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Plasmodium falciparum hrp2/3 deletions not identified among symptomatic subjects in the Democratic Republic of the Congo

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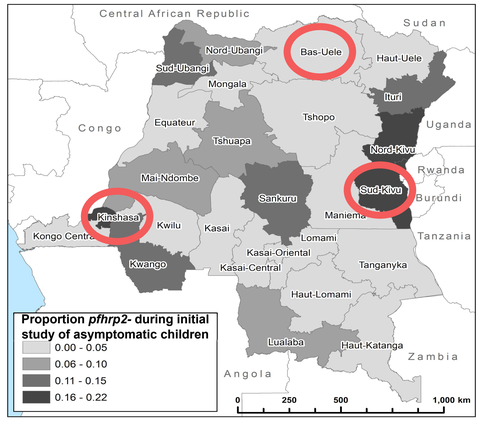
1. Background

- Over two-thirds of all malaria diagnoses in Africa are made using rapid diagnostic tests that detect histidine rich protein 2 (HRP2).[1]
- A recently described deletion mutation of the *P. falciparum* histidine-rich protein 2 and/or 3 (*pfhrp2/3*) genes allows it to escape detection by common rapid diagnostic tests (RDTs).[2, 3]
- Increasing reports from Africa indicate that parasites with this mutation are common in select locations.[4]
- We previously found a 6.4% national prevalence of *pfhrp2*-negative parasites among asymptomatic children in the DRC.[5]
- Initial hypothesis: *Pfhrp2*-deleted parasites are responsible for missed clinical cases of falciparum malaria in the DRC.

2. Methods

1. Sampled **3,628 symptomatic adults and children in the DRC** from three provinces.



2. Screened a **subset of 427 RDT-/microscopy+ samples** and 428 RDT+/microscopy+ controls using multiple PCR assays to detect **deletion mutations in the *pfhrp2* and *pfhrp3* genes**.[6]
3. Whole-genome sequenced parasites with putative deletions based on initial PCR testing using selective whole-genome amplification and Illumina HS2500 short-read sequencing.
4. Confirmed PCR and whole-genome sequencing results using Luminex HRP2 antigen testing.[7]
5. Performed species-specific real-time PCR on a randomly selected **subset of 1,000 samples** to evaluate the impact of non-falciparum malaria on RDT performance.

References: 1) WHO. World Malaria Report 2015, 2) Gamboa D et al. PLOS One 2010, 3) Cheng Q et al. Malar J 2014., 4) WHO. *P. falciparum hrp2/3* gene deletions: Conclusions and recommendations of a Technical Consultation. 2016. 5) Parr JB et al. J Infect Dis 2017, 6) Parr JB et al. Malar J 2018, 7) Rogier E et al. PLOS One 2017, 8) Baker J et al. J Infect Dis 2005.

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3. Results

No *pfhrp2*- or *pfhrp3*-deleted parasites were identified (0%)

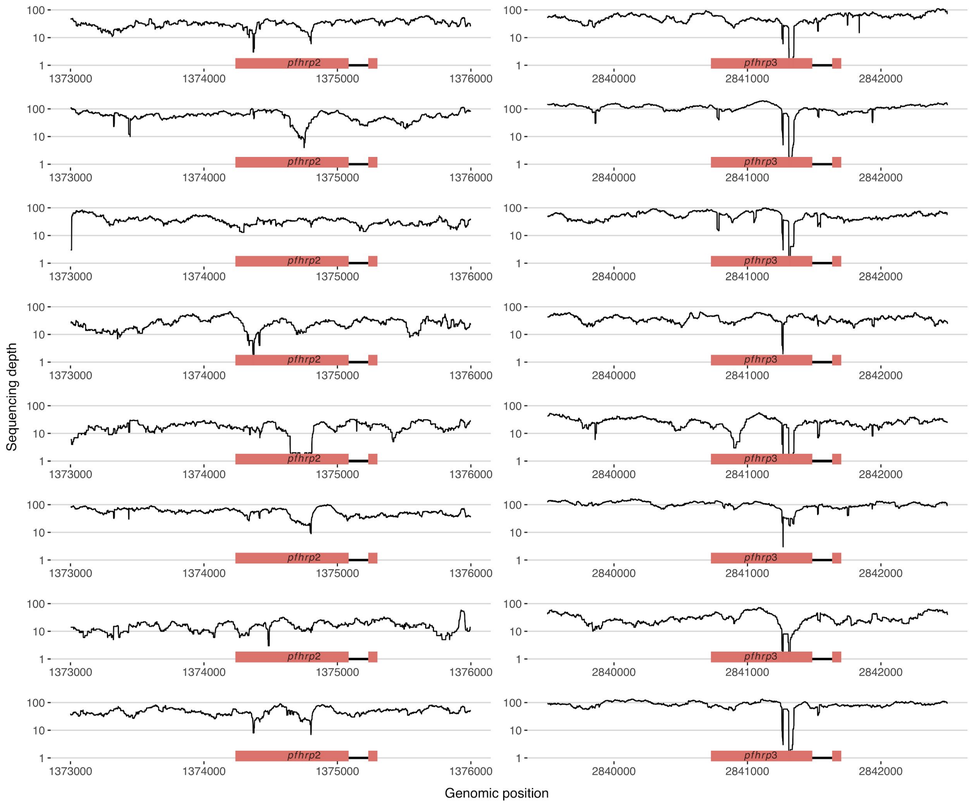

Figure 1. Whole-genome sequencing of 5 *pfhrp2-/pfhrp3-*, 2 *pfhrp2-/pfhrp3+*, and 1 *pfhrp2+/pfhrp3-* parasites originally identified by single-step PCR confirmed intact genes. Each row is one parasite. Parasitemias ranged from 1,778 to 38,200 parasites/uL by microscopy. Follow-up Luminex HRP2 testing and nested PCR confirmed the presence of antigen and DNA targets, respectively.[8]

False-negative RDTs occurred in the setting of high HRP2 antigenemia

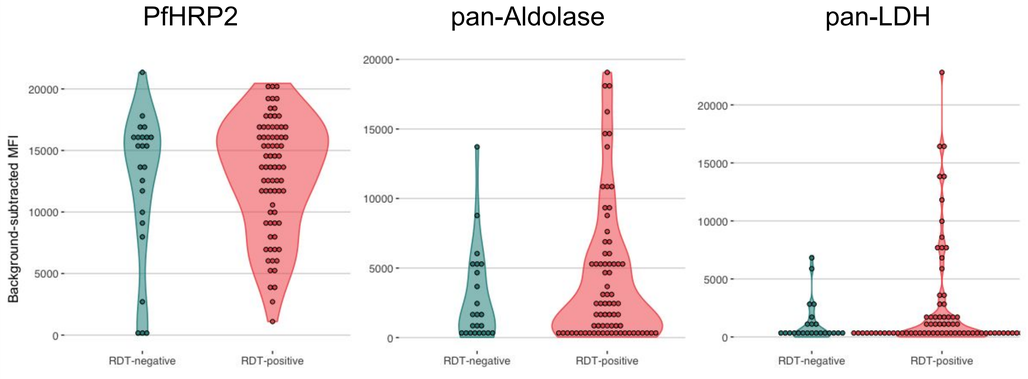

Figure 2. A Luminex bead-based immunoassay for three parasite antigens confirmed circulating PfHRP2 antigen in the majority of RDT- but PCR+/microscopy+ isolates tested. Testing was performed on a subset of 98 samples, including 23 RDT-negative and 74 RDT-positive controls. PfHRP2 levels were higher than pan-Aldolase and pan-LDH.

Malaria prevalence was high (57%) and non-falciparum co-infection common (15%) in symptomatic subjects

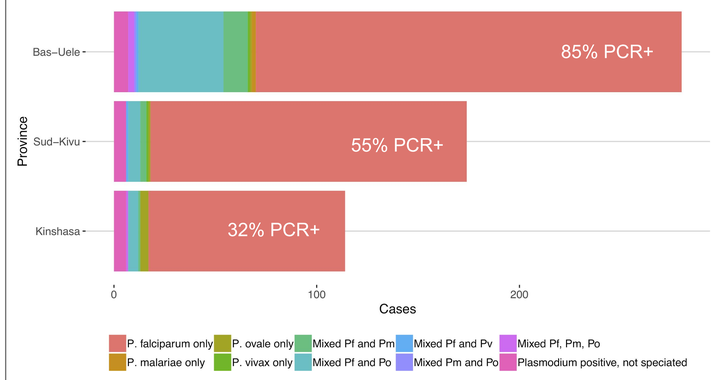

Figure 3. *Plasmodium* PCR prevalence and species-specific real-time PCR results for *P. falciparum, ovale, malariae, and vivax* among 1,000 samples tested. Malaria prevalence varied by region. Most non-falciparum cases occurred as mixed infections. 4 cases of *P. vivax* were identified.

HRP2-based RDT performance was satisfactory and superior to microscopy

	PCR+	PCR-
RDT+	404	36
RDT-	134	426

	PCR+	PCR-
Micro+	287	88
Micro-	251	374

Figure 4. RDTs were 75% sensitive and 92% specific compared to 18S rDNA PCR. Microscopy was 53% sensitive and 81% specific. Among a random subset of 1,000 samples tested, agreement between RDTs and PCR was good (kappa 0.66), while agreement between microscopy and PCR was only fair (kappa 0.33).

4. Conclusions

- **Symptomatic malaria due to *pfhrp2*- or *pfhrp3*-deleted *P. falciparum* was not observed in the DRC.**
- Molecular testing for *pfhrp2/3* deletions is complex. Testing using multiple approaches improves confidence in deletion calls. PfHRP2/3 antigen testing is a useful tool.
- These findings contrast with our prior study of asymptomatic children in the DRC, raising the possibility of differences in parasite virulence and/or previously undescribed PCR inhibitors.
- **Ongoing HRP2-based RDT use in the DRC is appropriate** for detection of falciparum malaria.
- Symptomatic malaria due non-falciparum species is common but usually occurs in mixed infections with *P. falciparum*.

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