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Evaluating the Prevalence of Malaria Parasite Infection among Adults in Wetlands Using Nested PCR and High Resolution Melting Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Wetlands serve as breeding grounds for mosquitoes, the vector of malaria *Plasmodium*. Effective detection of *Plasmodium* infection requires a very sensitive method.
Aim: The aim of the present study was to evaluate the prevalence of malaria parasite infection in Sagbama LGA, Bayelsa State using nested PCR and high resolution melting analysis (HRMA) technique in comparison with microscopy.

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Methods: A community based cross sectional study design was employed to randomly select 206 study participants. DNA was extracted from 200 ɥl of whole blood of each participant and a set of primers was used to target and amplify the 18S rRNA gene of *Plasmodium falciparum* in both molecular methods.

Result: The prevalence of *Plasmodium falciparum* infection by microscopy was 33.01% (5.8% asymptomatic, 17% mild and 10.2% severe malaria).The prevalence of malaria parasite infection by nested genomic PCR was 71.36% (asymptomatic, 42.20% mild, 18.93%and severe malaria, 10.19%). Prevalence of malaria parasite infection by high resolution melting analysis 92.71% (asymptomatic, 63.59%, mild, 18.93% and severe, 10.19%). All the study participants that were positive for microscopy and nested genomic PCR were also positive for high resolution melting analysis. There were 59.71% and 21.36% false negatives by microscopy and nested PCR respectively. McNemar`s test for pair-wise performance comparisons among the different methods was statistically significant ($p < 0.001$). High parasite density (19927 ± 749 parasites/µl blood) and hyper parasite density (103667 ± 5214 parasites/ɥl of blood) were found among asymptomatic and severe malaria subjects (P < 0.000).

Conclusion: In conclusion, a high malaria parasite infection prevalence of 92.72% was found in Sagbama LGA. This requires urgent attention especially asymptomatic malaria. HRMA was the most sensitive molecular method and is therefore recommended for future molecular detection of malaria infection.

Keywords: HRM-analysis; Malaria; nested PCR; Bayelsa State.

1. INTRODUCTION

Malaria is a widespread disease that is transmitted in tropical and subtropical regions of the world. Globally, an estimated 619 000 malaria related deaths and 247 million malaria cases were reported in 2020, with Nigeria having the highest prevalence of malaria [1]. Niger Delta, Nigeria is the largest wetland in Africa and the third largest mangrove swamp forest in the world [2]. It is located in South-South Nigeria with States like Bayelsa located in the core of Niger Delta, Nigeria [3]. Swamps support high transmission of malaria because they serve as breeding grounds for mosquitoes, the vector of *Plasmodium* species [4]. Thus, a current and comprehensive up-to-date evaluation of the trends in malaria infection in wetlands will contribute greatly to malaria control and elimination [1,4]. *Plasmodium falciparum* is the deadliest and most predominant *Plasmodium* species in Nigeria, followed by the less pathogenic and less common *Plasmodium malariae* and *Plasmodium ovale. Plasmodium vivax*, the second most deadly is almost absent in sub-Saharan Africa but is most dominant outside sub-Saharan Africa [1].

Aside from malaria which presents with febrile signs and symptoms, asymptomatic malaria has been reported as a major hindrance to malaria elimination [5,6]. Asymptomatic malaria is defined as the presence of any density of malaria parasite in the blood without the clinical symptoms of malaria and has been described as the hidden reservoir sustaining malaria transmission [5]. Total malaria elimination requires the detection and eradication of the human reservoir of malaria parasite using very sensitive method [5].The limit of detection of malaria microscopy is approximately 30 parasites/ɥl of blood [6]. Thus, both microscopy and RDT misses the detection of *Plasmodium* when parasite densities are low [7]. Also, microscopy is time consuming. The duration per slide for malaria microscopy is approximately 60 minutes [7,8].

Several molecular methods for *Plasmodium falciparum* infection detection with limit of detection between 1 – 5 parasites/ɥl of blood have been developed over the years [6,7]. However, they differ according to sample processing, reaction temperature, reaction to result time, ease of use, limit of detection, cost, required equipment and pre-amplification quantification or detection methods [5,8]. Tropical wetlands, are important hydrological features that serve as breeding habitats, for mosquitoes, the vectors of *Plasmodium* [4]. Thus, mapping and monitoring the magnitude of *Plasmodium falciparum* infection cases in tropical wetlands using very sensitive molecular methods will facilitate early detection of hidden carriers of the parasite and active cases of malaria not detected by microscopy. This will inform early treatment, thereby reducing the health care burden of the disease [5,8]. To date however, the most

sensitive molecular method among the several molecular methods for malaria diagnosis has not been established. Studies on comparison of molecular methods for malaria diagnosis has yielded conflicting results. Therefore, the aim of the present study was to determine the most sensitive molecular method between nested PCR and high resolution melting analysis (HRMA) for the evaluation of the prevalence of malaria parasite infection in Sagbama LGA, Bayelsa State.

2. MATERIALS AND METHODS

2.1 Study Location and Design

The study location was Sagbama LGA, Bayelsa State, Nigeria. Bayelsa State is a core State in Niger Delta Nigeria [9] and is located on latitude 5.152239 and longitude 6.192479. It is a wetland that lies between the well-watered oil-palm freshwater bushy swamps and salt-water creeks and mangrove swamps. The Ijaw speaking tribe are the major indigenes in the state [10]. A household survey using a cross sectional multistage random sampling study design was employed to randomly select 206 subjects for the study [11]. Study participants (aged ≥18 years) were grouped into uninfected, asymptomatic (infected but non febrile, haemoglobin concentration < 11 – 10 g/dl), mild (infected and febrile with haemoglobin concentration 8 - 10 g / dl) and severe (infected and febrile with haemoglobin concentration <8 g / dl) malaria groups [12].

2.2 Sample Size Determination

Sample size was calculated using the Cochran formula below [13].Where: n_0 was the sample size

$$n_0 = \frac{Z^2pq}{e^2}$$

e was 0.05, the desired level of precision (i.e. the margin of error). P was 0.85 (85%), the (estimated) proportion of the population having *Plasmodium falciparum* infection in Bayelsa State based on a previous pilot study. q was 0.1.15 (1 – P). Z-value was found in a Z table. Z-value at 95% confidence interval was 1.96.

Therefore $n_0 = ((1.96)^2 (0.85)\ (0.15))\ /\ (0.05)^2 =$ 195.92. Assuming a nonresponse rate of 5%, the sample size was increased to 206.

2.3 Blood Sample Collection and Microscopy

Whole blood (5 ml) was drawn by venipuncture from each participant into a Vacutainer® EDTA tube. Thick and thin blood films were prepared and read by two experienced microscopists following the World Health Organization malaria microscopy standard operating procedures using an oil immersion lens (100×). Malaria parasites were counted against 200 white blood cells. Parasite density of positive thick film was calculated using the formula below [8].

Parasite density (Parasites/μL blood) = (Number of asexual parasites counted x 8000 white cells/μL) / Number of white cells counted

2.4 Classification of Parasite Density

Low (< 1000 parasites/μl blood), moderate (1000 – 4999 parasites/μl blood), high (5000 – 99,999 parasites/μl blood), and hyperparasitaemia (≥ 100,000 parasites/μl blood) [14].

2.5 DNA Extraction and Purification

gSYNC™ extraction kit (Geneaid, Biotech. Ltd, New Taipei, Taiwan) was used for genomic DNA extraction following the manufacturer's protocol. Agarose gel electrophoresis was carried out to determine the integrity of extracted DNA using 0.7% agarose gel (stained with 5 μl of 10 mg/ml Ethidium Bromide) in 100 ml of 1X Tris Acetate EDTA buffer (pH 8.0), followed by visualization under a UV transilluminator. DNA purity (absorbance at 260 nm / absorbance at 280 nm) and DNA concentration (absorbance at 260 nm) were also determined.

2.6 Nested PCR for *Plasmodium falciparum* Infection Detection

5x HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Estonia) was used for nested PCR following the manufacturer`s protocol. Previously used primer pair by Zhao et al., [6] (F: 5`TCAAAGATTAAGCCATGCAAGTGA3` and R: 5`CCTGTTGTTGCCTTAAACTTC3`) were used to amplify 18S ribosomal RNA (rRNA) gene of *Plasmodium* genus in the first PCR reaction while another primer pair forward: 5`TTAAACTGGTTTGGGAAAACCAAATATATT3` and reverse: 5`ACACAATGAACTCAATCATGACTACCCGTC3 `was employed to amplify the species specific

18S rRNA gene of *Plasmodium* falciparum in the second PCR reaction. The product of the first PCR served as the template for the second PCR reaction. PCR was carried out in aProFlex^{TM} 96-well PCR Thermal Cycler System (Applied Biosystems, Foster City, California, USA). Cycling conditions were: an initial activation at 95^{0}C for15 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 65^{0}C for 20 s, and elongation at 72^{0}C for 20 s. Water replaced DNA template for negative control and PCR was carried out in duplicates. Agarose gel electrophoresis was carried out on the products of the second PCR. Expected molecular size of *Plasmodium falciparum* 2nd PCR fragment was 205 base pairs. Matching positions between sample bands and positive control band was confirmatory of a positive case [5].

2.7 HRM-analysis for the Detection of *P. falciparum*

HOT FIREPol® EvaGreen® HRM Mix-ROX (SolisBiodyne, Tartu Estonia) was used for HRM-analysis following the manufacturer`s protocol. A pair of primers (forward: 5`TTAAACTGGTTTGGGAAAACCA AATATATT3` and reverse 5`ACACAATGAACTCAATC ATGACTCCCGTC 3`) previously used by Zhao et al., [6] was employed to amplify the 18S rRNA gene of *Plasmodium falciparum.*PCR was carried out in a StepOnePlus™ Real-Time PCR Thermal Cycler Systems with a high resolution melt software v 3.0.1 installed (Applied Biosystem, Foster City, USA). Amplification conditions were: an initial activation at 95 ^{0}C for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 65 ^{0}C for 20 s, and extension at 72 ^{0}C for 20 s. A post-amplification high resolution melting curve was prepared from 65 ^{0}C to 90 ^{0}C for 10 min. Positive controls and negative controls were included in the assay and the reaction was carried out in duplicates. Samples with melting curves aligning with the melting curve of positive control (with a temperature shift interval of ±0.3 ^{0}C) and with fluorescent signal within 30 cycles (C_t) were considered positive for malaria parasite infection [5].

2.8 Statistical Analysis

SPSS version 27 was used for data analysis. Results were expressed as percentages and mean ± standard deviation.Chi Square test and independent t-tests were used to compare means and categorical data respectively. McNemar's exact test was used for pair-wise comparison among the proportions of positive detections for the different methods. Significant level for all analysis was set at $p < 0.05$ [15,16].

3. RESULTS AND DISCUSSION

3.1. Malaria Infection Prevalence in Sagbama LGA, Bayelsa State, Nigeria by Microscopy

Presented in Fig. 1 is the prevalence of malaria parasite infection detected by microscopy in Sagbama LGA, Bayelsa State, Nigeria. The prevalence of *Plasmodium falciparum* infection by microscopy was 33.01% (5.8% asymptomatic, 17% mild and 10.2% severe malaria). Based on microscopy result, over half of the study participants were uninfected. The microscopy-based prevalence of malaria parasite infection found in the present study was higher than findings of a previous study among adults [17]. Limited attention is given to research on malaria parasites among adults. However, the present study suggests the need for more attention to be targeted at adults infected with malaria parasites especially in Niger Delta Wetlands in Nigeria.

3.2 Malaria Parasite Density in Bayelsa State, Nigeria

Presented in Table 1 is themalaria *Plasmodium falciparum* density in Sagbama LGA, Bayelsa State, Nigeria. As shown in Table 1, high and hyperparasitemia was found among the study population. A significant difference in parasitemia level was also found between asymptomatic, mild and severe malaria groups ($p< 0.05$). Among asymptomatic malaria subjects, high parasitemia was found yet they did not show any sign nor symptoms of malaria. A previous study in Columbia found that the malaria parasite density levels observed in populations with severe malaria was lower than >50,000 parasites/µl, which isthe officially established values by World Health Organization for severe malaria [18]. This suggests that parasite density criterion is not really a relevant criterion for the definition of severe cases in Sagbama and it certainly not be used to make a clinical decision about the severity of the disease in this region. The present study found no association between parasite density and sex. This is in disagreement

with findings of a previous study were an association between sex and parasite density was found [19], suggesting that gender variation exist in different geographic location in relation to parasite density.

3.3 Integrity and Concentration of Purified Genomic DNA

Fig. 2 shows a section of genomic DNA samples extracted and purified from the whole blood of residents in Sagbama LGA, Bayelsa State. Overall, two hundred and six DNA samples were extracted and purified. The DNA bands in Fig. 2 a), b), and c) are sharp, clear and above the 10 kilo base pair molecular marker, indicating that the extracted and purified DNA were not degraded. The concentration of the purified genomic DNA was 45 ± 0.02 µg/ml, while the purity was 1.81 ± 0.01.The extracted DNA bands obtained in this present study was of a higher integrity compared with extracted DNA bands obtained in a previous study [20]. However, similar extracted DNA integrity and yield was obtained in another previous study carried out by Gong and Li [21].The 206 extracted and purified DNA samples were analysed for the presence of *Plasmodium falciparum* using nested genomic PCR and real time PCR high resolution melting analysis.

3.4 Prevalence of Malaria Parasite Infection Detected by Nested Genomic PCR in Sagbama LGA, Bayelsa State, Nigeria

Fig. 3 shows a section of agarose gel electrophoretogram of *Plasmodium falciparum* positive samples detected by nested genomic PCR in Sagbama LGA, Bayelsa State, while, Fig. 4 shows the prevalence of *Plasmodium falciparum* infection by nested genomic PCR. The prevalence of malaria parasite infection detected by nested genomic PCR was 71.36% (asymptomatic, 42.20% mild, 18.93% (n = 39) and severe malaria, 10.19% (n = 21) (10.19%)]. Nested genomic PCR showed that less than one quarter of the study population were uninfected.

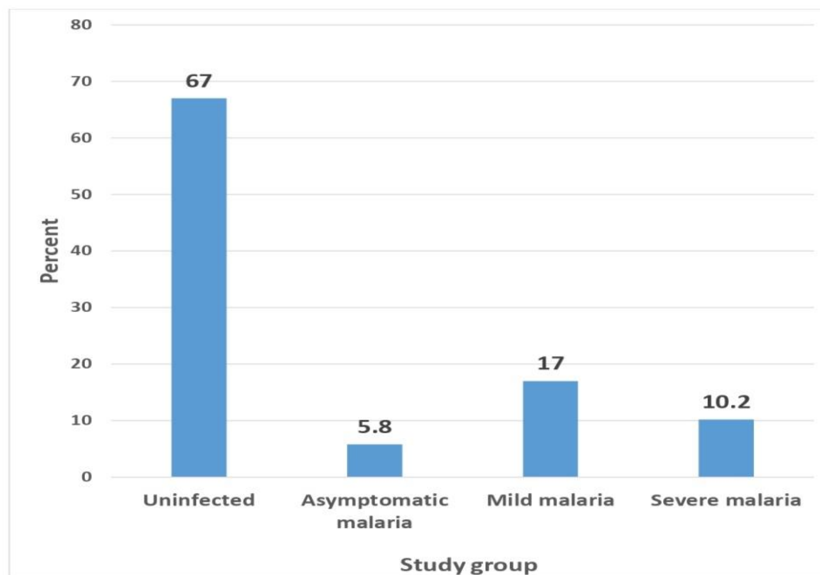

Fig. 1. Malaria infection prevalence in Sagbama LGA, Bayelsa State, Nigeria by microscopy

Table 1. Malaria parasite density of the study population in Bayelsa State, Nigeria

Study group	Malaria parasite density (parasites/µl of blood)		
	Male	Female	Total
Uninfected	0	0	0
Asymptomatic	20065.29 ± 164.73a	19733.40 ± 485.24a	19927.00 ± 748.95a
Mild malaria	62240.67 ± 4896.62b	72406.35 ± 5045.62b	68049.63 ± 3620.10b
Severe malaria	104406.00 ± 2065.63c	102995.45 ± 1164.87c	103667.14 ± 5213.93c

Values with different superscript on a column are significantly

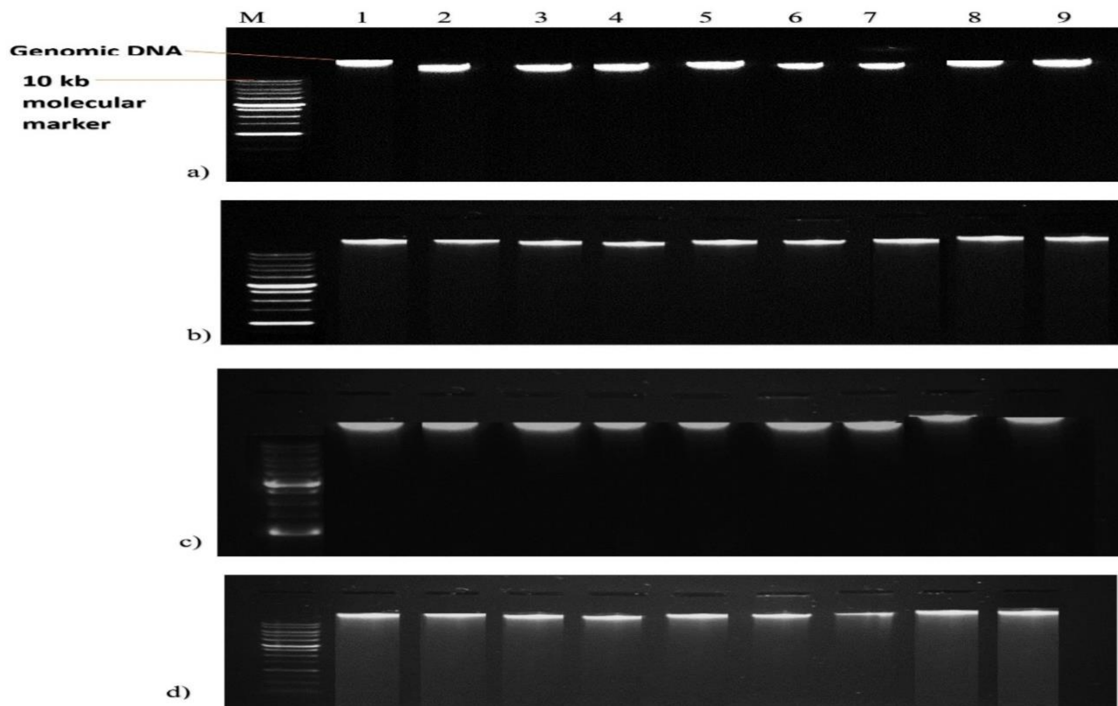

Fig. 2. Genomic DNA from 200 µl whole blood samples of the a) uninfected group b) asymptomatic group c) mild malaria group and d) severe malaria group among residents in Sagbama LGA, Bayelsa State. Extracted genomic DNA(s) were well above the 10 kb molecular marker (M), indicating non-degraded DNA

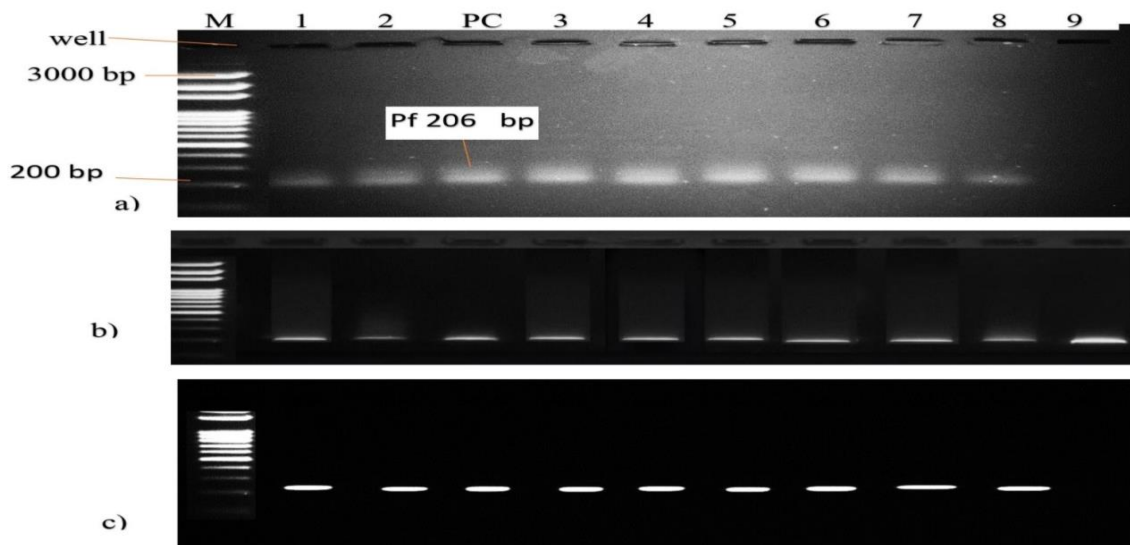

Fig. 3. A section of nested genomic PCR products for a) asymptomatic malaria group b) mild malaria group and c) severe malaria group in Sagbama LGA, Bayelsa State

PC; Positive Control, NC; Negative Control, M; 100 base pairs DNA ladder (molecular marker),Pf; Plasmodium falciparum 18S rRNA gene fragment

All study participants that were positive under malaria microscopy were also positive under nested-genomic PCR in addition to 79 new cases of malaria parasite infection previously undetected by microscopy. Using nested PCR as reference method, microscopy yielded 38.4% false negatives. A previous study showed that nested PCR was sixfoldmore sensitive compared

with microscopy for asymptomatic malaria detection [6]. Another study showed that microscopy yielded 13% false negatives when compared with nested PCR for the detection of febrile cases of malaria with 6% error in species diagnosis [22]. This suggests the need for a more sensitive method to be used as gold standardfor malaria determination.

3.5 Prevalence of Malaria Parasite Infection by High Resolution Melting Analysis in Sagbama, Bayelsa State, Nigeria

Fig. 5 shows a section of the melt curves of *Plasmodium falciparum* positive samples in Sagbama LGA, Bayelsa State. The melt curves of the positive samples aligned at an average melting temperature (Tm) of 74.74 ± 0.34 ^{0}C. In addition, Fig. 6. shows the total count of the melt curves that aligned at 74.74 ± 0.34 ^{0}C. Based on the count, prevalence of malaria parasite infection by high resolution melting analysis in Sagbama LGA, Bayelsa State, Nigeria was 92.71% (191 individuals) [asymptomatic, 63.59% (131 individuals) and mild, 18.93% (39 individuals) and severe, 10.19% (21 individuals).

All the study participants that were positive for microscopy and nested genomic PCR were also positive for high resolution melting analysis. Based on the result for HRM analysis, a higher prevalence of malaria infection was found in the present study compared with previous studies [5,8,23]. This highlights the need for malaria interventions to be directed to dwellers in wetlands especially in Nigeria and Africa at large. A high prevalence of asymptomatic malaria, higher than findings of previous studies [5,22] was also found in the present study using HRM analysis. Given that asymptomatic carriers can still transmit the infection through mosquitoes and asymptomatic malaria may also progress to malaria sickness, the need for attention and interventions to be directed at asymptomatic malaria group is implicated in the present study. Asymptomatic malaria subjects only have partial immunity to malaria due to continuous exposure but do not have immunity to the infection [5,8,23]. Although various medicinal plants and herbs have been reported for the treatment of various diseases [24,25,26], however, herbs and medications for the treatment of asymptomatic malaria is limited in literature.

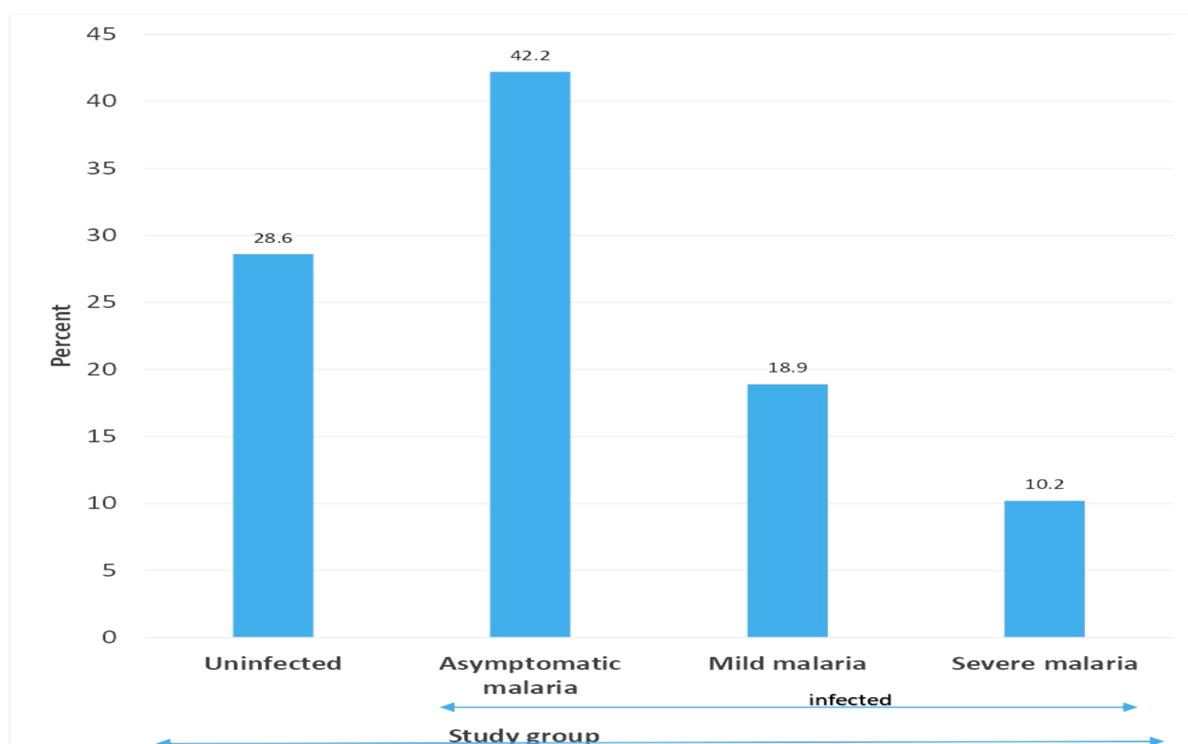

Fig. 4. Prevalence of Malaria parasite infection by nested genomic PCR diagnostic method in Sagbama LGA, Bayelsa State, Nigeria

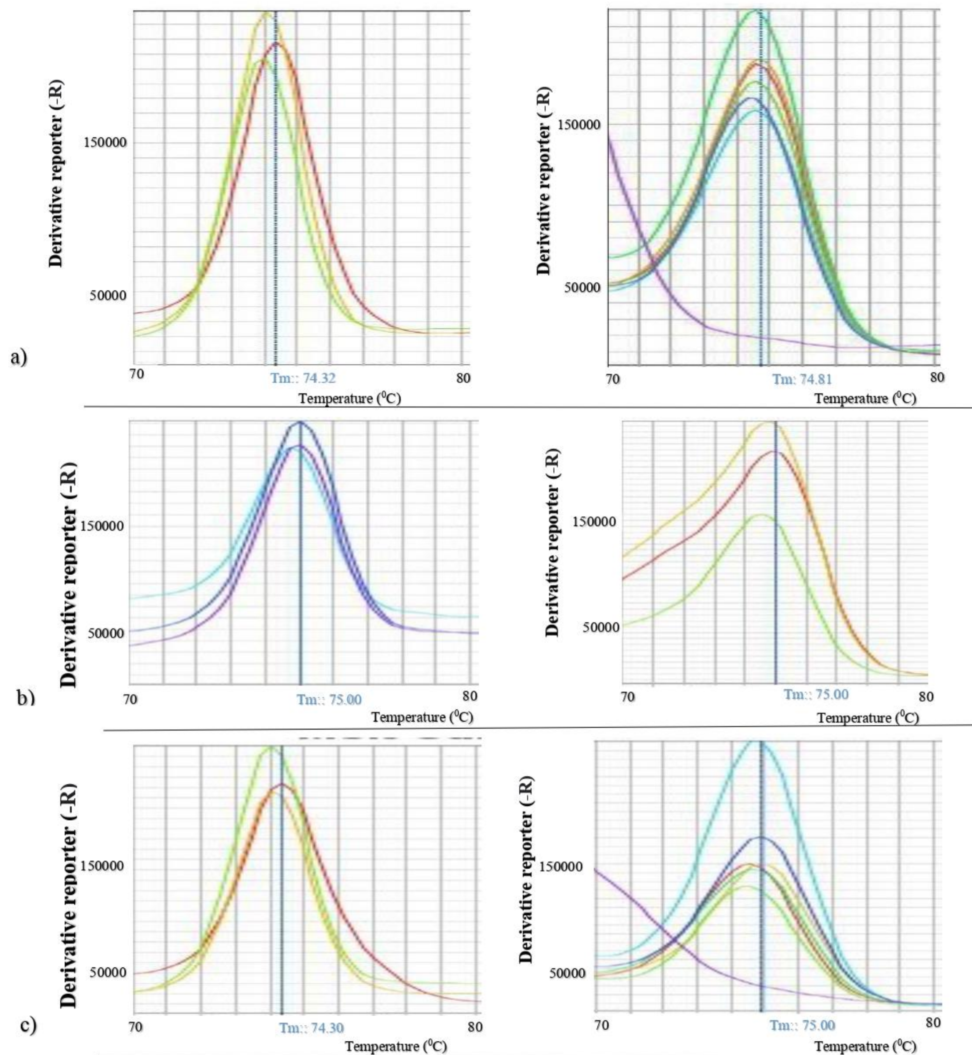

Fig. 5. A section of melt curves of *Plasmodium falciparum* positive samplesfor a) asymptomatic, b) mild, and c) severe malaria in Sagbama LGA, Bayelsa State, Nigeria

Tm = melting temperature. Melting curves of Plasmodium falciparum positive samples aligned at a melting temperature of 74.74 ± 0.34 ^{0}C

3.6 Comparative Performance of the Three Diagnostic Methods

Table 2 shows the result for the pair-wise comparison of the positive cases detected by the different methods. Pair-wise comparison between the proportions of positive cases for the different methods was significant at $p < 0.001$. Table 3 shows the sensitivity and specificity of each detection method. Microscopy yielded 58.25% false negatives while nested genomic PCR yielded 19.9% false negatives. High resolution melting analysis had the highest sensitivity.Although microscopy is the gold standard for diagnosing malaria [8],it howeveryielded false negatives in the present study as well as in previous studies [5,22]. The two molecular methods used in the present study were able to detect a higher prevalence of malaria parasite infection compared with microscopy. However, the proportion of malaria parasite infection detected by HRM analysis was significantly higher than the proportion detected by nested PCR. HRM analysis was more sensitive as it greatly enhanced the detection of submicroscopic malaria infections in the present study, was less laborious and less time consuming compared with nested PCR which involved two separate PCR reactions, agarose gel electrophoresis and visualization of agarose gel under a transilluminator.

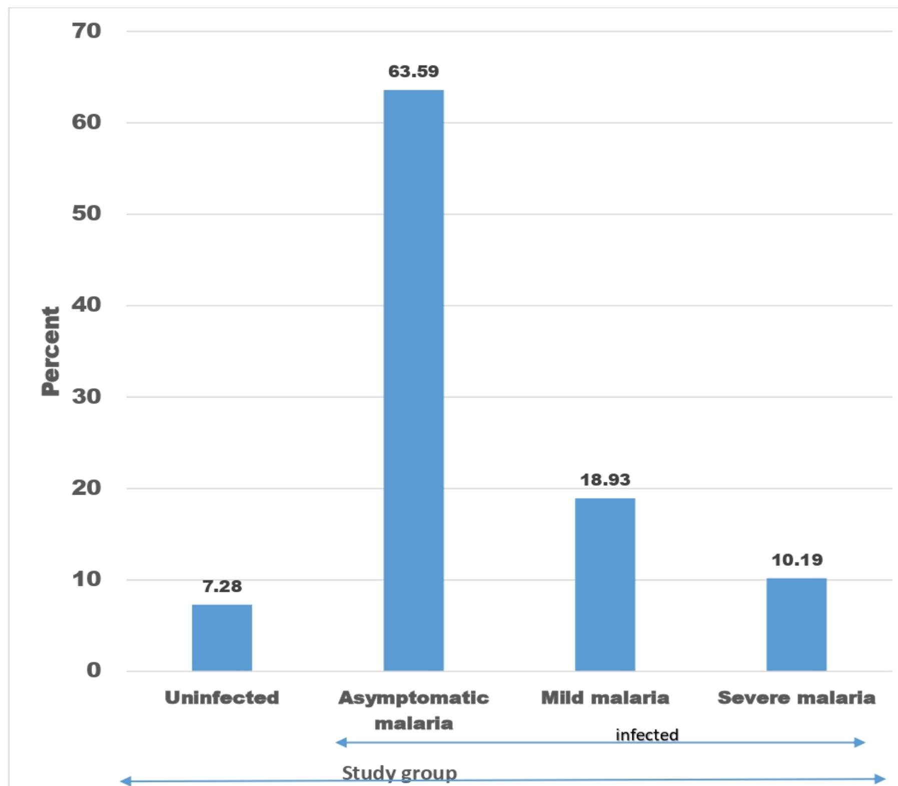

Fig. 6. Prevalence of malaria parasite infection by high resolution melting analysis diagnostic method in Sagbama LGA, Bayelsa State, Nigeria

Table 2. Pairwise comparison of the performance ofmicroscopy, nested PCR and HRMA for the detection of malaria parasite infected subjects in Bayelsa State, Nigeria

	Microscopy N (%)	Nested PCR N (%)	HRMA N (%)
Uninfected	138 (67.00)	59 (28.64)	15 (7.28)
***Infected**	68 (33.00)a	147 (71.36)b	191 (92.72)c
Asymptomatic	12 (5.80)a	87 (42.26)b	131(63.62)c
Malaria	56 (27.20)	60 (29.10)	60 (29.10)

**Pair-wise comparison of positive cases for the different methods was significant at $p < 0.001$. Values with different superscript are significantly different, HRMA; High resolution melting analysis, N; number of Participants*

Table 3. Comparison of the sensitivity and specificity of the detection methods

	Microscopy N (%)	Nested PCR N (%)	HRMA N (%)
True positives (TP)	68 (33.00)	147 (71.36)	191 (92.72)
False negatives (FN)	123 (59.71)	44 (21.36)	0 (0.00)
True negatives (TN)	15 (7.28)	15 (7.28)	15 (7.28)
False positive (FP)	0 (0.00)	0 (0.00)	0 (0.00)
Sensitivity (%)	35.60	76.96	100
Specificity (%)	100	100	100

Values with different superscript are significantly different. HRMA; High resolution melting analysis, N; number of participants. Sensitivity and specificity were calculated using HRMA as reference method.Sensitivity =(TP / TP + FN) x 100; specificity = (TN / TN + FP) x 100 [27]

4. CONCLUSION

A 92.72% prevalenceof malaria parasite infection was found in Sagbama LGA, Bayelsa State, Nigeria in the present study. Also, real time PCR high resolution melting analysis was the most sensitive molecular method for malaria parasite detection and it is therefore suggested as a

standard method for future malaria parasite infection detection, especially for asymptomatic malaria detection. Inclusion of asymptomatic malaria elimination in malaria control and intervention programs is recommended.

CONSENT AND ETHICAL APPROVAL

Ethical approval for the study was obtained from the Research Ethics Committee in Bayelsa State Primary Health Care Authority in Sagbama LGA. Informed consent was obtained from each participant prior to commencement of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Malaria, Geneva, Switzerland; 2023.
2. Wetlands International. Conserving and restoring wetlands in Nigeria`s Niger River Delta.
   Available:https://www.wetlands.org/casestudy/conserving-and-restoring-wetlands-in-nigerias-niger-river-delta/
   Access on 2018, May 31
3. Ogbole FA. Urinalysis for dehydration, kidney injury and urinary tract infection assessment in rural riverside, Bayelsa State, Nigeria. IJISRT. 2021;6(12):806–810.
4. Midekisa A, Senay GB, Wimberly MC. Multisensor earth observations to characterize wetlands and malaria epidemiology in Ethiopia. Water Resour. Res. 2014;50:8791–8806.
5. Ouma FF, Nateghpour M, Haghi AM, Mohebali M, Farivar L, Hosseini-Safa A, Mosawi SH. Application of High-Resolution Melting (HRM) technique towards the detection of asymptomatic malaria in a malaria endemic area of Southeastern Iran under elimination program. J Arthropod Borne Dis. 2020;14(4):353-362.
6. Yoon J, Jang WS, Nam J, Mihn DC, Lim CS. An automated microscopic malaria parasite detection system using digital image analysis. Diagnostics (Basel). 2021; 11(3):527.
7. World Health Organization. Global Malaria Programme: Microscopy, Rapid diagnostics test and Nucleic acid amplification-based diagnostics. Geneva, Switzerland; 2016.
8. Zhao Y, Zhao Y, Lv Y, Liu F, Wang Q, Li P, Zhao Z, Liu Y, Cui L, Fan Q, Cao Y. Comparison of methods for detecting asymptomatic malaria infections in the China–Myanmar border area. Malar J. 2017;16(159).
9. Ogbole FA, Harold BA. Association of undiagnosed pre-diabetes and type-2 diabetes mellitus with interleukin-2 mRNA expression among adults in Bayelsa State Nigeria. IJRR. 2023;10(5):210 – 215.
10. Ogbole FA, Oyelana O. Health risk assessment of the drinking water from Sagbama river, Bayelsa state, Niger Delta, Nigeria. IJSER. 2020;11(9):1455-1460.
11. Ogbole FA, Fatusi AO. Co-occurrence of health risk behaviours for communicable and non-communicable diseases in a tertiary institution in South West Nigeria. IJRSI. 2020;7(1):10-14.
12. Sumbele IUN, Kimbi HK, Ndamukong-Nyanga JL, Nweboh M, Anchang-Kimbi JK, Lum E, Nana Y, Ndamukong KKJ. Malarial anaemia and anaemia severity in apparently healthy primary school children in urban and rural settings in the mount Cameroon area: Cross sectional survey. Plos One. 2015;10(4):1-17.
13. Charan TB. How to calculate sample size for different study designs in medical research." Indian J. Psychol Med. 2013; 35(21):121-126.
14. Chipwaza B, Sumaye RD. High malaria parasitemia among outpatient febrile children in low endemic area, East-Central Tanzania in 2013. BMC Research Notes. 2020;13:251.
15. Ogbole FA, Moroyei BE. Upregulation of Interleukin-2 among hypertensive subjects in Bayelsa state, Nigeria. International Journal of Research and Review. 2023; 10(5):557-565.
16. Ogbole FA, Sanugba EE. Distribution of body mass index and abdominal obesity in Bayelsa State with associated interleukin-2 gene expression. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2023;14(4):1-10.
17. Jenkins R, Omollo R, Ongecha M, Sifuna P, Othieno C, Ongeri L, Kingora J, Ogutu B. Prevalence of malaria parasites in adults and its determinants in malaria endemic area of Kisumu County, Kenya. Malar J. 2015;14:263.

18. Padilla-Rodríguez JC, Olivera MJ, Guevara-García BD. Parasite density in severe malaria in Colombia. Plos One. 2020;15(6).
19. Awosolu OB, Yahaya ZS, Farah Haziqah MT. Prevalence, parasite density and determinants of *Falciparum* malaria among febrile children in some Peri-Urban communities in Southwestern Nigeria: A cross-sectional study. Infect Drug Resist. 2021;14:3219-3232.
20. Fabrizia B, Cristina C, Benedetta C, Grazia CM, Argante B. Induction of apoptosis by quercetin: Different response of human chronic myeloid (K562) and acute lymphoblastic (HSB-2) leukemia cells. Molecular and Cellular Biochemistry. 2007;296:137-149.
21. Gong R, Li S. Extraction of human genomic DNA from whole blood using a magnetic microsphere method. International Journal of Nanomedicine 2014;9:3781–3789.
22. Zhou X, Huang JL, Njuabe MT, Li SG, Chen JH, Zhou XN. A molecular survey of febrile cases in malaria-endemic areas along China-Myanmar border in Yunnan province, People`s Republic of China. Parasite. 2014:21-27.
23. Chua HK, Lim SC, Ng CC, Lee PC, Lim YL, Lau TP. Development of high resolution melting analysis for the diagnosis of human malaria. Scientific Reports. 2015;5:15671.
24. Ogbole FA, Crown OO, Olayeriju OS, Olaleye MT, Akindahunsi AA. Antidiabetic effect of methanolic extract of *Garcinia kola* leaves on streptozotocin-induced diabetic rats. GSJ. 2019;7(4):634–641.
25. Ogbole FA, Crown O, Olayeriju OS, Olaleye MT, Akindahunsi AA. Hepatoprotective and antidyslipidemic effect of methanolic extract of *Garcinia kola* leaves on streptozotocin-induced diabetic rats. IJEAST. 2019;4(4):1-5.
26. Omagha R, Idowu ET, Alimba CG, Otubanjo AO, Adeneye AK. Survey of ethnobotanical cocktails commonly used in the treatment of malaria in Southwestern Nigeria. Futur. J. Pharm. Sci. 2021;7(152): 1-13.
27. Van Honacker E, Coorevits L, Boelens J, Verhasselt B, Van Braeckel E, Bauters F, et al. Sensitivity and specificity of 14 SARS-CoV-2 serological assays and their diagnostic potential in RT-PCR negative COVID-19 infections. ActaClin Belg. 2022;77(2):315-320.

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