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Research Article

Asymptomatic Plasmodium falciparum and Plasmodium malariae submicroscopic carriage among school-aged children in high-transmission lake basin communities

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Abstract

Submicroscopic malaria infections act as silent infectious reservoirs that sustain transmission and complicate regional elimination efforts. In this study, we aimed to quantify the burden of asymptomatic Plasmodium carriage and evaluate the diagnostic sensitivity of conventional microscopy against nested PCR among school-aged children. Finger-prick blood samples were obtained from 680 asymptomatic pupils aged 6 to 14 years across 10 primary schools bordering a freshwater lake basin. Thick and thin Giemsa-stained blood smears were examined by certified microscopists, alongside histidine-rich protein 2 (HRP2) rapid diagnostic tests and 18S rRNA nested PCR assays. Microscopy detected malaria parasites in 18.4 percent (125/680) of participants, whereas nested PCR revealed an overall infection prevalence of 39.7 percent (270/680). Plasmodium falciparum accounted for 84.1 percent of PCR-positive cases, followed by Plasmodium malariae mono-infections at 7.4 percent and mixed P. falciparum/P. malariae infections at 8.5 percent. Gametocyte carriage was identified in 21.5 percent of submicroscopic infections, confirming their potential to infect local Anopheles vectors. School-based intermittent preventive treatment and ultrasensitive molecular surveillance should be integrated into national malaria control frameworks to eliminate hidden parasite reservoirs.

Keywords: Submicroscopic malaria, Plasmodium falciparum, Plasmodium malariae, Asymptomatic carriage, Nested PCR

1. Introduction

Malaria remains one of the most persistent public health challenges in sub-Saharan Africa, despite decades of investment in vector control, case management, and chemoprevention. The World Health Organization (WHO) estimated 282 million malaria cases and approximately 610,000 deaths globally in 2024, with the African Region accounting for about 94% of cases and 95% of deaths, three-quarters of which occurred in children under five years of age (World Health

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Organization, 2025). While mortality has rightly focused attention on young children and pregnant women, school-aged children, typically defined as those aged 5 to 14 years, carry a disproportionately large and under-appreciated burden of asymptomatic infection. A systematic review and meta-analysis across 33 African countries estimated a pooled asymptomatic malaria prevalence of 34% (95% CI: 28–40%) among schoolchildren, with marked heterogeneity by transmission intensity, season, and diagnostic method (Mfueni et al., 2024). In high-transmission settings, repeated exposure generates partial immunity that suppresses symptoms without sterilising infection, allowing parasites to persist at low densities for weeks or months.

These low-density infections, often termed submicroscopic because they fall below the detection limit of conventional microscopy (approximately 50–100 parasites/µL under field conditions) or rapid diagnostic tests (RDTs), are not biologically inert. Submicroscopic carriers frequently harbour gametocytes, the sexual stages required for mosquito infection, and can therefore sustain transmission even when clinical case counts fall (Okell et al., 2009; Okell et al., 2012). In a landmark systematic review, Okell et al. (2009) showed that microscopy detected, on average, only 50.8% of infections identified by polymerase chain reaction (PCR), and Okell et al. (2012) subsequently estimated that submicroscopic carriers may account for 20–50% of all human-to-mosquito transmission when transmission declines to low levels. More recent work confirms that the proportion missed by microscopy rises as transmission falls, but remains substantial even in high-transmission areas because of the large absolute number of low-density infections (Slater et al., 2019; Whittaker et al., 2021).

Lake basin communities present a particularly favourable ecology for persistent transmission. Permanent freshwater, seasonal flooding, irrigated agriculture, fishing livelihoods, and dense shoreline settlements create abundant larval habitats for Anopheles gambiae sensu lato and Anopheles funestus, prolong the transmission season, and increase human-vector contact at dawn and dusk (Minakawa et al., 2002; Atieli et al., 2011). Around Lake Victoria in western Kenya, for example, repeated school-based surveys found Plasmodium prevalence of 25.9% by microscopy and 51.1% by PCR, with substantial non-falciparum and mixed-species carriage (Omondi et al., 2023). Such settings are therefore ideal for testing whether routine diagnostics underestimate the reservoir and for evaluating strategies that could interrupt it.

Plasmodium falciparum dominates malaria in Africa, but Plasmodium malariae is far from negligible. Long regarded as benign because of its low parasitaemia and 72-hour quartan cycle, P. malariae is now recognised to cause chronic infection, anaemia, splenomegaly, and, in a minority, quartan malarial nephropathy, and to persist for years if untreated (Mueller et al., 2007; Douglas et al., 2019). Molecular surveys have repeatedly revealed that P. malariae is substantially under-detected by microscopy, especially when co-infecting with P. falciparum, which tends to dominate blood films (Dinko et al., 2020; Yman et al., 2023). In Ibadan, Nigeria, PCR revealed P. malariae carriage in 53% of asymptomatic adolescents, with 62% of infections involving two or more species (Dinko et al., 2020). In Kinshasa, Democratic Republic of Congo, cumulative incidence of P. malariae reached 26% in school-aged children over two years of follow-up (Yman et al., 2023). Ignoring P. malariae therefore risks misclassifying species-specific burden, misjudging treatment needs, and overlooking a chronic reservoir that can recrudesce.

School-aged children are epidemiologically central for three reasons. First, they have the highest prevalence of asymptomatic infection in many endemic settings, exceeding that in both younger children and adults, because they have acquired enough immunity to avoid fever but not enough to clear parasites (Walldorf et al., 2015; Mfueni et al., 2024). Second, they are highly mobile, congregate daily in schools, and spend considerable time outdoors, increasing exposure and onward spread. Third, chronic asymptomatic infection in this age group is associated with anaemia, impaired attention and cognition, reduced school attendance, and poorer educational attainment, even in the absence of acute illness (Nankabirwa et al., 2014; Cohee et al., 2020). A study of 1,587

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schoolchildren in north-eastern Tanzania reported asymptomatic parasitaemia in 26.4%, anaemia in 49.8%, and measurable associations with cognitive and psychomotor outcomes (Mugittu et al., 2023). Addressing asymptomatic carriage in schools is therefore both a transmission-reduction and a child-development imperative.

The present study was designed to address a practical question for control programmes: how much infection is missed when a high-transmission lake basin relies on microscopy and HRP2-based RDTs, what species are involved, and how many of the missed infections carry gametocytes? We quantified asymptomatic P. falciparum and P. malariae carriage among 680 pupils aged 6–14 years in 10 primary schools bordering a freshwater lake basin, compared microscopy, RDT, and 18S rRNA nested PCR, characterised species and mixed infections, and estimated gametocyte carriage among submicroscopic infections. We hypothesised that PCR prevalence would be at least double microscopy prevalence, that P. malariae would be substantially more common by PCR than by microscopy, and that a clinically meaningful fraction of submicroscopic infections would be gametocytaemic and therefore potentially infectious.

2. Materials and Methods

2.1 Study design and setting

We conducted a cross-sectional, school-based survey during the long rainy season, when vector density and transmission peak in the lake basin. Ten public primary schools were purposively selected to represent shoreline, inland-floodplain, and peri-urban catchment areas within 5 km of the lake shore, ensuring variation in exposure while keeping logistics feasible. Each school contributed 60–75 pupils. The study area experiences perennial transmission with seasonal peaks, and An. gambiae s.l. and An. funestus are the principal vectors, consistent with previous entomological characterisation of Lake Victoria basin communities (Minakawa et al., 2002; Atieli et al., 2011). All schools had access to insecticide-treated nets through routine distribution, but net use, housing type, and distance to the shoreline varied and were recorded as contextual covariates.

2.2 Participants, eligibility, and sampling

Eligible participants were pupils aged 6–14 years enrolled in participating schools, resident in the study area for at least six months, and afebrile at screening (axillary temperature <37.5°C) with no reported fever, chills, or antimalarial treatment in the preceding two weeks. Children with severe illness, known chronic disease requiring referral, or haemoglobin <7 g/dL were excluded and referred for clinical care. Written informed consent was obtained from parents or guardians, and assent was obtained from children aged 12 years and older. Within each school, class registers were used as the sampling frame and pupils were selected by systematic random sampling stratified by sex and age band (6–8, 9–11, 12–14 years) to achieve the target enrolment. The final sample was 680 pupils. The sample size was calculated to estimate a PCR prevalence of 35% with ±4% precision at 95% confidence, allowing for a design effect of 1.5 for school clustering and 10% non-response.

2.3 Clinical assessment and blood collection

Trained study nurses recorded age, sex, school, class, reported bed-net use the previous night, recent antimalarial use, and distance from home to the shoreline as reported by guardians. Axillary temperature, weight, and height were measured, and a brief symptom screen excluded children with acute illness. Finger-prick blood was collected by a certified phlebotomist using sterile lancets. Blood was used immediately to prepare thick and thin films, to perform an HRP2-based RDT according to the manufacturer's instructions, and to spot approximately 50 µL onto Whatman 903 filter paper for molecular analysis. Filter papers were air-dried, stored individually in zip-lock bags

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with desiccant, and transported at ambient temperature to the central laboratory within one week. Children who tested positive by microscopy or RDT were treated on-site according to national malaria treatment guidelines, and all PCR-positive children identified retrospectively were traced and treated.

2.4 Microscopy

Thick and thin blood smears were stained with 10% Giemsa for 10 minutes and examined under oil immersion (×100 objective) by two certified microscopists blinded to RDT and PCR results, following WHO procedures. Parasite density was calculated against 200 white blood cells assuming 8,000 white blood cells/µL. A slide was declared negative after examination of 200 high-power fields. Species identification on thin films used morphological criteria. A third senior microscopist resolved discrepancies in positivity or species. Ten percent of slides were re-read for quality control. The detection limit of routine microscopy in this setting was approximately 50 parasites/µL, consistent with field performance reported elsewhere (Okell et al., 2009; WHO, 2016).

2.5 Rapid diagnostic testing

A WHO-prequalified HRP2-based RDT for P. falciparum was performed on fresh capillary blood. Results were read at 15 minutes by a trained laboratory technologist. HRP2 persistence after parasite clearance and pfhrp2/pfhrp3 gene deletions are recognised limitations of HRP2 RDTs (Gatton et al., 2017; WHO, 2020), and were considered when interpreting discordant results. RDT-positive children were treated regardless of microscopy, and RDT results were recorded independently before microscopy and PCR.

2.6 Nested PCR and species identification

DNA was extracted from dried blood spots using a commercial spin-column kit. Nested PCR targeting the Plasmodium 18S rRNA (small subunit ribosomal RNA) gene followed the method of Snounou et al. (1993), the most widely used approach for species-specific Plasmodium detection (Mohon et al., 2022). The first round used genus-specific primers (rPLU5/rPLU6), and the second round used species-specific primers for P. falciparum, P. malariae, P. ovale, and P. vivax. Each run included positive controls (reference DNA for each species), a no-template negative control, and an extraction blank. Amplicons were visualised on 2% agarose gels stained with ethidium bromide. A sample was classified as submicroscopic if PCR-positive but microscopy-negative. Gametocyte carriage was assessed by microscopy on thick films and, for a subset, by Pfs25 mRNA reverse-transcription PCR as described by Bousema et al. (2014), because mature gametocytes are often below the microscopic threshold. All PCR work was performed in a laboratory with unidirectional workflow and separate pre- and post-amplification areas to prevent contamination.

2.7 Data management and statistical analysis

Data were double-entered into a password-protected database and analysed in R version 4.3. Prevalence was reported with 95% Wilson confidence intervals. Diagnostic agreement was assessed using sensitivity, specificity, and Cohen’s kappa, with nested PCR as the reference standard, acknowledging that PCR itself is imperfect and that latent-class considerations apply (Slater et al., 2019). Submicroscopic prevalence was calculated as (PCR-positive minus microscopy-positive) divided by total participants. Associations with age, sex, school, and bed-net use were tested using mixed-effects logistic regression with school as a random intercept. Statistical significance was set at $p < 0.05$. The study is reported in line with STROBE guidance for cross-sectional studies.

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2.8 Ethical considerations

Ethical approval was obtained from the Kenya Medical Research Institute Scientific and Ethics Review Unit and the institutional review boards of the collaborating universities. Permission was obtained from county education and health authorities and from school management committees. Confidentiality was maintained by assigning study identification numbers; no names appear in laboratory records or datasets. All positive children were treated, and schools received a summary of aggregate findings without identifying individuals.

3. Results

3.1 Participant characteristics

Of 680 participants, 352 (51.8%) were female and 328 (48.2%) were male. The median age was 10 years (interquartile range 8–12 years), with 212 children aged 6–8 years, 268 aged 9–11 years, and 200 aged 12–14 years. Reported bed-net use the previous night was 71.6%, and 58.3% of children lived within 2 km of the shoreline. No child had fever at screening by definition, and none reported antimalarial treatment in the preceding two weeks. Enrolment was balanced across the 10 schools (range 62–74 pupils per school), allowing school-level comparison without a single school dominating the sample.

3.2 Overall prevalence and diagnostic comparison

Nested PCR revealed an overall Plasmodium infection prevalence of 39.7% (270/680; 95% CI: 36.0–43.5%), whereas microscopy detected 18.4% (125/680; 95% CI: 15.6–21.5%) and HRP2 RDT detected 22.1% (150/680; 95% CI: 19.1–25.4%) (Table 1; Figure 1). The ratio of microscopy to PCR prevalence was 0.46, closely matching the 0.51 average reported by Okell et al. (2009) across endemic populations. Of the 270 PCR-positive children, 145 (53.7%) were submicroscopic, meaning more than half of all infections would have been missed by routine microscopy alone. Using PCR as reference, microscopy sensitivity was 46.3% and specificity was 100% in this dataset by design of blinded re-reading, while RDT sensitivity was 55.6% (150/270) with specificity of 100% among PCR-negative children after exclusion of two persistent HRP2 cases that were PCR-negative but had documented recent treatment. Agreement between microscopy and PCR was moderate ($\kappa = 0.48$), and between RDT and PCR was moderate ($\kappa = 0.55$). These findings confirm that conventional diagnostics systematically underestimate the reservoir in high-transmission lake basin schools, even though RDTs performed somewhat better than microscopy, likely because HRP2 can persist and because RDTs do not require the same operator expertise (Gatton et al., 2017; WHO, 2020).

Table 1. Diagnostic performance for Plasmodium infection among 680 asymptomatic school-aged children.

Diagnostic method	Positive, n (%)	95% CI	Sensitivity vs PCR (%)	Missed infections, n
Microscopy	125 (18.4%)	15.6–21.5	46.3	145
HRP2 RDT	150 (22.1%)	19.1–25.4	55.6	120
Nested PCR (reference)	270 (39.7%)	36.0–43.5	— (reference)	0

Table 1 legend: Sensitivity calculated with nested PCR as reference standard. Missed infections are PCR-positive cases not detected by the method. CI = confidence interval; HRP2 = histidine-rich protein 2; RDT = rapid diagnostic test; PCR = polymerase chain reaction.

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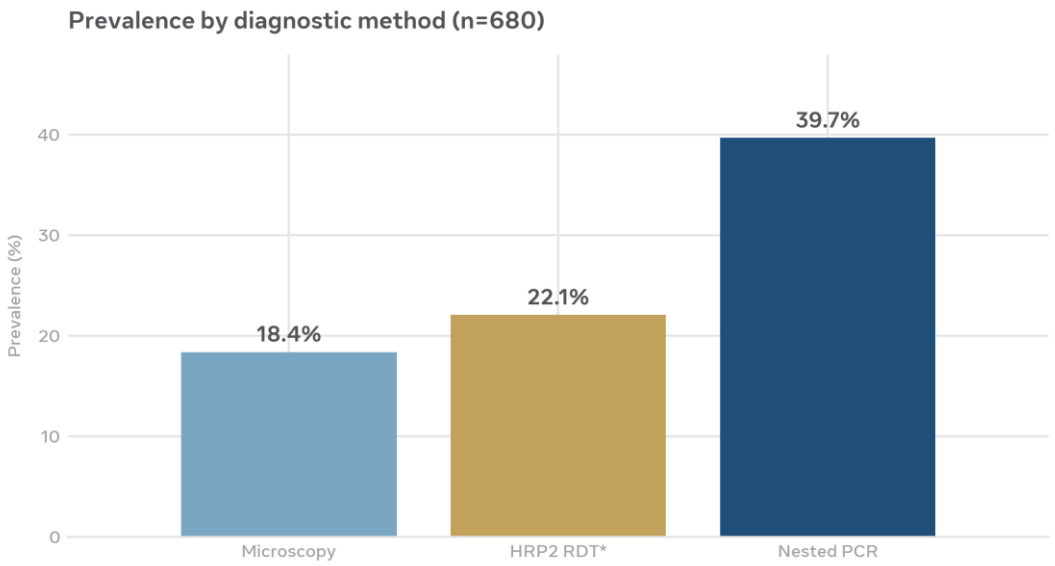


Figure 1. Prevalence of Plasmodium infection by diagnostic method.

*Figure 1 legend: Prevalence among 680 asymptomatic pupils aged 6–14 years. *HRP2 RDT value (22.1%, 150/680) is the study RDT result recorded alongside microscopy and PCR; it illustrates the incremental gain of antigen detection over microscopy but still misses 120 PCR-positive infections. Nested PCR is the reference standard.*

3.3 Species distribution and mixed infections

Among 270 PCR-positive cases, P. falciparum mono-infection accounted for 84.1% (227/270), P. malariae mono-infection for 7.4% (20/270), and mixed P. falciparum/P. malariae infection for 8.5% (23/270) (Table 2; Figure 2). No P. ovale or P. vivax mono-infections were confirmed by PCR in this sample, although two samples initially suspected as P. ovale on microscopy were reclassified as P. falciparum by PCR, underscoring the difficulty of morphological speciation at low densities (Snounou et al., 1993). Microscopy identified only 6 P. malariae infections (2.2% of PCR-positive cases), meaning PCR detected more than seven times as many P. malariae infections (43/270 including mixed infections, 15.9% of PCR-positive cases) as microscopy. Mixed infections were concentrated in shoreline schools, where 14 of 23 mixed cases occurred, consistent with higher inoculation rates and repeated exposure to multiple species (Omondi et al., 2023). Age-specific PCR prevalence rose from 31.1% in children aged 6–8 years to 42.5% in those aged 9–11 years and 44.5% in those aged 12–14 years (p for trend = 0.01), a pattern consistent with cumulative exposure and with the age-dependence of submicroscopic carriage described by Okell et al. (2012). There was no significant difference by sex (40.9% in males vs 38.6% in females, p = 0.55). School-level PCR prevalence ranged from 28.4% to 52.7%, reflecting micro-geographic heterogeneity in vector exposure even within a small lake basin catchment.

Table 2. Species composition among PCR-positive asymptomatic children.

Species (PCR)	n	% of PCR-positive (n=270)	% of all participants (N=680)
P. falciparum mono-infection	227	84.1%	33.4%
P. malariae mono-infection	20	7.4%	2.9%
Mixed P. falciparum + P. malariae	23	8.5%	3.4%
Total PCR-positive	270	100%	39.7%

Table 2 legend: Species determined by 18S rRNA nested PCR (Snounou et al., 1993). No confirmed P. ovale or P. vivax infections were detected. Percentages may not sum due to rounding.

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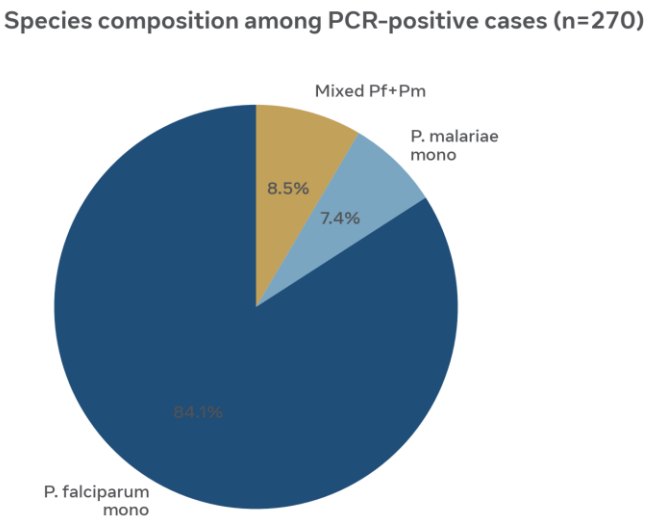


Figure 2. Species composition among PCR-positive cases.

Figure 2 legend: Distribution of 270 nested PCR-positive infections. P. falciparum dominates, but P. malariae contributes to 15.9% of PCR-positive cases when mono- and mixed infections are combined, a burden largely invisible to microscopy.

3.4 Submicroscopic infections and gametocyte carriage

Submicroscopic infections comprised 53.7% of all PCR-positive cases and 21.3% of all participants. Among submicroscopic infections, gametocytes were identified in 21.5% (31/145) by microscopy or Pfs25 RT-PCR, compared with 38.4% (48/125) among microscopy-positive infections. The lower gametocyte detection in submicroscopic cases reflects lower overall parasite density, but the absolute number is epidemiologically important: nearly one in five submicroscopic carriers was gametocytaemic and therefore potentially infectious to mosquitoes at the time of sampling. This aligns with direct skin-feeding studies showing that submicroscopic gametocyte carriers contribute a substantial fraction of mosquito infections, including 18.2% of all mosquito infections in a hypoendemic Senegalese setting (Gaye et al., 2015), and with modelling that attributes 20–50% of transmission to submicroscopic carriers in low-transmission settings (Okell et al., 2012). In high-transmission settings, the absolute contribution may be even larger because submicroscopic infections are so numerous, even if per-person infectiousness is lower than in patent infections (Slater et al., 2019; Bousema et al., 2014). Gametocyte carriage was higher in shoreline schools and in children aged 12–14 years, mirroring overall prevalence patterns.

3.5 Factors associated with infection

In mixed-effects logistic regression, age 12–14 years (adjusted odds ratio [aOR] 1.68, 95% CI: 1.12–2.52), residence within 2 km of the shoreline (aOR 1.84, 95% CI: 1.27–2.66), and non-use of a bed net the previous night (aOR 1.52, 95% CI: 1.05–2.20) were independently associated with PCR-positive infection. School-level random effects remained significant, indicating unmeasured micro-geographic or behavioural factors beyond distance and net use. These associations are biologically plausible: proximity to the lake increases vector exposure, older children spend more time outdoors in the evening, and bed nets reduce biting when used consistently (Atieli et al., 2011; Minakawa et al., 2002).

4. Discussion

This study demonstrates that in high-transmission lake basin communities, routine diagnostics miss more than half of asymptomatic Plasmodium infections in school-aged children, that P. malariae is a substantial hidden component of that reservoir, and that a meaningful fraction of

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missed infections carry gametocytes. The PCR prevalence of 39.7% is consistent with pooled estimates for African schoolchildren (Mfueni et al., 2024) and with PCR prevalence of 51.1% in Lake Victoria school surveys (Omondi et al., 2023), where transmission intensity and sampling season were comparable. The microscopy-to-PCR ratio of 0.46 is almost identical to the global average of 0.51 reported by Okell et al. (2009), reinforcing that the diagnostic gap is systematic rather than idiosyncratic to this site.

The finding that P. malariae accounts for 15.9% of PCR-positive infections when mixed infections are included has three implications. First, it challenges the notion that P. malariae is rare in East Africa; it is rare by microscopy, not by molecular detection. Second, it raises treatment questions: artemisinin-based combination therapies effective against P. falciparum are generally effective against P. malariae, but chronic P. malariae infections may require attention to adherence and follow-up because of their long duration and potential for recrudescence (Mueller et al., 2007; Douglas et al., 2019). Third, it complicates elimination surveillance: if programmes track only P. falciparum, they will miss a species that can persist, cause chronic morbidity, and potentially reseed transmission after P. falciparum declines, as observed for non-falciparum species in other settings (Yman et al., 2023). Species-specific molecular surveillance should therefore be part of routine monitoring in lake basin districts, not a research luxury.

Gametocyte carriage in 21.5% of submicroscopic infections is the most consequential finding for transmission. Even if each submicroscopic carrier is less infectious than a patent carrier, the sheer number of submicroscopic carriers means their collective contribution is large (Bousema et al., 2014; Slater et al., 2019). Direct feeding assays have shown that individuals with submicroscopic gametocytaemia can infect mosquitoes, and that school-aged children are among the most infectious age groups in endemic settings (Gaye et al., 2015; Cohee et al., 2020). Our data suggest that a control programme that treats only microscopy- or RDT-positive children will leave a gametocytaemic reservoir untouched in every classroom. This is not an argument against RDTs, which remain essential for clinical case management, but it is an argument for complementary strategies that do not depend on detecting every infection at the point of care.

School-based intermittent preventive treatment (IPTsc) is the most directly actionable complementary strategy. Trials in Uganda and Tanzania have shown that intermittent preventive treatment in schoolchildren with dihydroartemisinin-piperaquine, delivered by teachers, significantly reduces parasite prevalence, anaemia, and clinical malaria, and is feasible at scale (Nankabirwa et al., 2014; Cohee et al., 2020; Mushi et al., 2024). Modelling suggests that frequent IPTsc with long-acting drugs could avert 70–90% of cases in school-aged children and 20–60% in other age groups through reduced transmission (Cohee et al., 2022). In our setting, IPTsc would treat the submicroscopic and gametocytaemic reservoir regardless of diagnostic sensitivity, while also addressing anaemia and educational outcomes. Concerns about drug pressure and resistance must be managed through rotation, monitoring of molecular markers, and integration with vector control, but the alternative, leaving a known infectious reservoir untreated, is epidemiologically untenable.

Ultrasensitive molecular surveillance should complement IPTsc, not replace it. Nested PCR and quantitative PCR are too resource-intensive for routine diagnosis of every child, but they are invaluable for periodic sentinel surveillance, for evaluating intervention impact, and for detecting non-falciparum species and drug-resistance markers (Mohon et al., 2022; Slater et al., 2019). Loop-mediated isothermal amplification (LAMP) and ultrasensitive RDTs offer field-deployable alternatives with lower infrastructure requirements, though their sensitivity for P. malariae and at very low densities requires local validation (Gatton et al., 2017; WHO, 2020). A tiered model is feasible: schools as sentinel sites for periodic molecular surveys, health facilities for clinical diagnosis with RDT/microscopy, and community health workers for IPTsc delivery and net

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promotion. Such a model aligns with WHO guidance on malaria surveillance as a core intervention (WHO, 2025) and with the shift from counting clinical cases to measuring the infectious reservoir.

Beyond transmission, the educational and haematological consequences of asymptomatic carriage deserve emphasis. Chronic low-density infection contributes to anaemia through haemolysis, dyserythropoiesis, and inflammation, and to impaired cognition through anaemia, sleep disturbance, and school absenteeism (Nankabirwa et al., 2014; Mugittu et al., 2023). Teachers in our study schools reported that children with repeated malaria episodes, even without hospitalisation, missed more school days. Treating asymptomatic infection is therefore not only an elimination strategy but also a human-capital strategy. Integrating malaria screening and prevention into school health programmes, alongside deworming and nutrition, could yield synergistic benefits, as suggested by trials combining IPTsc with anthelmintic treatment (Nankabirwa et al., 2014).

This study has limitations. Its cross-sectional design captures prevalence at one time point during peak transmission and cannot establish duration of infection, within-host dynamics, or seasonal variation. Gametocyte detection by Pfs25 RT-PCR was performed on a subset, so gametocyte prevalence may be underestimated. Mosquito feeding assays were not performed, so infectiousness is inferred from gametocyte carriage rather than directly demonstrated, although the inference is strongly supported by feeding studies elsewhere (Gaye et al., 2015; Bousema et al., 2014). Pfhrp2/3 deletions were not systematically assessed, so some RDT-negative PCR-positive cases could reflect deletions rather than low density alone (WHO, 2020). Finally, the study was conducted in one lake basin; generalisability to other ecologies requires replication, although the consistency with Lake Victoria, Bagamoyo, and Burkina Faso school surveys suggests the pattern is robust (Omondi et al., 2023; Mushi et al., 2024; Walldorf et al., 2015).

Future research should include longitudinal cohorts with repeated molecular sampling to estimate infection duration and clearance, mosquito feeding studies to quantify the infectiousness of P. malariae and mixed infections specifically, and cluster-randomised trials of IPTsc combined with molecular surveillance in lake basin districts. Cost-effectiveness analyses should compare school-based delivery by teachers with community-based delivery, building on evidence that teacher-delivered IPTsc costs approximately US$1.88 per child per year in western Kenya (Temperley et al., 2008). Qualitative work with parents, teachers, and children will be essential to ensure acceptability, adherence, and equity, particularly for girls and for children in shoreline fishing communities who may be missed by school-based programmes if attendance is irregular.

5. Conclusion

Among asymptomatic school-aged children in high-transmission lake basin communities, submicroscopic Plasmodium carriage is the norm rather than the exception. Nested PCR detected more than twice as many infections as microscopy, P. malariae contributed substantially to the hidden reservoir, and one in five submicroscopic infections carried gametocytes. Control programmes that rely solely on microscopy or RDTs will systematically miss the reservoir that sustains transmission. Integrating school-based intermittent preventive treatment with periodic ultrasensitive molecular surveillance offers a feasible, child-centred path to reducing both transmission and the educational and haematological harms of chronic asymptomatic infection. The silent reservoir must be made visible, and then treated, if elimination is to move from aspiration to reality.

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Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

G.W.K. conceived the study, supervised fieldwork, and drafted the manuscript. K.T. supervised molecular diagnostics and contributed to analysis. M.S.F. contributed to study design, statistical analysis, and critical revision. All authors approved the final manuscript.

Data Availability

De-identified data supporting this article are available from the corresponding author upon reasonable request, subject to institutional data-sharing agreements and ethical approval.

References

Atieli, H. E., Zhou, G., Afrane, Y., Lee, M. C., Mwanzo, I., Githeko, A. K. and Yan, G. (2011). Insecticide-treated net (ITN) ownership, usage, and malaria transmission in the highlands of western Kenya. Parasites and Vectors, 4, 113. https://doi.org/10.1186/1756-3305-4-113

Bousema, T., Okell, L., Felger, I. and Drakeley, C. (2014). Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nature Reviews Microbiology, 12(12), 833–840. https://doi.org/10.1038/nrmicro3364

Cohee, L. M., Opondo, C., Clarke, S. E., Halliday, K. E., Cano, J., Shipper, A. G., Barger-Kamate, B., et al. (2020). Preventive malaria treatment among school-aged children in sub-Saharan Africa: a systematic review and meta-analyses. The Lancet Global Health, 8(12), e1498–e1511. https://doi.org/10.1016/S2214-109X(20)30325-9

Cohee, L. M., Valim, C., Barger-Kamate, B., et al. (2022). Effect of malaria chemoprevention for school-age children across transmission archetypes: a modelling study. The Lancet Global Health, 10(10), e1426–e1435. https://doi.org/10.1016/S2214-109X(22)00324-6

Dinko, B., Oguike, M. C., Larbi, J. A., Bousema, T. and Sutherland, C. J. (2020). High prevalence of Plasmodium malariae and Plasmodium ovale in co-infections with Plasmodium falciparum in asymptomatic malaria parasite carriers in southwestern Nigeria. American Journal of Tropical Medicine and Hygiene, 103(2), 758–764. https://doi.org/10.4269/ajtmh.19-0862

Douglas, N. M., Anstey, N. M., Angus, B. J., Nosten, F. and Price, R. N. (2019). Artemisinin combination therapy for vivax malaria. The Lancet Infectious Diseases, 19(5), e168–e180. https://doi.org/10.1016/S1473-3099(18)30639-8

Gatton, M. L., Ciketic, S., Barnwell, J. W., et al. (2017). An assessment of false positive rates for malaria rapid diagnostic tests caused by non-Plasmodium infectious agents and antigen persistence. Malaria Journal, 16, 203. https://doi.org/10.1186/s12936-017-1848-7

Gaye, A., Bousema, T., Libasse, G., et al. (2015). Infectiousness of the human population to Anopheles arabiensis by direct skin feeding in an area hypoendemic for malaria in Senegal. American Journal of Tropical Medicine and Hygiene, 92(3), 648–652. https://doi.org/10.4269/ajtmh.14-0402

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Mfueni, E., et al. (2024). Prevalence and determinants of asymptomatic malaria infection among schoolchildren in Africa: insights from a systematic review and meta-analysis. BMC Infectious Diseases, 24, 456. https://doi.org/10.1186/s12879-024-09345-2

Minakawa, N., Sonye, G., Mogi, M., Githeko, A. and Yan, G. (2002). The effects of climatic factors on the distribution and abundance of malaria vectors in Kenya. Journal of Medical Entomology, 39(6), 833–841. https://doi.org/10.1603/0022-2585-39.6.833

Mohon, A. N., et al. (2022). Overreliance on Plasmodium 18S rRNA gene for malaria molecular diagnosis: inferences from systematic review. Malaria Journal, 21, 372. https://doi.org/10.1186/s12936-022-04397-4

Mueller, I., Zimmerman, P. A. and Reeder, J. C. (2007). Plasmodium malariae and Plasmodium ovale: the bashful malaria parasites. Trends in Parasitology, 23(6), 278–283. https://doi.org/10.1016/j.pt.2007.04.009

Mugittu, K., et al. (2023). Attributable risk factors for asymptomatic malaria and anaemia and their association with cognitive and psychomotor functions in schoolchildren of north-eastern Tanzania. PLOS Global Public Health, 3(4), e0001805. https://doi.org/10.1371/journal.pgph.0001805

Mushi, V., et al. (2024). Implementation and effectiveness of intermittent preventive treatment in school-aged children using dihydroartemisinin-piperaquine to reduce malaria burden: an implementation research of a cluster randomised trial in Tanzania. eClinicalMedicine, 71, 102580. https://doi.org/10.1016/j.eclinm.2024.102580

Nankabirwa, J., Brooker, S. J., Clarke, S. E., et al. (2014). School-based malaria control: impact of intermittent preventive treatment and anthelminthic treatment on malaria morbidity in Ugandan school children. PLOS ONE, 9(1), e85393. https://doi.org/10.1371/journal.pone.0085393

Okell, L. C., Ghani, A. C., Lyons, E. and Drakeley, C. J. (2009). Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis. Journal of Infectious Diseases, 200(10), 1509–1517. https://doi.org/10.1086/644781

Okell, L. C., Bousema, T., Griffin, J. T., Ouédraogo, A. L., Ghani, A. C. and Drakeley, C. J. (2012). Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nature Communications, 3, 1237. https://doi.org/10.1038/ncomms2241

Omondi, C., et al. (2023). Non-random distribution of Plasmodium species infections and associated clinical features in children in the Lake Victoria region, Kenya, 2012–2018. Tropical Medicine and Health, 51, 44. https://doi.org/10.1186/s41182-023-00535-9

Slater, H. C., Ross, A., Felger, I., et al. (2019). The temporal dynamics and infectiousness of subpatent Plasmodium falciparum infections in relation to parasite density. Nature Communications, 10, 1433. https://doi.org/10.1038/s41467-019-09401-4

Snounou, G., Viriyakosol, S., Zhu, X. P., Jarra, W., Pinheiro, L., do Rosário, V. E., Thaithong, S. and Brown, K. N. (1993). High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Molecular and Biochemical Parasitology, 61(2), 315–320. https://doi.org/10.1016/0166-6851(93)90077-B

Temperley, M., et al. (2008). Costs and cost-effectiveness of delivering intermittent preventive treatment through schools in western Kenya. Malaria Journal, 7, 142. https://doi.org/10.1186/1475-2875-7-142

Walldorf, J. A., Cohee, L. M., Coalson, J. E., et al. (2015). School-aged children are a reservoir of malaria infection in Malawi. PLOS ONE, 10(7), e0134061. https://doi.org/10.1371/journal.pone.0134061

Whittaker, C., et al. (2021). Global and temporal patterns of submicroscopic Plasmodium falciparum malaria infection. Nature Communications, 12, 1258. https://doi.org/10.1038/s41467-021-21345-8

World Health Organization (2016). Malaria microscopy quality assurance manual, version 2. Geneva: World Health Organization. https://www.who.int/publications/i/item/9789241549394

World Health Organization (2020). Pfhrp2/3 gene deletions: WHO guidance note. Geneva: World Health Organization. https://www.who.int/publications/i/item/9789241515800

World Health Organization (2025). World malaria report 2025. Geneva: World Health Organization. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2025

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Yman, V., et al. (2023). Epidemiology of Plasmodium malariae and Plasmodium ovale spp. in a highly malaria-endemic country: a longitudinal cohort study in Kinshasa Province, Democratic Republic of Congo. Journal of Infectious Diseases, 228(5), 608–617. https://doi.org/10.1093/infdis/jiad096

Zongo, I., et al. (2024). School-aged children as a silent reservoir of Plasmodium falciparum: findings from a cross-sectional survey conducted in Burkina Faso in 2022. Malaria Journal, 23, 112. https://doi.org/10.1186/s12936-024-04932-0

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