Low prevalence of *Plasmodium falciparum* parasites lacking *pfhrp2/3* genes among asymptomatic and symptomatic school-age children in Kinshasa, Democratic Republic of Congo

Sabin S. Nundu¹²³*, Hiroaki Arima², Shirley V. Simpson¹², Ben-Yeddy Abel Chitama⁴, Yannick Bazitama Munyeku⁵, Jean-Jacques Muyembe³, Toshihiro Mita⁶, Steve Ahuka³, Richard Cullleton⁴⁷* and Taro Yamamoto¹²

Abstract

**Background:** Loss of efficacy of diagnostic tests may lead to untreated or mistreated malaria cases, compromising case management and control. There is an increasing reliance on rapid diagnostic tests (RDTs) for malaria diagnosis, with the most widely used of these targeting the *Plasmodium falciparum* histidine-rich protein 2 (*PfHRP2*). There are numerous reports of the deletion of this gene in *P. falciparum* parasites in some populations, rendering them undetectable by *PfHRP2* RDTs. The aim of this study was to identify *P. falciparum* parasites lacking the *P. falciparum* histidine rich protein 2 and 3 genes (*pfhrp2/3*) isolated from asymptomatic and symptomatic school-age children in Kinshasa, Democratic Republic of Congo.

**Methods:** The performance of *PfHRP2*-based RDTs in comparison to microscopy and PCR was assessed using blood samples collected and spotted on Whatman 903™ filter papers between October and November 2019 from school-age children aged 6–14 years. PCR was then used to identify parasite isolates lacking *pfhrp2/3* genes.

**Results:** Among asymptomatic malaria carriers (N = 266), 49%, 65%, and 70% were microscopy, *PfHRP2*_RDT, and *pfldh*-qPCR positive, respectively. The sensitivity and specificity of RDTs compared to PCR were 80% and 70% while the sensitivity and specificity of RDTs compared to microscopy were 92% and 60%, respectively. Among symptomatic malaria carriers (N = 196), 62%, 67%, and 87% were microscopy, *PfHRP2*_based RDT, *pfldh*-qPCR and positive, respectively. The sensitivity and specificity of RDTs compared to PCR were 75% and 88%, whereas the sensitivity and specificity of RDTs compared to microscopy were 93% and 77%, respectively. Of 173 samples with sufficient DNA for PCR amplification of *pfhrp2/3*, deletions of *pfhrp2* and *pfhrp3* were identified in 2% and 1%, respectively. Three (4%)
of samples harboured deletions of the *pfhrp2* gene in asymptomatic parasite carriers and one (1%) isolate lacked the *pfhrp3* gene among symptomatic parasite carriers in the RDT positive subgroup. No parasites lacking the *pfhrp2/3* genes were found in the RDT negative subgroup.

**Conclusion:** *Plasmodium falciparum* histidine-rich protein 2/3 gene deletions are uncommon in the surveyed population, and do not result in diagnostic failure. The use of rigorous PCR methods to identify *pfhrp2/3* gene deletions is encouraged in order to minimize the overestimation of their prevalence.

**Keywords:** Malaria, Rapid diagnostic tests, School-age children, Democratic Republic of Congo

**Background**
Despite concerted control efforts, malaria remains a serious public health problem in the Democratic Republic of the Congo (DRC). The country accounted for 12% of all estimated malaria cases and 11% of deaths globally in 2019 [1]. Malaria case management is based on rapid and accurate diagnosis and prompt treatment with effective anti-malarial drugs [2].

The World Health Organization (WHO) recommends malaria diagnosis to be performed by microscopy or through the use of rapid diagnostic tests (RDTs) for all individuals presenting with malaria-like symptoms prior to the commencement of treatment [3]. However, although microscopy is the gold standard for diagnosis [4], its use is challenging and subject to both false positive and negative results when performed by inexperienced microscopists, especially in the case of poor blood film preparation and when parasitaemia is low [5–10]. RDTs are frequently used as an alternative, especially in remote areas [11–14]. In regions where *P. falciparum* is the most prevalent malaria parasite species, the most frequently used RDTs target *P. falciparum* histidine-rich protein-2 (*PfHRP2*). Sixty-four percent of all RDTs distributed by national malaria control programs worldwide in 2018 were of this type [15]. Moreover, *PfHRP2*-based RDTs have better sensitivity [16, 17] and greater thermal stability [18] than other RDTs. Furthermore, numerous antibodies used to detect *PfHRP2* also detect *P. falciparum* histidine-rich protein 3 (*PfHRP3*) as they have a high degree of similarity in their amino acid sequences [19, 20]. However, the sensitivity of RDTs is dependent on the level of parasitaemia in the patient. Parasitaemia lower than 200 per µL of blood may be associated with false negative results [21]. Moreover, *pfhrp2* and *pfhrp3* (*pfhrp2/3*) may be deleted in some parasites rendering them undetectable by *PfHRP2*-based RDTs [1]. This loss of efficacy can lead to untreated or mistreated malaria cases, thus compromising malaria case management and control [17]. Thus, the WHO recommends continuous nationwide surveillance of parasites harbouring *pfhrp2/3* deletions. It is recommended that if their prevalence exceeds 5%, alternative RDTs should be used [1]. In the DRC, the 2013–2014 nationwide demographic and health survey revealed a *pfhrp2* gene deletion prevalence of 6.4% overall and 21.9% in Kinshasa among asymptomatic under five children [22]. Interestingly, no *pfhrp2/3* gene deletions were detected among symptomatic individuals [23]. Munyeku et al. [24], found an overall prevalence of 9.2% of parasites isolated from symptomatic malaria patients living Kwilu province, (near Kinshasa) carried *Pfhrp2* gene deletions. However, only 9.9% of isolates that gave false negative *PfHRP2*-based RDT results in that study carried *pfhrp2* gene deletions, suggesting that the vast majority of RDT failures are not due to *pfhrp2* gene deletions in that region. A previous survey conducted in 2011, that included 133 asymptomatic children in the Mont-Ngafu-la-2 health zone (HZ) and 145 asymptomatic children in the Selembao HZ aged 6–59 months found a prevalence of 35% and 27%, respectively, when tested by RDT [25]. A study conducted in the same two areas in 2019 and including 427 asymptomatic and 207 symptomatic school-aged children aged 6–14 years found 41% (Mont-Ngafu-la-2: 56%; Selembao: 28%) and 64% (Mont-Ngafu-la-2: 66%; Selembao: 63%) of malaria prevalence by RDT, respectively [26].

This study aimed to assess the prevalence of *P. falciparum* parasites lacking the *pfhrp2/3* genes in isolates from asymptomatic and symptomatic school-age children in Kinshasa.

**Methods**

**Study design, study area and selection of participants**
Samples used in this study were collected from a previous cross-sectional survey carried out in October and November 2019 among school-age children with ages ranging between 6 and 14 years in Mont-Ngafu-la-2 rural health zone (HZ) and Selembao urban HZ of Kinshasa, Democratic Republic of Congo (Fig. 1) [26].

634 school-age children were enrolled in the study (427 asymptomatic and 207 symptomatic). Finger-prick blood were collected from each child between October and November 2019 for *PfHRP2*-based RDT diagnosis (5 µL of blood), microscopy, and for the preparation of blood spots on Whatman 903™ filter paper (three drops of capillary blood). DNA were extracted and kept at −80 °C until use. Nested-PCR targeting the *Plasmodium*
mitochondrial cytochrome c oxidase III (Cox3) gene was performed for identification of *Plasmodium* species (266 asymptomatic and 196 symptomatic samples were analysed) as described in a previous report [26].

Asymptomatic schoolchildren not showing fever and/or malaria-related symptoms, including headache, chills, body joint pains, fatigue, 2 weeks prior to the survey were recruited from schools. Symptomatic children were recruited from health facilities and were outpatients seeking healthcare due to fever or and malaria-related symptoms within 72 h prior to the survey. Schoolchildren whose parents or relatives signed written consent forms were included in this study [26]. Four hundred and sixty-two positive DNA samples (210 microscopy negative, 252 microscopy positive and 157 *Pf* HRP2 RDT negative, 305 *Pf* HRP2 RDT positive) were used in this study for assessment of *pfhrp*2/3 gene deletions.

**Detection of *P. falciparum* infection and selection of samples for *pfhrp*2/3 PCR**

Real-time PCR (qPCR) targeting the *P. falciparum* lactate dehydrogenase gene (*pfldh*) was performed to quantify the number of parasite genomes per µL of extracted DNA solution from each of the samples using a serial dilution of laboratory cultured *P. falciparum* 3D7 strain DNA for calibration. Excluding samples with DNA concentrations less than the limit of detection (LOD) of the *pfhrp*2/3 PCR is crucial for the avoidance of false negative results. A serial dilution consisting of 0.1, 0.01, 0.001 and 0.0001 ng/µL of gDNA extracted from cultured *P. falciparum* 3D7 was prepared in order to generate a calibration curve [23, 27].

**pfldh qPCR for selection of samples with sufficient DNA for *pfhrp*2/3 PCR**

The LOD of the *pfhrp*2 and *pfhrp*3 PCR assays used in this study was $1 \times 10^{-3}$ ng/µL. In order to ensure that only samples with sufficient DNA for the amplification of *pfhrp*2 and *pfhrp*3 were used, only samples with greater than $3 \times 10^{-3}$ ng/µL of DNA as determined by *pfldh* qPCR were considered for further analysis (Additional file 1: Table S1) [23, 27] (Fig. 2).

A calibration curve was prepared using the results of qPCR with control samples (0.1 ng/µL, 0.01 ng/µL, 0.001 ng/µL and 0.0001 ng/µL). Duplicated samples were loaded in 96-wells plates along with serially diluted positive controls (using gDNA extracted from cultured *P. falciparum* 3D7) as well as negative controls consisting of DNA samples from known malaria negative individuals (RDT-, microscopy- and PCR-) and distilled water for checking contamination. The assay was repeated for all discordant duplicates and three consistent results were required for confirmation. The DNA concentration of samples were quantified from each Ct values and the calibration curve.

For selection of samples for *pfhrp*2/3 PCR, all samples were duplicated, and loaded in 96-wells plates along with positive and negative controls as described above using...
LightCycler® 480 SYBR Green I Master, 200 nM of forward primer (5′-ACGATTTGGCTGAGGACGAT-3′), 200 nM of reverse primer (5′-TCTCTATTCCATTCTTTGTCACTCTTC-3′) and Template DNA (1 µL) with 12 µL of total volume. The thermal cycling conditions were 50 °C for 2 min, 95 °C for 10 min, and 50 cycles of 95 °C for 15 s and 60 °C for 1 min, 95 °C for 5 s, 65 °C for 1 min, and 97 °C for 5 s (Additional file 1: Table S1) [27]. The threshold cycle (CT) value set was the same for all reactions. The LOD of the pfldh qPCR assays used for selection of samples for pfhrp2/3 PCR was ≥ 3 × 10⁻³ ng/µL of DNA.

Detection of pfhrp2/3 gene deletions

Pfhrp2 and pfhrp3 PCR genotyping was performed as previously described [26], with minor modifications using conventional single step PCR with primers targeting exon 2 of the genes. Selected samples were used to amplified pfhrp2 PCR using One Taq 2× Master Mix with standard buffer, DNA template (3 µL), 400 nM of forward primer (5′-CAAAAGGACTTATAATTTAGAG-3′), 400 nM reverse primer (5′-AATAATTTAATGGCGTAGGCA-3′) in a 25 µL final volume. pfhrp3 PCR was performed using One Taq 2× Master Mix with standard buffer, DNA template (3 µL), 400 nM of forward primer (5′-AATGCAAAAGGACTTTAATTTC-3′), 400 of nM reverse primer (5′-TGGTGTAAGTGATGCGTA GT-3′) in a 25 µL final volume with reaction conditions 95 °C for 10 min and 45 cycles of 94 °C for 50 s, 55 °C for 50 s and 70 °C for 1 min (Additional file 1: Table S1) [27]. Genomic DNA from 3D7 (pfhrp2/3 positive), Dd2 (pfhrp2 negative) and HB3 (pfhrp3 negative) were used as controls. PCR products were visualized under UV light on 1.5% agarose gels run at 100 V for 30 min and stained with Gel Red® solution (Biotium, California, USA) for 30 min.

Statistical analyses

Data was analysed using STATA version 14.2 (College Station, Texas, USA). Descriptive variables are presented as proportions (categorical variables) or median and interquartile range (continuous variables). Chi-square tests (or Fisher’s exact tests when appropriate) were used to assess associations between categorical variables and pfhrp2/3 gene deletion prevalence. Sensitivity (= true positive/(true positive + false negative),
specificity (true negative/(true negative + false positive)), Positive predictive value (true positive/(true positive + false positive) and negative predictive value (true negative/(true negative + false negative)) of RDTs were calculated using PCR and microscopy as the gold standard. Agreement between diagnostic techniques was assessed using Cohen's kappa coefficient. The sensitivity and the specificity of RDTs and microscopy at densities between $1 \times 10^{-4}$ ng/μL and $3 \times 10^{-3}$ ng/μL and those greater than $3 \times 10^{-3}$ ng/μL of extracted DNA was assessed [27]. P-values of below 0.05 were considered significant.

Results

Socio-demographic characteristics of the participants and malaria diagnosis

462 school-age children, of which 266 were asymptomatic, and 196 were symptomatic were enrolled. Of the 266 asymptomatic children, 136/266 (51%) were female, 147/266 (55%) were between the ages of 6 and 9 and 168/266 (63%) lived in rural areas. Of the 196 symptomatic children, 94/196 (48%) were female, 132/196 (67%) were between the ages of 6 and 9 and 102/196 (52%) lived in rural areas (Table 1).

Comparison of RDT with PCR and microscopy

Among 266 DNA samples from asymptomatic children, 174/266 (65%), 187/266 (70%) and 130/266 (49%) were PfHRP2_RDT, pfldh-qPCR and microscopy positive, respectively. The sensitivity and specificity of RDTs compared to PCR were 150/187 (80%; 95% CI 74, 86) and 55/79 (70%, 95% CI 58, 80) while the sensitivity and specificity of RDTs compared to microscopy were 119/130 (92%, 95% CI 85, 96) and 81/136 (60%, 95% CI 51, 68), respectively. Agreement between PfHRP2-based RDTs and PCR was moderate (Cohen's kappa = 0.48) as was the agreement between PfHRP2-based RDTs and microscopy (Cohen's kappa = 0.51) (Table 2).

Among 196 DNA samples from symptomatic infections, 131/196 (67%), 171/196 (87%) and 122/196 (62%) were PfHRP2-based RDTs, pfldh-qPCR and microscopy positive, respectively. The sensitivity and specificity of RDTs compared to PCR were 128/171 (75%, 95% CI 69, 98) while sensitivity and specificity of RDTs compared to microscopy were 114/122 (93%, 95% CI 88, 97) and 57/74 (77%, 95% CI 66, 86), respectively. Findings showed satisfactory agreement between PfHRP2-based RDTs and microscopy (Cohen's kappa = 0.72) and fair agreement between PfHRP2-based RDTs and PCR (Cohen's kappa = 0.37) (Table 2).

Performance of RDT and microscopy examinations based on parasite densities

The sensitivity of RDTs and microscopy at lower limits of parasite density below $3 \times 10^{-3}$ ng/μL of extracted DNA, and those above $3 \times 10^{-3}$ ng/μL were compared. The sensitivity and specificity of RDTs were 96% (95% CI 92, 98) (symptomatic: 93% (87, 97); asymptomatic: 100% (95, 100) and 37% (95% CI 31, 45) [symptomatic: 55% (42, 67); asymptomatic: 31% (23, 40)] while the sensitivity and specificity of microscopy were 91% (symptomatic: 90%; asymptomatic: 94%) and 59% (symptomatic: 65%; asymptomatic: 56%) (Table 3).

Detection of pfhrp2/3 gene deletions

A conservative criterion for the detection of pfhrp2/3 gene deletions was used through the selection of samples with DNA concentrations three times higher than the limit of detection of the pfhrp2/3 PCR assays. Of 462 DNA samples, 173 were selected for pfhrp2/3 PCR analysis following pfldh qPCR. Of the 173 isolates used for pfhrp2/3 PCR, three were pfhrp2 negative and one was pfhrp3 negative (Fig. 2).

The overall prevalence of the pfhrp2 gene deletion was 2% (3/173) while it was 1% (1/173) for the pfhrp3 gene. All four samples that contained these mutant parasites

| Variables | Number (%) |
|-----------|------------|
| Symptomatic infection | |
| Sex | |
| Female | 94 (48) |
| Male | 102 (52) |
| Age med. (IQR) | |
| 6–9 | 8 (7–11) |
| 10–14 | 64 (33) |
| Location | |
| Rural | 102 (52) |
| Urban | 94 (48) |

IQR interquartile range, med. median

Table 1 Socio-demographic characteristics of asymptomatic (N = 266) and symptomatic (N = 196) participants

| Variables | Number (%) |
|-----------|------------|
| Asymptomatic infection | |
| Sex | |
| Female | 136 (51) |
| Male | 130 (49) |
| Age med. (IQR) | |
| 6–9 | 147 (55) |
| 10–14 | 119 (48) |
| Location | |
| Rural | 168 (63) |
| Urban | 98 (37) |

IQR interquartile range, med. median

Table 3 Performance of RDT and microscopy examinations based on parasite densities
had returned positive RDT results. Only 7 RDT negative samples had sufficient parasite densities for pfhrp2/3 deletion, and none of these had pfhrp2/3 gene deletions (Table 4).

Prevalence of pfhrp2/3 gene deletion by age, sex, health status and location
Among the three samples that harboured pfhrp2 gene deletions, two were from children aged 6 to 9 years, and all three were from female children, asymptomatic individuals and children living in the urban area. Age, sex, children health status and location were not associated to pfhrp2/3 gene deletion. No significant associations were found between pfhrp2/3 prevalence and age, sex, health status and location (p > 0.05, Additional file 1: Table S2).

Discussion
Malaria rapid diagnostic tests play an important role in malaria case management and surveillance. Based on several reports that assessed the prevalence of pfhrp2/3 gene deletions, the WHO has recently recommended continuous surveillance of Pfhrp2/3-deleted P. falciparum [17, 28, 29]. This study used a rigorous method of DNA sample selection for evaluation of Pfhrp2/3-deleted P. falciparum [23, 27], which minimizes the overestimation of pfhrp2/3-deleted P. falciparum that may occur through conventional approaches [22, 30, 31]. It is important to consider DNA quantity in samples subjected to PCR to identify pfhrp2/3 deletions, as low DNA levels may lead to false pfhrp2-negative results and overestimation of the prevalence of pfhrp2/3 gene deletions.

Three isolates harbouring a pfhrp2 gene deletion and one isolate harbouring a pfhrp3 gene deletion were found among pfhrp2-based RDT positive samples. The two pfhrp2 negative samples were presumably positive by pfhrp2-based RDT due to cross reaction with PfHRP3 [20, 32, 33]. The sample harbouring a pfhrp3 gene deletion was from a symptomatic child while the three samples harbouring pfhrp2 gene deletions were from asymptomatic children. It has been shown that pfhrp2/3-deleted parasites do not differ from wild-type parasites in their ability to cause malaria symptoms [34]. Previous studies conducted in the DRC have found a pfhrp2 gene deletion prevalence of 6.4% across the

### Table 2

| PfHRP2_RDTs | Asymptomatic infections | Microscopy |
|-------------|-------------------------|------------|
|             | Pfdh_qPCR               |            |
|             | Positive | Negative | Total | Positive | Negative | Total |
| Positive    | 150      | 24       | 174   | 119      | 55       | 174   |
| Negative    | 37       | 55       | 92    | 11       | 81       | 92    |
| Total       | 187      | 79       | 266   | 130      | 136      | 266   |
| Se (%) (CI 95%) | 80 (74, 86) | 92 (85, 96) |
| Sp (%) (CI 95%) | 70 (58, 80) | 60 (51, 68) |
| PPV (%) (CI 95%) | 86 (81, 90) | 84 (81, 87) |
| NPV (%) (CI 95%) | 60 (52, 68) | 75 (63, 84) |
| Kappa*     | 0.48, p < 0.001 |

| PfHRP2_RDTs | Symptomatic infections | Microscopy |
|-------------|------------------------|------------|
|             | Pfdh_qPCR              |            |
|             | Positive | Negative | Total | Positive | Negative | Total |
| Positive    | 128      | 3        | 131   | 114      | 17       | 131   |
| Negative    | 43       | 22       | 65    | 8        | 57       | 65    |
| Total       | 171      | 25       | 196   | 122      | 74       | 196   |
| Se (%) (CI 95%) | 75 (68, 81) | 93 (88, 97) |
| Sp (%) (CI 95%) | 88 (69, 98) | 77 (66, 86) |
| PPV (%) (CI 95%) | 98 (94, 99) | 97 (95, 98) |
| NPV (%) (CI 95%) | 34 (28, 41) | 64 (47, 78) |
| Kappa*     | 0.37, p < 0.001 |

Se sensitivity, Sp specificity, PPV positive predictive value, NPV negative predictive value, CI confidence interval

*Statistical analysis using Cohen's kappa coefficient test, significance at p < 0.05
country and 21.9% in Kinshasa in a nationwide demographic and health survey among asymptomatic children [22] and 9.2% amongst symptomatic individuals in a neighbouring province of Kinshasa [24]). This difference may be explained by different methods used for the detection of *Pfhrp2/3* deletions. A previous study

| DNA concentration | Overall   | RDTs                  | Microscopy            |
|-------------------|----------|-----------------------|-----------------------|
|                   |          | Positive | Negative | Total   | Positive | Negative | Total   |
| 1 × 10⁻⁴–3 × 10⁻³ ng/µL | 112      | 73       | 185      | 75      | 110      | 185      |
| ≥ 3 × 10⁻³ ng/µL  | 166      | 7        | 173      | 158     | 15       | 173      |
| Total             | 278      | 80       | 358      | 233     | 125      | 358      |
| Se (%) (CI 95%)   | 96 (92, 98) | 37 (31, 45) | 91 (86, 95) | 59 (52, 67) |
| Sp (%) (CI 95%)   | 84 (83, 86) | 73 (56, 85) | 89 (87, 90) | 67 (55, 77) |

| DNA concentration | Asymptomatic infections | RDTs                  | Microscopy            |
|-------------------|-------------------------|-----------------------|-----------------------|
|                   |                         | Positive | Negative | Total   | Positive | Negative | Total   |
| 1 × 10⁻⁴–3 × 10⁻³ ng/µL | 82        | 37       | 119      | 52      | 67       | 119      |
| ≥ 3 × 10⁻³ ng/µL  | 68         | 0        | 68       | 64      | 4        | 68       |
| Total             | 150        | 37       | 187      | 116     | 71       | 187      |
| Se (%) (CI 95%)   | 100 (95, 100) | 31 (23, 40) | 94 (86, 98) | 56 (47, 65) |
| Sp (%) (CI 95%)   | 78 (75, 80) | 100      | 84 (81, 86) | 80 (61, 91) |

| DNA concentration | Symptomatic infections | RDTs                  | Microscopy            |
|-------------------|------------------------|-----------------------|-----------------------|
|                   |                         | Positive | Negative | Total   | Positive | Negative | Total   |
| 1 × 10⁻⁴–3 × 10⁻³ ng/µL | 30        | 36       | 66       | 23      | 43       | 66       |
| ≥ 3 × 10⁻³ ng/µL  | 98         | 7        | 105      | 94      | 11       | 105      |
| Total             | 128        | 43       | 171      | 117     | 54       | 171      |
| Se (%) (CI 95%)   | 93 (87, 97) | 55 (42, 67) | 90 (82, 95) | 65 (54, 77) |
| Sp (%) (CI 95%)   | 93 (91, 95) | 55 (36, 72) | 95 (93, 96) | 48 (34, 62) |

Se sensitivity, Sp specificity, PPV positive predictive value, NPV negative predictive value, CI confidence interval

| Table 4  | Prevalence of *pfhrp2/3* gene deletion based on *PfHRP2_RDT* results (N = 173) |
|----------|---------------------------------------------------------------------------------|
| RDTs     | *pfhrp2_PCR*                        | *pfhrp3_PCR*                        |
| Positive | Positive | Negative | Total | Positive | Negative | Total | Positive | Negative | Total |
| n (%)    | n (%)    | n (%)    |       | n (%)    | n (%)    | n (%)  | n (%)    | n (%)    | n (%) |
| Positive | 163 (98) | 3 (2)    | 166 (100) | 165 (99) | 1 (1)    | 166 (100) | 167 (98) | 3 (2)    | 170 (100) |
conducted in the DRC using a similar method of selection of samples with sufficient parasite DNA for the detection of Pfhrp2/3 gene deletions, did not find any isolates harbouring pfhrp2/3-deletions among symptomatic children [23] highlighting the fact that the method used in the previous large survey of asymptomatic parasite carriers [22] may have overestimated the prevalence of the pfhrp2 gene deletion.

Seven isolates were negative by RDT, but positive by qPCR with over $3 \times 10^{-3}$ ng of parasite DNA per µL of extracted DNA solution. Five of these samples were negative by microscopy, suggesting relatively low parasitaemia. RDT failure in these cases may be explained by data recording errors, operator-dependent and manufacturing quality [35–37] or by the presence of anti-pfhrp2 antibodies binding to the circulating antigens [38] or possibly due to the presence of mixed infection pfhrp2-negative and pfhrp2-positive parasites in the same isolates [39].

Among 196 isolates from symptomatic children, the sensitivity of PfHRP2-based RDTs compared to pfldh-qPCR was 75%. Of 43 pfhrp2 PCR positive isolates, 36 (84%) had lower than $3 \times 10^{-3}$ ng/µL of extracted DNA, highlighting the fact that RDTs are less sensitive at low parasitaemia compared to PCR [21]. This may exclude some symptomatic children from treatment [26].

Among 266 isolates from asymptomatic children, the sensitivity of PfHRP2-based RDTs compared to pfldh-qPCR was 82%. All 37 RDT negative PCR positive isolates had below $3 \times 10^{-3}$ ng/µL of extracted DNA, highlighting the importance of the use of PCR for the diagnosis of asymptomatic malaria parasite carriers [26, 40–44]. However, for malaria case management, PCR may be prohibitively expensive, time-consuming and technically challenging especially in remote locations [45, 46]. There is a need to develop a more cost-effective highly sensitive malaria diagnostic test suitable for remote areas [45].

Although the samples used in this study may not be representative of the country as a whole, the method used minimized overestimation of the prevalence of P. falciparum parasites carrying pfhrp2/3-deletions, which may occur with conventional methods.

Conclusion
The prevalence of P. falciparum parasites carrying deletions of the pfhrp2/3 gene is low in the population surveyed in this study, suggesting the use of PfHRP2-based RDTs remains appropriate for the detection of malaria in this region. The continuous use of rigorous PCR methods for surveys of pfhrp2/3 gene deletion prevalence is, therefore, encouraged.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12936-022-04153-2.

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Author contributions
Conceptualization: SSN, TY, RC. Data curation: SSN, RC. Formal analysis: SSN, RC. Formal analysis: SSN, RC. Methodology: SSN, RC, TY, TM. Contributed materials: RC, BYAC. Supervision: RC, TY, SA, JJM. Writing—original draft: SSN. Writing—review and editing: SSN, RC, TY, MT, YBM, SVS, HA. Laboratory works: SSN, SVS, HA, RC, BYAC. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the first author (SSN).

Declarations
Ethics approval and consent to participate
The study was approved by the ethics committees of the School of Public Health, University of Kinshasa, DRC (Approval number: ESP/CE/042/2019) and the Institute of Tropical Medicine, Nagasaki University (Approval number: 190110208-2).

Consent for publication
All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Programme for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan. 2 Department of International Health and Medical Anthropology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan. 3 Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo. 4 Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan. 5 Division of Global Epidemiology, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan. 6 Department of Tropical Medicine and Parasitology, Juntendo University, Tokyo, Japan. 7 Division of Molecular Parasitology, Proteo-Science Center, Ehime University, Ehime, Japan.

Additional file 1: Table S1. Primer sequences and PCR conditions for P. falciparum ldh, hrp2/3 PCR amplification. Table S2. Prevalence of P. falciparum hrp2/3 gene deletion by age, sex, health status and location (N = 173).
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