Update on *Plasmodium falciparum* hrp2/3 gene deletions

Jane Cunningham – MPAC 22–24 March 2017
Overview

• Summary of reports: *pfhrp2/3* gene deletions reports: Central/South America, Africa, Asia

• Progress report on action items (1-7) from MPAC – Sept 2016
Central and South America
Parasites Lacking HRP2/3 in Central and South America

Gamboa et al 2010
Maltha et al 2012
Akinyi et al 2013
Houze et al 2011
Trouvay et al 2013
Akinyi et al 2015
Murillo et al 2015
Abdallah et al 2015
Dorado EJ et al 2016
Rachid Viana GM et al 2017

Global Malaria Programme

Slide courtesy of Q. Cheng, AMI
Spatial heterogeneity: Brazil

<table>
<thead>
<tr>
<th>Site</th>
<th>Pfhrp2 deletion</th>
<th>Pfhrp3 deletion</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>95% confidence interval</td>
<td>N (%)</td>
</tr>
<tr>
<td>Acre, Brazil</td>
<td>25/79 (31.6%)</td>
<td>21.6–43.1</td>
<td>30/79 (38.0%)</td>
</tr>
<tr>
<td>Para, Brazil</td>
<td>0/59 (0%)</td>
<td>NA</td>
<td>30/59 (50.9%)</td>
</tr>
<tr>
<td>Rondonia, Brazil</td>
<td>2/60 (3.3%)</td>
<td>0.4–11.5</td>
<td>11/60 (18.3%)</td>
</tr>
<tr>
<td>Bolivia</td>
<td>1/25 (4.0%)</td>
<td>0.1–20.4</td>
<td>17/25 (68.0%)</td>
</tr>
</tbody>
</table>

* - a total of 23 double negative for pfhrp2 and pfhrp3 were detected in Acre (21 samples) and Rondonia (2 samples).

Africa
Pfhrp2/3 deletion reports

Published:
Mali (2012)
Senegal (2013)
Ghana (2016)
DRC (2016)
Rwanda (2017)

Unpublished (2016):
Eritrea (pre-submission)
Mozambique (submitted)
Zambia
Uganda
Reports: West Africa

Pfhrp2 PCR-negative reports:

**Mali** (Koita et al. AJTMH 2012), first African report
- **2.1%** (n=10) of 480 asymptomatic, micro-positive subjects

**Senegal** (Wurtz et al. Malar J 2013)
- **2.2%** (n=3) of 136 symptomatic, micro-positive subjects
- **12.8%** pfhrp3 negative

**Ghana** (Amoah et al. Malar J 2016)
- 124 asymptomatic pfhrp2-negative subjects
Pfhrp2 PCR-negative reports:

**Zambia** (unpublished) - 2009-2013
- Community based surveys - Choma and Nchelenge
- 8 asymptomatic, RDT-/PCR+ subjects
- 0-4.7%

**Mozambique** (submitted for pub) – 2010-2016
- Cross sectional community survey – n=9124
- Pfhrp2/3 gene analysis for RDT-/micro+ or PCR+ - n=164; many samples excluded due to poor quality DNA
- 69 samples analyzed - 1 asymptomatic subject had pfhrp2 deletion
Uganda (unpublished – Nsobya ASTMH talk #1261)

- Household survey 2012-2013
  - 1.6% (n=25) of 1493 smear-pos/PCR-pos subjects were pfhrp2 PCR-negative
    - Of 96 RDT-neg/microscopy-pos subjects, only 56/96 (58%) confirmed PCR + : of these 56 : 25 (45%) pfhrp2 PCR-negative, 39 (70%) pfhrp3 PCR-negative, 19 (34%) had double deletions
  - 3 sites: MOI 1.0-2.0, mean 225-700p/μL, EIR 3.8-125
  - 44/56 samples with deletions were from Tororo

Rwanda (Kozycki et al. Malar J (2017) 16:123)

- DHS 2014-2015
  - 1.0% (n=32) of 3291 smear-pos subjects were pfhrp2 PCR-negative
    - Of 322 were RDT-neg/PCR-pos, 32 pfhrp2 PCR-negative
    - 3 sites: EIR <1-21, slide positivity 0-4.4% in children

(* excluding Eritrea)
Reports: Central Africa

DRC (Parr et al. J. Inf Dis. 2016)

- DHS survey
- **6.4% (n=149) prevalence** among asymptomatic, PCR-pos subjects had a *pfhrp2* deletion.
  - Only 5 (3.4%) of these 149 also had a *pfhrp3* deletion
- **First national survey**
  - Deletions more common in areas of low malaria prevalence
- **Population genetics**
  - Deleted parasites are genetically distinct from controls
Eritrea (unpublished – Berhane ASTMH poster #879)
pfhrp2/pfhrp3 deletions in Eritrea - 2016

Rapid diagnostic tests failing to detect *Plasmodium falciparum* infections in Eritrea: an investigation of reported false negative RDT results

Araia Berhane¹, Mulugeta Russom², Iyassu Bahta³, Filmon Hagos⁴, Michael Ghirmal⁵ and Selam Uqubay⁶

Berhane et al. Malar J (2017) 16:105
DOI 10.1186/s12936-017-1752-9

- RDTs implemented in 2006: SD Bioline Pf/Pv 05FK80
- False negative RDTs reported in 2014-2015

Eritrea MOH team: Araia Berhane; Selam Mihreteab; Salih Mohamed; Filmon Hagos

Australian Army Malaria Institute-QIMR Berghofer: Karen Anderson, Qin Cheng

WHO: Jane Cunningham, Anderson Chinorumba

**Ghindae Hospital**

- *pfhrp2*: 19%
- *pfhrp3*: 8%

**Massawa Hospital**

- *pfhrp2*: 58%
- *pfhrp3 Exon 1-2*: 29%

- **n = 26**
  - 1,381-89,120 P/µL

- **n = 24**
  - 32 – 25,760 P/µL

pfhrp2 -pfhrp3 - : 42-81%
Negative Reports

Ghana (unpublished)

- 0 pfhrp2 deletions found among 165 asymptomatic, PCR-pos/RDT-neg subjects

Kenya (unpublished)

- 0 pfhrp2 deletions found among 50 asymptomatic, PCR-pos subjects

Unpublished K. Beshir, LSHTM
Asia
pfhrp2/pfhrp3 deletions in India

- Conducted in Dec 2010 in Bilaspur district of Chhattisgarh in Central India.
- 48 LM confirmed Pf, with densities: 1800 – 54,448/µL
- 2/48 RDT negative: CB18 and CB21
- CB18 and CB21 failed to amplify pfhrp2, but were successful with amplification of 3 single copy genes

Kumar et al 2013
Prevalence of *pfhrp2* and/or *pfhrp3* Gene Deletion in *Plasmodium falciparum* Population in Eight Highly Endemic States in India

Praveen Kumar Bharti¹, Himanshu Singh Chandel¹, Amreen Ahmad¹, Sri Krishna¹, Venkatachalam Udhayakumar², Neeru Singh¹*

- HRP2 deletion: 2.4% (36/1521)
  Range: 0-25%, 2.4 95% CI: 1.6-3.3
- HRP3 deletion: 1.8% (27/1521)
- Both HRP2/3: Range: 0–8% (1.6, 95% CI; 1.0–2.4)

July – Dec 2014
16 sites in eight malaria endemic states in India

Bharti et al 2016
pfhrp2/pfhrp3 deletions: China-Myanmar border

- May 2011 - Dec 2012
- 87 LM confirmed Pf patients from China-Myanmar border, with densities: 40 to 105,920/µL
- 4/87 samples from Myanmar failed to amplify any pfhrp2 fragments
- 3/4 samples also failed to amplify pfhrp3
- All 4 samples amplified 3 single copy genes
A Case of *Plasmodium falciparum* hrp2 gene mutation in Bangladesh

Maisha Khair Nima¹, Thomas Hougard¹, ², Mohammad Enayet Hossain¹, Mohammad Golam Kibria¹, Abu Naser Mohon¹, ³, Rajibur Rahman¹, Rashidul Haque¹, Mohammad Shafiu Alam⁴

¹International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka 1212, Bangladesh; ²Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, Minnesota, USA; ³Department of Microbiology & Infectious Disease, Cumming School of Medicine, University of Calgary, Alberta, Canada

24 year old male from Kamalganj Upazilla Health Complex in Sylhet

- *P. falciparum* infection confirmed by 18S rRNA PCR
- PCR yielded no visible amplification product for exon 1 of *pfhrp2* gene; exon 2 amplification yielded DNA fragment

No mention of single copy gene amplification….
## Countries where we have found HRP2/3 deletions

<table>
<thead>
<tr>
<th>Samples</th>
<th>Percent of samples with deletions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HRP2</td>
</tr>
<tr>
<td>Cambodia</td>
<td>856</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>24</td>
</tr>
<tr>
<td>Kenya</td>
<td>122</td>
</tr>
<tr>
<td>Laos</td>
<td>121</td>
</tr>
<tr>
<td>Malawi</td>
<td>262</td>
</tr>
<tr>
<td>Papua Indonesia</td>
<td>79</td>
</tr>
<tr>
<td>Peru</td>
<td>28</td>
</tr>
<tr>
<td>Senegal</td>
<td>59</td>
</tr>
<tr>
<td>Thailand</td>
<td>888</td>
</tr>
<tr>
<td>Vietnam</td>
<td>216</td>
</tr>
</tbody>
</table>

_unpublished: R. Amato, D. Kwiatkowski, R Pearson_
‘Report card’
WHO should promote a harmonized approach to investigating, surveying and reporting *pfhrp2/3* gene deletions through the provision of standard protocols (including sample size calculations) and operating procedures.

A protocol to determine *pfhrp2* gene deletion prevalence among symptomatic individuals with a *Plasmodium falciparum* infection attending public health facilities is being finalized.

***** identify areas with evidence of HRP2 gene deletion prevalence above 5% *****

Key characteristics:

- Province/state will serve as the sampling domain
- Cross-sectional consisting of a systematic random sample of public health facilities selected from a sampling frame of a complete list of all facilities, stratified by facility type and including a measure of facility size, in each province (with transmission).
- All individuals attending the selected facilities with fever and confirmed malaria infection by quality assured pan or pf-pLDH RDTs or microscopy.
- HRP2 (-)/pan or pf-pLDH RDT (or microscopy) (+) patients will be consented for collection of dried blood spot for PCR confirmation of *P. falciparum* infection and *pfhrp2/3* genes
- Brief questionnaire
Sample sizes for determining if the observed HRP2 deletion prevalence is above or below the 5% threshold at the survey domain (province) level

<table>
<thead>
<tr>
<th>Estimated proportion of HRP2 deletion (outcome 1: total HRP2- &amp; pan-pLDH+ / total LDH+)</th>
<th>Column 2</th>
<th>Column 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1%</td>
<td>150</td>
<td>15</td>
</tr>
<tr>
<td>1%</td>
<td>150</td>
<td>15</td>
</tr>
<tr>
<td>2%</td>
<td>150</td>
<td>15</td>
</tr>
<tr>
<td>3%</td>
<td>350</td>
<td>20</td>
</tr>
<tr>
<td>4%</td>
<td>1,550</td>
<td>155</td>
</tr>
<tr>
<td>5%</td>
<td>2,280 (assume = 5%)</td>
<td>228</td>
</tr>
<tr>
<td>6%</td>
<td>2,280</td>
<td>228</td>
</tr>
<tr>
<td>7%</td>
<td>660</td>
<td>66</td>
</tr>
<tr>
<td>8%</td>
<td>330</td>
<td>33</td>
</tr>
<tr>
<td>9%</td>
<td>210</td>
<td>21</td>
</tr>
<tr>
<td>&gt;9%</td>
<td>150</td>
<td>15</td>
</tr>
</tbody>
</table>

Dear [Recipient],

The table above illustrates the number of individuals needed for each estimated proportion of HRP2 deletion to determine if the observed prevalence is above or below the 5% threshold at the survey domain (province) level. For instance, if the observed diagnostic prevalence of HRP2 deletion is 1%, 150 individuals are needed to conclude that 90% CI does not include 5%. Similarly, if the observed prevalence is above 9%, 150 individuals suffice and enrollment may stop.

This table is crucial for planning and executing diagnostic surveys to ensure accurate and reliable results in malaria control programs.

Sincerely,

[Your Name]
Additional survey tools

- Facility tally sheet
- Consent template
- Assent template
- Report forms (patient and laboratory): age, sex, location, travel, antimalarials, RDT, PCR, including electronic data entry tool
- Illustrative study budget
- Tabulation plan for HPP2 prevalence
- Sample size estimator
### Reference laboratories

#### Molecular studies
PCR confirmation of Plasmodium and species ID and hrp2/3 PCR
DNA Sequencing: whole genome, targeted

<table>
<thead>
<tr>
<th>Institution</th>
<th>Country</th>
<th>Scientist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian Army Malaria Institute</td>
<td>Australia</td>
<td>Dr. Q. Cheng</td>
</tr>
<tr>
<td>Institut Pasteur</td>
<td>Cambodia</td>
<td>Dr. Didier Menard</td>
</tr>
<tr>
<td>National Institute for Research in Tribal Health (NIRTH)</td>
<td>India</td>
<td>Dr. Neeru Singh</td>
</tr>
<tr>
<td>MRL/LSHTM</td>
<td>UK</td>
<td>Dr. Khalid Beshir/Colin Sutherland</td>
</tr>
<tr>
<td>CDC</td>
<td>USA</td>
<td>Dr. Venkatachalam Udhayakumar (Kumar)</td>
</tr>
<tr>
<td>University North Carolina</td>
<td>USA</td>
<td>Dr. Steven Meshnick/Jonathan Parr</td>
</tr>
</tbody>
</table>

### Immunoassay (optional)
Elisa
Luminex (HRP2, aldolase, pLDH)

Bead-based immunoassay allows sub-picogram detection of histidine-rich protein 2 from *Plasmodium falciparum* and estimates reliability of malaria rapid diagnostic tests

Published: February 13, 2017 • [http://dx.doi.org/10.1371/journal.pone.0172139](http://dx.doi.org/10.1371/journal.pone.0172139)
pfhrp2/3 surveys and surveillance activities should first target countries where deletions or concerns have been identified, and the neighbouring countries.

- WHO actively supporting the design and planning of surveys for pfhrp2/3 gene deletions in the states/provinces of Ethiopia and Sudan bordering Eritrea.

- Target implementation during the high-transmission season (September/October 2017).

- Sampling will be powered in order to obtain precise estimates of pfhrp2/3 gene deletions at the province level.

- If < 5% threshold conversion to sentinel site surveillance.
WHO should integrate information about *pfhrp2/3* gene deletions into the global mapping database

- A review was conducted of all published (and some unpublished) reports of *pfhrp2/3* gene deletions, and data were extracted to inform the online global mapping database under development.

- Reports on presence and absence of *pfhrp2/3* gene deletions will be included.

- The review yielded data from 20 countries.

- Since the last MPAC meeting, new reports of *pfhrp2/3* gene deletions have emerged from Rwanda, Uganda, Bangladesh and Mozambique.
The published recommended procedures for investigating and accurately reporting *pfhrp2/3* deletions are comprised of three steps: establishing initial evidence, establishing confirmatory evidence, and establishing prevalence (Cheng Q et al., *Malaria Journal* 2014 13:283).

Revise to recommend

- confirmatory evidence include PCR for *pfhrp3* in addition to PCR for *pfhrp2*, as HRP3 proteins can show cross-reactivity in HRP2-based RDTs;
- analysis of flanking genes for *pfhrp2* (and *pfhrp3*);
- the confirmation of absent HRP2 antigen (by ELISA or second brand of RDT) are optional.

WHO information note has been updated to reflect these modifications.

Need to publish in the peer review literature - specifically data quality of recent reports, accurate reporting and thresholds that trigger change.
WHO should establish a consortium to provide technical support in investigating suspected false-negative RDTs due to pfhrp2/3 deletions, to establish appropriate surveillance systems, and to elaborate on factors influencing the emergence and spread of pfhrp2/3 deletions.

- Network of laboratories has been established to support investigations for pfhrp2/3 gene deletions
  - More resources will be required
- GMP/SUR supporting development of standard survey protocol for determining prevalence of pfhrp2/3 gene deletions – should facilitate incorporation into routine surveillance activity;
Tests with both HRP2 and pLDH antibodies on the same test line should be prioritized for assessment by WHO prequalification, including a laboratory evaluation against pfhrp2/3 single- and double-deleted parasites (culture and clinical samples) to determine whether the tests meet recommended performance criteria.

- Archived materials (7 samples from Peru) and culture adapted *P.falciparum* isolates that do not express HRP2 have been identified
- Prospective collection of wildtype pfhrp2 deleted parasites is ongoing in Peru (Universidad Peruana de Cayetano Heredia).
- Round 8 of WHO malaria RDT product testing will include a panel of hrp2 deleted parasites (≈30 samples)
  - 9/35 (26%) products in round 8 target pf-pLDH for detection of *P.falciparum*
    - Manufacturers are responding !!
- Unfortunately, based on round 7 results one pf-pLDH-combination RDT that previously meet procurement criteria, no longer does and only one new product does meet criteria.
Develop a plan of action for surveillance and response that can be supported by partners and implemented in countries.

**Action plan** should be rooted in an understanding of the extent and spread of deletions and clinical impact?

- **Contents outlined**
  - State of knowledge and research gaps
  - Surveillance plan
  - Managing the response & assessing the economic costs
    - Case detection and case management strategies at trigger points (dual testing, etc.)
    - Risk communication with countries/national programmes;
  - Engagement with the diagnostics industry;
  - Procurers (cost constraints, complexity of procuring >1 RDT type and full product replacement);
  - Changes required to WHO Product Testing;
  - Interaction with regulatory/qualifying bodies;
  - Resource mobilization

- **Consultant identified**
- **Ad hoc MPAC review** June-July 2017