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Unveiling the magnitude of submicroscopic parasitemia and gametocytaemia in different malaria endemic areas of Mainland Tanzania

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Abstract

Background: The prevalence of *Plasmodium falciparum* in community samples is one of the cornerstones for describing malaria transmission. Prevalence measurements greatly depend on the diagnostic techniques applied and can be biased and inaccurate when parasite densities are low since parasites often remain undetected by the routinely used diagnostic tool, light microscopy. Highly sensitive molecular tools can detect submicroscopic infections and can serve to evaluate the performance of diagnostic tools used in field surveys. The study investigated local differences in the prevalence of submicroscopic infections and gametocyte carriage at different sites of varying endemicity. It investigated age trends in submicroscopic carriage of both asexual parasites and gametocytes from Tanzanian communities.

Methods: A community survey involved 1820 individuals from four regions of varying endemicity. Finger prick blood was collected by light microscopy, mRDT, and 18S rRNA qPCR for parasite detection. Gametocytes were detected by both light microscopy and qRT-PCR targeting transcripts of the gametocyte-specific expressed marker *pfs25*. Submicroscopic infections were those positive by qPCR but not by light microscopy.

Results: *P. falciparum* prevalence by qPCR varied from 37% at the site of high endemicity to 0.6% at the site of low endemicity. Of these, 52.7% (174/330) of qPCR-positive samples were submicroscopic infections. The submicroscopic carriage did not show a clear relationship with endemicity patterns and was 73% (11/15) in low, 63% (5/8), in moderate, and 51.1% (156/305) in high endemic sites. Molecular-determined gametocyte prevalence at each location closely followed the asexual parasite prevalence. But most (96%; 277/288) of gametocyte carriers were submicroscopic.

Conclusion: Molecular parasite detection revealed a high prevalence of submicroscopic carriage in Tanzania, particularly among adults. Submicroscopic infections were recorded in all endemic settings but were more prevalent in the high-endemic sites, where nearly half of the infections were not detected by routine light microscopy. Such data on submicroscopic parasitemia and gametocytemia from multiple sites within a country can help to better assess the human infective reservoir for onward transmission and the relative contribution of asymptomatic populations to the ongoing malaria transmission.

Keywords: Malaria, submicroscopic malaria, gametocyte prevalence, endemicity, qPCR, microscopy

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Introduction

Measurement of malaria parasite prevalence rates in endemic communities is one of the most important metrics for describing levels of malaria endemicity. Prevalence rates complement entomological measures of transmission that are much more difficult to obtain, particularly in low-transmission settings. Monitoring changes in prevalence rates is useful for measuring the outcomes of malaria interventions and for informing control strategies.

As a consequence of intensified malaria interventions, a decline in malaria prevalence has been observed in many areas, including highly endemic regions in sub-Saharan Africa (WHO, 2023). The East African countries have also recorded a decline in malaria prevalence in recent years (Whittaker et al., 2021) Tanzania, in particular, has recorded a decline in the national average of malaria prevalence from 18% in 2008 to 7% in 2023 (MoH et al., 2018; MoH, 2023).

A decline in malaria prevalence and thus, exposure to *Plasmodium falciparum* could affect parasite densities in the population and the sensitivity of the commonly used malaria diagnostic tools. In view of a worldwide malaria reduction, it is important to investigate the potential changes in the performance of light microscopy, the traditional malaria diagnostic tool, in various epidemiological settings. In particular, the use of molecular diagnosis has gained increasing importance in the current efforts to scale up malaria control and move towards malaria elimination owing to its higher sensitivity and reliability than the traditional malaria diagnostic tools (Britton et al., 2016; Hofmann et al., 2018; Lazrek et al., 2023; Oyegoke et al., 2022; Shamseddin et al., 2022).

Several molecular-epidemiological studies in Tanzania have reported a large proportion of submicroscopic infections in North-East Tanzania (Kaaya et al., 2022; Shekalaghe et al., 2007) the coastal area (Schindler et al., 2019) and the lake zone (Manjurano et al., 2011, 2023; Rapp et al., 2023). These are all underestimates of the actual prevalence of sub-microscopic carriage rates because many infections have densities that are too low even for reliable detection by PCR. This suggests that low parasite densities account for the low positivity rates by light microscopy in community samples, particularly from low transmission settings (Desmurget et al., 1998) Such a relationship would imply that light microscopy, the primarily used detection method, might provide insufficient sensitivity for malaria surveillance if the prevalence of malaria further declines in the future.

For other Tanzanian regions, in particular, the prevalence of submicroscopic infections in moderate and high malaria transmission settings has not been investigated so far. The comparative analysis of four (4) Tanzanian regions of greatly differing malaria endemicity aimed at filling this gap. In parallel to investigating *P. falciparum* prevalence across Tanzania, the prevalence of gametocytes was also assessed by light microscopy and molecular assays. Gametocyte quantification is useful for describing the human infectious reservoir for mosquitoes.

This multi-site study aimed to determine regional variations in the percentage of submicroscopic parasitemia and explore the efficacy of light microscopy in malaria surveillance across the entire gamut of local transmission intensities. This also provides a useful and more precise description of the malaria prevalence in the country. Determining the extent of submicroscopic parasitemia, particularly in areas of very low transmission and urban areas in Tanzania, aims to test the hypothesis that in such settings, most *P. falciparum* infections are detectable only by molecular methods. The secondary objective was to document the molecular gametocyte prevalence in the different transmission settings and to confirm their presence in samples of very low or urban transmission, despite their apparent absence by light microscopy.

Methods and study design

Study sites

The study was conducted in four regions in Tanzania, which were classified as urban, low, medium and high endemic settings according to district prevalence data recorded by the

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Tanzania HIV and Malaria indicator surveys of 2008 (MoH, 2008): Coastal Region (high), Tanga (moderate), Dar Es Salaam (urban Low), and Iringa (Low).

Study design

Cross-sectional surveys were conducted at all study sites between May and August 2013. 1820 individuals aged (>6 months to 90 years) were enrolled. Demographic information was collected for all consented individuals. Similarly, finger prick blood was collected, and malaria screening was performed by Pan PLDH/Pf (HRP2) SD Bioline® Rapid Diagnostic Tests (mRDT). ACT was administered as prescribed on the spot to those who tested positive for malaria by RDT.

In addition, thick blood smears were also prepared in order to diagnose malaria using light microscopy. For molecular analysis, about 100–150µL of whole blood from a finger prick was collected in EDTA tubes and kept at ambient temperature until later that same day, when 100µL of EDTA blood was transferred to microtubes containing 500µL of RNA Protect® reagent and mixed. All blood samples were stored at -20°C until further molecular analysis was performed in the molecular laboratory at the Swiss Tropical Public Health Institute in Basel, Switzerland. Ribo-Nucleic Acid (RNA) and Deoxy-Nucleic Acid (DNA) were co-extracted in a 96-well plate format. Details of nucleic acid (RNA and DNA) extraction methods have been described previously in (Mwingira et al., 2014; Wampfler et al., 2013). The obtained nucleic acids were stored temporarily at 20°C storage prior to Polymerase Chain Reaction (PCR) analysis.

Ethical clearance

Approval for the cross-sectional study was obtained from the Institutional Review Board of the Ifakara Health Institute (IHI), Dar Es Salaam (No. 13-2013). Approval was also sought from the District Medical Officers and the local governing bodies of respective administrative wards, streets, and hamlet levels. Before blood sampling, informed written consent was obtained from all participants or parents/guardians of all children.

Light microscopy

Thick films were made from finger-prick blood, air-dried, and stained with 10% Giemsa in the IHI laboratory. Parasite densities were quantified by counting the number of asexual and gametocytes per 200 and 500 leukocytes, respectively. The results were converted to parasites/µL, assuming a total of 8000 WBC/µL blood.

P. falciparum Asexual and gametocyte detection

P. falciparum asexual stages were analyzed by a DNA-based *Pf* 18S rRNA qPCR assay as described elsewhere (Wampfler et al., 2013). *P. falciparum* gametocyte detection was performed on RNA from all samples irrespective of their DNA-based positivity but only after confirming the presence of parasite RNA in the sample. Transcripts of the gametocyte-specific expressed gene (*pfs25* mRNA gene), were reverse transcribed and the resulting *pfs25* cDNA was amplified by quantitative polymerase chain reaction (qPCR) in a single reaction (Wampfler et al., 2013)Standard curves of assay-specific plasmids in triplicates were analyzed on each plate together with the test samples to convert Ct values into template copy numbers/µL blood.

Data analysis

Data were entered and analyzed by STATA® version 13, Texas USA. Descriptive statistics was used to present frequencies, percentages, and averages of the variables, tables, and graphs. Comparison of malaria prevalence rates by light microscopy, mRDT, and qPCR were determined and compared between the sites using chi-square. The age of participants was categorized into 8 groups as follows: <1, 1-2, 3-4, 5-9, 10-19, 20-39,40-59, 60+ years. Samples with missing age data were excluded from the analysis. Submicroscopic parasitemia/gametocytaemia was

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defined as samples that were parasite/gametocyte positive by molecular detection but not by light microscopy.

Results

Demographic information

There were 1820 participants, of whom 58.1% (n = 1058) were female. The participants' ages ranged from 5 months to 91 years, with a mean age of 30.7 years (Table 1). The majority of participants were from the Coast region (44.84%; n = 816), while the fewest hailed from the Tanga region (12.91%; n = 232).

Table 1: Demographic information for participants (N=1820)

Variable	Category	n	Frequency(%)
Region	Dar es Salaam	440	24.18
	Iringa	329	18.08
	Pwani	816	44.84
	Tanga	235	12.91
Sex	Female	1058	58.13
	Male	762	41.87
Age (Years)	0 to 5	295	16.21
	6 to 15	335	18.41
	16 to 25	221	12.14
	26 to 35	247	13.57
	36 to 45	237	13.02
	46 to 55	186	10.22
	55+	299	16.43

Malaria prevalence and asexual parasite density

The overall *Plasmodium falciparum* malaria prevalence rates determined by two diagnostic tests were 18.1% (n = 330; CI = 16.3–19.9%) by qPCR and 11.8% (n = 216; CI = 10.3–13.3%) by light microscopy. The prevalence rates by qPCR varied substantially between geographic sites, ranging from approximately 37% in the Coast region to 0.6% in Iringa. Light microscopy. mirrored the molecular (qPCR) findings (Figure 1). The discrepancy between prevalence rates recorded by qPCR and light microscopy was largest in the Coast region (high malaria endemic), where about 40% of total parasite infections were undetected by light microscopy. However, in Dar Es Salaam (Urban), where light microscopy recorded a prevalence of 6.6% (29/440), which was 2-fold higher than that by qPCR of 3.4% (15/440) (Figure 1; Supplementary Tab S1). The two diagnostic methods differed only marginally in prevalence rates recorded in Iringa (low endemicity) and Tanga (moderate endemicity). This implies that submicroscopic infections prevail at high endemic sites but are rare in low and moderate transmission sites, where light microscopy performed more or less similar to molecular assay.

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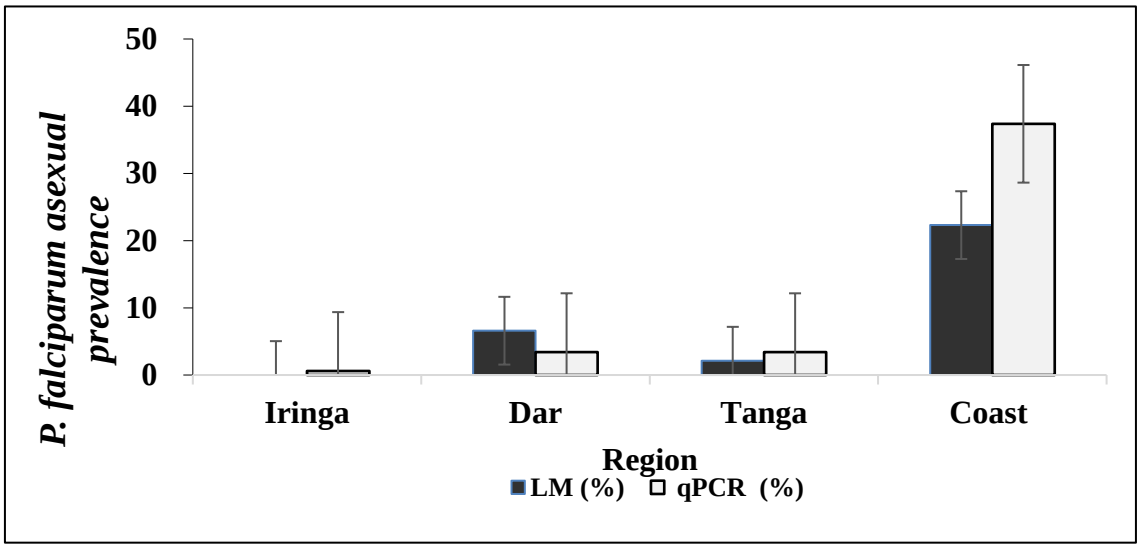

Figure 1: *Plasmodium falciparum* malaria prevalence by light microscopy and qPCR diagnostic methods by region (* = LM = Light microscopy, qPCR = Quantitative PCR)

To investigate whether higher asexual parasite densities in areas of low and moderate transmission could account for the good light microscopy diagnosis observed, we analyzed parasite counts by light microscopy. The geometric mean parasite density by light microscopy was 4407 parasites/µL blood (95% CI: 3131-6203 parasites/µL blood and 317 parasites/µL blood; 95% CI: 269-374 parasites/µL blood) in the coastal region (high) and Dar (low) endemic sites, respectively (Figure 2). This implies a 14-fold difference in the mean asexual parasite density measured by light microscopy between the lowest and highest endemic sites. However, the numbers of *P. falciparum* positive samples in the low and moderate endemic settings were very low, so the estimates of the mean parasite load at these sites may not be robust enough to provide a conclusion.

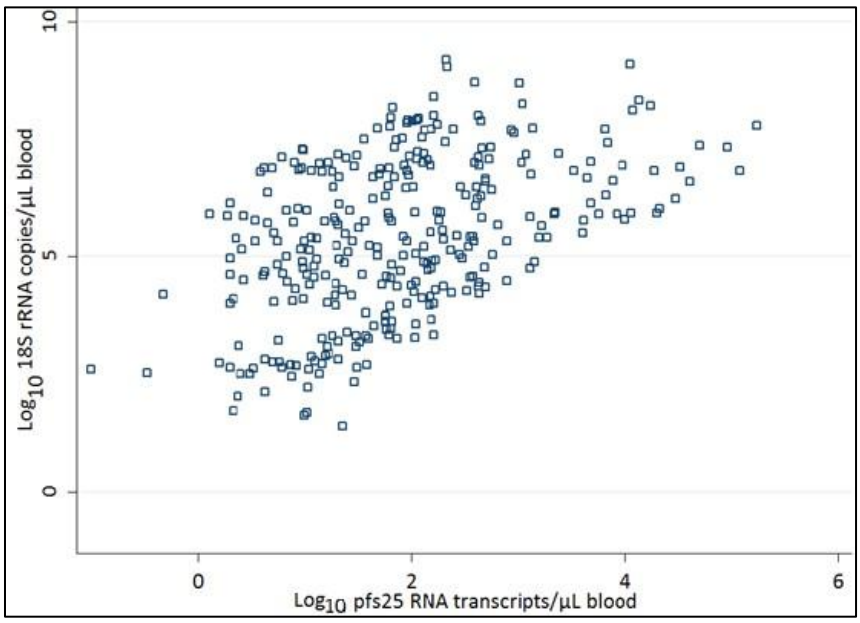

Figure 2: Correlation of *P. falciparum* asexual parasite load (expressed as Log_{10} 18S rRNA copy numbers/µL blood) and gametocyte load by (expressed as Log_{10} *pfs25* copy numbers/µL blood) in samples positive for both molecular assays.

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Plasmodium falciparum gametocyte prevalence and density

P. falciparum gametocytes (sexual parasites) were diagnosed in all samples by light microscopy and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis. The overall molecular gametocyte prevalence in samples from the four (4) sites was 15.8% (288/1820; 95% CI 14.1–17.5%). Specifically, 87.2% (288/330) and 0.82% (15/1820; 95% CI 0.47–1.2%). of *P. falciparum*-positive samples carried gametocytes by qRT-PCR and light microscopy, respectively. The molecular *P. falciparum* gametocyte prevalence ranged from <1% in Iringa and Dar Es Salaam to 43.6% in the Coast region (Supplementary Table S1). Generally, light microscopy identified only 5.2% (15/288) of all gametocyte carriers identified by qRT-PCR. The overall mean gametocyte density was 52 gametocytes/µL blood, ranging from 30-90 gametocytes/µL blood by light microscopy. In the coastal region, the high endemic site displayed a geometric mean of 54 gametocytes/ µL blood, while only one positive sample in Dar, an urban low endemic site, had a geometric mean of 32 gametocytes/µL blood. A positive linear correlation (r2 = 0.47) was observed between the P. falciparum parasite load depicted by the log-transformed 18S rRNA copies/µL blood and the gametocyte load in the same sample (Figure 2).

Submicroscopic asexual and gametocyte malaria prevalence

Overall, 52.7% (174/330) of samples positive for asexual *P. falciparum* were submicroscopic. At the site of lowest endemicity (Iringa), light microscopy did not detect any positive samples, whereas two (2) *P. falciparum*-positive samples were found by qPCR. The low numbers of positive samples preclude any conclusion on submicroscopic prevalence in the two low-endemicity sites. In contrast, high numbers (51.1%; 156/305) of asexual stage submicroscopic carriers were observed in the Coast region. Generally, a trend of increase in the proportion of asexual submicroscopic carriage with malaria endemicity was observed (Table 2).

Table 2: Proportion of submicroscopic asexual parasite carriers in different malaria endemic regions of Tanzania (N=1820)

Region	Sample size	qPCR positives n (%)	LM positives in qPCR positives x/n (%)	Proportion submicroscopic carriers y/n (%)	Prevalence of submicroscopic carriers (n/N) (%)
Iringa	329	2 (0.6)	0	2/2 (100)1)	2/329* (n.a.)
Dar	440	15 (3.4)	4/15 (26.7)	11/15 (73.3)	11/440 (2.5)
Tanga	235	8 (3.4)	3/8 (37.5)	5/8 (62.5)	5/235 (2.1)
Coast	816	305 (37.4)	149/305 (48.9)	156/305(51.1)	19/316 (19.1)

n.a. Number too low to support any firm conclusion.

Light microscopy-based gametocyte detection was 15-fold lower than molecular diagnosis in the high coastal region. Contrary, in the low and moderate malaria-endemic areas (Iringa, Dar es Salaam, and Tanga), light microscopy did not detect any gametocytes, while qRT-PCR detected gametocytes in all sites. Overall, 96% (277/288) of all P. falciparum gametocyte-positive samples were submicroscopic since they were detected by molecular methods only. Malaria endemicity did not affect the prevalence of submicroscopic gametocytemia in a major way (Table 3).

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Table 3: Proportion of submicroscopic gametocyte carriers in different endemic regions of Tanzania (N=1820)

Region	Sample size	*pfs25* positive n (%)	Microscopy positive in *pfs25* positive x/n (%)	Proportion submicroscopic gametocyte carriers y/n (%)	Submicroscopic gametocyte carriers y/N (%)
Iringa (Low)	329	1 (0.3)	0	1/1 (100)	1/239 (n.a)
Dar (Low and Urban)	440	3 (0.68)	0	3/3 (100)	3/440 (n.a)
Tanga (Moderate)	235	15 (6.4)	0	15/15 (100)	15/235 (6.4)
Coast (high)	816	269 (33.0)	11(4.1)	258/269 (95.9)	258/816 (31.6)

n.a.) Number too low to support any firm conclusion

Age trends in prevalence and submicroscopic carriage of P. falciparum parasites and gametocytes

Molecular data from all sites were combined, to investigate age trends in parasite density, prevalence, and submicroscopic carriage. In age groups 1-2 years, 3–4 years, and 5–9 years, both high prevalence rates (Figure 3) and high densities were observed (Figure 4). The highest prevalence rate by qPCR peaked at 5–9 years. A similar observation was shown by light microscopy. Parasite density by light microscopy and PCR was highest at 3–4 years, followed closely by age groups (1-2 years and 5–9 years). A decline in both prevalence rates and parasite load by light microscopy and qPCR was observed in children older than 10 years and adults.

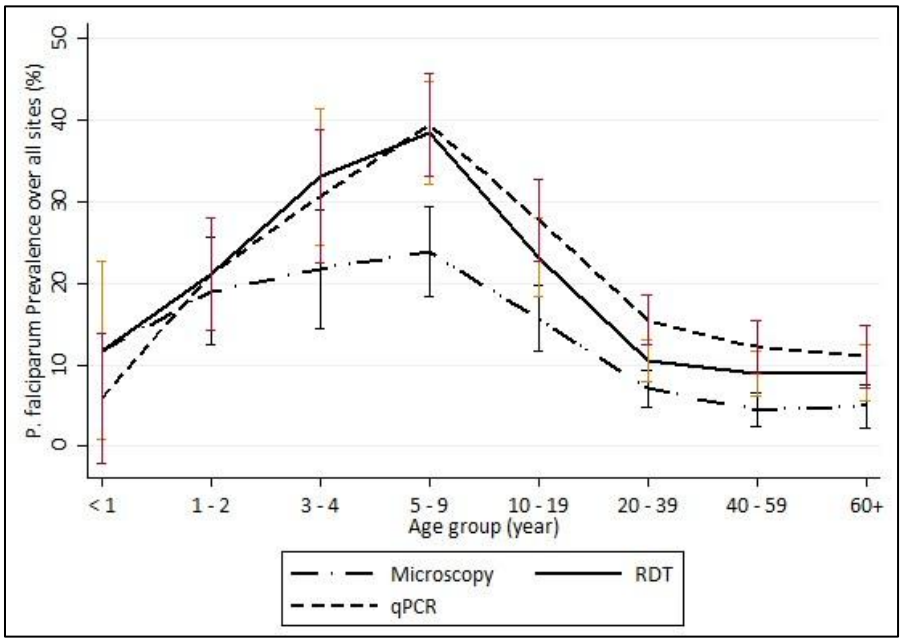

Figure 3: Age trends in *P. falciparum* prevalence diagnosed by LM, RDT and qPCR for the combined data set from all study sites.

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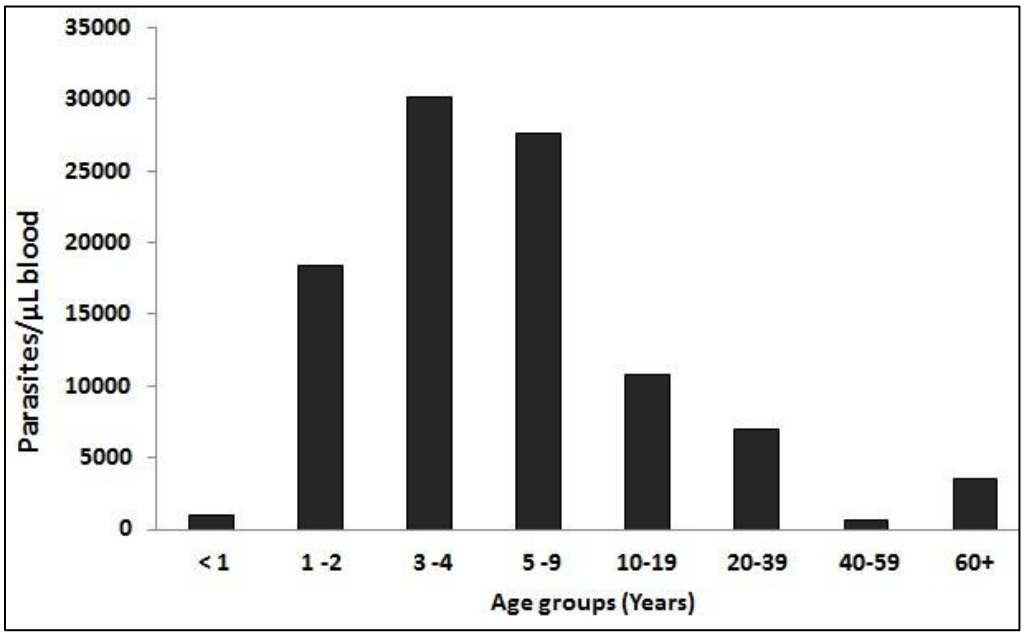

Figure 4: Mean microscopic asexual parasite density (parasites/µL blood) by age

The prevalence of submicroscopic infections increased steadily over the younger age groups and peaked at 20–39 years, followed by a decline in the oldest age groups (Figure 5). Thus, the age trend in submicroscopic infections was non-monotonous, with a clear increase in the younger age groups.

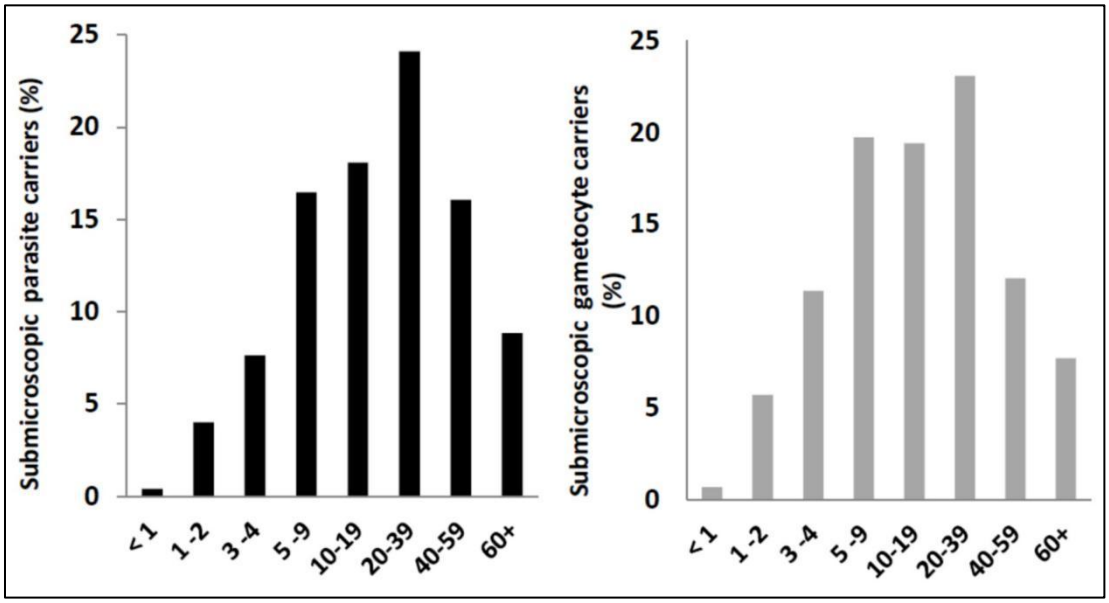

Figure 5: Percentage of submicroscopic parasite carriers (left) and percentage of submicroscopic gametocyte carriers (right) by age.

Discussion

Considerable variations in *P. falciparum* parasite and gametocyte prevalence rates across the regions with varying malaria endemicity were revealed. The strength of this study is the good comparability of the data generated at each site. Using generalized protocols, parasite detection by light microscopy, mRDT, and qPCR was performed on the entire data set by the same field and laboratory team. This approach minimizes technical variability and has major advantages over a comparison of data from unrelated studies performed with different techniques. The observed differences between the study sites could thus be attributed to micro-geographical factors,

particularly those that favour the development and survival of mosquito vectors (Challe et al., 2024; Traoré et al., 2024). In Tanzania, for instance, highland areas, such as Iringa region, with low temperatures of about 15°C and an altitude >1600m, are known to have low or no malaria transmission (Mboera et al., 2008; MoH, 2023). Urban areas of Dar Es Salaam have much less malaria (MoH, 2023; Strøm et al., 2013), compared to the rural regions of Tanga (Hayuma et al., 2021; Mmbando et al., 2010; MoH, 2023) and the Coast region (Khatib et al., 2012; MoH, 2023) that are well documented and known to be moderate to highly endemic (MoH, 2023; MoH et al., 2018; Thawer et al., 2020).

In the low and moderate malaria-endemic areas, the prevalence rates obtained by the three (3) independent diagnostic tools did not differ substantially. This implies that most infections were captured even by light microscopy and mRDTs. In contrast, the high endemicity site observed significant discrepancies between light microscopy and qPCR. This is because the highly sensitive detection limit of 0.34 parasites/µL by the 18S rRNA qPCR (Hofmann et al., 2015) significantly exceeds the limit of detection of light microscopy, which is around 50–100 parasites/µL (Lazrek et al., 2023; Mwenda et al., 2021). Thus, the current study confirmed that molecular detection yielded the highest prevalence rates at all sites except Dar Es Salaam, where light microscopy recorded the highest malaria prevalence estimates. The 14/440 samples that were positive only by light microscopy could be attributed to technical problems at this site or to erroneous readings of thick smears. Under conditions of low endemicity, light microscopy sometimes creates false positives. High levels of false positives by light microscopy have been previously reported from other areas of low endemicity in Dar es Salaam (Kahama-Maro et al., 2011; Strøm et al., 2013) and elsewhere (Fançony et al., 2013; Wongsrichanalai et al., 2007).

This study confirms the predominance of submicroscopic *P. falciparum* infections in Tanzania, with more than half (52.7%) of all molecular-detected infections not detected by light microscopy. This is consistent with data from the Usambara , Korogwe, and Handeni in Tanga, the Kilimanjaro region, Mwanza and Coast region in Tanzania, where up to 50% of the total infections were submicroscopic (Hayuma et al., 2021; Kaaya et al., 2022; Manjurano et al., 2011, 2023; Rapp et al., 2023; Shekalaghe et al., 2007). The results are also in line with an earlier meta-analysis that indicated light microscopy detects, on average, only 50% of total infections determined by PCR (Okell et al., 2009). Submicroscopic infections in this study were more prevalent in older children and adults, peak prevalence at 20-39 years.

A similar observation was reported from North-Eastern and Coastal Tanzania, where older children were up to 3-times more likely to carry submicroscopic infections compared to younger children (Manjurano et al., 2011; Sumari et al., 2017) Additionally, a meta analysis on by Eijk et al.,revealed that submicroscopic carriage peaked at ≥ 30 years among pregnant women in Africa (Eijk et al., 2023). Similarly, a meta-analysis by Okell et al.,showed that submicroscopic infections were common in adults, owing to the ability of acquired immunity to control parasite densities to levels under the detection limit of light microscopy (Desmurget et al., 1998; Okell et al., 2009)Contrary to this meta-analysis, the current study did not observe an increase of submicroscopic carriage with decreasing endemicity sites, and data on submicroscopic carriage did not show substantial differences between sites of different endemicities.

The detection limit of light microscopy very often does not permit the diagnosis of low-density infections. Thus estimates generated by it will depend significantly on the average parasite density of the population studied. The current study found no significant differences in parasite densities generated by light microscopy across the transmission settings. This indicates that parasite densities in high and low endemic settings might be well controlled by acquired immunity because of previous exposure to malaria parasites. Additionally, it has been argued that a recent decline in transmission in formerly highly endemic areas keep parasite densities low despite reduced transmission (Björkman & Morris, 2020; Desmurget et al., 1998; Drakeley et al., 2018; Gatton & Cheng, 2010; Golassa et al., 2013; Lindblade et al., 2013; Nguyen et al., 2018; Sturrock et al., 2013). Similarly, a study in Tanzania indicates an inverse relation between parasite

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densities and endemicity, suggesting that submicroscopic infections are highly likely to occur in high endemic areas where exposure to infection is more frequent and densities are low (Mosha et al., 2013). The latter and the current data are inconsistent with the analysis by Okell et al., 2012, which indicates that in low endemic settings with a *P. falciparum* prevalence of <10% the majority of infections (88%) should be submicroscopic, while in high endemic settings with a prevalence >75% the submicroscopic carriage was estimated to be as low as 25%. In the current study, there were very few positive malaria cases in low endemic sites (e.g., only 2 infections by qPCR at the lowest endemicity site). Thus, not sufficient data is generated to draw firm conclusions on the submicroscopic carriage.

Additionally, errors in slide reading contributed to this effect. These limitations became clear given the few qPCR-positive samples even in low (positive samples: 2/329 and 15/440) and moderate (positive samples 8/235 and 56/316) transmission settings. The study observed high *P. falciparum* prevalence rates by both light microscopy and qPCR in young children, with a peak in children aged 5-9 years. This was expected as high incidence rates for clinical malaria and infections of high parasite densities mainly occur in children. Similar studies in Ghana and Tanzania have also recorded this age shift from under five to a peak in malaria prevalence at 5 - 9 years (Abate et al., 2022; Drakeley et al., 2018; Kigozi et al., 2020; Lindblade et al., 2013; Lubinda et al., 2021; Mosha et al., 2013; Salgado et al., 2021).

Another objective of this study was to investigate whether malaria endemicity affects the prevalence of submicroscopic gametocyte carriers. As expected, applying molecular tools for gametocyte detection was far better than light microscopy. The molecular assay detected 15.8% gametocyte carriers compared to <1% by light microscopy. Generally, 96% of all the gametocyte-positive samples were submicroscopic. The occurrence of a high proportion of submicroscopic gametocytemia (95-100% at all sites), irrespective of endemicity, is of concern since several studies involving mosquito-feeding experiments have indicated that hosts with submicroscopic gametocytaemia can transmit malaria (Churcher et al., 2013; Coalson et al., 2018; Coleman et al., 2004; Lin et al., 2014, 2016; Muirhead-Thomson, 1951; Schneider et al., 2006).

Despite the great benefits of molecular tools for parasite detection, PCR will unlikely replace the two standard diagnostic tools in the field, light microscopy and mRDT, which will continue to be heavily used in resource-limited settings (Mwingira et al., 2014). However, PCR can play an essential role in assessing the quality of light microscopy. By routinely conducting PCR-based studies in parallel to light microscopy diagnosis in some malaria surveys, valuable estimates of this otherwise undetected parasite burden can be generated, and help make informed decisions on malaria elimination strategies. Therefore, with a country-wide cross-sectional survey combined with PCR diagnosis, the current study could assess the light microscopy quality and unveil the magnitude of submicroscopic malaria infections for Tanzania's widely differing transmission intensities.

The fact that some regions in East Africa (Whittaker et al., 2021) and particularly in Tanzania, have undergone an epidemiological shift towards lower malaria endemicity over the last decade (MoH, 2023; MoH et al., 2018) calls for continuous monitoring of the magnitude of submicroscopic carriage, especially among asymptomatic infections.

Conclusion

This study confirms a high prevalence of *Plasmodium falciparum* submicroscopic parasitemia and gametocytemia in Tanzania. The molecular parasite detection assays unveiled 52% of infections and 96% of gametocyte carriers that would have been missed if the survey had relied solely on light microscopy. Submicroscopic infections were observed in all endemic settings and did not show a specified trend with decreasing endemicity. These results can inform control strategies by drawing attention to asymptomatic individuals, mostly adults with primarily sub-microscopic infections, whose contribution to onward transmission should not be ignored. Thus,

the qPCR-based point prevalence rates for areas of different endemicity contribute to a solid basis for planning and monitoring elimination efforts in Tanzania.

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Authors' contribution

FWM:conceived the study concept and design, collected data, performed analyses, data interpretation and wrote and approved the manuscript.

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Competing interests

The author declares no conflict of interest.

Availability of data and materials

All data generated or analyzed during this study are achieved and available under reasonable request.

References

Abate, A., Assefa, M., & Golassa, L. (2022). Five-Year Trend of Malaria Prevalence in Mojo Town, Central Ethiopia: Shifting Burden of the Disease and Its Implication for Malaria Elimination: A Retrospective Study . *Infection and Drug Resistance*, *15*, 455–464. https://doi.org/10.2147/IDR.S348203

Björkman, A., & Morris, U. (2020). Why Asymptomatic Plasmodium falciparum Infections Are Common in Low-Transmission Settings. *Trends in Parasitology*, *36*(11), 898–905. https://doi.org/10.1016/j.pt.2020.07.008

Britton, S., Cheng, Q., & McCarthy, J. S. (2016). Novel molecular diagnostic tools for malaria elimination: A review of options from the point of view of high-throughput and applicability in resource limited settings. *Malaria Journal*, *15*(1), 88. https://doi.org/10.1186/s12936-016-1158-0

Challe, D. P., Francis, F., Seth, M. D., Tupa, J. B., Madebe, R. A., Mandara, C. I., Kigadye, E. S., Makene, V. W., & Ishengoma, D. S. (2024). *Prevalence and risk factors associated with malaria infections at a micro-geographic level in three villages of Muheza district, north-eastern Tanzania* (p. 2024.03.04.24303697). medRxiv. https://doi.org/10.1101/2024.03.04.24303697

Churcher, T. S., Bousema, T., Walker, M., Drakeley, C., Schneider, P., Ouédraogo, A. L., & Basáñez, M.-G. (2013). Predicting mosquito infection from Plasmodium falciparum gametocyte density and estimating the reservoir of infection. *eLife*, *2*, e00626. https://doi.org/10.7554/eLife.00626

Coalson, J. E., Cohee, L. M., Buchwald, A. G., Nyambalo, A., Kubale, J., Seydel, K. B., Mathanga, D., Taylor, T. E., Laufer, M. K., & Wilson, M. L. (2018). Simulation models predict that school-age children are responsible for most human-to-mosquito Plasmodium falciparum transmission in southern Malawi. *Malaria Journal*, *17*, 147. https://doi.org/10.1186/s12936-018-2295-4

Coleman, R. E., Kumpitak, C., Ponlawat, A., Maneechai, N., Phunkitchar, V., Rachapaew, N., Zollner, G., & Sattabongkot, J. (2004). Infectivity of Asymptomatic Plasmodium-Infected Human Populations to Anopheles dirus Mosquitoes in Western Thailand. *Journal of Medical Entomology*, *41*(2), 201–208. https://doi.org/10.1603/0022-2585-41.2.201

Desmurget, M., Pélisson, D., Urquizar, C., Prablanc, C., Alexander, G. E., & Grafton, S. T. (1998). Functional anatomy of saccadic adaptation in humans. *Nature Neuroscience*, *1*(6), 524–528. https://doi.org/10.1038/2241

Drakeley, C., Gonçalves, B., Okell, L., Slater, H., Drakeley, C., Gonçalves, B., Okell, L., & Slater, H. (2018). Understanding the Importance of Asymptomatic and Low- Density Infections for Malaria Elimination. In *Towards Malaria Elimination—A Leap Forward*. IntechOpen. https://doi.org/10.5772/intechopen.77293

Eijk, A. M. van, Stepniewska, K., Hill, J., Taylor, S. M., Rogerson, S. J., Cottrell, G., Chico, R. M., Gutman, J. R., Tinto, H., Unger, H. W., Yanow, S. K., Meshnick, S. R., Kuile, F. O. ter, Mayor, A., Eijk, A. M. van, Stepniewska, K., Hill, J., Taylor, S. M., Rogerson, S. J., … Mayor, A. (2023). Prevalence of and risk factors for microscopic and submicroscopic malaria infections in pregnancy: A systematic review and meta-analysis. *The Lancet Global Health*, *11*(7), e1061–e1074. https://doi.org/10.1016/S2214-109X(23)00194-8

Fançony, C., Sebastião, Y. V., Pires, J. E., Gamboa, D., & Nery, S. V. (2013). Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: A comparison using PCR as the gold standard. *Malaria Journal*, *12*, 284. https://doi.org/10.1186/1475-2875-12-284

Gatton, M. L., & Cheng, Q. (2010). Interrupting Malaria Transmission: Quantifying the Impact of Interventions in Regions of Low to Moderate Transmission. *PLOS ONE*, *5*(12), e15149. https://doi.org/10.1371/journal.pone.0015149

Golassa, L., Enweji, N., Erko, B., Aseffa, A., & Swedberg, G. (2013). Detection of a substantial number of sub-microscopic Plasmodium falciparum infections by polymerase chain reaction: A potential threat to malaria control and diagnosis in Ethiopia. *Malaria Journal*, *12*(1), 352. https://doi.org/10.1186/1475-2875-12-352

Hayuma, P. M., Wang, C. W., Liheluka, E., Baraka, V., Madebe, R. A., Minja, D. T. R., Misinzo, G., Alifrangis, M., & Lusingu, J. P. A. (2021). Prevalence of asymptomatic malaria, submicroscopic parasitaemia and anaemia in Korogwe District, north-eastern Tanzania. *Malaria Journal*, *20*(1), 424. https://doi.org/10.1186/s12936-021-03952-3

Hofmann, Gruenberg, M., Nate, E., Ura, A., Rodriguez-Rodriguez, D., Salib, M., Mueller, I., Smith, T. A., Laman, M., Robinson, L. J., & Felger, I. (2018). Assessment of ultra-sensitive malaria diagnosis versus standard molecular diagnostics for malaria elimination: An in-depth molecular community cross-sectional study. *The Lancet. Infectious Diseases*, *18*(10), 1108–1116. https://doi.org/10.1016/S1473-3099(18)30411-0

Hofmann, N., Mwingira, F., Shekalaghe, S., Robinson, L. J., Mueller, I., & Felger, I. (2015). Ultra-Sensitive Detection of Plasmodium falciparum by Amplification of Multi-Copy Subtelomeric Targets. *PLOS Medicine*, *12*(3), e1001788. https://doi.org/10.1371/journal.pmed.1001788

Kaaya, R. D., Matowo, J., Kajeguka, D., Tenu, F., Shirima, B., Mosha, F., & Kavishe, R. (2022). The Impact of Submicroscopic Parasitemia on Malaria Rapid Diagnosis in Northeastern Tanzania, an Area with Diverse Transmission Patterns. *Infectious Disease Reports*, *14*(6), Article 6. https://doi.org/10.3390/idr14060082

Kahama-Maro, J., D'Acremont, V., Mtasiwa, D., Genton, B., & Lengeler, C. (2011). Low quality of routine microscopy for malaria at different levels of the health system in Dar es Salaam. *Malaria Journal*, *10*, 332. https://doi.org/10.1186/1475-2875-10-332

Khatib, R. A., Skarbinski, J., Njau, J. D., Goodman, C. A., Elling, B. F., Kahigwa, E., Roberts, J. M., MacArthur, J. R., Gutman, J. R., Kabanywanyi, A. M., Smith, E. E., Somi, M. F., Lyimo, T., Mwita, A., Genton, B., Tanner, M., Mills, A., Mshinda, H., Bloland, P. B., … Kachur, S. P. (2012). Routine delivery of artemisinin-based combination treatment at fixed health facilities reduces malaria prevalence in Tanzania: An observational study. *Malaria Journal*, *11*(1), 140. https://doi.org/10.1186/1475-2875-11-140

Kigozi, S. P., Kigozi, R. N., Epstein, A., Mpimbaza, A., Sserwanga, A., Yeka, A., Nankabirwa, J. I., Halliday, K., Pullan, R. L., Rutazaana, D., Sebuguzi, C. M., Opigo, J., Kamya, M. R., Staedke, S. G., Dorsey, G., Greenhouse, B., & Rodriguez-Barraquer, I. (2020). Rapid shifts in the age-specific burden of malaria following successful control interventions in four regions of Uganda. *Malaria Journal*, *19*(1), 128. https://doi.org/10.1186/s12936-020-03196-7

Lazrek, Y., Florimond, C., Volney, B., Discours, M., Mosnier, E., Houzé, S., Pelleau, S., & Musset, L. (2023). Molecular detection of human Plasmodium species using a multiplex real time PCR. *Scientific Reports*, *13*(1), 11388. https://doi.org/10.1038/s41598-023-38621-9

Lin, J. T., Saunders, D. L., & Meshnick, S. R. (2014). The role of submicroscopic parasitemia in malaria transmission: What is the evidence? *Trends in Parasitology*, *30*(4), 183–190. https://doi.org/10.1016/j.pt.2014.02.004

Lin, J. T., Ubalee, R., Lon, C., Balasubramanian, S., Kuntawunginn, W., Rahman, R., Saingam, P., Heng, T. K., Vy, D., San, S., Nuom, S., Burkly, H., Chanarat, N., Ponsa, C., Levitz, L., Parobek, C., Chuor, C. M., Somethy, S., Spring, M., … Saunders, D. L. (2016). Microscopic Plasmodium falciparum Gametocytemia and Infectivity to Mosquitoes in Cambodia. *The Journal of Infectious Diseases*, *213*(9), 1491–1494. https://doi.org/10.1093/infdis/jiv599

Lindblade, K. A., Steinhardt, L., Samuels, A., Kachur, S. P., & Slutsker, L. (2013). The silent threat: Asymptomatic parasitemia and malaria transmission. *Expert Review of Anti-Infective Therapy*, *11*(6), 623–639. https://doi.org/10.1586/eri.13.45

Lubinda, J., Haque, U., Bi, Y., Shad, M. Y., Keellings, D., Hamainza, B., & Moore, A. J. (2021). Climate change and the dynamics of age-related malaria incidence in Southern Africa. *Environmental Research*, *197*, 111017. https://doi.org/10.1016/j.envres.2021.111017

Manjurano, A., Lyimo, E., Kishamawe, C., Omolo, J., Mosha, J., Donald, M., Kazyoba, P., Kapiga, S., & Changalucha, J. (2023). Prevalence of G6PD deficiency and submicroscopic malaria parasites carriage in malaria hotspot area in Northwest, Tanzania. *Malaria Journal*, *22*(1), 372. https://doi.org/10.1186/s12936-023-04801-1

Manjurano, A., Okell, L., Lukindo, T., Reyburn, H., Olomi, R., Roper, C., Clark, T. G., Joseph, S., Riley, E. M., & Drakeley, C. (2011). Association of sub-microscopic malaria parasite carriage with transmission intensity in north-eastern Tanzania. *Malaria Journal*, *10*(1), 370. https://doi.org/10.1186/1475-2875-10-370

Mboera, L. E., Kamugisha, M. L., Rumisha, S. F., Kisinza, W. N., Senkoro, K. P., & Kitua, A. Y. (2008). Malaria and mosquito net utilisation among schoolchildren in villages with or without healthcare facilities at different altitudes in Iringa District, Tanzania. *African Health Sciences*, *8*(2), 114–119.

Mmbando, B. P., Vestergaard, L. S., Kitua, A. Y., Lemnge, M. M., Theander, T. G., & Lusingu, J. P. (2010). A progressive declining in the burden of malaria in north-eastern Tanzania. *Malaria Journal*, *9*(1), 216. https://doi.org/10.1186/1475-2875-9-216

MoH. (2008). *Tanzania—Tanzania HIV/AIDS and Malaria Indicator Survey 2007-2008*. https://www.nbs.go.tz/tnada/index.php/catalog/20/study-description

MoH. (2023). *Tanzania Demographic and Health Survey and Malaria Indicator Survey*.

MoH, NBS/Tanzania, N. B. of S.-, OCGS/Zanzibar, O. of the C. G. S.-, & ICF. (2018). *Tanzania Malaria Indicator Survey 2017—Final Report*. https://dhsprogram.com/publications/publication-mis31-mis-final-reports.cfm

Mosha, J. F., Sturrock, H. J., Greenhouse, B., Greenwood, B., Sutherland, C. J., Gadalla, N., Atwal, S., Drakeley, C., Kibiki, G., Bousema, T., Chandramohan, D., & Gosling, R. (2013). Epidemiology of subpatent Plasmodium falciparum infection: Implications for detection of hotspots with imperfect diagnostics. *Malaria Journal*, *12*(1), 221. https://doi.org/10.1186/1475-2875-12-221

Muirhead-Thomson, R. C. (1951). Distribution of Anopheline Mosquito Bites Among Different Age Groups. *Br Med J*, *1*(4715), 1114–1117. https://doi.org/10.1136/bmj.1.4715.1114

Mwenda, M. C., Fola, A. A., Ciubotariu, I. I., Mulube, C., Mambwe, B., Kasaro, R., Hawela, M. B., Hamainza, B., Miller, J. M., Carpi, G., & Bridges, D. J. (2021). Performance evaluation of RDT, light microscopy, and PET-PCR for detecting Plasmodium falciparum malaria infections in the 2018 Zambia National Malaria Indicator Survey. *Malaria Journal*, *20*(1), 386. https://doi.org/10.1186/s12936-021-03917-6

Mwingira, F., Genton, B., Kabanywanyi, A.-N. M., & Felger, I. (2014). Comparison of detection methods to estimate asexual Plasmodium falciparum parasite prevalence and gametocyte carriage in a community survey in Tanzania. *Malaria Journal*, *13*(1), 433. https://doi.org/10.1186/1475-2875-13-433

Nguyen, T.-N., Seidlein, L. von, Nguyen, T.-V., Truong, P.-N., Hung, S. D., Pham, H.-T., Nguyen, T.-U., Le, T. D., Dao, V. H., Mukaka, M., Day, N. P., White, N. J., Dondorp, A. M., Thwaites, G. E., & Hien, T. T. (2018). The persistence and oscillations of submicroscopic Plasmodium falciparum and Plasmodium vivax infections over time in Vietnam: An open cohort study. *The Lancet Infectious Diseases*, *18*(5), 565–572. https://doi.org/10.1016/S1473-3099(18)30046-X

Okell, L. C., Ghani, A. C., Lyons, E., & Drakeley, C. J. (2009). Submicroscopic Infection in Plasmodium falciparum-Endemic Populations: A Systematic Review and Meta-Analysis. *The Journal of Infectious Diseases*, *200*(10), 1509–1517. https://doi.org/10.1086/644781

Oyegoke, O. O., Maharaj, L., Akoniyon, O. P., Kwoji, I., Roux, A. T., Adewumi, T. S., Maharaj, R., Oyebola, B. T., Adeleke, M. A., & Okpeku, M. (2022). Malaria diagnostic methods with the elimination goal in view. *Parasitology Research*, *121*(7), 1867–1885. https://doi.org/10.1007/s00436-022-07512-9

Rapp, T., Amagai, K., Sinai, C., Basham, C., Loya, M., Ngasala, S., Said, H., Muller, M., Chhetri, S., Yang, G., François, R., Odas, M., Mathias, D., Juliano, J., Lin, F.-C., Ngasala, B., & Lin, J. (2023). Micro-heterogeneity of transmission shapes the submicroscopic malaria reservoir in coastal Tanzania. In *medRxiv: The preprint server for health sciences*. https://doi.org/10.1101/2023.09.06.23295089

Salgado, C., Ayodo, G., Macklin, M. D., Gould, M. P., Nallandhighal, S., Odhiambo, E. O., Obala, A., O'Meara, W. P., John, C. C., & Tran, T. M. (2021). The prevalence and density of asymptomatic Plasmodium falciparum infections among children and adults in three communities of western Kenya. *Malaria Journal*, *20*(1), 371. https://doi.org/10.1186/s12936-021-03905-w

Schindler, T., Jongo, S., Studer, F., Mpina, M., Mwangoka, G., Mswata, S., Ramadhani, K., Sax, J., Church, L. W. P., Richie, T. L., Tanner, M., Hoffman, S. L., Abdulla, S., & Daubenberger, C. (2019). Two cases of long-lasting, sub-microscopic Plasmodium malariae infections in adults from coastal Tanzania. *Malaria Journal*, *18*(1), 149. https://doi.org/10.1186/s12936-019-2787-x

Schneider, P., Bousema, T., Omar, S., Gouagna, L., Sawa, P., Schallig, H., & Sauerwein, R. (2006). (Sub)microscopic Plasmodium falciparum gametocytaemia in Kenyan children after treatment with sulphadoxine-pyrimethamine monotherapy or in combination with artesunate. *International Journal for Parasitology*.

Shamseddin, J., Ghanbarnejad, A., Zakeri, A., Abedi, F., Khojasteh, S., & Turki, H. (2022). Molecular Method Is Essential to Identify Asymptomatic Malaria Reservoirs: A Successful Experience in the Malaria Elimination Program in Iran. *Diagnostics*, *12*(12), Article 12. https://doi.org/10.3390/diagnostics12123025

Shekalaghe, S. A., Bousema, J. T., Kunei, K. K., Lushino, P., Masokoto, A., Wolters, L. R., Mwakalinga, S., Mosha, F. W., Sauerwein, R. W., & Drakeley, C. J. (2007). Submicroscopic Plasmodium falciparum gametocyte carriage is common in an area of low and seasonal transmission in Tanzania. *Tropical Medicine & International Health: TM & IH*, *12*(4), 547–553. https://doi.org/10.1111/j.1365-3156.2007.01821.x

Strøm, G. E. A., Haanshuus, C. G., Fataki, M., Langeland, N., & Blomberg, B. (2013). Challenges in diagnosing paediatric malaria in Dar es Salaam, Tanzania. *Malaria Journal*, *12*, 228. https://doi.org/10.1186/1475-2875-12-228

Sturrock, H. J. W., Hsiang, M. S., Cohen, J. M., Smith, D. L., Greenhouse, B., Bousema, T., & Gosling, R. D. (2013). Targeting Asymptomatic Malaria Infections: Active Surveillance in Control and Elimination. *PLOS Medicine*, *10*(6), e1001467. https://doi.org/10.1371/journal.pmed.1001467

Sumari, D., Mwingira, F., Selemani, M., Mugasa, J., Mugittu, K., & Gwakisa, P. (2017). Malaria prevalence in asymptomatic and symptomatic children in Kiwangwa, Bagamoyo district, Tanzania. *Malaria Journal*, *16*(1), Article 1. https://doi.org/10.1186/s12936-017-1870-4

Thawer, S. G., Chacky, F., Runge, M., Reaves, E., Mandike, R., Lazaro, S., Mkude, S., Rumisha, S. F., Kumalija, C., Lengeler, C., Mohamed, A., Pothin, E., Snow, R. W., & Molteni, F. (2020). Sub-national stratification of malaria risk in mainland Tanzania: A simplified assembly of survey and routine data. *Malaria Journal*, *19*(1), 177. https://doi.org/10.1186/s12936-020-03250-4

Traoré, N., Singhal, T., Millogo, O., Sié, A., Utzinger, J., & Vounatsou, P. (2024). Relative effects of climate factors and malaria control interventions on changes of parasitaemia risk in Burkina Faso from 2014 to 2017/2018. *BMC Infectious Diseases*, *24*(1), 166. https://doi.org/10.1186/s12879-024-08981-2

Wampfler, R., Mwingira, F., Javati, S., Robinson, L., Betuela, I., Siba, P., Beck, H.-P., Mueller, I., & Felger, I. (2013). Strategies for Detection of Plasmodium species Gametocytes. *PLoS ONE*, *8*(9), e76316. https://doi.org/10.1371/journal.pone.0076316

Whittaker, C., Slater, H., Nash, R., Bousema, T., Drakeley, C., Ghani, A. C., & Okell, L. C. (2021). Global patterns of submicroscopic Plasmodium falciparum malaria infection: Insights from a systematic review and meta-analysis of population surveys. *The Lancet Microbe*, *2*(8), e366–e374. https://doi.org/10.1016/S2666-5247(21)00055-0

WHO. (2023). *World malaria report 2023*. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023

Wongsrichanalai, C., Barcus, M. J., Muth, S., Sutamihardja, A., & Wernsdorfer, W. H. (2007). A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). *The American Journal of Tropical Medicine and Hygiene*, *77*(6 Suppl), 119–127.

Supplementary Table

Supplementary Table S1: *Plasmodium falciparum* asexual and gametocyte prevalence detected by LM, RDT and molecular assays by study site

		Asexual Parasite Prevalence		Gametocyte Prevalence	
Region	Sample size	LM positive (%)	qPCR Positive (%)	LM	*Pfs25* RT-PCR
Iringa	329	0(0.0)	2 (0.6)	0 (0.0)	1 (0.3)
Dar	440	29 (6.6)	15 (3.4)	1 (0.23)	3 /(0.6)
Tanga	235	5 (2.1)	8 (3.4)	0 (0.0)	15 (6.4)
Coast	816	182(22.3)	305 (37.4)	14 (1.72)	269 (33.0)