ケニア・ヴィクトリア湖周辺地域におけるマラリア迅速診断キット偽陰性を引き起こす*P. falciparum* histidine rich protein2 (PfHRP2)およびPfHRP3遺伝子欠損原虫の同定

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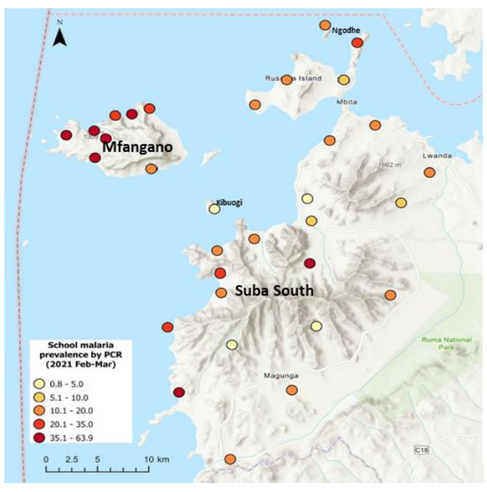
Background

Malaria prevalence in Lake Victoria region, Homa Bay County

- ***Plasmodium* prevalence by PCR ～ 37.1%.**
- **The prevalence of P. falciparum is the highest.**
- **Malaria prevalence is heterogeneous, with the highest rates on the mainland (Suba South) and on Mfangano Island.**

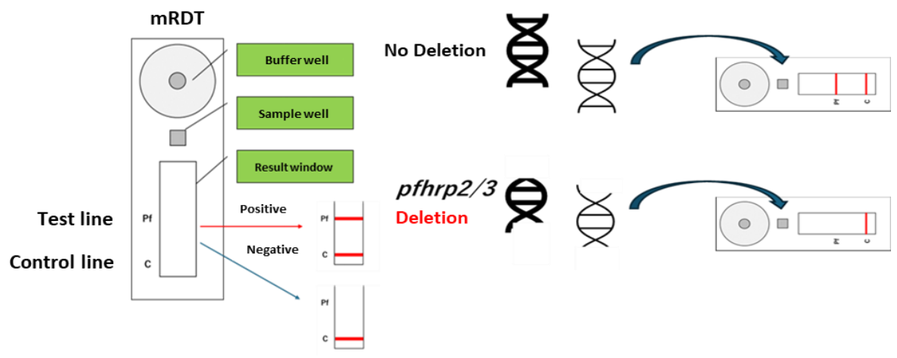


- **Malaria diagnostic tools within the study area**

✓ **In the study areas, due to its high sensitivity and simplicity of use, diagnosis by malaria rapid diagnostic test (mRDT) .**

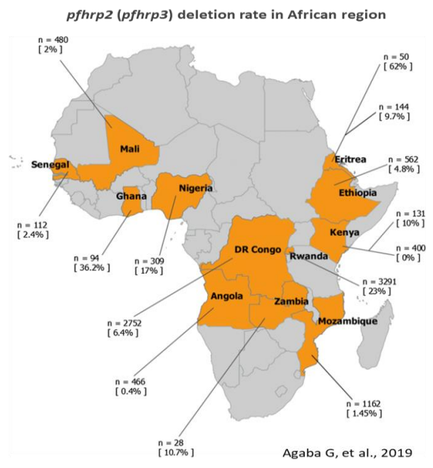
✓ **The use of a pfHRP2-based RDT is recommended by the WHO for the diagnosis of *P. falciparum*.**

- **RDT false negative cases in Africa due to *pfhrp2/3* double deletion**



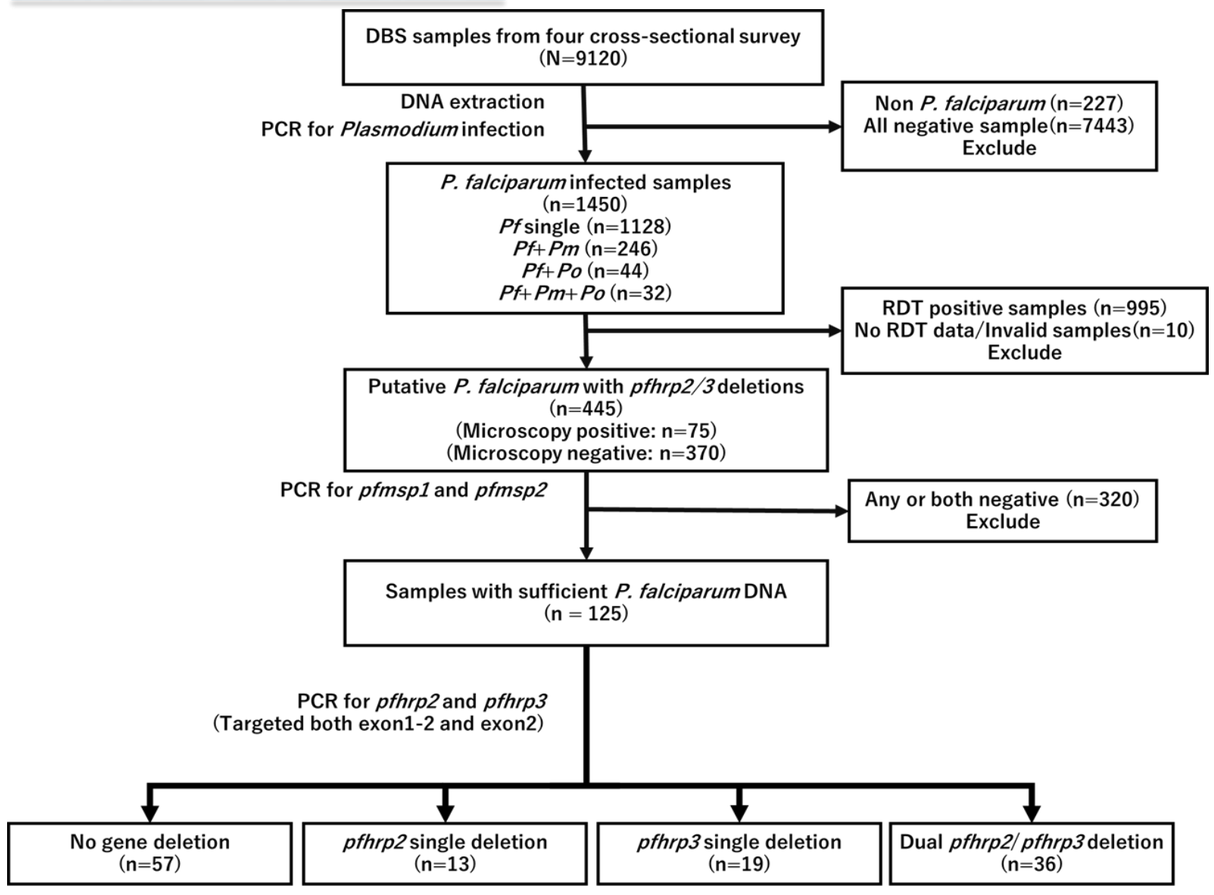
- ***P. falciparum* lacking *pfhrp2* and *pfhrp3* cases in Africa.**

***P. falciparum* lacking *pfhrp2* and *pfhrp3* found in patients from coastal Kenya, but many in endemic areas have submicroscopic and asymptomatic *P. falciparum* infections that remain undiagnosed.**



Study aim: To analyze the presence of *P. falciparum* with *pfhrp2/3* gene deletions among infected individuals in the Lake Victoria region of Homa Bay County, Western Kenya.

Materials and Methods



Pfmsp1 and *pfmsp2* amplification by PCR

To confirm the quality and quantity of *P. falciparum* DNA and determine the multiplicity of infection (MOI)

Analysis of *pfhrp2/3* gene deletion by PCR

Multiple deletion types in each gene

PCR amplifications were performed for regions between exons 1 and 2 (including introns) and entire exon 2.

Data Analysis

Comparing MOI : Mann-Whitney and Kruskal-Wallis tests
Comparing proportions : Pearson's chi-square and Fisher's exact tests
Determining association between *pfhrp2/3* deletions and clinical/demographic attributes: logistic regression models

Result

Table 1. *P. falciparum* with *pfhrp2* and *pfhrp3* deletions in Homa Bay County, Kenya 2018-2020

	RDT-negative, *cox3* PCR positive (N=445)	Both *pfmsp1/2* successfully amplified n, (%) (n=125)	*Pfhrp2* single deletion (n=13)	*Pfhrp3* single deletion (n=19)	*Pfhrp2/3* double deletion (n=36)	No deletion (n=57)
Survey Period						
2018-Sep	132	40 (30.3)	2	7	12	19
2019-Jan	89	22 (24.7)	1	4	5	12
2019-Sep	107	40 (37.4)	5	4	11	20
2020-Jan	117	23 (19.7)	5	4	8	6
Survey Place						
Suba South	116	39 (33.6)	1	5	16	17
Mfangano	265	73 (27.5)	10	11	18	34
Ngodhe	54	10 (18.5)	1	3	2	4
Kibuogi	10	3 (30.0)	1	0	0	2
Microscopy						
Negative	370	82 (65.6)	9	14	27	32
Fever						
≥37.5℃	87	29 (33.3)	2	5	6	16

- **Of 125 samples analyzed, 13 and 19 samples had *P. falciparum* with *pfhrp2* single deletion and *pfhrp3* single deletion, respectively.**
- **36 samples had *P. falciparum* with *pfhrp2/3* double deletions.**
- ***P. falciparum* with double deletions were found in all surveyed years.**

Table 2. MOI and *pfhrp2/3* gene deletions in *P. falciparum* infections in Kenya, 2018-2020

MOI	*Pfhrp2* deletion	*Pfhrp3* deletion	*Pfhrp2/3* double deletion	No deletion	P-value
1	10	14	31	33	
2	2	4	4	21	
3	0	1	1	3	
4	1	0	0	0	
Mean MOI (SD)	1.38 (0.87)	1.32 (0.58)	1.17 (0.45)	1.47 (0.60)	0.16

- **Monoclonal infections accounted for 70.4% (88/125) of the samples**
- **The mean MOI was slightly higher among microscopic infections (1.39 ± SD 0.57) than submicroscopic infections (1.33 ± SD 0.61), although the difference was not statistically significant (p = 0.98).**

Table 3. Association between *pfhrp2/3* deletions and predictors

Predictors	*Pfhrp2* single deletion OR (95% CI)	*Pfhrp3* single deletion OR (95% CI)	*Pfhrp2/3* double deletion OR (95% CI)
Age	1.034 (0.981 - 1.090)	1.008 (0.945 - 1.074)	0.996 (0.949 - 1.046)
Sex	0.746 (0.215 - 2.588)	1.278 (0.427 - 3.827)	0.880 (0.384 - 2.016)
Polyclonal infection	0.647 (0.153 - 2.734)	0.867 (0.251 - 3.001)	0.286 (0.098 - 0.835)
Asymptomatic infection	0.844 (0.151 - 4.697)	1.678 (0.333 - 8.455)	1.120 (0.365 - 3.438)
Submicroscopic infection	1.872 (0.488 - 7.172)	1.740 (0.501 - 6.047)	1.761 (0.692 - 4.483)
Study site	0.557 (0.297 - 1.045)	1.041 (0.702 - 1.545)	1.191 (0.876 - 1.619)
Study period	1.556 (0.853 - 2.837)	0.932 (0.567 - 1.532)	1.142 (0.781 - 1.670)
HL chi2†	5.57	10.75	10.69
(p-value)	(0.6955)	(0.2160)	(0.2199)

- **Polyclonal infections were significantly associated with a lower odds of *pfhrp2/3* double deletions (OR = 0.286, p = 0.022)**

Conclusion

This study reveals the presence of *pfhrp2/3* double deletions among *P. falciparum* infections in Homa Bay County, western Kenya.

✓ **Most of the samples with deletions were found in asymptomatic and submicroscopic infections which can nevertheless sustain transmission, raising the possibility that parasites with deletions will become more widespread.**

✓ **Co-infections of parasites with intact and deleted genes can mask the presence of *pfhrp2/3* deletions.**

These findings indicate…

- **The need to enhancement active molecular surveillance of these deletions to ensure effectiveness of PfHRP2-based mRDT.**
- **The need for further research to understand the genetic relationships between parasites with deletions, their prevalence, and their impact on malaria transmission dynamics.**

Study limitation

✓ **The detection limit of conventional PCR-based protocols for submicroscopic infection samples.**

Acknowledgement

We appreciate the study participants, field and laboratory assistants, and microscopists in Homabay County, Kenya; also Ms. Mayumi Fukui and Ikuko Kusuda for PCR.

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