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Malaria Prevalence, Associated Risk factors, and Molecular Surveillance of Plasmodium falciparum hrp2/hrp3 Gene Deletions in Northern Uganda

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Abstract

Background

Malaria remains a leading cause of mortality and morbidity in Uganda, where *Plasmodium falciparum* accounts for the 98% of malaria infections. Although malaria rapid diagnostic tests (mRDTs) based on histidine-rich protein 2/3 (PfHRP2/3) are widely used, deletions in these genes can compromise diagnostic accuracy and lead to false-negative results. This study investigated the prevalence of malaria, evaluated the performance of PfHRP2-based mRDTs, and assessed the occurrence of PfHRP2/3 gene deletions, along with associated risk and behavioral factors among symptomatic patients in Northern Uganda.

Methods

A cross-sectional study was conducted between June and August 2024 in six health facilities across the Acholi and Lango sub-regions. A total of 424 participants were enrolled using systematic random sampling. Malaria diagnosis was performed using mRDTs, microscopy, and polymerase chain reaction (PCR). Data on socio-demographic, behavioral, and risk factors were collected using structured questionnaire. Descriptive statistics were used to summarize study variables. Chi-square tests assessed associations between categorical variables and malaria infection status. Variables significant at Bivariable analysis were entered into multivariable logistic regression models to identify predictors of malaria infection, while $\log_{10}$-transformed parasite density data were analyzed using linear regression.

Results

The overall malaria prevalence in Northern Uganda was 30.2% (95% CI: 25.9–34.8) highest in rural districts, notably Dokolo HCIV (40%) and Aboke HCIV (31%) in Lango, and Anaka GH (30.3%) and Awach HCIV (32%) in Acholi. PfHRP2-based mRDTs demonstrated high diagnostic accuracy (98.53%; 95% CI: 94.79–99.82), and no PfHRP2/3 gene deletions were detected. Malaria positivity was highest among children aged 5–9 years (50%), followed by individuals aged 10–24 years (37.1%). Multivariable logistic regression analysis identified age, sex (female: AOR = 3.89; 95% CI: 1.61–10.2; p = 0.004), and travel history (AOR = 2.85; 95% CI: 0.98–8.66; p = 0.056) as key factors associated with malaria infection.

Conclusion

Malaria prevalence remains high in rural Northern Uganda, while PfHRP2-based mRDTs remain reliable due to the absence of PfHRP2/3 deletions. Demographic and behavioral factors significantly influence malaria risk, underscoring the need for continued molecular surveillance and targeted interventions to strengthen malaria diagnosis and control in Uganda.

Background

Worldwide, malaria cases increased from 247 million in 2022 to 263 million in 2023, with approximately 94% of cases concentrated in 29 African nations. The African Region continues to bear the greatest burden, with East Africa contributing a major share. Within the region, Uganda ranks third globally, accounting for 5% of global malaria cases and an annual incidence of 60.4 cases per 1,000 populations [1]. Ethiopia follows with 3.6% of global cases, while Tanzania, Burundi, and South Sudan report some of the highest incidence rates in the region, exceeding 250 cases per 1,000 populations [1]. In Uganda, malaria remains a major public health burden, accounting for approximately 30–40% of outpatient visits, 20% of hospital admissions, and about 10% of inpatient deaths [2]. The malaria-associated hospitalization rate is estimated at about 6%, which is nearly four times higher than the global average reported in 2023[1]. Malaria prevalence is unevenly distributed across the country, with the northern and eastern regions bearing the highest burden [2] *Plasmodium falciparum* is the predominant species, responsible for approximately 98% of infections.

Despite the implementation of key control strategies, including long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), intermittent preventive treatment in pregnancy (IPTp), and the “Test and Treat policy-malaria transmission [3–5] Variations in transmission intensity are influenced by factors such as urbanization, inconsistent intervention coverage and environmental conditions. Additionally, certain population groups, including travelers and vulnerable demographics face increased risks due to factors such as age, sex, locality, housing conditions, and proximity to mosquito breeding sites [6]. Continued surveillance through regular population-based surveys is therefore essential to inform policy decision and guide targeted malaria control interventions (Malaria Indicator Survey, 2018).

Microscopy is the primary gold standard for malaria diagnosis; however, its utilization remains a challenge in resource-limited settings due to high costs, limited access to electricity and a shortage of trained personnel [7]. While malaria rapid diagnostic tests (mRDTs) are essential in such settings because of their affordability and ease of use [7], their reliability is increasingly threatened by *pfhrp2/3* gene deletions [8]. These genetic mutations result in false-negative RDT results, allowing infections to go undetected, thereby fueling further transmission [8]. Although PfHRP2/3-based RDTs have significantly improved the detection of *P. falciparum*, including low-density infections, their reliability is increasingly undermined by poor storage conditions, operator errors, and supply chain disruptions contribute to reduced test performance [9]. However, unlike these quality-related issues, PfHRP2/3 gene deletions represent a biological threat that cannot be predicted without targeted molecular investigations. First reported in Peru, the prevalence of *pfhrp2* deletions rose from 13% between 1998 and 2001 to over 40% between 2003 and 2005 [10][11]. Subsequent studies have confirmed the presence of these deletions across South America, Asia, and Africa, prompting WHO to recommend routine global surveillance[12].

The reported prevalence varies widely, with estimates of approximately 43% in South-Central America, 3–4% in Africa, and 1–3% in Asia [13]. Notably, extremely high prevalence has been reported in Eritrea (up to 80%) and Ethiopia (4–95%). exceeding the WHO-recommended threshold of < 5% [14]. False

negative RDT results allow undetected parasites to persist, facilitating ongoing transmission. Evidence suggests that PfHRP2-deleted parasite strains may exhibit higher transmission efficiency, with transmission rates reported to reach up to 80% compared to approximately 30% in non-deleted strains [15, 16].Across Africa, Pfhrp3 deletions have been reported in Nigeria (13.3%) Sudan (3.6%), and South Sudan (5.9%), although dual Pfhrp2/3 deletions were not consistently detected in all settings [17]. Similarly, studies in the Democratic Republic of Congo (5.4%) and Tanzania(1.6%) have reported PfHRP2 gene deletion, alongside PfHRP3 deletions in approximately 50% of the samples analyzed [18]. In Uganda, the prevalence of *PfHRP2/3* gene deletions has been reported to range from 3.3–14.7%, with Eastern Uganda exceeding the WHO-recommended 5% threshold for reconsidering PfHRP2-based mRDT use [19]. Although Northern Uganda previously reported a relatively low prevalence (~ 0.2%) of *pfhrp2/3* deletions [20], the region remains a high malaria transmission setting compared to many other endemic regions in Uganda [4]. In addition, available data from Northern Uganda remain geographically limited and insufficient for continuous surveillance in the context of ongoing malaria transmission and widespread reliance on PfHRP2-based mRDTs.

False-negative mRDT results due to *pfhrp2/3* deletions can delay treatment, sustain transmission, and increase the risk of severe malaria outcomes[21]. Such diagnostic inaccuracies also undermine malaria case management, reduce confidence in diagnostic tools, and weaken malaria control efforts. These concerns highlight the need for continued molecular surveillance and regular evaluation of mRDT performance in high-transmission settings. This study therefore assessed malaria prevalence, evaluated the diagnostic performance of PfHRP2-based mRDTs, determined the occurrence of *pfhrp2/3* gene deletions, quantified parasite density, and examined demographic, behavioral, and other risk factors associated with malaria infection in Northern Uganda.

Methods

Study Area

The study was conducted in the districts of Nwoya, Gulu, Kole, Dokolo, as well as Gulu city in Acholi sub-region and Lira city in Lango sub-region (Fig. 1). Data collection was conducted in six health facilities: in Lango, Dokolo Health Centre IV, Aboke Health Centre IV, Lira Regional Referral Hospital, and in Acholi, Gulu Regional Referral Hospital, Awach Health Centre IV and Anaka General Hospital. These health facilities were purposively selected based on high malaria positivity rates (28–62%) as reported in the DHIS-2 data 2023, as well as their large catchment population within the Lango and Acholi sub regions.

Study Design and sample size determination

This was a descriptive cross-sectional study conducted between June and August 2024, a period corresponding to the peak rainy season and high malaria transmission in Uganda. Malaria infection was diagnosed using a combination of malaria rapid diagnostic tests (mRDTs), microscopy, and polymerase

chain reaction (PCR), which were also used to assess *PfHRP2/3* gene deletions. In addition, structured questionnaires were administered to collect data on socio-demographic characteristics and potential behavioral and other risk factors associated with malaria infection. The sample size was determined using the Kish–Leslie formula (1965) for cross-sectional studies, assuming an unknown malaria prevalence of 50% (P = 0.5), a 95% confidence level (Z = 1.96), and a 5% margin of error (d = 0.05);

$n = Z^2 *P*(1-P) / d^2$, n is the minimum required sample size, Z is the standard normal deviate corresponding to 1.96 for 95% confidence interval, P is the estimated prevalence of malaria in the population and d is the margin of error set at 0.05(5%). Substituting these values in the formula; $n = 1.96^2$ x0.5 (1-0.5)/0.05^2

= 384.16 participants

The study sample was adjusted to 385 participants, with an additional 10% (39 participants) included to account for non-response, resulting in a final sample size of 424 participants. These participants were proportionally distributed across study sites. A total of 424 patients presenting with malaria symptoms were subsequently enrolled from six selected health facilities across the two study regions.

Inclusion and exclusion criteria

Participants were eligible for the study if they had symptoms of malaria such as fever, loss of appetite and vomiting, diarrhea, weakness, headache, muscle or joint pains, chills and fatigue, anemia, dehydration, cerebral symptoms (coma/fits) and respiratory distress[22]. The study population consisted of individuals with confirmed malaria infection to ensure diagnostic rigor and minimize the inclusion of misdiagnosed clinical cases. Patients who were on antimalarial treatment or who had been treated with antimalarial within the past two weeks were excluded from the study.

Sampling procedure for the selection of the participants

Following clinical examination and referral for laboratory testing, informed consent was obtained from all illegible participant or their guardians, with written consent forms provided to confirm participation. Systematic random sampling was employed to recruit participants using a sampling frame based on the average monthly suspected malaria cases reported at each facility, ensuring proportional allocation to the sample size. Briefly, every N^{th} person (N= Study sampling frame /study area sample size) recorded in the outpatient register and meeting the selection inclusion criteria was enrolled in the study. In cases where the selected patient or guardian declined to provide consent, the next eligible person who consented was considered for inclusion. This process continued until the required sample size was attained at each health facility. Blood sample collection of from study participants was carried out at the following health facilities: Aboke Health Centre IV (58 participants), Anaka General Hospital (76), Awach

Health Centre IV (75), Dokolo Health Centre IV (110), Gulu Regional Referral Hospital (66), and Lira Regional Referral Hospital (39).

Blood specimen collection and examinations

Blood specimen collection

Blood samples were collected from eligible patients by finger prick, preferably from the ring or middle finger, after cleansing the puncture site with a 70% alcohol swab and allowing it to air dry. A sterile lancet was then used to collect sufficient blood for Malaria Rapid Diagnostic Tests (mRDTs), preparation of thick blood smears for microscopy, and dried blood spots (DBS) on filter paper for polymerase chain reaction (PCR) analysis. Prior to collection and testing, all devices including RDT kits, blood slides, and DBS cards were labeled with each patient's unique identification number to ensure accurate sample tracking. All sample collection and laboratory testing procedures were carried out by trained and qualified laboratory personnel under the supervision of senior laboratory technologists stationed at each study site.

Malaria rapid diagnostic test

Malaria test was conducted onsite using the SD Biosensor Standard Malaria Ag. Pf HRP-RDT test (lot number 507566101), which has a validated sensitivity of 99.38% (322/324, 95% CI: 97.79–99.93%) and specificity of 100% (256/256, 95% CI: 98.57–100%) [23]. Approximately 5 µL capillary blood sample obtained through a finger prick was transferred into the sample well of the test device using either a sterile loop or micropipette. Following this, 90 µL (three drops) of assay diluent was added into the designated buffer well, facilitating sample migration via capillary action. Test results were visually assessed within 15 to 20 minutes and recorded either as positive or negative depending on the outcome.

Malaria microscopic examination

A clean glass slide was labeled with the patient's unique identification number and recorded in the laboratory logbook. A finger-prick capillary blood sample was carefully dropped onto the labelled glass slide and spread to approximately 10 millimetre in diameter using an applicator stick to make a thick blood film. The slides were air-dried prior to staining with 10% Giemsa solution for 25 minutes [24] .Following staining, the slides were rinsed gently with clean water and allowed to air-dry once more before microscopic examination. The resulting blood films were subsequently examined microscopically under a 100x oil immersion objective lens to identify malaria parasites. A sample was considered positive when one or more asexual stages of *Plasmodium* parasite, including ring-form trophozoites, schizonts, or gametocytes were detected. A smear was classified as negative if no asexual *Plasmodium* stages were detected after approximately 10 minutes of examination across 200 oil immersion fields. Each slide was independently reviewed by a second microscopist, and any discrepancies in results were resolved by a third microscopist who was trained and certified by WHO. To ensure the quality and

reliability of microscopy results, quality control procedures included filtering the Giemsa stain and pre-testing known malaria-positive and malaria-negative smears using the same standard protocol applied to routine patient samples prior to examination of the study samples. Samples that previously tested negative with *PfHRP2*-based mRDTs were further analyzed using nested PCR assays targeting the *PfHRP2* and *PfHRP3* genes to detect possible gene deletions associated with false-negative RDT results.

Extraction of parasite DNA

DNA from malaria parasites was extracted from dried blood spot (DBS) samples, each consisting of a single 6 mm punch using a modified Tween-Chelex 100 protocol (Bio-Rad Laboratories, USA) as previously described by [25]. Briefly, DBS punches were incubated overnight at 4°C in 1 mL of 0.05% Tween-20 (Sigma) prepared in phosphate-buffered saline (PBS, Thermo Fisher Scientific). Once the filter paper discs turned white or cream, they were placed on a low-speed shaker to facilitate the release of parasite DNA from the red blood cells. The samples were then centrifuged for 5 minutes, after which the Tween-PBS solution was discarded. The discs were subsequently washed with 1 mL of 1X PBS and incubated for an additional 30 minutes at 4°C. Following centrifugation, PBS was discarded and 150 µL of 10% Chelex 100 resin (BioRad Laboratories, Hercules, CA, USA) prepared in distilled water was added to each sample. The mixture was then incubated at 95°C for 10 minutes in a heat block with intermittent vortexing, prior to the first two vortex cycles, the tubes were briefly uncapped to release built-up pressure. The samples were then centrifuged at high-speed for 5 minutes to sediment the Chelex resin and filter paper. The resulting supernatants, containing approximately 150 µL of DNA, were carefully transferred into clean micro centrifuge tubes and centrifuged again at 14,000 rpm for 10-minute. The final Chelex-free supernatants (about 50 µL) were then collected and stored at − 20°C until PCR analysis. Prior to the final PCR amplification, DNA integrity was evaluated by amplifying a target region from the extracted samples. The PCR cycle consisted of an initial denaturation step at 95°C for 5 minutes, followed by 30 amplification cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 2 minutes, and extension at 72°C for 2 minutes, concluding with a final 5 minutes extension at 72°C [26].

Confirmation of Plasmodium falciparum parasite DNA

Plasmodium species were identified at the Infectious Diseases Research Collaboration (IDRC) Laboratory in Tororo, Uganda using PCR-based methods. Initial detection was performed using genus-specific quantitative PCR (qPCR) targeting *Plasmodium* DNA, followed by species confirmation using nested PCR targeting the *Plasmodium* 18S rRNA gene, which allows differentiation of *Plasmodium* species, as described by [27].

PCR-based Genotyping of PfHRP2/3 deletions

Genotyping to detect *Plasmodium falciparum* histidine-rich protein 2 and 3 (*PfHRP2/3*) gene deletions was carried out using a nested PCR protocol. Primers specific to *PfHRP2* and *PfHRP3* (Table 1) were sourced from Microgen M Inc., Seoul, South Korea, while PCR reagents were procured from Africa's Genomics Company Inqaba Biotec East Africa, Uganda Branch. Amplification of exon 2 and its adjacent

regions of the *PfHRP2/3* genes were performed using nested PCR. In the first amplification round (Nest-1 PCR), primers targeted the repetitive sequences within the highly variable regions of the genes. The second amplification round employed primers focused on the intronic region to enhance specificity and sensitivity of detection. Expected amplicon sizes for the Nest-1 PCR ranged from 400 to 1050 base pairs, with absence or alteration of the expected bands suggestive of potential PfHRP2/3 gene deletions [18].

Table 1
Primers for *Plasmodium falciparum* Histidine-Rich protein 2/3 gene deletion

Primers for pfHRP2/3 deletion detection	
Primers for nest-1 PCR	Pfhrp2-F1 (5'-CAAAAGGACTTA ATTTAAATAAGAG-3')
	Pfhrp2-R1 (5'-AATAAATTTAATGGCGTAGGCA-3')
Primers for Nest-2 PCR	Pfhrp2-F2 (5'-ATTATTACACGAAACTCAAGCAC-3')
	Pfhrp2-R1 (5'-AATAAATTTAATGGCGTAGGCA-3').
Primers for Nest-1 PCR	Pfhrp3-F1 (5'-AATGCAAAAGGACTTAAT TC-3')
	Pfhrp3-R1 (5'-TGGTGTAAGTGATGCGTAGT-3')
Primers for Nest 2 PCR	Pfhrp3-F2 (5'-AAATAAGAGATTATTACACGAAAG-3')
	Pfhrp3-R1 (5'-TGGTGTAAGTGATGCGTAGT-3')

Data analysis

Prevalence of malaria, diagnostic accuracy and evaluation of parasite density

Descriptive analysis was performed to describe the participant's demographic characteristic and determine the proportion of parasitaemia in the samples. The diagnostic performance of PfHRP2-based malaria rapid diagnostic tests (mRDTs) and blood smear microscopy was evaluated using PCR as the reference standard. Diagnostic accuracy was assessed using standard epidemiological parameters including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Sensitivity was defined as the proportion of true malaria-positive cases correctly identified by the test (TP / [TP + FN]), whereas specificity represented the proportion of true negative cases accurately detected (TN / [TN + FP]). The PPV was calculated as the proportion of positive test results that were true positives (TP / [TP + FP]), while NPV was determined as the proportion of negative test results that were true negatives (TN / [TN + FN]). Parasite density was quantified using quantitative PCR, expressed per microliter.

Questionnaire data were compiled in an Excel spreadsheet, subjected to cleaned, and analyzed to identify risk and behavioral factors linked to malaria infection among confirmed cases. Data was entered

into a Microsoft Excel sheet, coded, checked for completeness and reconciled any possible errors and exported to R4.5.1 software for statistical analysis at the 5% level of significance.

Descriptive statistics were used to summarize malaria prevalence, parasite density, and participants' demographic characteristics across the six study sites in the Acholi and Lango sub-regions. Frequencies and percentages were used for categorical variables, while median and interquartile ranges (IQR) were used for continuous variables. Chi-square (χ^2) tests assessed associations between socio-demographic and behavioral factors and malaria infection status. Variables significant at $P < 0.05$ were entered into a multivariable generalized linear model to identify predictors of malaria infection at a 95% confidence interval.

Prior to regression analysis of parasite density, data normality was assessed using the Shapiro–Wilk test, which showed significant non-normality ($W = 0.492$, $p < 0.001$), with marked positive skewness (3.13) and kurtosis (9.75). Therefore, parasite density was $\log_{10}$-transformed [$\log_{10}$(parasite density + 1)] before regression analysis, resulting in improved normality ($W = 0.937$, $p < 0.001$).

Results

Socio-demographic attributes of the study group

A total of 424 blood samples were obtained from study participants in the Lango and Acholi sub-regions. Of these, 243 (57.3%) were female and 181 (42.7%) were male, giving a female-to-male ratio of 1.3:1. The mean age of participants was 21.2 ± 17.8 years. Females had a significantly higher mean age than males (23.2 ± 17.3 vs. 16.2 ± 18.2 years; $p = 0.00039$). Most participants were aged 10–24 years (n = 151), followed by those aged 0–5 years (n = 100) and 25–45 years (n = 94). The distribution of gender differed significantly across age groups (χ^2 test, $p < 0.001$).

Prevalence of malaria in selected health facilities in Acholi and Lango sub regions

The overall malaria prevalence was 30.2% (95% CI: 25.9–34.8). Prevalence was comparatively lower in the two regional referral hospitals in Gulu and Lira cities (Table 2), remaining below 20% in both facilities. Gulu Regional Referral Hospital (RRH) reported a prevalence of 19.7% (95% CI: 11.3–31.7), while Lira RRH recorded the lowest prevalence at 15.4% (95% CI: 6.4–31.2). In contrast, district-level health facilities exhibited substantially higher prevalence estimates, ranging from 28.9% to 40%. Dokolo and Gulu districts recorded the highest prevalence, at 40% and 32%, respectively.Overall, routine diagnostic methods demonstrated good performance in detecting malaria infections. However, two false-negative rapid diagnostic test (RDT) results were identified among samples from Anaka General Hospital.

Table 2

Prevalence of P. falciparum infection in the Health facilities in Lango and Acholi sub region

Diagnostic method	Acholi Region			Lango Region			
	Anaka GH	Awach HC IV	Gulu RRH	Aboke HC IV	Dokolo HC IV	Lira RRH	Total (N = 424)
RDT, n (%);95%CI	22 (28.9%; 19.4–40.6)	24 (32%; 22-43.9)	13 (19.7%; 11.3–31.7)	18 (31%; 19.9–44.7)	44 (40%; 30.9–49.8)	6 (15.4%; 6.4–31.2)	127 (30%; 25.7–34.6)
Microscopy, n (%);95%CI	23 (30.3%; 20.5–42)	24 (32%; 22-43.9)	13 (19.7%; 11.3–31.7)	18 (31%; 19.9–44.7)	44 (40%; 30.9–49.8)	6 (15.4%; 6.4–31.2)	128 (30.2%; 25.9–34.8)
PCR, n (%);95%CI	23 (30.3%; 20.5–42)	24 (32%; 22-43.9)	13 (19.7%; 11.3–31.7)	18 (31%; 19.9–44.7)	44 (40%; 30.9–49.8)	6 (15.4%; 6.4–31.2)	128 (30.2%; 25.9–34.8)
Negative but PCR positive	-	-	-	-	-	-	1 (0.2%; 0-1.5)
Positive but microscopy negative	-	-	-	-	-	-	0 (0%; 0-1.1)

Sensitivity and specificity of RDT and Microscopy

The sensitivity and specificity analysis demonstrated that both RDT and microscopy showed high performance when compared with polymerase chain reaction (PCR) as the reference standard. RDTs detected 99.2% of PCR-confirmed cases, while microscopy achieved 100% sensitivity. Although RDT showed a slightly wider confidence interval (95% CI: 95.1–100) compared to microscopy (95% CI: 96.4–100), both methods recorded positive predictive values of 100%, indicating that all positive results were true positives. Only 0.8% of PCR-positive cases were missed by RDTs, whereas microscopy detected all PCR-positive cases.

Table 3
Performance metrics for rapid diagnostic tests (RDTs) with microscopy as the reference standard

Metric	RDT
Sensitivity (95% CI)	88.2 (82.0-92.9)
Specificity (95% CI)	99.3(97.4–99)
PPV (95% CI)	98.5 (94.8–99.8)
NPV (95% CI)	93.7(90.3–96.2)
ROC area 0.94(0.91–0.96)	0.8
False Negative mRDT Rate (% of PCR Positive)	

Pfhrp2/3 Gene Deletions Among Patients with False-Negative Malaria RDT Results Following successful amplification of DNA samples using qPCR techniques, two samples with false-negative RDT results obtained from Anaka General Hospital (sample ID 62 and 107) were selected for further molecular analysis alongside four additional positive control samples (Fig. 2). Nested PCR was performed to detect possible deletions in the *PfHRP2* and *PfHRP3* genes, with both positive and negative controls included throughout the procedure. *PfHRP2* and *PfHRP3* samples. These two cases showed *PfHRP2* amplicon sizes of 600bp and 500bp, and *PfHRP3* levels of 400bp and 600bp, respectively. Although no pfhrp2/3 deletions were detected, the limited number of false negative samples analyzed may have limited the ability of the study to detect rare gene deletions within the study population.

Positive (PC) and negative control (NC) samples were tested alongside RDT false-negative samples (62 and 107), as well as three RDT-positive concordant samples (63, 108, and 109), to assess the potential cross-reactivity of Pf HRP3 RDT assays.

Parasite density analysis by District and Health Facility in Lango and Acholi sub region

The parasite density varied considerably across districts and health facilities (Table 4). Rural health facilities, including Dokolo, Awach (Gulu), Aboke (Kole), and Anaka (Nwoya) Districts, generally recorded moderate parasite densities among most patients, although a smaller proportion exhibited exceptionally high parasitemia. This variation was more pronounced in Gulu District, where patients attending Awach HC IV (n = 24) had a parasite density of 4,094.4 parasites/μL with a very wide interquartile range (IQR) of (1,900 – 22,584 parasites/μL), indicating considerable heterogeneity in parasite burden among patients. In contrast, facilities in Dokolo and Nwoya districts demonstrated relatively narrower distributions, though notable variability in parasite densities was still observed across patients. At Kole District, particularly Aboke HC IV (n = 18), parasite densities showed a more even distribution, with moderate parasitemia and less heterogeneity compared to other districts. Marked differences in parasite densities were also observed between the two regional referral hospitals. Although both facilities recorded median

parasite densities below 200parasites/μL, Gulu RRH showed evidence of a small subset of patients with extremely high parasite loads, reflected by a wide IQR of (10.8–55,322 parasites/μL). Conversely, Lira RRH demonstrated substantially lower parasite densities overall, with a median of 3.0 parasites/μL and an IQR of (1.2–532 parasites/μL), suggesting comparatively lower transmission intensity or earlier healthcare-seeking behavior among patients attending the facility.

Table 4
Parasite densities by district and health facilities among *P. falciparum* infected individuals in the Sub region

District	Health Facility	n	Median	Q1	Q3	IQR
Dokolo	Dokolo HC IV	46	1,213.0	480.0	77,922.0	77,442.0
Gulu	Awach HC IV	24	4,094.4	1,900.0	22,584.0	20,684.1
Gulu city	Gulu RRH	15	186.0	10.8	55,322.0	55,311.0
KOLE	Aboke HC IV	18	1,254.0	90.3	4,886.0	4,796.1
Lira City	Lira RRH	7	3.0	1.2	532.0	530.4
Nwoya	Anaka GH	26	1,845.0	6.1	39,366.0	39360.1

Values are presented as median parasite density with interquartile range (IQR). Parasite density categories were defined as: Low (< 1,000 parasites/μL) and moderate (1,000–10,000 parasites/μL).

Table 4:

Multiple Linear Regression Analysis of Parasite Density vs demographic characteristics and locations of health facilities

Using Aboke Health Centre IV (HC IV) and the youngest age group (0–11 months) as reference categories, multivariable linear regression was performed on $\log_{10}$-transformed parasite density to address non-normality (Shapiro-Wilk test after transformation after transformation: $W = 0.937$, $p < 0.001$; improved skewness to -0.36 and kurtosis to -0.94) (Table 5). The analysis revealed no statistically significant associations between parasite density and sex, age group, or health facility location with parasite density (all $p > 0.05$). Specifically male sex was not significantly associated with parasite density ($p = 0.506$), and none of the age groups differed significantly from the 0–11 months reference category (all $p > 0.26$). Although some health facilities showed numerically higher median densities compared to Aboke HC IV, these differences were not statistically significant.

Table 5
Multiple Linear Regression Analysis of Parasite Density vs demographic characteristics and locations of health facilities

Categories	Estimate	SE	Statistic	P-value	conf. low	conf. high
(Intercept)	19604.56	22709.49	0.86	0.39	-25343.88	64553.01
sex M	-2183.58	16529.85	-0.13	0.90	-34900.78	30533.63
Age groups: 12–23	-10469.60	18345.18	-0.57	0.57	-46779.86	25840.65
Age groups: 24–35	-34364.43	22466.01	-1.53	0.13	-78830.96	10102.11
Age groups: 36–47	-10227.14	37927.53	-0.27	0.79	-85296.34	64842.06
Age groups: 48–59	-32009.16	36805.63	-0.87	0.39	-104857.80	40839.49
Age groups: 60+	-25300.42	48889.95	-0.52	0.61	-122067.32	71466.48
Health facility: Anaka GH	67696.87	26128.27	2.59	0.01	15981.71	119412.03
Health facility: Awach HC IV	7670.96	25426.78	0.30	0.76	-42655.76	57997.68
Health facility: Dokolo HC IV	28916.90	22659.75	1.28	0.20	-15933.08	73766.89
Health facility: Gulu RRH	34619.90	28968.43	1.20	0.23	-22716.73	91956.54
Health facility: Lira RRH	-5596.13	37304.09	-0.15	0.88	-79431.38	68239.12

The analysis identified statistically significant results for variables with p-values less than 0.05, highlighting the impact of health facility location over demographic characteristics.

Aboke HC IV and the 0–11 month age group were used as reference categories because Aboke HC IV represented a mid-level facility with moderate prevalence and sample size, and the 0–11 months group represents a cohort with relatively lower cumulative exposure to malaria parasites and are less likely to have developed partial immunity; thus, comparing older groups against this cohort allows interpretation of how parasite density changes with age and acquired immunity.

Intraclass correlation coefficients for parasite quantity

To determine the extent to which variation in parasite density was attributed to clustering at health facility and regional levels, an intraclass correlation coefficient (ICC) analysis was performed (Table 6). Approximately 6.5% of the total variance in parasite load was obtained and this value explained for differences across health facilities within the two sub regions. The low ICC value of 6.5% indicates that parasite density is highly variable at the individual level and does not cluster meaningfully by health

facility or geographic region. This suggests that host-specific factors (immunity, infection stage, and genetics) are more important than location-based factors. In contrast, variation attributable to regional-level differences between the Lango and Acholi sub-regions was negligible and not statistically significant.

Table 6
Intra-class correlation coefficients (ICC) for parasite quantity in Lango and Acholi sub region in Northern Uganda

Variables	Adjusted ICC
Health Facility	0.0647 95% CI: [0, 0.3608]
Region	0.0

**ICC values interpreted using Koo & Li* [28] *classification: Poor (< 0.50), Moderate (0.50–0.75), Good (0.75–0.90), and Excellent (> 0.90).*

Risk and behavioral Factors Associated with Malaria infection

The analysis shows that regional disparities explain a greater proportion of the variation in the risk of infections than demographic or behavioral factors (Table 7). However, the latter were more important in determining the likelihood of health care seeking for malaria testing and treatment. Individuals from the higher-incidence Lango sub-region had an eight times risk of malaria infection compared to those from Acholi (OR = 8.125, 95% CI: 1.561–55.926, $p = 0.011$) (Table 7). Conversely, although a strong sex-related difference was observed in health care-seeking behavior with females being more than twice as likely to undergo malaria testing (approximately 45% vs. 20%), this study found that sex was not significantly associated with malaria infection. Therefore, females participants had a similar likelihood of malaria infection as males (OR = 1.005, 95% CI: 0.262–4.02, $p = 0.994$). Additionally, individuals who had traveled outside the catchment area within the past two weeks had a slightly higher treatment rate (~ 55%) compared to those non-travellers (~ 50%). As shown in Table 9, this was found not to be significantly associated with increased likelihood of malaria infection (OR = 2.725, 95% CI: 0.631–11.907, $p = 0.175$). Malaria testing rates were found relatively consistent across age groups, ranging between ~ 30% and ~ 40%. However, the risk of infection appeared to decline with increasing age. Specifically, individuals aged 24–35 years (OR = 0.149, 95% CI: 0.01–1.041, p = 0.055) and those aged 48–59 years (OR = 0.129, 95% CI: 0.001–1.608, p = 0.123) exhibited relatively lower odds of infection compared to those of the children. Furthermore, patients who reported using bed-nets tested for malaria more frequently (~ 40%) than those that relied on mosquito repellents or Fansidar prophylaxis (< 10%). There was no statistical significance association between the two malaria control measures that were assessed in this study for malaria risk of infections.

Table 7

Logistic Regression Analysis of Malaria Infection Risk Factors in Northern Uganda

Categories	OR (95% CI)	p value
(Intercept)	0.27 (0.001–16.342)	0.572
Sex M	1.005 (0.262–4.02)	0.994
Age groups: 12–23	0.389 (0.102–1.381)	0.145
Age groups: 24–35	0.149 (0.01–1.041)	0.055
Age groups: 36–47	0.197 (0.001–3.092)	0.288
Age groups: 48–59	0.129 (0.001–1.608)	0.123
Age groups: 60+	1.63 (0.01-36.598)	0.79
Region: Lango	8.125 (1.561–55.926)	0.011
Travel history: 2wksYes	2.725 (0.631–11.907)	0.175
Received malaria test within 2 weeks Yes	3.35 (0.959–13.686)	0.058
Prior treatment Yes	1.032 (0.202–5.37)	0.969
Comorbidity status: HIV	1.14 (0.002-1599.988)	0.968
Comorbidity status: Hyphertenson	0.278 (0-378.307)	0.702
Comorbidity status: No	0.208 (0.004–52.059)	0.5
Comorbidity status: Sickle cell	1.227 (0.003–551.01)	0.941
Malaria control method: Fansida/bednet	5.902 (0.035-161.102)	0.381
Malaria control method: Mosquito repellant	15.185 (0.082-545.878)	0.226

OR: Odds Ratio; CI: Confidence Interval; **Note**: *The 0–11 month's age groups were used as reference category.*

Discussion

Prevalence of malaria and Pf HRP2/3 deletions in Lango and Acholi sub regions

Our findings demonstrated that malaria prevalence in northern Uganda is strongly shaped by rural ecological conditions and disparities in health system capacity, resulting in substantially higher transmission in rural districts than in urban centers. In this study, the highest malaria burden was recorded in Dokolo district (40%), followed by Gulu District (32%), Kole (31%), Nwoya district (30.3%), Gulu City (19.7%), and Lira City (15.4%). Rural areas in the sub-regions are characterized by extensive

wetlands draining into Lake Kyoga and the River Nile, including broad tributaries, rivers and large swamps that provide favorable breeding habitats for *Anopheles* mosquito vectors. In contrast, urban wetlands are fewer, often modified through agricultural activities, and subject to sewage pollution, conditions less conducive to vector breeding and survival. The high malaria burden in rural settings may also be attributed to socio-economic and health service-related challenges, including poor housing conditions, delayed healthcare seeking, limited access to timely diagnosis and treatment, lower coverage and utilization of vector control interventions such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS), as well as reduced community awareness regarding malaria prevention practices. These findings align with previous studies by [29] which reported substantial spatial heterogeneity in malaria transmission across Uganda, with rural communities bearing a disproportionate burden. Similarly, reports from [8] have emphasized that malaria transmission patterns is often heterogeneous within countries due to differences in ecological, behavioral, and health system factors.

In this study, a higher proportion of malaria-positive cases were observed among females (57.3%) than males (42.7%), yielding a female-to-male ratio of 1.3 to 1. This disparity may be partly explained by differences in health-seeking behavior, as females are generally more likely to access health services for testing and care. Consequently, this may lead to higher observed prevalence compared among women compared to men who may underutilize health services and therefore remain underdiagnosed. Participants aged 10–24 years represented the largest malaria-positive group, with a statistically significant difference in age distributions between males and females (P = 0.00039). This higher prevalence in this age group likely reflects amplified exposure to mosquito bites due to outdoor activities such as schooling, social activities, and agricultural works, coupled with inconsistent use of preventive measures including insecticide-treated bed nets and repellants. In contrast, children under five years, despite being biologically more susceptible to malaria are often better protected through targeted interventions such as insecticide-treated nets and chemoprevention programs. Adolescents and young adults, however, are often underrepresented in targeted malaria control strategies and may harbor asymptomatic infections, thereby contributing to sustained community transmission. Similar age- and sex-based disparities in malaria prevalence have been reported in high-burden regions[30]..

The absence of PfHRP2/3 gene deletions in this study supports the continued reliability of PfHRP2-based RDTs in Northern Uganda, while underscoring the need for sustained, region-specific molecular surveillance due to global and national heterogeneity in deletion prevalence. These findings are consistent with prior studies in Northern Uganda, which reported a very low prevalence of PfHRP2 deletions (0.2% in Lango and none in Acholi) [20]. While national surveys in Uganda have reported PfHRP2/3 deletion rates ranging from 3–10%, with hotspots reaching up to 14.7% in Eastern Uganda, the current study supports the evidence that Northern Uganda remains a low-risk area [20, 31]. Globally, the prevalence of PfHRP2/3 gene deletions shows marked heterogeneity, with an average estimate of about 4% [13], but with extremely high levels reported in countries such as Ethiopia and Eritrea (62–80%)[14, 32], while remaining very low in Angola and the Democratic Republic of Congo (< 1%)[33, 34]. In Uganda, national surveys have reported moderate *Plasmodium falciparum* HRP2/HRP3 gene deletion rates ranging from 3% to 10%, with localized hotspots reaching up to 14.7% in the eastern regions, yet northern

Uganda continues to show minimal or undetectable deletions. This marked spatial variation underscores the heterogeneity of gene deletion patterns globally[16] and reinforces WHO's recommendation for region-specific surveillance strategies. Such targeted monitoring is essential, as localized outbreaks or spread of deletion-bearing parasite populations could compromise the reliability of HRP2-based RDT[8, 35] .

The effectiveness of PfHRP2-based RDTs, microscopy and PCR in malaria diagnosis

In this study, malaria RDTs demonstrated excellent diagnostic performance, with a sensitivity of 88.2% and specificity of 99.3% when microscopy was used as the gold standard. Only two false-negative RDT results were recorded among the 424 participants, and importantly, these were not due to *PfHRP2/3* deletions, which were entirely absent in the study population. These findings confirm the continued reliability and suitability of PfHRP2-based malaria rapid diagnostic tests (mRDTs) for accurate malaria case management in Northern Uganda, particularly within the Lango and Acholi sub-regions. The present findings are consistent with reports from other African settings that have demonstrated high diagnostic accuracy of PfHRP2-based mRDTs. For example, a study conducted in Senegal reported a diagnostic sensitivity of 95.2% and specificity of 93.7% for mRDTs when compared with PCR-based diagnosis[36]. The high specificity observed in the current study suggests a low likelihood of false positive results. However, previous studies have indicated that specificity might be reduced because HRP2 antigens may persist in the bloodstream even after the parasites have been eliminated, potentially leading to occasional false-positive outcomes and reduced specificity in some settings [37].

Furthermore, [37] reported that HRP antigenemia detected by PCR demonstrated a high sensitivity of 97.9% but a low specificity of 48.1%, underscoring the difficulty in distinguishing active malaria infections from persistent circulating HRP2 antigens in HRP-based diagnostic methods. This finding emphasizes the exceptional performance of mRDTs in this study, particularly given the high specificity (100%) observed, which may reflect lower background antigenemia, effective case selection or effective clinical procedures as similarly reported by [38].

In contrast, mRDT performance has been suboptimal in some settings. In Nigeria, [39] reported low sensitivity (29%) and specificity (89%) for Curestat™ RDTs compared to microscopy, largely due to low parasite densities that reduce detection of infections, particularly at low parasitaemia. In Uganda, [40] found higher sensitivity (95.8%) and specificity (90.1%) in private facilities but a low PPV (63%), indicating over-diagnosis, likely due to microscopist error and inconsistent smear interpretation, highlighting the need for strengthened quality assurance. Compared with these findings, mRDT performance in the present study was high, supported by the absence of *PfHRP2/3* deletions. However, the detection of occasional false-negative results underscores the need for continued molecular surveillance, confirmatory testing, and routine quality assurance, especially in RDT-negative suspected cases. Maintaining diagnostic accuracy will require integration of molecular tools, staff training, and sustained monitoring. Similarly, studies in Uganda have reported rare false-negative PfHRP2-based RDT

results, often linked to non-falciparum infections, reinforcing the need for multi-method diagnosis and ongoing surveillance of *PfHRP2/3* deletions [30]. These findings suggest that both RDTs and microscopy remain highly effective tools for malaria diagnosis in the study population, with microscopy showing marginally superior performance.Parasites density among malaria Confirmed Cases in the study districts in Northern Uganda

Parasite density among *Plasmodium falciparum*–infected individuals varied by health facility but not significantly by geographic location. Moderate parasite densities predominated in rural districts such as Dokolo, Awach (Gulu), Aboke (Kole), and Anaka (Nwoya), while a few patients, particularly at Awach HC IV, exhibited very high parasitaemia. This spatial heterogeneity is consistent with previous studies highlighting the role of local ecological and epidemiological factors in shaping parasite distribution [41]. The two regional referral hospitals (Gulu RRH and Lira RRH) showed contrasting parasite profiles, with Gulu RRH recording occasional very high parasite densities despite a generally low median. This suggests that referral facilities may manage more severe or complicated cases, consistent with [34]. Regression analysis further indicated that health facility location, rather than demographic factors such as age or sex, was a stronger predictor of parasite density, with Anaka General Hospital showing significantly higher parasite loads. The intraclass correlation coefficients confirmed this pattern, with minimal variation attributed to regional differences (0.00%) and moderate variation between facilities (6.5%)[28], underscoring the importance of localized transmission dynamics. Overall, these findings suggest that parasite density did not vary significantly according to geographical location of the health facility or demographic characteristics among the participants included in this study. This suggests that parasite density was more strongly influenced by individual host-specific factors such as immunity, stage of infection stage and genetic variability, rather than broader geographical/location or regional factors.

Although no PfHRP2/3 deletions were detected in this study, previous evidence links parasite genetic diversity to transmission intensity. Baker et al. (2010) reported that high transmission and recombination may promote PfHRP2/3 diversity and potential diagnostic evasion. Similarly,[34] showed genotype-dependent differences in parasite density, while modeling studies predict increasing selection for deletions in settings relying heavily on PfHRP2-based RDTs[42]. Overall, while deletions were absent in this study—possibly reflecting current transmission patterns and parasite density distributions—continued molecular surveillance remains essential given the potential for selection pressure from widespread RDT use to favor future emergence of pfhrp2/3 deletions [43][44].

Demographic, environmental and behavioral factors associated with malaria infection

This study identified geographic region as the strongest predictor of malaria infection, outweighing demographic and behavioral factors. Participants from the Lango sub-region had an eightfold higher odds of infection compared to those from the Acholi sub-region (OR = 8.125; 95% CI: 1.561–55.926; p = 0.011), indicating marked spatial clustering of transmission. This disparity may reflect ecological

conditions in Lango, including proximity to Lakes Kyoga and Kwania and extensive wetlands that promote mosquito breeding, as well as possible differences in implementation of vector control interventions such as indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs). These findings are consistent with studies by [31] in the Democratic Republic of Congo and [6] in Uganda, which similarly demonstrated that malaria transmission is highly localized and shaped by variation in climate, vector control coverage, housing conditions, and human movement. Arinaitwe et al. further noted that areas with limited IRS coverage experience higher transmission, particularly during periods of increased mobility, a pattern that may also partly explain higher infection levels in parts of Acholi such as Nwoya, where seasonal agricultural movement is common. These results suggest that incorporating finer-scale ecological and mobility-related variables in future studies could improve understanding of sub-regional differences in malaria transmission.

In this study, females accounted for a higher proportion of malaria infections (57.3%) compared to males (42.7%). However, despite higher testing rates among females (~ 45% vs. ~20%), there was no significant association between sex and malaria infection (OR = 1.005; 95% CI: 0.262–4.02; p = 0.994), suggesting that observed differences in case distribution likely reflect differences in health-seeking or testing patterns rather than true biological susceptibility. These findings are consistent with [45]) in Ethiopia, who reported no significant association between gender and *PfHRP2/3* deletions or malaria infection risk, although delayed care-seeking was noted to worsen outcomes in both sexes. In contrast, studies by [46] in Ethiopia and [47] in Cameroon reported higher infection rates among males, likely due to increased outdoor exposure and occupational activities. However, these risk patterns were not evident in the present study. Participants who reported travel outside the catchment area within the past two weeks had a moderately increased risk of malaria infection (OR = 2.725; 95% CI: 0.631–11.907; p = 0.175) and a slightly higher treatment rate (~ 55%) compared to non-travelers. Although not statistically significant, this trend aligns with [6]), who highlighted that human mobility increases malaria exposure, particularly when individuals travel to high-transmission areas without adequate protection such as LLINs, IRS, or chemoprophylaxis. The findings further support the concept of “imported malaria,” a concern in partially controlled transmission settings where population movement can reintroduce parasites into lower-transmission areas and potentially facilitate the spread of drug- or diagnostic-resistant strains, including potential *PfHRP2/3* deletions. This phenomenon has been widely documented in elimination settings and underscores the importance of targeting mobile populations in malaria control strategies[6, 48].

Age showed a negative association with malaria infection, particularly among the 24–35 and 48–59-year age groups, which had lower odds of infection (OR = 0.149 and 0.129, respectively). Although these associations were not statistically significant (p = 0.055 and 0.123), the trend is biologically plausible and consistent with acquired immunity from repeated exposure, as reported in Ghana [49] and Ethiopia [50][46]. Similarly, [13] noted that younger children often experience higher morbidity despite lower infection complexity, underscoring the importance of age-targeted malaria interventions, particularly for prevention and early treatment in children [2]. In contrast, participants aged 10–24 years accounted for the highest proportion of infections in this study, highlighting a substantial malaria burden among

adolescents and young adults, a group often underemphasized in malaria control programs that traditionally focus on children under five and pregnant women. Testing rates were relatively similar across age groups (~ 30–40%), despite higher infection levels in younger populations, raising concerns about potential missed or delayed diagnoses, particularly among children. Given that over 90% of malaria diagnoses in Northern Uganda rely on PfHRP2-based RDTs[20], timely access to testing remains critical for effective case management.

While this study found high sensitivity and specificity of HRP2-based RDTs and microscopy, other studies have reported diagnostic errors linked to poor-quality microscopy and limited laboratory experience[40], underscoring the need for continued training and community awareness to ensure accurate testing and diagnosis. Unlike studies in Ghana and Ethiopia, this study did not assess environmental risk factors such as housing quality and proximity to breeding sites, which have been shown to influence malaria risk [46, 47, 49] also reported paradoxical findings where LLIN users had higher infection rates, potentially due to bias or poor net condition. In this study, testing rates varied by prevention method, with higher rates among bed net users (~ 40%) and lower rates among users of mosquito repellents or Fansidar (< 10%).

In conclusion, malaria transmission remains substantial in rural Northern Uganda, and PfHRP2-based RDTs remain reliable due to the absence of *PfHRP2/3* deletions. Demographic and behavioral factors such as sex, travel history, and prevention practices influence malaria risk, highlighting the need for continued molecular surveillance and targeted interventions, particularly among adolescents and other high-risk groups, to strengthen malaria control efforts in Uganda.

Study Limitations

This study has some limitations. First, being a cross-sectional study conducted in only six selected healthcare facilities within the Lango and Acholi sub-regions, it is subject to selection bias and may not fully represent the extent of PfHRP2/3 deletions across the geographically and culturally diverse populations of northern Uganda. Furthermore, the sampling approach did not meet WHO's recommendation of at least ten randomly selected sites per region, thereby limiting the generalizability of the findings. The limited application of PCR analysis may have reduced the ability to detect infections that were missed by mRDTs or microscopy. In addition, the reliance on questionnaires introduces potential recall bias among participants. Finally, the relatively small number of infected individuals included in parasite density analyses may have reduced the statistical power to detect subtle facility-level or demographic associations. Despite the limitations, this study provides context specific information on the reliability of mRDT in detection of malaria, highlighting its importance for routine patient care in Northern Uganda.

Abbreviations

AOR Adjusted odd ratio

DBS Dry blood sample

HRP Histidine-rich protein

LLINs Long lasting treated nets

mRDT Malaria rapid diagnostic test

PCR Polymerase Chain reaction

WHO World health Organization

Declarations

Ethical approval and consent to participate

Ethical approved to conduct this study was obtained from Gulu University Research Ethics Committee (Approval GUREC-2024-793), Uganda national Council of Science and Technology (UNCST-HS4067ES). Permission to conduct study were sought from Gulu regional referral hospital research ethical committee (GRRH REC/2024/03/40), Lira regional referral hospital research ethical committee (LRRH/29/24), District health authorities of Kole, Gulu, Dokolo and Nwoya districts to conduct the study at the above health facilities. All patients/parent/guardians assented to and signed informed consent forms before enrolling for the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Note

Mean and standard deviation values were removed following formal normality assessment, which indicated that non-parametric measures (median and IQR) are more appropriate for this highly skewed distribution.

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Author Contribution

AF, ASP, HA,BA conceived and designed the study, collected data, performed initial data analyses and wrote initial draft manuscript. BM, RO, MGM, CA and PO read and critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Data Availability

The data analyzed for this study are available with the corresponding author upon reasonable request!

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Figures

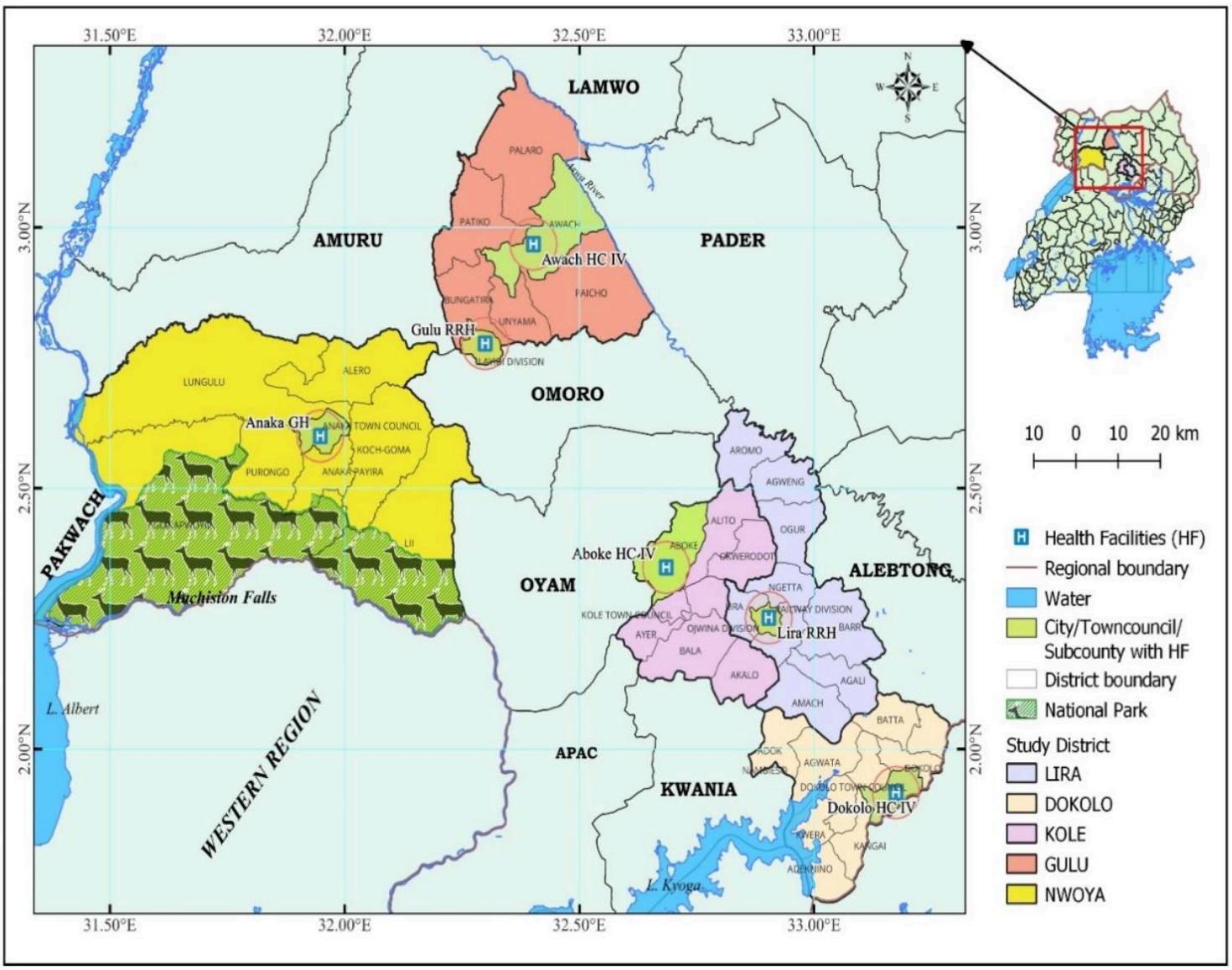


Figure 1

Location of Study Districts in the Lango and Acholi Sub-regions, Northern Uganda

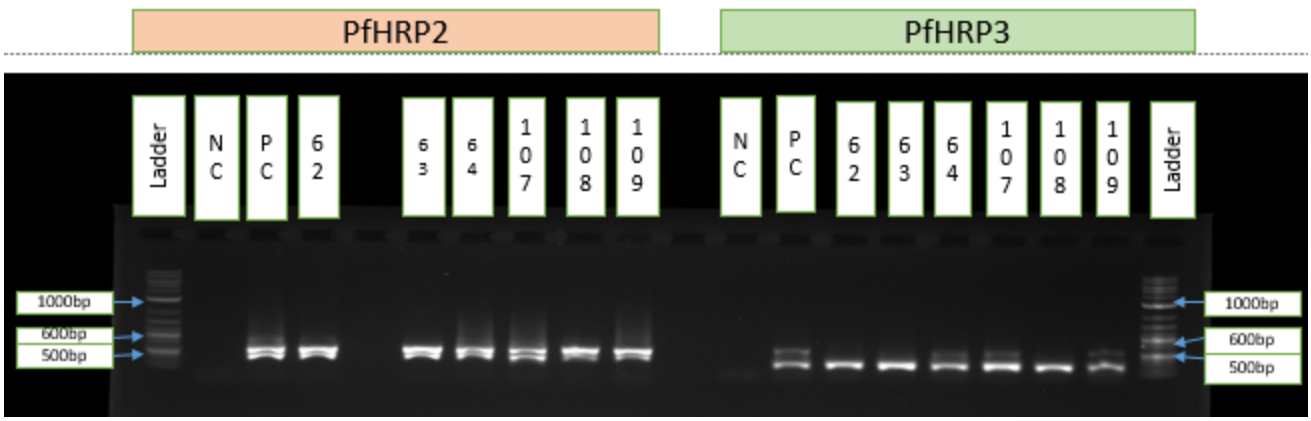


Figure 2

Nested PCR gel image for PfHRP-2 and PfHRP3 detection.

Positive (PC) and negative control (NC) samples were tested alongside RDT false-negative samples (62 and 107), as well as three RDT-positive concordant samples (63, 108, and 109), to assess the potential cross-reactivity of Pf HRP3 RDT assays.