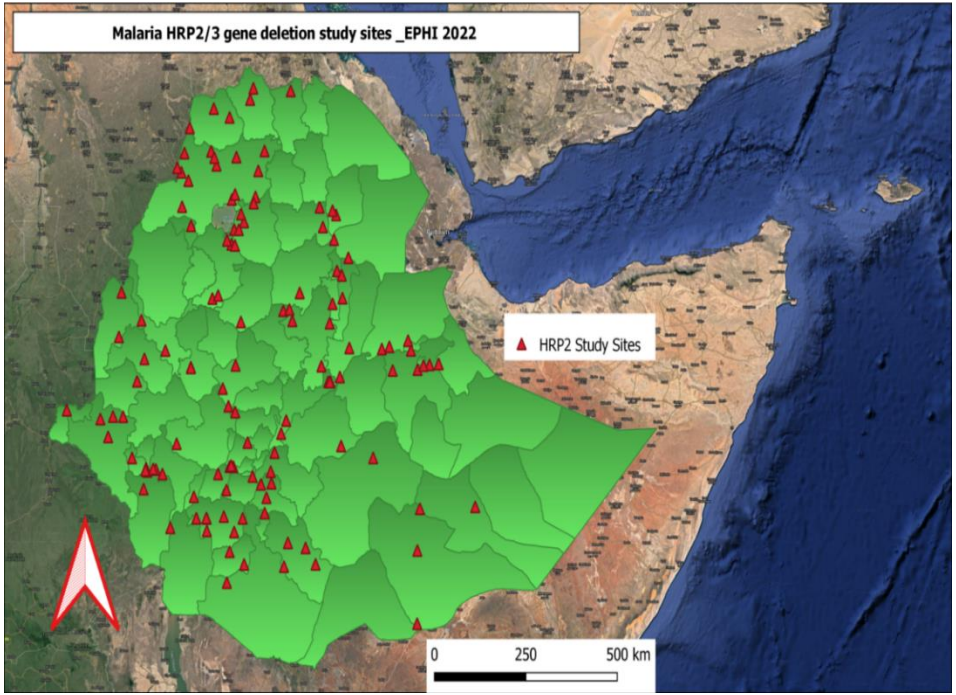
By 2021, National *PfHRP2 and HRP3* gene deletion survey in Ethiopia: 2nd Phase survey

Objective:

The overall of the objective of this study was to determine the national level prevalence of the *P. falciparum hrp2* and *hrp3* genes deletion in Ethiopia

phase two: HRP2/3 gene deletion survey site

Regions	Number of selected facilities per regions	Current Status
Somali	**7**	Done
Afar	**7**	Done
Tigray	**13**	Done
Gambella	**4**	Done
Oromia	**24**	Done
SNNPR	**21**	Done
Amhara	**31**	Done
B/Gumuz	**4**	Done
Sidama	**3**	Done
Total	**114**	Done



Screening methods

1st phase

- Carestart *Pf/Pv (HRP2/PLDH)*
- SD-Bioline *Pf (HRP2/PLDH)* RDTs

Discordant

- Carestart *Pf (HRP2)= Negative*
- SD-Bioline *pf/pf (HRP2/PLDH)* = negative/positive

2nd phase

- Carestart *Pf/Pv(HRP2/PLDH)*
- Microscope

Discordant

- *Pfhrp2*= negative
- Microscope =pf positive

Results

RDT/Microscopy discordant (n=144)

The *hrp*2/3 PCR assay,

- 68.7% (99/144) *pfhrp*2-/3-
- 3.5% (5/144) *pfhrp*2-/3+
- 14.5% (21/144) *pfhrp*2+/3- and
- 13.2 (19/144) hrp2+/3+

RDT/Microscopy concordant samples (n=260)

The *hrp*2/3 PCR assay,

- 2% (5/266) *pfhrp*2-/3-
- 0.77% (2/260) *pfhrp2-/3+*
- 51% (132/260) *pfhrp2+/3-*

Results….

In 2nd phase study overall, the *Pfhrp2* deletion in the total study population was 7.1% which is above the 5% WHO cutoff value

Conclusion and recommendations

A. By 2017/18: phase one

- pfhrp2/3 gene deletion was 9.7% bordering Eritrea, South Sudan, and Sudan

A. By 2020/21: phase two

- pfhrp2/3 gene deletion was 7.1% prevalence
  - Distribution was highly heterogeneous among Regions
  - Policy change was recommended for the NMEP

Recommendations and Policy implementation

- **By 2022,** a policy brief was prepared, and the guideline was revised!
- pfhrp2/ RDTs changed to Non-pfhrp2/3 RDTs
- Currently, procurement and distribution of pfpldh/pvpLDH kits is on progress

National malaria Elimination Program, MOH
September, 2022

Ministry of Health

MINISTRY OF HEALTH ETHIOPIA

Dereje Duguma (MD, MIH)
State Minister of Health

Treatment and diagnostic resistance is positively correlated?

nature microbiology

Analysis https://doi.org/10.1038/s41564-023-01461-4

Plasmodium falciparum resistant to artemisinin and diagnostics have emerged in Ethiopia

Received: 6 March 2023

Accepted: 26 July 2023

Published online: 28 August 2023

Check for updates

Abebe A. Fola[1,2,8], Sindew M. Feleke[3,8], Hussein Mohammed[3], Bokretsion G. Brhane[3], Christopher M. Hennelly[4], Ashenafi Assefa[3,4], Rebecca M. Crudal[1,2], Emily Reichert[5], Jonathan J. Juliano[4], Jane Cunningham[6], Hassen Mamo[7], Hiwot Solomon[3], Geremew Tasew[3], Beyene Petros[7], Jonathan B. Parr[4,10] & Jeffrey A. Bailey[1,2,10]

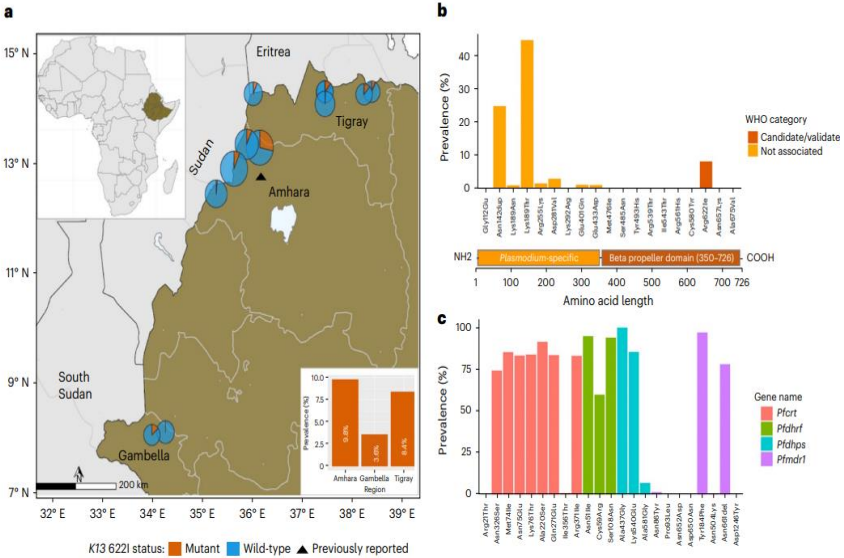


Fig. 1 | Prevalence of *K13* and key drug-resistance mutations in Ethiopia. **a**, Spatial distribution of *K13* 622I mutation at the district (pie charts) and regional (bar plot) levels. Colours indicate mutation status and pie chart size is proportional to sample size per district. The black triangle indicates the location where *K13* 622I mutation was reported previously. **b**, Prevalence of non-synonymous mutations across the *K13* gene, coloured according to WHO ACT resistance marker category. *K13* gene annotation shows 1–350 amino-acid residues in the poorly conserved *Plasmodium*-specific region and 350–726 residues in the beta propeller domain where validated resistance mutations are located. **c**, Prevalence of mutations across four key *P. falciparum* genes (colours) associated with commonly used antimalarial drugs.

Current status and way forward

Other continuous activities

- EPHI established multiplex qPCR pfhrp2/3 gene deletion assay
- **Surveillance:** Monitoring and follow up of pfhrp2/3 gene deletion at selected sites: On progress
- Evaluations of new **non-pfhrp2/3 RDTs** kits is on progress

Acknowledgment

- WHO, Geneva
- UNC at Chapel Hill, USA
- CDC, USA
- Global fund, MoH, Ethiopia
- Study participants
- EPHI colleagues

Thank you!

DR. AGABA BOSCO

Threat of HRP2 Deletion, Surveys and Implementation Experience from Uganda

Threat of pfhrp2/3 gene Deletion, Surveys and Implementation Experience from Uganda

DR. AGABA BOSCO (PhD)

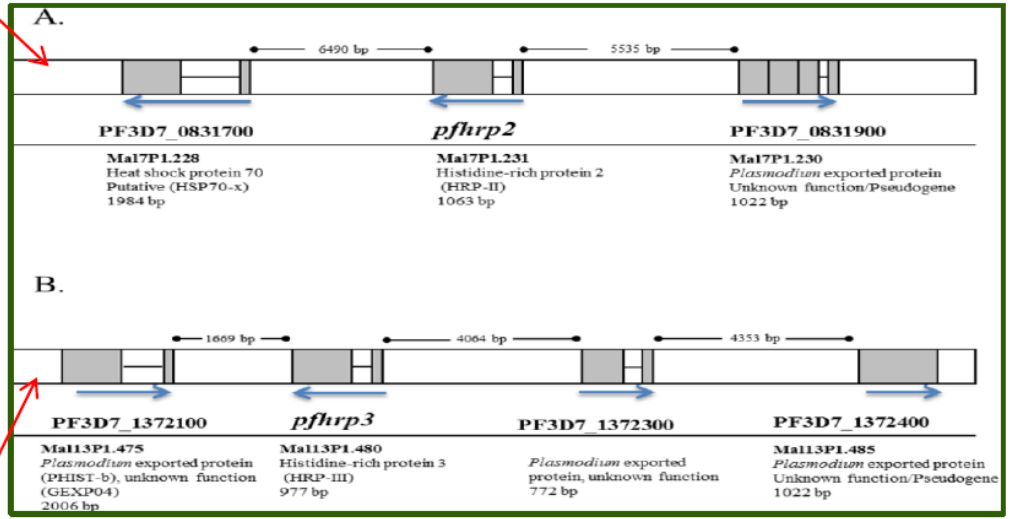
LSHTM/MRC, MUST, MALARIA CONTROL DIVISION, UGANDA

Outline

- Historical perspective and Description of terms
- Background
- Problem
- Surveys in Uganda and Results
- Field Implementation experience- Lessons & potential bottlenecks
- Key consideration for future
- Acknowledgement

Definition of terms & Historical Perspectives

1. ***pfhrp2*** **:** *Plasmodium falciparum* histidine rich protein 2 gene



Cheng et al

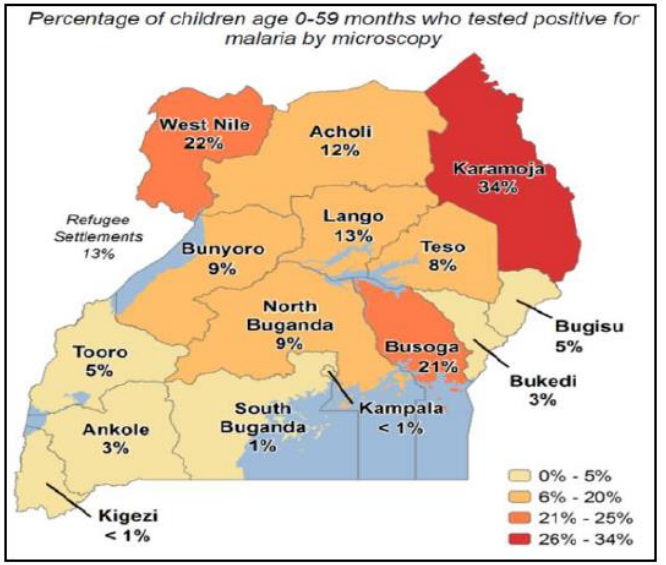
3. ***pfhrp3*** **:** *Plasmodium falciparum* Histidine rich protein 3 gene

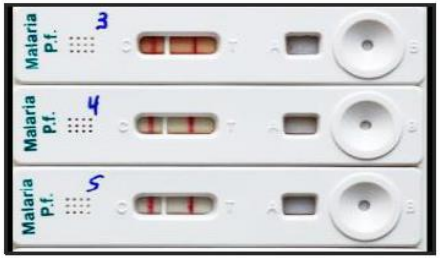
4. **HRP2**: Histidine Rich Protein 2 (Major target for RDTs)

- Deletion due to breakage at the unstable sub-telomeric regions of chromosome 8 & 13
- Globally, 1st Reported in field isolates in Amazon basin (2010)
- Now reported in Africa, India & elsewhere

BACKGROUND

- Malaria remains public health problem (MOH,2023)
- Accurate diagnosis- is a key intervention
- MOH policy recommends parasite-based diagnosis
- RDTs are the main diagnostic tools for malaria
- RDTs are threatened by *phrp*2 gene deletions
- Alternative RDTs exist (cost, sensitivity, stability)
- WHO recommends surveillance of gene deletions
- No routine surveillance system for deletions in most





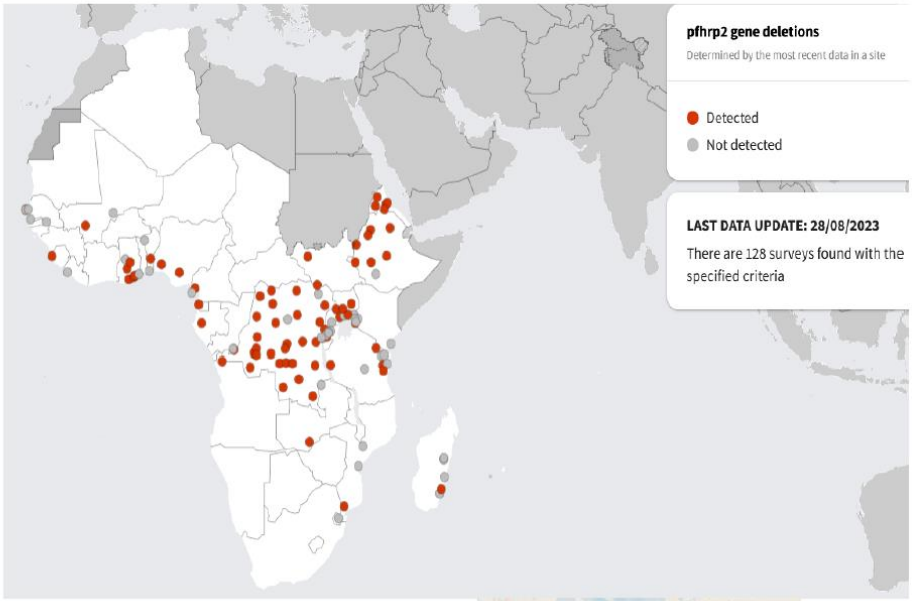
What's the Problem?

- The emergence of *pfhrp2/3* gene deletion threatens the utility of HRP2 RDTs.
- Yet HRP2 RDTs are the predominant malaria test- >85% of testing (DHIS2)
- Parasites with deletion do not express HRP2 Target Protein antigen
- In the absence of HRP2 expression, parasites are missed by HRP2 RDTs
- Missed diagnosis may lead to increased malaria transmission/morbidity/mortality
- Alternative RDTs exist (expensive, less sensitive, poor stability, are less abundant)

Global Threat Maps (WHO, 2023)

Global: spread and surveys conducted

Africa: Spread and surveys conducted



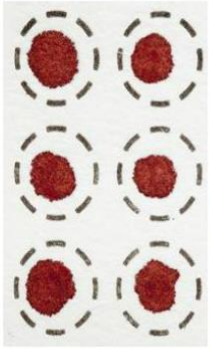
WHO Ref: https://apps.who.int/malaria/maps/threats/

Pfhrp2/3 Surveys in Uganda- (SURVEY 1)

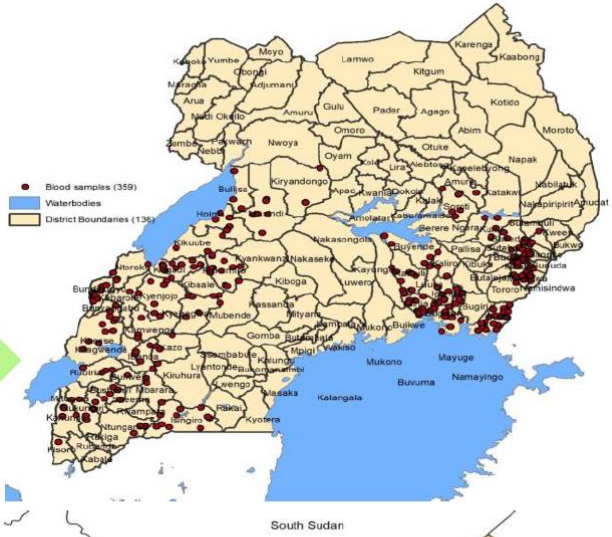
Design: Cross-sectional across 48 districts

Population: Symptomatic, 2-10 yrs

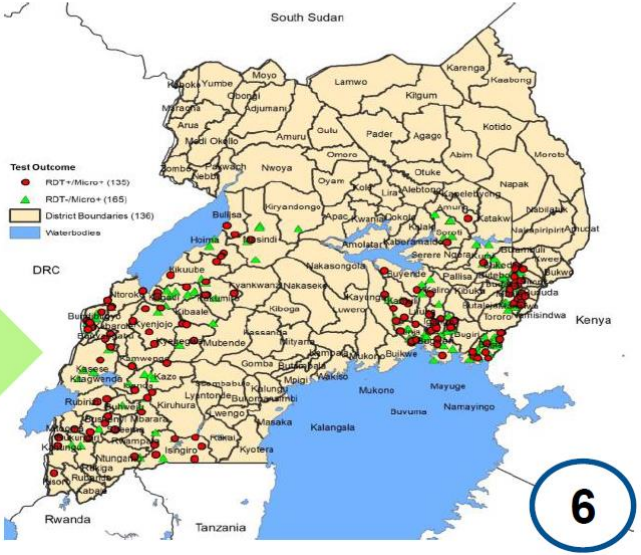
Tested: RDTs and Blood Smear, DBS



Survey Sites



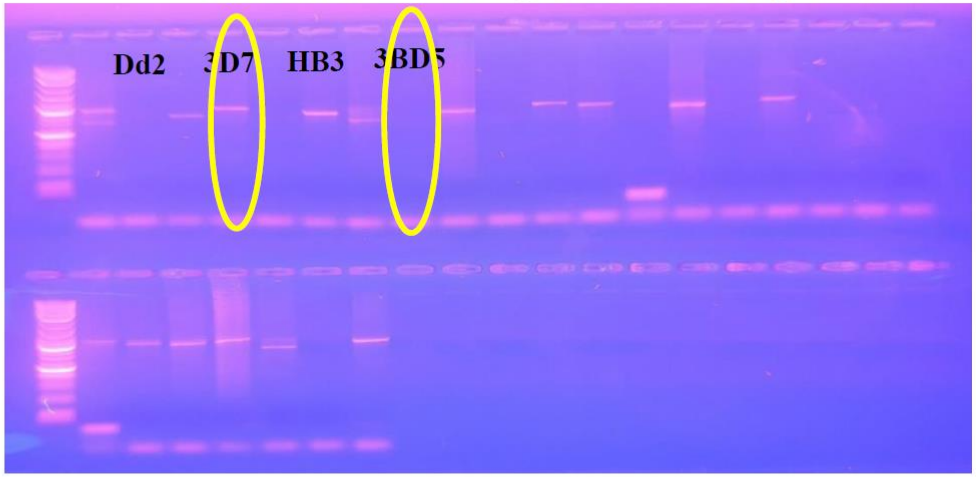
Discordant & Concordant Samples



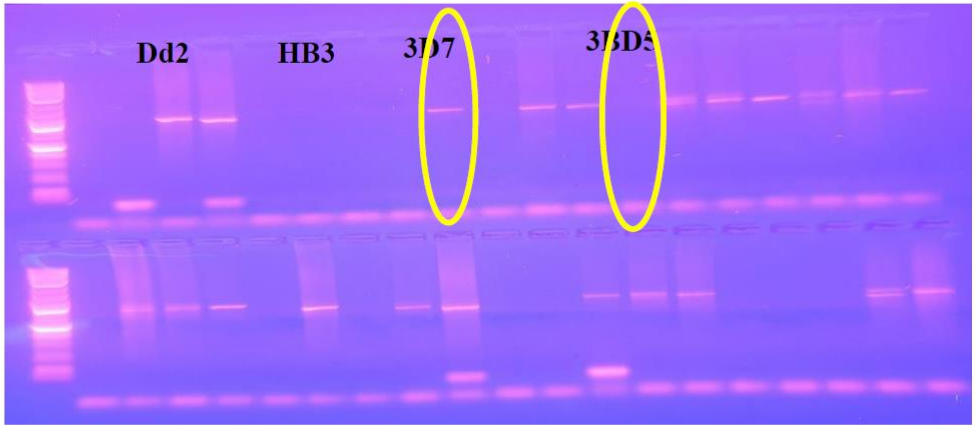
Lab assays for survey 1

Parasite strain	*Pfhrp2* status	*Pfhrp3* status
P. f 3D7	(+)	(+)
P. f Dd2	(-)	(+)
P. f HB3	(+)	(-)
P. f 3BD5	(-)	(-)
Human negative	(-)	(-)

HRP2 exon 2 Plate1 gel 1 30052019-Agaba Oct/19



HRP3 exon 2 Plate1 gel 1 30052019-Agaba Oct/19



Ladder: NEB 1 kB Plus: PfHrp2 Exon 2 is 600 to 1000 bp; PfHrp3 exon 2 is 250 to 600 bp.

1 .DNA Extraction

2. PCR set up

3. DNA Amplification

6. Data entry & Analysis

5. DNA electrophoresis

4. Amplification

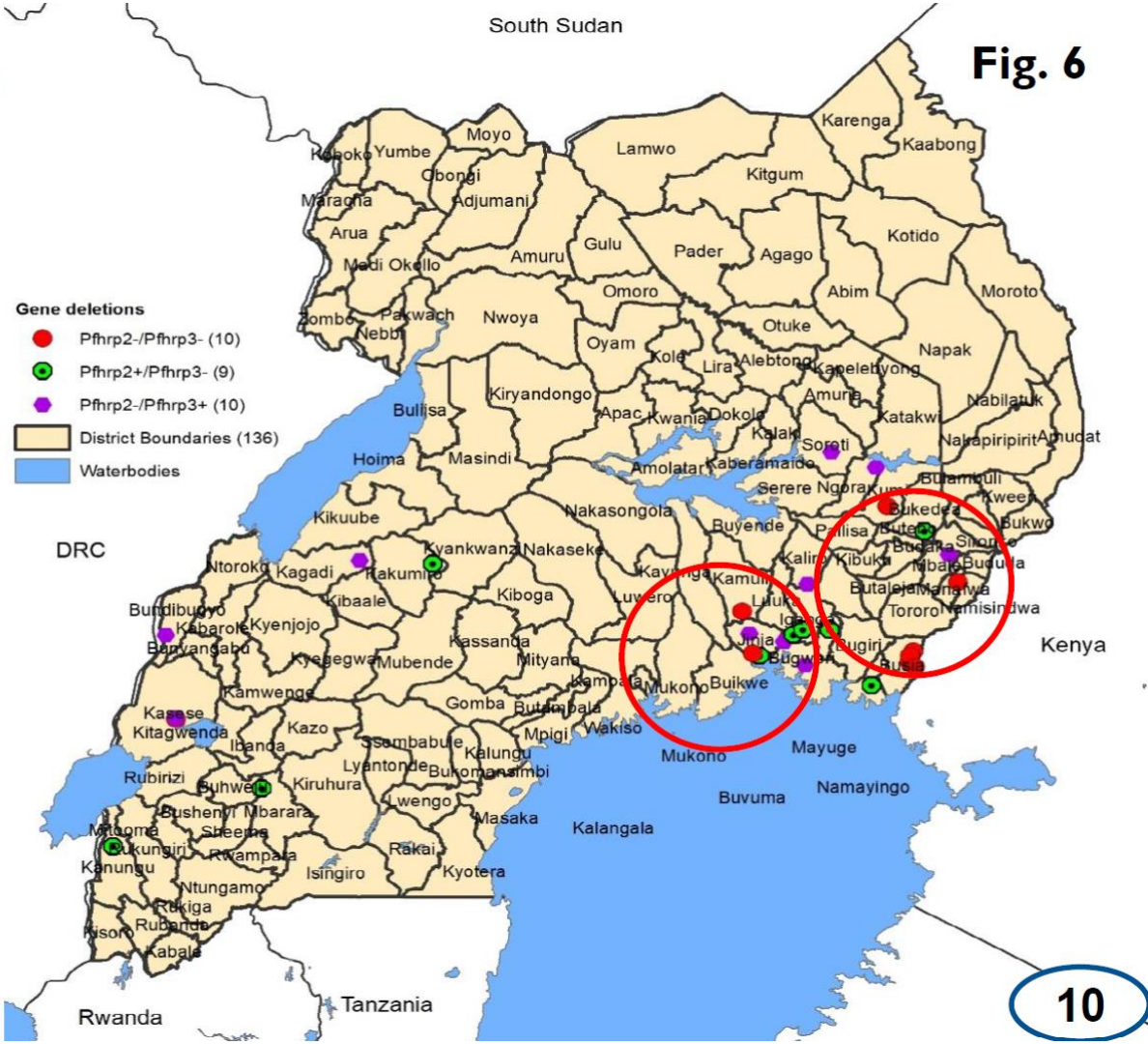
Proportion of Parasites with deletions in Survey 1

Gene deletion`	Overall		Blood Smear & PCR positive (*n*=300)				PR 95% CI;	p-value
	Total (N=300)		RDT Negative N=165		RDT Positive N=135			
	n	**% (95%)**	**n**	**% (95%)**	**n**	**% (95%)**		
Any deletion	29	9.7 (6.6-13.6)	24	14.5 (9.5, 20.9)	5	3.7 (1.2, 8.4)	3.91 (1.5,10.0);	0.002
pfhrp2-/pfhrp3+	10	3.3 (1.6, 6.0)	9	5.5 (2.5, 10.1)	1	0.7 (0.0, 4.1)	7.85 (1.0, 57.4);	0.021
pfhrp2+/pfhrp3-	9	3.0 (1.4, 5.6)	5	3.0 (1.0, 6.9)	4	3.0 (0.8, 7.4)	1.00 (0.3, 3.7);	1.000
pfhrp2-/pfhrp3-	10	3.3 (1.6, 6.0)	10	6.1 (2.9, 10.9)	0	0 (0, 2.7)	N/A	0.004
pfhrp2+/pfhrp3+	186	61.7 (55.9, 67.2)	61`	37.0 (29.6, 34.5)	124	91.9 (85.9, 95.9)	0.40 (0.3, 0.5);	0.001

[1]Significant Proportion (P=0.001) different from WHO recommend cut-off (5%)

Geographical Spread of deletions

- Double deletions (pfhrp2&3) clustered in mid and eastern Region
- Pfhrp2/3- single deletions occurred in both regions
- Overall, deletions were more frequent in Eastern

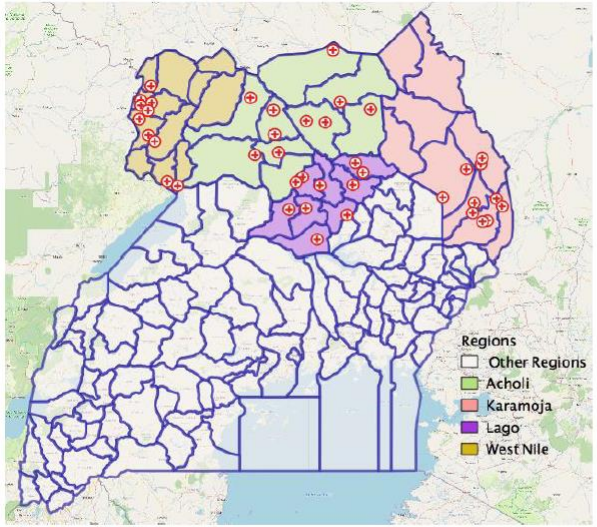


Survey 2: Pfhrp2/3 Surveys in Uganda (WHO Protocol)

Surveillance Objectives

- Estimate prevalence of suspected false-negative HRP2 RDT results among **symptomatic patients** with *P. f*
- Determine prevalence of *pfhrp2/3* deletions in symptomatic falciparum patients with a false negative RDT
- Estimate prevalence of non-*falciparum* species that can lead to false negative results with HRP2-RDTs

Project sites

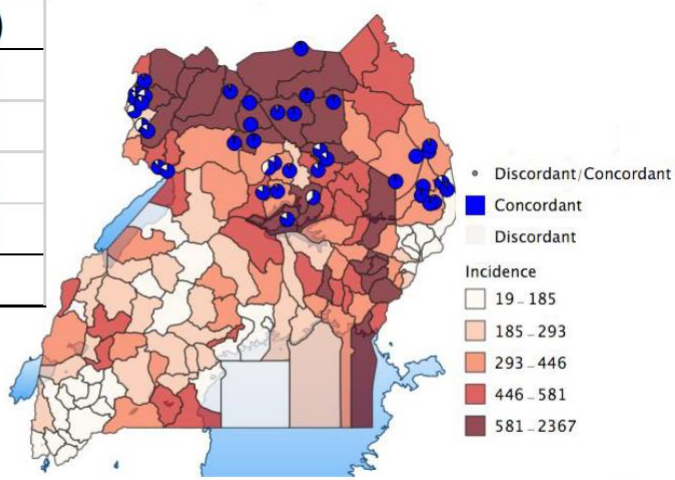


Samples per region (n)

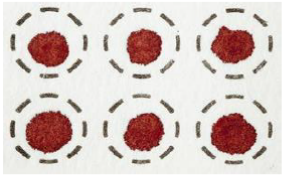
Region	DBS (N)	Positive (pLDH)
Acholi	549	378 (68.0%)
Lango	583	372 (63.8%)
W. Nile	676	386 (57.1%)
Karamoja	627	370 (59.0%)
Total	**2,435**	**61.60%**

Northern Region is ahigh transmission setting

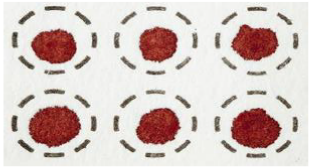
Discordant: HRP2-/pLDH+



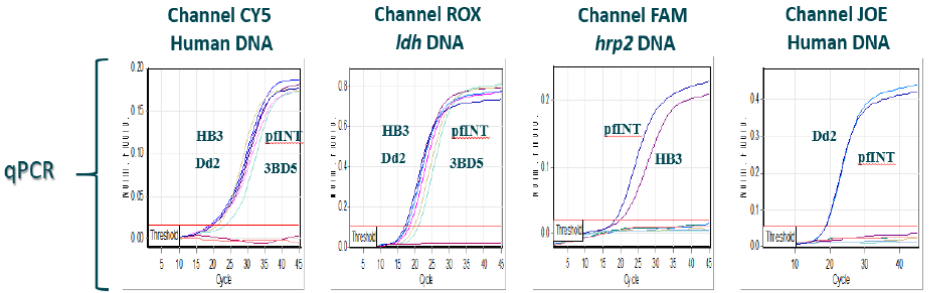
Lab Assays- Survey 2: Real-time Multiplex qPCR



DNA extracted from DBS



Amplifies a fragment each of *pfhrp2*, *pfhrp3*, *pfldh* and human tubumin (*htb*) genes simultaneously



No deletion	Positive	Positive	Positive	Positive
Deletion	Positive	Positive	Negative	Negative
No parasite	Positive	Negative	Negative	Negative
No *hum* DNA (Invalid)	Negative	-	-	-
controls	all	all	PfINT and HB3	PfINT and Dd2

Ref: Khalid

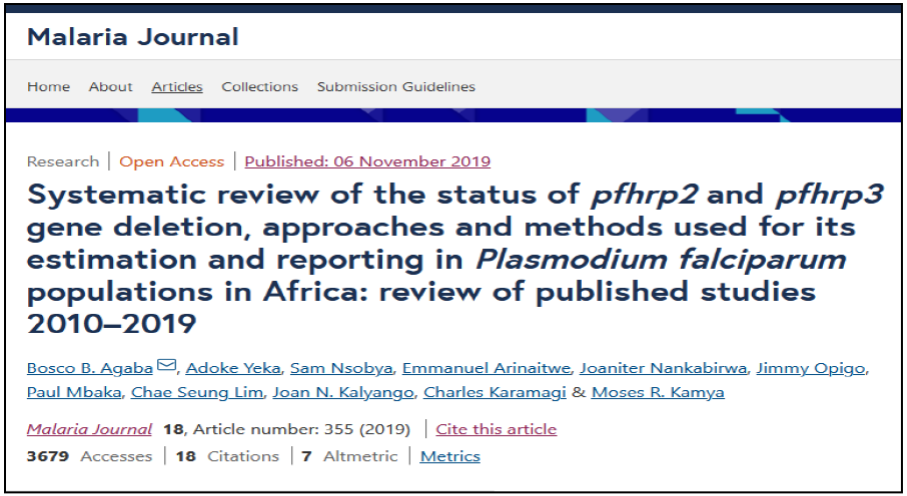
Summary of Survey 2 Results

Location	Valid PCR	Samples without deletion		Samples with confirmed pfhrp2 gene deletion	
		n	%	n	%
Acholi	93	93	100.0%	0	0.0%
West Nile	113	113	100.0%	0	0.0%
Karamoja	93	93	100.0%	0	0.0%
Lango	117	116	99.1%	1	0.86%
Total	416	415	99.8%	1	0.24%

***This sample also showed absence of HRP2 on ELISA

To confirm absence of pfhrp2 gene;

- **Show presence of presence of parasite-*pfldh***
- **Prove non-expression of HRP2 protein**
- **Amplification of *msp1* and *2***
- **Controls must pass- 3D7, 3BD5, Dd2, HB3**



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Systematic review of the status of *pfhrp2* and *pfhrp3* gene deletion, approaches and methods used for its estimation and reporting in *Plasmodium falciparum* populations in Africa: review of published studies 2010–2019

Bosco B. Agaba, Adoke Yeka, Sam Nsobya, Emmanuel Arinaitwe, Joaniter Nankabirwa, Jimmy Opigo, Paul Mbaka, Chae Seung Lim, Joan N. Kalyango, Charles Karamagi & Moses R. Kamya

Malaria Journal **18**, Article number: 355 (2019) | Cite this article

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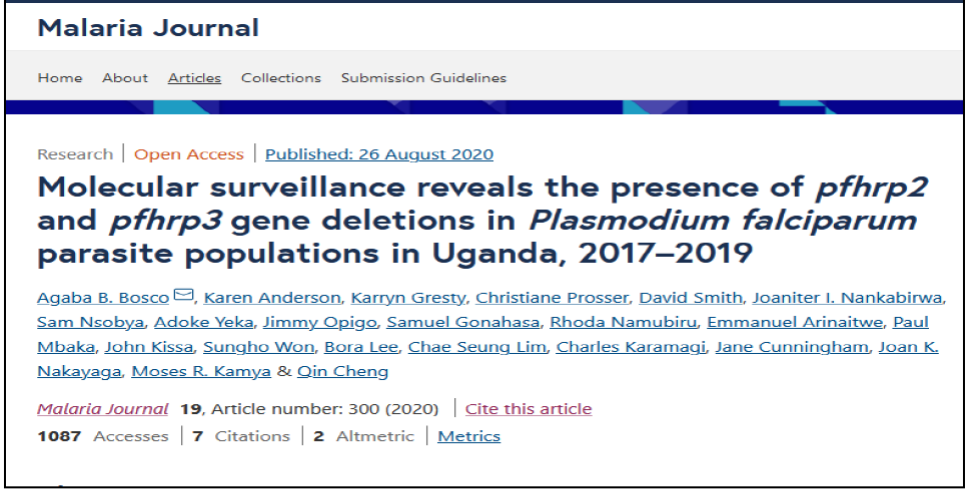

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Molecular surveillance reveals the presence of *pfhrp2* and *pfhrp3* gene deletions in *Plasmodium falciparum* parasite populations in Uganda, 2017–2019

Agaba B. Bosco, Karen Anderson, Karryn Gresty, Christiane Prosser, David Smith, Joaniter I. Nankabirwa, Sam Nsobya, Adoke Yeka, Jimmy Opigo, Samuel Gonahasa, Rhoda Namubiru, Emmanuel Arinaitwe, Paul Mbaka, John Kissa, Sungho Won, Bora Lee, Chae Seung Lim, Charles Karamagi, Jane Cunningham, Joan K. Nakayaga, Moses R. Kamya & Qin Cheng

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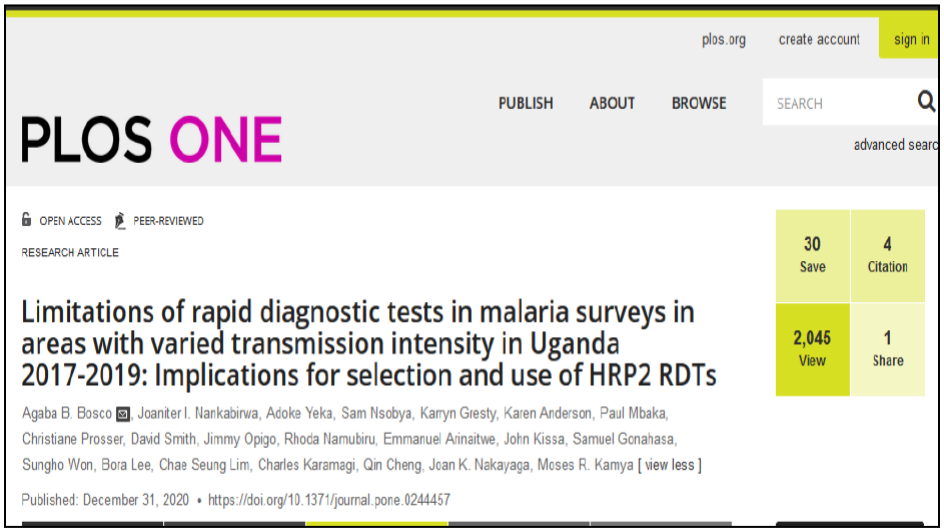

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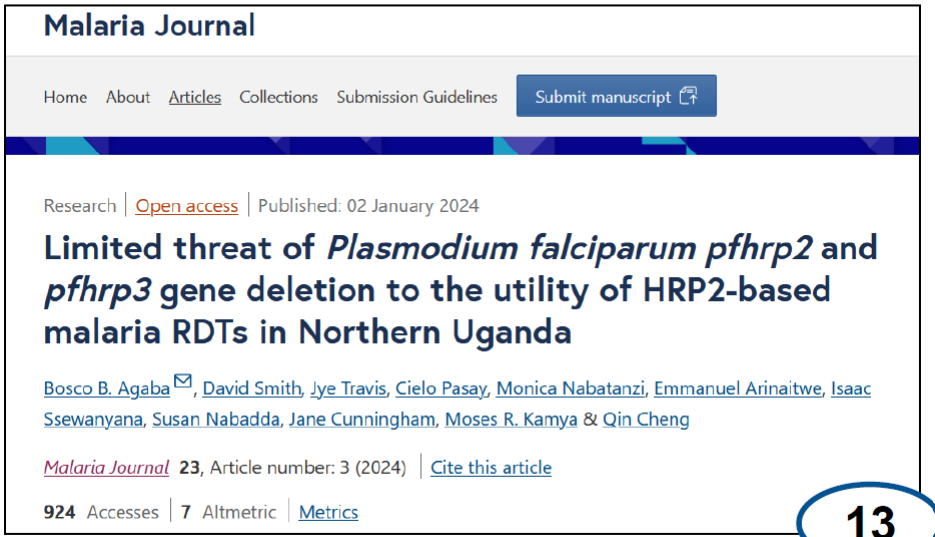
Limitations of rapid diagnostic tests in malaria surveys in areas with varied transmission intensity in Uganda 2017-2019: Implications for selection and use of HRP2 RDTs

Agaba B. Bosco, Joaniter I. Nankabirwa, Adoke Yeka, Sam Nsobya, Karryn Gresty, Karen Anderson, Paul Mbaka, Christiane Prosser, David Smith, Jimmy Opigo, Rhoda Namubiru, Emmanuel Arinaitwe, John Kissa, Samuel Gonahasa, Sungho Won, Bora Lee, Chae Seung Lim, Charles Karamagi, Qin Cheng, Joan K. Nakayaga, Moses R. Kamya [view less]

Published: December 31, 2020 • https://doi.org/10.1371/journal.pone.0244457



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Limited threat of *Plasmodium falciparum pfhrp2* and *pfhrp3* gene deletion to the utility of HRP2-based malaria RDTs in Northern Uganda

Bosco B. Agaba, David Smith, Jye Travis, Cielo Pasay, Monica Nabatanzi, Emmanuel Arinaitwe, Isaac Ssewanyana, Susan Nabadda, Jane Cunningham, Moses R. Kamya & Qin Cheng

Malaria Journal **23**, Article number: 3 (2024) | Cite this article

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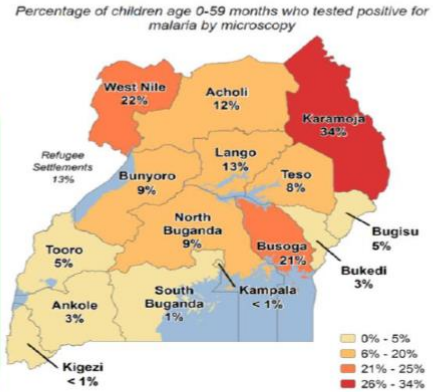
Sequence of steps followed to implement

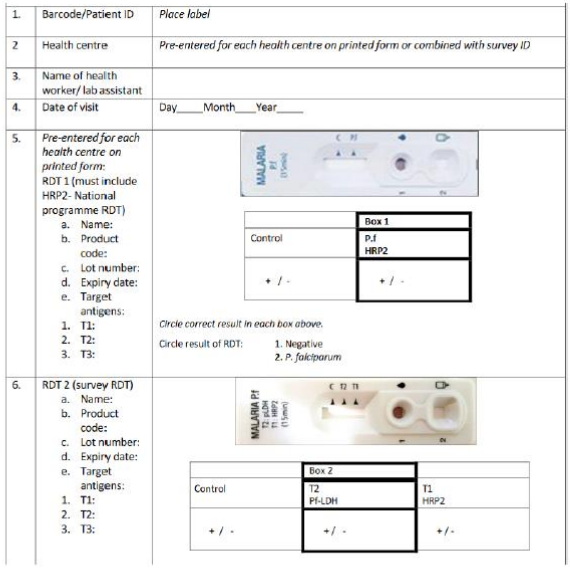
- Stakeholder engagement
- Identify survey areas/coverage
- Quantify the need (resources, supplies,)
- Resources mobilization
- Protocol (IRB, investigators, data tools (questionnaires, consent, translations)
- Constitute survey teams ***
- Lab testing – if available or shipment (MTA)
- Data analysis, Reporting & Dissemination

Important Field Considerations with the pfhrp2/3 Surveys

1. Positioning of sites- concerns of false negative, ensuring representativeness



2. Interchange of RDTs if using two RDTs



3. DBS- Use correct filter paper, Train field techs on spots

4. Microscopy competency if using Blood smears

5. Work within existing structures

6. Survey supervision

Potential hrp2 surveys implementation Bottlenecks

- Capacity to run surveys and molecular testing
- Surveys largely remain in "project" mode
- Communicating hrp2 deletion results where prev <5%
- Introduction of alternative tests alongside HRP2 RDTs
- MTA processes for those intending to ship

Deployment of alternative tests alongside HRP2 in requires efficient distribution system

Potential issues to consider for the future

- RDTs alternative target antigens
- Limited no. of approved non-HRP2 RDTs
- Harmonize detection methods across countries
- Integration into routine surveillance
- Leverage on existing capacity within facilities
- Adhere to WHO protocol
- Resources- grants and domestic resource

Surveillance template protocol for *pfhrp2/pfhrp3* gene deletions

World Health Organization

Area assessed	WHO recommendation	% of articles that complied
Design	Cross-sectional	100
Participants	Symptomatic	35.7
Sampling	Spread and distributed	42.8
Lab methods	Assessed quality DNA	21.4
Reporting	Both *pfhrp2* and *pfhrp3*	71.4

WHO international lab network to support *pfhrp2/3* surveillance

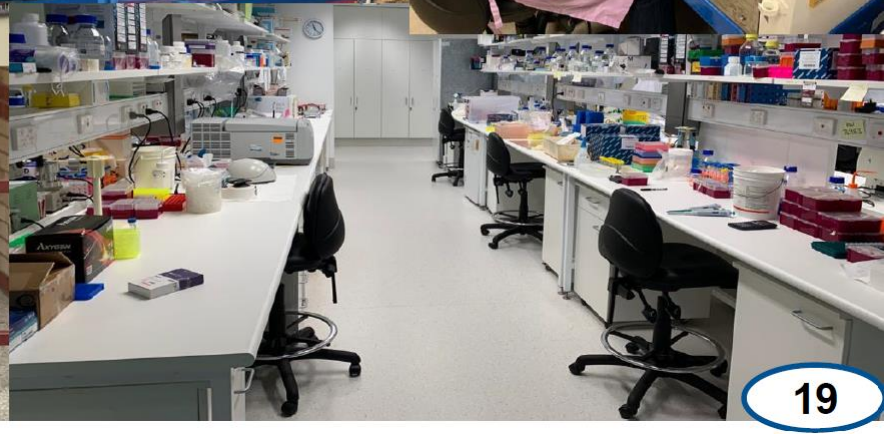
Plans to expand the network? If not what's the implication?

Institute	Country	Lead	PCR/qPCR for Speciation	Molecular analysis to confirm gene deletions				Serological analysis to confirm lack of HRP2 expression		Other molecular tests	
				Conventional PCR	Multiplex qPCR	Digital PCR	WGS/ Genomics	ELISA	Bead-based assay	MOI/ Origin	K13 mutations
LSHTM	UK	Dr. Khalid Beshir	Y	Y	Y	N	Y	N	N	Y (not routinely)	Y
UNC	USA	Dr. Jonathan Parr	Y	Y	Y	N	Y	N	Y (not routinely)	Y	Y
ADFMIDI	Australia	Dr Qin Cheng	Y	Y (moved away)	Y	Y (not routinely)	N	Y	N	Y	Y
CDC	USA	Dr Eric Rogier/?	Y	Y	N	N	N	N	Y	Y	Y
UCAD	Senegal	Prof Daouda Ndiaye	Y	Y	Y	N	Y	Y	Y	Y	Y
UPCH	Peru	Dr Dionicia Gamboa	Y	Y	N	N	Y	Y	Y	Y	Y
NIMR	India	Dr Praveen Bharti	Y	Y	Y	N	Y	Y	N	Y	Y
AHRI	Ethiopia	Dr Fitsum Girma									
UND	USA	Dr Christian Koepfli									

LSHTM: London School of Hygiene and Tropical Medicine
UNC: University of North Carolina
ADFMIDI: Australian Defence Force Malaria and Infectious Disease Institute
CDC: Centres for Disease Control
UCAD: Université Cheikh Anta Diop de Dakar
UPCH: Universidad Peruana Cayetano Heredia
NIMR: National Institute of Malaria Research
AHRI: Amauer Hansen Research Institute
UND: University of Notre Dame

Ref: Prof. Qin et al, Pfhrp2/3 CoP

WHO Collaborating Institution



Acknowledgement

Co-investigators

- Prof. Moses Kamya
- Prof. Chae Seung Lim

The WHO Collaborating Center

- Prof. Qin Cheng (Australia)
- Dr. Jane Cunningham (Geneva)

Malaria Control Division, Uganda

The Funders

- The NIH-FIC
- BMGF
- Global Emerging Infections Surveillance

Australian Army Malaria Institute

IDRC
INFECTIOUS DISEASES RESEARCH COLLABORATION

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pfhrp2/3 gene deletions
Mobilizing and providing peer and technical support

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MEDICAL & SCIENTIFIC AFFAIRS MANAGER, AFRICA, ABBOTT

Closing Remarks

Abbott