

Malaria diagnosis among children attending some primary health care centers in Keffi, Nigeria: A cross-sectional study

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Abstract

Malaria is a febrile illness caused by the bite of an infected female *Anopheles* mosquito transmitting the *Plasmodium* species. It is still an endemic public health disease especially in Sub-Saharan Africa, South-East Asia and South America. Nigeria, was reported to have the highest burden of the disease with 27% of the global malaria burden. 2ml of blood samples were collected from 220 children aged 0-17 years attending five Primary Health Centers (PHCs Angwan Wuje, Kofar-Pada, Guata, Angwan- Kaswa and Yelwa) in Keffi Local Government Area (LGA) to detect malaria parasites. First response Rapid Diagnostic Test (RDT) kits were used for the initial screening while thin and thick blood films were made and stained with Geimsa stain for microscopy. The prevalence of malaria using rapid diagnostic test kits (RDT) was 34.5% (76/220) while that for microscopy was 70% (154/220). The age range most affected for RDT and microscopy was 13-17 years (27.6% vs 29.9%) followed closely by 4-6years (25% vs 20.8%) and 7-12years (25% vs 19.5%), 1-3 years were (13.2% vs 15.6%) and < 1year (9.2%. vs 14.2%). Females were more infected than males at (57.9% vs 42.1%), but age and sex were not statistically significant ($p>0.05$). The age range most infected were those between 13-17years using both methods. The younger children 0-3 years were mostly protected with physical barriers like insecticide treated nets (ITNs) and its use was significant at $p<0.05$; 4-6years were the next with a high percentage of being infected as kids become more active and probably the uncomfortableness of ITNs make them restive. We advocate for more health promotion campaigns, enlightenment and preventive tips to be made as jingles, taught to parents and the growing children at schools, markets, health centers etc. so they don't serve as reservoirs. More policies e.g. environmental sanitation and seasonal malaria testing, treatment and prevention practices should be made and sustained by government to enhance the fight against malaria as global aid is dwindling.

Keywords: Rapid diagnostic test kits; Malaria microscopy; Prevalence; Physical barriers

1. Introduction

Malaria caused by the bite of the female *Anopheles* mosquito is still a global threat affecting Sub-Saharan African, South-East Asia and South American regions the more. Of the major *Plasmodia* species (*Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* *Plasmodium knowlesi* and *Plasmodium vivax*), *Plasmodium falciparum* is the most dangerous and endemic in the Sub-Saharan African region [1].

The World Health Organization (WHO) estimated that there were 263 million cases of malaria globally in 2023, with global death burden of 597,000 [1]. African region had a significant share of the cases; Nigeria is said to have about 27% of the global share of the deaths [1,2].

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sub-Saharan Africa accounts for the majority of malaria cases and deaths, with countries like Nigeria, the Democratic Republic of Congo and Mozambique being significantly affected [1,2]. *Plasmodium falciparum* is the most prevalent and virulent specie, mostly found in sub-Saharan Africa. It causes uncomplicated malaria but is also responsible for the majority of severe malaria cases and deaths globally [3,4]. It accounts for 99.7% of the estimated malaria cases in the WHO African region, 50% of the cases in the WHO South-East Asia region, 71% of the WHO Eastern Mediterranean region and 65% of the WHO Western Pacific Region [4]. The other malaria species are not as life threatening as *P. falciparum* [3,1].

Plasmodium falciparum assumes different forms during its life cycle. The human infective stage is the sporozoite from the salivary gland of the mosquito. The sporozoites grow and multiply in the liver to form merozoites. These merozoites invade the red blood cells (erythrocytes) to form trophozoites, schizonts and gametocytes during which the symptoms of malaria are produced. In the mosquito, the gametocytes undergo sexual reproduction to form a zygote from which ookinets are formed. Ookinets produce oocysts which in turn form sporozoites that remain in the salivary gland of the mosquito ready to take a blood meal so as to be released in the blood stream of the host and the cycle continues [5, 6].

The parasite is known to be transmitted by infected female *Anopheles* mosquitoes, *Anopheles gambiae* and *Anopheles funestus* are the primary vectors in Africa [7]. A latest threat known as *Anopheles stephensi* have been reported by the CDC; “this mosquito can thrive in both urban and rural areas and is resistant to most insecticides commonly used in malaria control” [5]. An additional 126 million people is estimated to be at risk of malaria if this vector continues to expand in Africa [5]. Transmission of malaria often peaks during and after the rainy season when mosquito populations are highest and the environment being humid enhances the growth of the mosquitoes. Human activities, such as agriculture and urbanization, can influence the breeding habitats of mosquitoes, impacting transmission dynamics [8].

Children are mostly affected due to their developing immune system; complications can be severe leading to acute renal failure, coma and death if not treated appropriately. The objectives of this study are to identify the prevalence of malaria among children attending Primary Health Centers (PHC) in Keffi, Nasarawa State Nigeria, diagnostic accuracy of rapid diagnostic test kits (RDTs) compared to microscopy in diagnosis of malaria and use of physical barriers like long lasting insecticide treated nets for preventive purposes. RDT and microscopy methods have their merits and demerits that can be harnessed differently to favor work on field analysis (RDT for its simplicity and ease of use) while for research, clinical bases (microscopy with trained and retrained personnels) are advocated. Primary health centers were used for this work as they are the first point of call for parents and guardians of children in a semi- urban area like Keffi. PHCs in Nigeria cover a range of essential services like maternal and child health, immunizations, health education, management of common illnesses e.g. malaria [9]. It is accessible and affordable to the locals compared to the secondary and tertiary health institutions which are for referrals.

2. Materials and methods

2.1. Study area

The study area used for this research work was Keffi Local Government Area (L.G.A.). Keffi L.G.A. is a suburban commercial town located in Nasarawa State, North central Nigeria. Keffi is 50 kilometers from Abuja, the Federal Capital Territory of Nigeria. Institutions like the Nasarawa State University, Federal Medical Center are located here and this attracts people from different works of life around this ancient city. Apart from the civil servants, occupations like farmers and traders abound there too.

It covers a total land mass of about 28,735km² bounded by Kaduna State to the north, Abuja and Kogi States to the west, Benue to the south, Plateau and Taraba State to the east [10]. The dry and wet seasons are the two main seasons in the area and the average humidity is 42%. It has a population of about 1,983,910 (2006 census) projected at 3.2% annual growth rate giving it an estimated population of 2,523,395 (2016) [11].

We used a cross-sectional design for the study and was conducted in five different primary health care centers (PHC), 44 participants per PHC, totally 220. (PHC Angwan-Wuje, PHC Kofar-Pada, PHC Guata, PHC Angwan Kaswa and PHC Yelwa) in Keffi Local Government Area (L.G.A.), Nasarawa State, Nigeria from June- August, 2024 which falls during the rainy season.



Figure 1 Map of Nasarawa State showing Keffi town [12]

PHCs are supported by the federal, state governments and donor agencies which makes it affordable to the locals. They have days stipulated for infant immunizations, health enlightenment and promotions on different diseases are taught there. So we keyed into it and spoke to them on Malaria- early detection, prevention and treatment, the importance of identifying it early especially in young children.

2.2. Ethical Consideration

Ethical approval for the study were obtained from Federal Medical Center Keffi, Nasarawa State Health Research Ethics Committee FMC/KF/HREC/02643/24, and the State Secretariat for Primary Health Care Development Agency DHS/07/24. Consent of the parents or legal guardians of the children were also gotten in writing before the study started.

2.3. Sample Size

A total of 220 blood samples were collected from children less than 17years attending the five selected primary health centers with complaints of headache, fever, malaise and vomiting. Selection of PHC was done randomly to eliminate bias.

Sample size was determined using Fisher's formular for estimating the minimum number of participants needed to obtain reliable results in studies of disease prevalence [13]. The sample size was calculated based on expected prevalence of malaria and the desired confidence level. The formular used to estimate the required sample size is as follows:

$$n = \frac{Z^2 \cdot P \cdot (1 - P)}{d^2}$$

Z represents the desired confidence level which is 1.96 for a 95% confidence interval, P represents the estimated prevalence rate of malaria in the population assumed to be (50%) 0.5 to account for maximum variability when the prevalence rate is unknown thereby ensuring a conservative estimate [14], d is the margin of error set at 7%, reflecting the desired level of precision in the estimate.

2.3.1. Calculation

Substituting the values into the formula:

$$n = (1.96)^2 \cdot 0.5 (1 - 0.5) / (0.07)^2$$

$$= 3.84 (0.25) / 0.0049$$

$$= 194$$

$$\text{Attrition factor} = 7 \% \text{ of } 194 = 13.58 + 194$$

$$= 207.58$$

The number was round up to the nearest ten, giving it a calculated value of 210. Additional 10 participants were added to this number to give it a more representative figure 44 per PHC making it 220. This ensures the sample has enough statistical power to make comparison between RDTs and microscopy in children attending these PHCs.

2.3.2. Sample Collection and Processing

2ml of blood sample was collected by venipuncture from each participant. The site was first sterilized with 95% ethanol and allowed to dry, then sample collected. Ethylene diamine tetra-acetic acid (EDTA) was labelled with unique identifiers for each child while a risk factor assessment questionnaire administered to the parent or guardian of the younger children.

9 packs of First Response Malaria Antigen *P.falciparum* (HRP2) rapid diagnostic kits (Premier Medical Corporation Private Limited, India) were used in testing all the participants. The kits were used according to manufacturer's instructions. Then blood dropped on a new microscope slide; thick and thin films were made and Geimsa stained. Thin film was methanol fixed only and both allowed to air dry. It was examined using x40 and then, at 100x/hpf oil immersion for parasite and specie identification [15]. The slides were read by trained medical laboratory personnels to ensure accuracy and precision in diagnosis.

2.3.3. Microscopic Analysis

Microscopic examination was used as the gold standard for examination of slides for the diagnosis of malaria. For each sample, 2ul and 6ul were dropped on same slide for a thin and thick film and labelled appropriately with a unique number corresponding to their questionnaire and consent form. Thin film was methanol fixed and thick film allowed to air dry; they were then Geimsa stained following established protocols [7]. Initial microscopic examination focused on the thick films which are used to detect the malaria parasites then specie identified with the thin film preparation on further examination. Reexamination of 10% of the slides by an independent quality control microscopist was done to ensure diagnostic precision and accuracy [4,7]. This served to remove errors and improve the study's reliability.

2.3.4. Rapid Diagnostic Test (RDT) Analysis

First Response Malaria Antigen *P.falciparum* (HRP2) rapid diagnostic kits (Premier Medical Corporation Private Limited, India) known for its robustness and has undergone WHO prequalification tests, were used immediately following the manufacturer's instructions to maintain accuracy and consistency. The RDT is highly sensitive to *Plasmodium falciparum* with a reported product specifications of: 100% sensitivity, 100% specificity and results can be read in 20-30 minutes time [16]. So, its suitable for field and clinical analysis.

2.4. Statistical Analysis

Statistical analysis of the data was conducted to evaluate the significance of variables, with the Chi-square test employed to assess relationships between categorical variables. Descriptive statistics, like percentages were used to summarize the collected data effectively.

Statistical Package for the Social Sciences version 22.0 were used to analyze the data. A p-value of ≤ 0.05 was considered statistically significant, giving a threshold for identifying meaningful differences or associations while a p-value of \geq was seen as not statistically significant association. Also, sensitivity, specificity, positive predictive value and negative predictive value were calculated using standard formulae to evaluate the diagnostic performance of the methods used.

3. Results

These results show the malaria propensity according to locations, age groups and the two types of testing used. From table 1, the different PHC locations and the positive results are shown; PHC Angwan Kaswa is seen to have the highest RDT prevalence at 40% and microscopy at 74%, this shows the scores are relatably high. Children living around this

area close to a market might be part of the reason for this high malaria prevalence as clusters of dirt, muddy pools of water can be found around because it was a rainy period. PHC Angwan Wuje and Yelwa had same prevalence of 64% using microscopy while RDT was 28% and 34% respectively. PHC Guata, had the least RDT value of 24% but the microscopy was higher at 58% highlighting microscopy of being able to pick the malaria parasites at very low volumes unlike RDT. Kofar-Pada was close to Guata at RDT of 26% and microscopy value of 29%.

Table 2 demonstrates the different age groups, gender and their association with the two diagnostic techniques in malaria detection. Age range 13-17 years is the highest affected (27.6% RDT), while microscopy is also high at 29.9%, though the range is a bit wider than the others to give the study more statistical power in comparing with other age groups. This is followed by age group 4-6 and 7-12 at 25% each for RDT while for microscopy, 4-6 years group having slightly higher value at 20.8% and 19.5%. This shows the evolving nature of children, movement from one place to another might increase their chances of coming in contact with mosquito bites. Interesting to note is the age group <1 year having the lowest positivity rate of RDT 9.2% and microscopy value at 14.2%. This shows that mothers have more care for the younger children, using physical barriers as preventive measures especially when they are younger and don't have the willpower to move around much. Gender showed that females (57.9%), were more infected than males (42.1%) for RDT, and microscopy values show that females 60.4% were also more infected than males 39.6%. This shows that both methods used also showed gender difference affecting more females than males. Females were more in number in this study (60.9%), than males, so the higher infection rate might be justified here as statistically it was not significant, both sexes having equal chances to be infected. The use of physical barriers e.g., long lasting insecticide treated nets, needs patience especially in this tropical, warm environment to protect from mosquito bites. This shows the importance of more enlightenment and health education to parents and the children in early detection, prevention and treatment to reduce morbidity and mortality from untreated or severe cases.

Table 3 compares the diagnostic performance of the two techniques utilized in this study by analyzing their sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV). This comparison shows the effectiveness of screening programs thereby having a comprehensible assessment of the accuracy and usefulness in malaria diagnosis. In this study, RDT shows a sensitivity of 57.5% and specificity of 62%, this reflects its ability to identify moderate positive cases and also identify negative cases as non-malarial cases. The Positive Predictive Value (PPV) was 46% and Negative Predictive Value 72%, indicating the probability that a positive result was fairly positive and a higher probability that a negative RDT malaria test was negative. The microscopy results show a remarkably sharp difference from the RDT having a sensitivity of 93.9% and specificity of 50%. This demonstrates the ability of microscopy to identify most positive malaria cases as true positives, this shows its ability as the gold standard of malaria parasite identification while having a lower ability to identify negative cases. The PPV was 70% while the NPV was 86.8%, this reflects a good likelihood that positive malaria results were accurate and also showed a relatively higher chance that a negative malaria test result was actually negative using microscopy. While microscopy had a higher sensitivity than RDT, both have relatively close specificity, PPV and NPV which may imply that both methods can be utilized comparatively depending on the environment of testing whether field, research or clinical setting, (but for clinical, microscopy is encouraged) to enhance diagnosis and make informed decisions on treatment.

Table 1 Prevalence of Malaria among Children according to location of studies using Rapid Diagnostic Tests and Microscopy

Variables	Groups (PHCs)	Total	RDT +ve (%)	Microscopy +ve (%)
Location	Angwa Wuje	44	14 (28.0)	32 (64.0)
	Yelwa	44	17 (34.0)	32 (64.0)
	Kofa Pada	44	13 (26.0)	24 (48.0)
	Guata	44	12 (24.0)	29 (58.0)
	Angwa Kaswa	44	20 (40.0)	37 (74.0)
	Total	220	76	154
		Chi-square	4.63	5.56
		p-value	0.33	0.23

Angwan Kaswa had highest prevalence showing environment and its role in malaria infection though $p > 0.05$

Table 2 Association between Rapid Diagnostic Tests and Microscopic positivity with demographic characteristics of participants (Age and Sex)

Variables	Groups(yrs)	No examined (%)	RDT positive (%)	χ^2	P-value	Microscopy positive (%)	χ^2	P-value
Age groups	< 1	33 (15)	7 (9.2)	6.32	0.18	22(14.2)	6.5	0.17
	1-3	37 (16.8)	10(13.2)			24 (15.6)		
	4-6	41 (18.6)	19 (25)			32 (20.8)		
	7-12	50 (22.7)	19 (25)			30 (19.5)		
	13-17	59 (26.8)	21(27.6)			46 (29.9)		
	Total	220 (100)	76 (100)			154 (100)		
Sex	Female	134(60.9)	44(57.9)	0.55	0.46	93 (60.4)	0.03	0.86
	Male	86 (39.1)	32(42.1)			61 (39.6)		

P value was > 0.05 in this study so association between RDT, microscopy and demographic parameters were not statistically significant

Table 3 Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) of both diagnostic methods used

Characteristics	RDT	Microscopy
Sensitivity	57.5%	93.9%
Specificity	62%	50%
PPV	46.3%	70%
NPV	72%	86.8%

Microscopy was more sensitive but RDT was a little more specific to diagnosis of *Plasmodium falciparum*.

Table 4 Use of Insecticide Treated Nets (ITN)

Use of ITNs	Response (Y) %	No. examined	RDT +ve (%)	Microscopy +ve
< 1year	30 (22.4)	33	7 (9.2)	22 (14.2)
1-3	34 (25.4)	37	10 (13.2)	24 (15.6)
4-6	20 (14.9)	41	19 (25.0)	32 (20.8)
7-12	23 (17.2)	50	19 (25.0)	30 (19.5)
13-17	27 (20.1)	59	21 (27.6)	46 (29.9)
Total	134 (100)	220	76 (100)	154 (100)

Y- Yes; Respondents use of ITNs was statistically significant at $p < 0.05$

4. Discussion

Malaria has continued to be a public health threat especially in the sub-Saharan Africa region. Nigeria has a disproportionate share of the infection (27%) [1], our climatic conditions and environment being a significant constant factor facing this challenge. This study seeks to carry out malaria diagnosis using the most common and affordable methods in our region: (RDT and microscopy); a comparative analysis of *P. falciparum* malaria rapid diagnostic tests and microscopy of children attending selected primary health centers in Keffi, Local Government Area, Nasarawa State, Nigeria were carried out. The findings depict malaria prevalence, association between malaria positivity between the different test methods and diagnostic efficiency. The overall prevalence of malaria in this study was 52.3%. This is in opposition to a study carried out in another facility which had a prevalence of below 15% and the study was carried out

in Keffi but in another location and using different RDTs [14]. This disparity might be due to location, environmental conditions, storage or batch of RDT used [6, 8, 17]. Another study carried out in the South-Eastern region of Nigeria showed an overall malaria prevalence of 26.7% but children in low socio-economic class had a significantly higher prevalence rate of 32.7% which is also similar to what is seen in this study [18]. According to the different Primary Health Centers (PHC), the different prevalences were seen to be affected by locations as the ones close to the market (PHC Angwan Kaswa RDT 40%, Microscopy 74%) higher values as opposed to the ones in a cleaner and drier environment. This is in line with another study, which proposes through generalized additive modelling that sufficient precipitation provides comfortable mosquito habitats for vector species, which ensures adequate humidity that propagates the survival of mosquitoes [19]. Also, it is seen that increased rainfall, flooding, increased temperature, precipitation etc. which are all climate change conditions increase vector-borne diseases like malaria, dengue fever, Lyme disease and West Nile virus which can lead to epidemics so stronger control strategies are advocated [17, 19].

The prevalence of malaria using rapid diagnostic tests was 34.5% while using microscopy the prevalence was 70%, this shows the disparity in diagnosis and limitation of RDT not being able to identify lower parasite density. In different parts of Nigeria, there are various reports of malaria prevalence like Comfort *et al.*, reported 14% for microscopy and 4.7% for RDTs in Keffi [14]. Another study in Sokoto, reported 60.4% [20], Nwaneli *et al.*, reported total prevalence of 46.2% in Nnewi [18], Onyishi *et al.*, in Nsukka reported 72.8% [21]; these show that timing and location can also affect malaria transmission. These values indicate that we should not relax on previous gains of malaria reduction but should work harder with control strategies (especially seasonal malaria interventions) early detections, prompt treatment of cases to achieve even if it's a pre-elimination mode in Nigeria.

Malaria positivity across the different age ranges <1, 1-3, 4-6, 7-12 and 13-17 years were 9.2%, 13.2%, 25%, 25%, 21% (RDT) while for microscopy it was 14.2%, 15.6%, 20.8%, 19.5% and 29.9%. This is in agreement with the work of Nwaneli *et al.*, that showed that malaria positivity was increased as the age of the children increased [18]. But from this age range, age 13-17 years, had a constant increase in both RDT and microscopy followed by 4-6 years, this showed that children 6 years and below might be more infected if appropriate care is not given to them, and the older children 13-17 years though assuming acquired immunity at this age might lose it and can expose them to drug resistant species if not detected and treated on time [22]. Females were more infected than males 57.9% vs 60.4% for RDT and microscopy, while males were 42.1% vs 39.6%, females were significantly more in number but sex was not a statistically significant association with malaria ($p > 0.05$). This is also seen in a study carried out in Ghana where females were more in number and more infected but not statistically significant [23]. The First Response RDT used in this study gave a fairly good representation almost half the microscopy values, which makes its ease and simplicity good for field work but with confirmation of most negative cases should be done by trained malaria microscopists. This is as opposed to other RDTs used in other studies by [14, 24]. They had lower positive rates using different RDTs, but microscopy results were higher as seen in this study. This means that some positive samples might be missed using some batches of RDTs. Some participants on medications or submicroscopic ratios might also be missed [22]. This might also be as a result of storage or temperature effects on the RDT kits which might lead to more false negative results [24].

The sensitivity, specificity Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the tests were used to evaluate the diagnostic efficiency of the two techniques used. The sensitivity of RDT was 57.5% while microscopy was 93.9%, Specificity was 62% and 50%, PPV 46.3% and 70% and NPV 72% and 86.8%. This is in line with the studies of Runmokon *et al.*, that had sensitivity of 97.08% in a study of two primary health and malaria in children in Port-Harcourt, Nigeria. Also, Comfort *et al.*, had high sensitivity for the microscopy works as opposed to the Ghana study that had sensitivity of microscopy as 39.3% and RDT 55.7% but both tests had comparable specificity of 98.2% and 98.3%, similar PPV 95.7% vs 94.5% but higher NPV (75.3% vs 69.0%) [23]. The NPV value of Opoku *et al.*, was similar to the result we got in this study as above depicting that negative results were actually negative similar to RDT and microscopy.

Plasmodium antigens produce special proteins called histidine rich proteins that are used in targeting or detection in rapid diagnostic kits [25, 26]. The presence of these proteins has been found to be deleted in some strains of *Plasmodium* due to breakage and rejoining at the unstable sub-telomeric regions of chromosome 8 and 13 for *pfhrp2* and *pfhrp3* genes so that the parasite can't be detected early enough (false-negative result) hence encouraging its transmission and resistance patterns [26]. The identification of more microscopy samples may be attributed to this. HRP2 and HRP3 are mostly common with *Plasmodium falciparum* and are both similar in structure. The structural similarity between them is responsible for the cross-reactivity of monoclonal antibodies against PfHRP2 with those of PfHRP3. Unlike PfHRP1 and PfHRP3, PfHRP2 is produced in large concentrations throughout the parasite life cycle [26].

5. Conclusion

Several reports have attributed microscopy as the gold standard method of malaria identification though more modern technologies like polymerase chain reaction, artificial intelligence is gaining ground (but the expensive nature is a drawback and can't be used as routine investigation). The comparison of rapid diagnostic test strips and microscopy is seen in this study as favoring microscopy as the acceptable gold standard identifying malaria parasites earlier and even in low parasitemia. This though requires training and retraining by certified laboratory personnels. In low- and middle-income countries where malaria is still endemic, strengthening manpower by trainings, infrastructure availability like electricity is necessary for microscopy to be efficiently carried out. Rapid diagnostic kits are affordable, accessible and can be easily used on the field but detecting low parasitemia coupled with the *hrp 2/3* deletions can be an issue so negative results should be confirmed by microscopy. Climatic change which we are noticing now can increase temperature, rainfall and humidity leading to proliferation of malaria-carrying mosquitoes then malaria transmission increases more. We advocate for more physical barriers using mosquito nets and window net screens at homes, environmentally safe indoor insecticide sprays, improved dip stick testing devices and subsidies for new malaria combination therapies after testing. Thus, early detection, treatment and prevention is pertinent in the fight against this ancient scourge. This will enable faster identification, treatment time and increase community, family wellbeing and the globe at large.

Compliance with ethical standards

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Author contributions

US and DI conceived the work, US and FS conducted sample collection and RDT. FS and VO carried out microscopy, US wrote the first draft of the manuscript, DI and AI edited the draft manuscript. All authors read and agreed with the final version of the manuscript

Disclosure of conflict of interest

The authors declare no conflict of interest in carrying out this research.

Statement of ethical approval

The study was approved by the Federal Medical Center Research and Ethics Committee FMC/ Prior to sample collection.

Statement of informed consent

Informed consent was gotten from each parent or guardian of the children.

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