RESEARCH ARTICLE

Where have all the parasites gone? Unusual *Plasmodium falciparum* monoparasitaemia in a cross-sectional malariometric survey in northern Nigeria [version 1; peer review: 3 approved with reservations]

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Abstract

Background: Malaria is caused by one of five currently known *Plasmodium* parasite species causing disease in humans. While modelling has provided information of the vector, the same is not entirely the case for the parasite. The World Malaria reports of 2014 to 2016 reported 100% of confirmed cases from Nigeria being due to *Plasmodium falciparum*. Generally, about 98% of cases of uncomplicated malaria in most regions surveyed in Nigeria recently is due to *P. falciparum*, with the remainder being due to *P. malariae*. This study aimed to determine the proportions of *Plasmodium* parasites causing uncomplicated malaria in Wamakko Local Government Area of Sokoto State, north-western Nigeria.

Methods: The study was a descriptive, cross-sectional study conducted during the rainy season and dry season in north-western Nigeria. The area has a 'local steppe' climate and Sudanian Savannah vegetation. Sampling was via multistage cluster sampling. Selected participants were examined for pallor, palpable splenomegaly and signs of complicated malaria. Blood samples were also taken for rapid diagnosis of malaria and thick and thin films to identify parasitaemia and the parasite species. Participants found to have malaria were treated with Artemether/Lumefantrine and those with complicated malaria were referred to the nearest hospital.

Results: We found a parasite prevalence of 34.8% overall, which was higher in the rainy season (49.3%) than in the dry season (20.2%). There was monoparasitaemia of *Plasmodium falciparum* throughout the study area, irrespective of the clinical status of the participant. Mapping of the parasite was extended throughout the Local

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Any reports and responses or comments on the

Government Area and the State.

Conclusions: Despite the intermediate endemicity in the area. *P. falciparum* monoparasitaemia affirms theories of disappearance of other parasite species, either due to faltering control of *P. falciparum* or more efficient control of other species.

Keywords

Malaria, Nigeria, Plasmodium falciparum, PfPR2-10

article can be found at the end of the article.

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Introduction

Malaria is caused by one of five currently known *Plasmodium* species causing diseases in humans. These are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Scientists have modelled the anopheles mosquito vector extensively based on its characteristics, but not so much the parasite¹. An absence of the Duffy antigen on red blood cells of West Africans has long been postulated to be responsible for the absence of *P. vivax* in these areas, but cases of *P. falciparum*, *P. malariae* and *P. ovale*, in order of decreasing occurrence, have been found. The World Malaria Reports of 2014² and 2015³ reported 100% of confirmed cases in Nigeria as being due to *P. falciparum*. It is generally thought to account for about 98% of all malaria cases, with *P. malariae* accounting for the rest, often as a co-infection with *P. falciparum*⁴. This figure likely over-estimates the proportion of cases as *P. falciparum* is responsible for most severe cases of malaria; these are the cases most commonly reported alongside confirmed cases of malaria, which are tracked by passive surveillance in Nigeria. It may also be because of limited expertise in identifying other species of *Plasmodium*.

Available data from across Nigeria shows a mixed picture; large areas across northern Nigeria in 1967/68 found an average proportion of 22% of malaria parasitaemia due to *P. malariae* in a study at a time when most of Nigeria was considered holoendemic for malaria. *P. malariae* was often seen in coinfection with *P. falciparum*, particularly among younger age groups. *P. ovale* was responsible for 5% of malaria infections, being more common in children under five years of age, and *P. falciparum* ranged between 84.4% and 90.5% across age groups⁵. The proportion of *P. malariae* was high, probably because the data was obtained from a survey of both asymptomatic and symptomatic participants.

More recently, in 2010, prior to the commencement of nationwide LLIN distribution, a study including 4209 individuals in Jos, northern Nigeria, found a *P. malariae* rate as low as 1.6%, with *P. falciparum* responsible for 98.7% of infections, sometimes in co-infection with *P. malariae*⁴. Children aged less than 10 years and all individuals in every third household were selected for this abridged malaria indicator survey (MIS). Crucially, however, no *P. vivax* or *P. ovale* species were seen either in this location or another in south-eastern Nigeria, which was shown to have a higher rate of *P. malariae* infections (around 30%) and lower rate of *P. falciparum* infections (68.1%) in a study also conducted in 2010⁶. This study, however, included 2,936 individuals from 1400 clusters in Abia state, spread out across the state. Quality control measures in the identification of parasite species were implemented in the study, including a WHO-certified malaria microscopist, giving credibility to the results obtained⁶. In south-western Nigeria, a study in Ikorodu in 2012 recruited 1,496 participants of all ages, which included 237 children under the age of five years and 509 children aged 15 years and below. Microscopy and DNA evaluation was used to determine parasite species, which found that 93.6% of participants had *P. falciparum* infection, with the remainder being *P. malariae*⁷. This is in contrast to previous studies in 1976 around the same location in south-western Nigeria, which found that 13% to 16% of

parasitaemia was due to *P. malariae*, with 62 to 76% being due to *P. falciparum*⁸. These proportions were the age-group specific parasite prevalence in the study, which included mostly children aged five to 10 years (1,500 participants) in an attempt to compare spleen and parasite rates among individuals with sickle cell trait and those with normal adult haemoglobin. It also found *P. ovale* in 2% to 3% of participants in 5–10 and 2–4 year age groups, respectively. These findings, although limited in scope, perhaps suggest a changing trend, with the disappearance of *P. ovale* from Nigeria over time. *P. falciparum* is an undisputed leader in all the studies performed. Its high proportion perhaps accounts for high rates of malaria-related anaemia in most studies, explained by the ability of the parasite to invade and destroy both young and senescent red blood cells^{7,9}.

We conducted this study to determine relative proportions of parasites causing clinical malaria in Sokoto, north-western Nigeria.

Methods

Ethical statement

Ethical approval was obtained from the Independent Ethics committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria with ethical approval number UDUTH/HREC/2014/No. 246. Ethical approval was also obtained from the Independent Ethical committee of the Sokoto State Ministry of Health with the approval number SMH/1580/VIV. Permission was also obtained from the district heads of the included communities in the study to visit the communities.

Study setting

The study was conducted in Wamakko Local Government Area of Sokoto State, located in the north-western geopolitical zone of Nigeria. It has an area of 732.146km², with a projected population of 260,860 by 2019¹⁰. It is located at coordinates 13°2'16"N 5°5'37"E. The geography of the area is predominantly flat plains with Sudan Savannah-type vegetation and it stands at an altitude of 292m above sea level, near to the confluence of the Sokoto and Rima rivers. Its climate is tropical, described as local steppe climate.

Study design

The study was a two-point, cross-sectional prospective descriptive study conducted during the rainy season and dry season. In April and November 2016, we screened and recruited participants simultaneously until we reached the target population.

We determined the minimum sample size using Cochran's formula¹¹, assuming a prevalence of 50% based on a previous survey¹², and 500 participants gave a power of at least 80% to show reliable results.

Sampling technique

As is the norm for MIS's, multistage cluster sampling in proportion to size was employed. The primary clusters were four randomly selected wards of the eleven political wards within the Local Government Area (LGA) based on the population within each of the wards, with secondary clusters being eight settlements

unevenly selected from the four wards; proportionate to size, making up the total sample size. Based on the assumptions of a 70% response rate and that 80% of households include at least one child less than five years of age, in accordance with a previous Nigeria MIS in 2010¹³, approximately 892 households were required to meet the target of at least 500 participants per season based on the assumptions stated earlier. This was surpassed by the estimated number of households within the eight settlements selected. All children in the secondary clusters who fulfilled the inclusion criteria, and whose parents consented to participate, were included in the study.

Sample population

Participants were visited at their homes and while in the household, after identifying the household head. They were provided with information regarding the study and all eligible children within the household invited to participate. Those parents who accepted signed or thumb-printed the informed consent form. All children in the selected settlements who met the age criteria of two to 10 years, with or without symptoms of malaria, were recruited for the study, provided they had been residents of the study area for at least two weeks. They were, however, excluded if they were suspected to have taken any medication with antimalarial properties within the two weeks prior to enrolment³⁶. Recruitment was carried out on consecutive days until the entire village was covered. Each participant was evaluated once except for those who had parasitaemia without symptoms, who were followed up by a field assistant for up to 48 hours for the development of symptoms. The period of recruitment was about a month in each season.

Procedures

We conducted the study procedures at a central location in each of the study villages. Field assistants went from house-to-house and recruited the participants and then brought the consenting participants to the central location. A paediatrician screened potential participants for eligibility and the caregivers of eligible participants were required to sign informed consent forms. He performed a physical examination for each participant and graded splenomegaly according to Hackett's criteria¹⁴. The WHO criteria for severe malaria was used¹⁵. Using a single use lancet, we collected capillary blood by pricking the index finger of the child's left hand. A drop of blood was collected each for a thick and thin malaria parasite film for estimation of parasite density and species identification, respectively.

Concomitantly, during the same session, we did rapid diagnosis of malaria using a drop of blood with CareStart® Malaria HRP2 rapid detection tests (RDTs) (Access Bio, Inc., model G0141), which can detect *P. falciparum*.

Each day, we transported the samples to the paediatric department laboratory of the Usmanu Danfodiyo University Teaching Hospital and fixing of thick films was done with methanol. Thin films were stained immediately and stored in the lab. We analysed the samples in a completely anonymized manner in pairs of thick and thin films; examining the thin film if we found the thick film

positive for malaria. The study numbers were the only identifiers for the thick films, which were kept apart from the thin films. A trained malaria microscopist performed the analysis, under the supervision of a medical parasitologist. We examined at least 10 fields before a slide was declared negative for malaria parasites.

The tail segment of the thin films was viewed to identify the species of malaria parasite, using the typical description of parasite species, having been trained on parasite identification¹⁶.

Quality control of the diagnosis of the parasitaemia was provided by a trained medical microbiologist re-examining 10% of the slides selected at random. A discrepancy of 10% or more would have necessitated reanalysis of all the thick films and the thin films subsequently. The discrepancy was 3% (kappa score of 0.71) and as such, this was not necessary.

Study participants with malaria, determined by the presence of at least one symptom and a positive RDT or thick blood film, were treated by the study paediatrician at home with Artemether-Lumefantrine. Children were dosed according to standard dosing¹⁷ but only the first dose was directly observed.

Statistical analysis

We analysed the data using SPSS version 22. We determined the prevalence of malaria by parasitaemia and RDT by determining proportions. We used descriptive statistics to determine averages and proportions. Participants with missing data were excluded from the analysis. We carried out sub-group analysis for age, gender and season and used kappa analysis to control the quality of malaria diagnosis.

Results

Participants included

We screened a total of 1136 participants for inclusion in the study after they consented to participation in the study. We excluded 109 because they had been treated with antimalarials in the two weeks prior to enrolment and excluded 10 from the analysis due to incomplete data. We included 1017 participants in the analysis (Figure 1)¹⁸.

The age-sex distribution showed that all ages were equally represented in the study, as shown in Table 1.

Prevalence of malaria parasitaemia

We found an overall prevalence of malaria for the study of 34.8% using microscopy and 33.8% using RDT as shown in Table 2. There was an agreement between the two diagnostic methods, as shown by the kappa statistic ($p < 0.001$).

Age-specific prevalence rate of malaria parasitaemia

We saw the highest age-specific prevalence among participants aged two years, with the lowest among ten-year olds. Table 3 also shows a significant association between the age of the participants and prevalence of malaria parasitaemia ($p = 0.000$).

Figure 1. Flow chart for inclusion in analysis for clinical malaria.

Table 1. Age and gender distribution of the subjects included in the study.

Age (completed years)	n	Gender	
		Male, n (%)	Female, n (%)
2	123	60 (5.9)	63 (6.2)
3	117	63 (6.2)	54 (5.3)
4	110	54 (5.3)	56 (5.5)
5	107	52 (5.1)	55 (5.4)
6	111	62 (6.1)	49 (4.8)
7	111	73 (7.2)	38 (3.7)
8	110	50 (4.9)	60 (5.9)
9	113	54 (5.3)	59 (5.8)
10	115	57 (5.6)	58 (5.7)
Total	1017	525 (51.6)	492 (48.4)

Table 2. Prevalence of malaria parasitaemia among children aged 2–10 years using microscopy and rapid detection test (RDT).

Test result		Thick film		
		Positive	Negative	Total (%)
RDT	Positive	295	49	344 (33.8)
	Negative	59	614	673 (66.2)
	Total (%)	354 (34.8)	663 (65.2)	1017 (100.0)

Kappa agreement $\kappa = 0.764$; $p < 0.001$.

Table 3. Age-specific prevalence of malaria parasitaemia.

Age (completed years)	n	Malaria parasitaemia	
		Positive Freq (%)	Negative Freq (%)
2	123	62 (50.4)	61 (49.6)
3	117	57 (48.7)	60 (51.3)
4	110	37 (33.6)	68 (66.4)
5	107	39 (36.4)	68 (63.6)
6	111	38 (34.2)	73 (65.8)
7	111	32 (28.8)	79 (71.2)
8	111	30 (27.3)	80 (72.7)
9	110	35 (31.0)	78 (69.0)
10	113	24 (20.9)	91 (79.1)
Total	1017	354 (34.8)	663 (65.2)

$\chi^2 = 38.453$; $df = 8$; $p < 0.01$.

Parasite species causing malaria

279 participants were found across the seasons to have clinical malaria and all of them had *P. falciparum* malaria, irrespective of the season and nature of their clinical presentation. The relative proportions of parasites are depicted in Figure 2. The most common presenting feature among these was fever (92%),

Figure 2. Parasite species causing clinical malaria.

followed by vomiting (38%), refusal to feed/poor appetite (32%) and body weakness (25%).

Of the clinical cases of malaria, 9.6% had complicated malaria, as indicated by the WHO criteria for severity¹⁹. The number of severe malaria cases was significantly lower in the dry season than the rainy season, as shown in [Table 4](#). Different participants had various combinations of the criteria for severity, although the common criteria were hyperpyrexia, prostration and persistent vomiting.

Comparison of prevalence between the seasons

The prevalence of malaria parasitaemia during the rainy season was significantly higher than the dry season, with prevalence rates of 49.3% and 20.2%, respectively, all due to *P. falciparum*.

Parasite density across the seasons

The mean parasite density was much higher during the rainy season (1006.13) than during the dry season (405.45). The details are shown in [Table 5](#).

Discussion

The prevalence of malaria in this study, when compared with serial MIS’s performed in 2010¹³ and 2015²⁰ shows a progressive reduction; from 48.1% to 37.1% for north-western Nigeria, and a prevalence of 46.6% for Sokoto in 2015 compared with 34.8% for our study. Prevalence in the MIS’s was measured among children aged six to 59 months and is probably higher than for the children included in this study because including the children from 6–10 years is likely to reduce the overall prevalence, as is excluding those aged six months to two years, who generally have a higher prevalence rate²¹.

Table 4. Prevalence of severe malaria across seasons.

Season	N	Severe malaria Freq (%)	Other cases Freq (%)
Rainy season	511	24 (4.7)	487 (95.3)
Dry season	506	2 (0.4)	504 (99.6)
Total	1017	26 (2.6)	991 (97.4)

$\chi^2 = 18.883$; df = 1; p = 0.000.

Table 5. Parasite density across the seasons (parasites/ μ L).

Season	Mean	SD	Minimum	Maximum	Interquartile range
Rainy	1006.13	495.8	16	284000	840
Dry	405.45	209.48	16	204000	640
Overall	833.1	443.8	16	284000	1064

Although there were studies performed in the past in Sokoto, they are limited in comparison to the present study by virtue of having been conducted in a different age group, or hospital in lieu of community setting and the seasons in which these studies were conducted. The prevalence of 34.8% found here was higher than the 27.9% found by Abdullahi *et al.*¹² in Sokoto; however, samples in the previous study were collected from patients visiting two hospitals within the metropolis and was thus not community-based. Furthermore, all ages from 0 to 65 years were included in the study, which is likely to further dilute the findings and give a falsely low prevalence because the

incidence of malaria is generally lower among adolescents and adults, as indicated in the study. The overall picture supports the suggestion of a reduction in the prevalence of malaria, likely owing to better access to malaria prevention and increasing urbanisation; both of which cause a decline in malaria parasite rates generally²².

The prevalence found in this study is also lower in comparison to the 45.4% prevalence rate found in a study by Jiya *et al.*²³, conducted in Sokoto between 2007 and 2009. Additionally, it was lower than the prevalence of 49.6% found among children under the age of five years in the same study. Considering both age-specific prevalence rates, there is a reduction in prevalence, although being a hospital-based study, the prevalence for the former study is likely to be higher than the current. It is, however slightly, higher than the projected national average of 29% for 2015, with wide inter-regional differences²⁴. The Nigerian MIS of 2015 found a higher prevalence of 46.6% than this study, although the age of included participants ranged from six to 59 months, which will limit the comparability of results from this study due to the different age ranges of participants²⁰.

The prevalence by age in this study roughly indicated a progressive decline with age. The highest age-specific prevalence was among two-year-olds (50.4%), with a statistically significant difference among the age groups. This finding is in conformity with the steady-state assumption and is similar to findings in previous studies that showed higher prevalence among younger age-groups.

With respect to the parasite species causing uncomplicated malaria, all parasitaemia in this study was found to be due to *P. falciparum*. This is in keeping with recent studies in Adamawa²⁵ and Cross River states⁹ in 2011 and 2013, respectively. Another study from Ihiala, in Anambra state of south-eastern Nigeria, found *P. falciparum* mono-parasitaemia even though this study considered all types of malaria, both severe and uncomplicated²⁶. This is, however, unlikely to affect the findings, as most cases of severe malaria in this area are due to *P. falciparum*²⁶. An earlier report from Sokoto between 2005 and 2006, carried out at Usmanu Danfodiyo University Teaching Hospital by Jiya and Sani^{23,27} likewise did not find any *Plasmodium* species apart from *P. falciparum*, although they only considered cases of severe malaria, which are unlikely to be due to a different species of *Plasmodium* within Nigeria. Meanwhile, only three cases out of 582 (0.01%) were positive for *P. malariae* in another study by Nwaorgu and Orajaka²⁸ in Awka, south-eastern Nigeria. The

finding of *P. falciparum* mono-parasitaemia supports the fact that *P. falciparum* is the dominant species of *Plasmodium* in Sub-Saharan Africa, with the tendency to exclude other forms of parasitaemia, as expounded by Lucas and Gilles²⁹ in 1998 with time and sustained malaria control activities.

Other studies have shown the presence of other forms of parasitaemia, notably with *P. malariae* either as mono-infection or coinfection with *P. falciparum*. In Abia and Plateau states (2010), *P. malariae* accounted for 32.0% and 1.4% of malaria infections, respectively⁶. In a study in north-central Nigeria, 6.1% of examined participants had *P. malariae* infection³⁰ and as high as 41% and 4%, respectively, had *P. malariae* and *P. ovale* infections in a historical study in Garki, Abuja (1968)³¹. Outside Nigeria, there has been a shift towards mono-parasitaemia with *P. falciparum* as well, and this has been documented in the Horn of Africa³² and Benin in West Africa³³. Some authors have suggested it be an evidence of failing control measures but this is at variance with data from this study, which shows a reduction in prevalence from previous data, including a reduction in the number of severe cases of malaria, which were very few in this study.

Severe malaria was seen in 26 of the 1017 participants analysed in this study, with an overall prevalence of 2.6%. It was higher during the rainy than the dry season, probably due to higher prevalence of the disease and higher parasitaemia, as earlier discussed. This is lower than expected from other hospital-based studies for which children presenting to the hospital are more likely to be ill than those found in a community-based survey such as this. In one such study in Ilorin by Olanrewaju and Johnson²⁹ found that a third of all children admitted with malaria had a severe form of malaria.

Data availability

Underlying data

Figshare: complete data.xlsx. <https://doi.org/10.6084/m9.figshare.11590542.v1>¹⁸.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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[PubMed Abstract](#) | [Publisher Full Text](#)

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Version 1

Reviewer Report 18 September 2020

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Linda Eva Amoah

Immunology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

Abstract/Introduction:

1. What is monoparasitaemia? Do you mean mono species parasitaemia?
2. The first sentence of the abstract/introduction should be revised as malaria in other animals is caused by other *Plasmodium* species.
3. The reference WHO 2014 and 2016 are not current, what is the current data?
4. What is meant by 'parasite mapping' and why is this important to the reader?
5. Check the text and remove spaces before full stops as well include a space before the falciparum in *P. falciparum*.
6. 'not so much the parasite'? I do not understand what this means. There is no modeling in this study so the second sentence in the introduction is not relevant, especially as it is very confusing.
7. What is the definition of 'confirmed cases'?
8. What does 'crucially' mean?

Methods:

1. Is April the peak of the malaria season? The description of malaria indices in the study site is not sufficient.
2. The blood draw procedure can be rewritten to enhance clarity. Overall, how many drops of blood were collected? And what processes/procedures were performed?

Tables:

1. Tables 1, 2, 3, and 5 can easily be merged. It would reduce the repetition.
2. Table 2 can be stratified by age as the other tables.
3. Table 4 should be age stratified and other cases separated into asymptomatic and uncomplicated cases.

Figure 2:

1. The title is wrong as it includes 'no parasites'. No parasites do not cause malaria.
2. There are items in the legend that are not on the graph, it would be best to remove and just state in the foot notes that none of those other species were identified.

Results:

1. Was the parasite density at the two time points significantly different?

Discussion:

1. It would be very easy to compare the results from the current study with the data in the MIS by comparing similarly aged children. The statement can be revised if the analysis is redone for children aged 5 years and below.
2. I think the last paragraph should be a summary of the major findings and provide information related to the main aim or relevance to the title but it is presently not so.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Recombinant DNA technology and Malaria transmission, Plasmodium species

identification, gametocytes and serology as well as host genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Sep 2020

Usman Nasir Nakakana, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria

Abstract/Introduction:

1. What is monoparasitaemia? Do you mean mono species parasitaemia?
Yes, this is clarified in the text
2. The first sentence of the abstract/introduction should be revised as malaria in other animals is caused by other *Plasmodium* species.
It has been rephrased for clarity
3. The reference WHO 2014 and 2016 are not current, what is the current data? **The data was collected in 2016 and written up in 2017, the data is referencing the time around which the data was collected to ensure comparability, considering the constantly changing data**
4. What is meant by 'parasite mapping' and why is this important to the reader?
Parasite mapping refers to the description of geographical spread of the parasite species and how it can be extended to other parts of the state, based on climatic and geographic information.
5. Check the text and remove spaces before full stops as well include a space before the falciparum in *P. falciparum*.
Checked and corrected
6. 'not so much the parasite'? I do not understand what this means. There is no modeling in this study so the second sentence in the introduction is not relevant, especially as it is very confusing.
Reference to modelling is deleted, the statement has been rephrased
7. What is the definition of 'confirmed cases'? confirmed cases is defined as those with a positive malaria parasite test and clinical symptoms.
This has been elaborated
8. What does 'crucially' mean?
Changed to 'notably'

Methods:

1. Is April the peak of the malaria season? The description of malaria indices in the study site is not sufficient.
Some information has been provided
2. The blood draw procedure can be rewritten to enhance clarity. Overall, how many drops of blood were collected? And what processes/procedures were performed?
It has been re-written to increase clarity

Tables:

1. Tables 1, 2, 3, and 5 can easily be merged. It would reduce the repetition. **Table 3 contains more data including data on the age distribution**

2. Table 2 can be stratified by age as the other tables.

The data is provided in table 3

3. Table 4 should be age stratified and other cases separated into asymptomatic and uncomplicated cases.

The data is provided in table 3

Figure 2:

1. The title is wrong as it includes 'no parasites'. No parasites do not cause malaria.
2. There are items in the legend that are not on the graph, it would be best to remove and just state in the foot notes that none of those other species were identified.

The figure has been changed entirely in version 2

Results:

1. Was the parasite density at the two time points significantly different? **There was no test of significance performed but it is much higher in the rainy than the dry season.**

Discussion:

1. It would be very easy to compare the results from the current study with the data in the MIS by comparing similarly aged children. The statement can be revised if the analysis is redone for children aged 5 years and below.
The prevalence of malaria is described as it relates to the parasite species distribution and the impact of malaria endemicity. The metric for endemicity is the prevalence of falciparum malaria among children 2 to 10 years of age, which is the age group we studied. We also did not collect any data in children 6 months to 2 years and so even if we consider children under 5 years, we still would not be able to compare the data directly with MIS data.
2. I think the last paragraph should be a summary of the major findings and provide information related to the main aim or relevance to the title but it is presently not so.
It has been revised

Competing Interests: No competing interests reported

Reviewer Report 15 September 2020

<https://doi.org/10.5256/f1000research.23106.r69658>

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Richard Mwaiswelo

¹ Department of Microbiology, Immunology and Parasitology, Hubert Kairuki Memorial University, Dar es Salaam, Tanzania

² Department of Parasitology and Medical Entomology, Muhimbili University of Health and Allied

Sciences, Dar es Salaam, Tanzania

This article presents findings from a cross-sectional study conducted in Wamakko Local Government Authority, in Sokoto State in Nigeria with a broad objective of determining the prevalence of *Plasmodium* parasite species causing uncomplicated malaria in human in the area. The diagnosis of malaria was performed using mRDTs (for the detection of the presence of infection) and thick (for assessing parasite density) and thin (for species identification) smears. The key finding is *Plasmodium falciparum* mono-infection in the study area.

Major Comment

The authors have failed to answer the following questions so as to convince readers, and thus should be addressed: What is the aim of this study? Why do you want to understand the prevalence of different malaria parasite species present in that part of Nigeria? How does the understanding of the prevalence of the parasite species contribute to the management, control and prevention of malaria in general? Try to explain this so as to lay down the significance of this study.

Abstract

Background

- The first sentence.....'Malaria is caused by one of five currently known Plasmodium parasite species'...the sentence is not clear. Where do the authors refer to where malaria is caused by that one of the five species?
- What information on vector does the modeling provide? The sentence is redundant; I suggest to be omitted.
- The sentence.....'This study aimed to determine proportions of Plasmodium parasites'.....is not in line with the true aim of the study. It would sound clear if it was stated that the aim was to determine the proportions of *Plasmodium* species infecting humans in the study area.

Methods

- 'Selected participants were examined for signs of complicated malaria'. However, there is nowhere in the abstract where it has been stated that this was either a hospital or community based study, and whether only symptomatic or both symptomatic and asymptomatic individuals for malaria infection were included in the study. How were the study participants selected, randomly or conveniently? What was the age limit of the study participants?
- '.....and thick and thin films to identify parasitemia and parasite species'. Which one of the tests was used to identify parasitemia, and which one was used to identify species? Be specific.
- '..... with complicated malaria were referred to the nearest hospital'.....but you are silent about those individuals with uncomplicated malaria, were they treated?

Results

- 'There was monoparasitemia of *Plasmodium falciparum*'..... I suggest to rewrite the

sentence and read..... There was Plasmodium falciparum mono-infection throughout.....

- On the other hand, detection of parasite species was based on microscopy thin smears, where there any efforts to detect the parasite species using more sensitive methods such as PCR?
- 'Mapping of the parasite.....' This sentence is not part of the results but rather of the methods, move it to the methods section.

Conclusion

- Omit the sentence.....'Despite the intermediate endemicity in the area'..... otherwise, how does the endemicity determine the presence of a certain malaria parasite specie?

Introduction

- The first sentence is confusing, same as in the abstract. See the above comment.
- The third sentence.....'scientists have modelled the Anopheles.....but not so much on the parasite'. This sentence is misleading, and which characteristics are you referring to? The sentence itself is redundant and I suggest to be omitted.
- This figure likely overestimated the proportion of cases.....tracked by passive surveillance in Nigeria'. The sentence is not clear, rephrase it to increase clarity.
- These proportions were the age-group specific parasite prevalence.....sickle cell trait and those with normal adult hemoglobin. Rephrase this sentence to increase clarity.
- 'Plasmodium falciparum is an undisputed leader'..... I don't think the word leader suits well in this sentence, I suggest the use of the word 'predominant/prevalent'.
- There is no aim of the study in the whole introductory section.

Methods

Study settings

- How many rainfall seasons are there in the study area? When does the rainfall season start and end? What are the major economic activities in the area? What are the major vectors? What are the major malaria control tools employed in the area?

Study design

- Where the participants selected randomly or conveniently?
- Was this a community-based or health facility-based study?

Sample population

- Rewrite sentences 1 and 2 to increase clarity.....'after identifying the household head. They were provided with information regarding the study'..... To who was the information provided, the household head, or everyone in the household? Who gave the consent?

- 'Residents of the study area for at least two weeks'.....with this very short time of being a residency in the study area, how sure are you that the malaria parasite species found in the assessed individuals have not originated out of your study area (where the assessed individual was living before moving into the study community)?
- Except for those who had parasitemia without symptoms.....followed up..... for up to 48 hours for the development of symptoms. Is it ethical to not treat an infected individual and only wait for symptoms to occur?

Procedures

- Does this mean that the field assistant recruited the study participants prior to obtaining consent from parents/guardians? Is this ethical?
- 'We examined at least 10 fields before a slide was declared negative'.....what is the standard number of fields that are required to be read before declaring a slide negative?

Statistical analysis

- 'We determined the prevalence of malaria by parasitemia and RDT by determining proportions'. The sentence is not clear, rewrite it.

Results

- Combine tables 1 and 3
- Combine tables 4 and 5
- Figure 2.....the prevalence of other parasite species is 0%, I suggest you find another method for presenting this, otherwise the figure is not convincing.
- The following sub-sections can be combined: Prevalence of malaria, age-specific and sex-specific prevalence of malaria, the prevalence of malaria by seasons, and parasite density by seasons. All these can be under one sub-section 'Malaria prevalence'.
- You should have at least 3 sub-sections to include: Included participants, Prevalence of malaria, and Plasmodium parasite species.

Parasite density across the season

- '.....density was much higher during rainy....., but the p-value is not given and the authors states that details are shown in Table 5, this is not proper.

Discussion

- Evaluation of the prevalence of malaria in the study area was not the main objective of the study, but rather the evaluation of the prevalence of *Plasmodium* species infecting humans in the study area was the main objective. Why then the first paragraph concentrate on the prevalence of malaria rather than of the infecting parasite species?
- The second sentence in the first paragraph is not clear, and what are you trying to suggest by 'as is excluding those aged 6 months to 2 years? How is malaria prevalence stratified by age, I mean what is the standard?

- The discussion has concentrated much on malaria prevalence rather than on the main objective of the study, which is the prevalence of Plasmodium species infecting humans in the study area.
- The conclusion is missing.

References

- Reference 2. World Health Organization..... Nigeria. Geneva, 2014. Remove the name Nigeria.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Malaria

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 01 September 2020

<https://doi.org/10.5256/f1000research.23106.r69662>

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Manuela Runge

Northwestern University, Evanston, IL, USA

Ifeoma Ozodiegwu

Northwestern University, Evanston, IL, USA

The paper presents findings from a cross-sectional study conducted in one LGA (Wamakko) in Sokoto State in Nigeria that aimed to determine the proportions of *plasmodium* parasites causing uncomplicated malaria. The study included data collection in both, rainy as well as the dry season. The diagnosis of malaria in study participants was done via rapid tests and dried blood spots, which in the study closely aligned with each other (3% difference). The key finding is monoparasitaemia in the study population with all of the malaria infections caused by *plasmodium falciparum*.

However, it is not exactly clear why this study was performed. The authors did not make a clear statement of the gap in the literature or how their study complements the literature. The studies that they cited suggests that the monoparasitemia as it relates to malaria in Nigeria is a known fact. Is their goal to confirm this for a different geographical location? A more clearly written aims and study motivation will convince readers of the importance of their study

Major comments

1. Please elaborate on your methods.
 - If cluster sampling proportionate to size was used, list all the wards and settlement in a table with their population sizes. This can be included in the supplement.
 - How was cluster selection done? Did you stratify by ward and settlement sizes before random sampling? Be explicit about what was done and how it was done.
2. Please use the discussion section to discuss the implications of your study for treatment and control and the wider scientific community. What are the study limitations? The bulk of this section should not be spent comparing your findings with other studies. We would refer the authors to this [link](#) on how to write a discussion section. There are other resources online as well.
3. In the discussion, studies are cited that report monoparasitaemia, which seems circular/contradictory to the title of '*unusual*' monoparasitaemia, as the discussion indicates that this could have been expected? The authors may consider rephrasing the title or to add a stronger argumentation in the introduction and discussion (see major comment 1).
4. The conclusions are missing in the main text, but included in the abstract.
5. The potential reasons for the disappearance "*either due to faltering control of P. falciparum or more efficient control of other species*" (last part of the abstract conclusion) could be discussed more in the discussion as one would expect from the title.
6. Severe versus clinical malaria. The study aim states a focus on uncomplicated malaria, however, Table 4 only shows severe malaria, which should also show uncomplicated malaria (or instead). The WHO criteria for severe malaria were used, does that include criteria for uncomplicated malaria if not which criteria were used? (Paragraph on Procedures, line 9). How were severe cases treated in the study did they also receive ALu at home or were referred to the next hospital or health facility? Moreover, it would be relevant to show

uncomplicated malaria per age in addition to parasitaemia in Table 3.

Minor comments

1. Previous studies reported the highest parasitaemia among school-aged children ¹ and prevalence curves that peak among school children while incidence curves peak in children under the age of five ². The article here found that parasitaemia is highest at age two and then decreases by age. A sentence for clarification on this would be helpful that explain the decrease over age?
2. The authors found 65% to be non-malaria fevers, this is interesting and the authors may consider highlighting it as a finding and adding it to the discussion (Fig 2).
3. *"Scientists have modelled the anopheles mosquito vector extensively based on its characteristics, but not so much the parasite"* the reference to this study and to niche models of vectors is confusing, as the author's study is not doing niche modelling or geographic mapping of parasites. It would seem more appropriate to have a description of the geographical occurrence of parasites leading over to the next sentence.
4. Missing reference for the Duffy antigen sentence line 6 to 9 in the first paragraph (*"An absence of the Duffy antigen on red blood cells of West Africans has long been postulated to be responsible for the absence of P. vivax in these areas, but cases of P. falciparum, P. malariae and P. ovale, in order of decreasing occurrence, have been found"*).
5. *"Participants were visited at their homes and while in the household"* Perhaps should be "while being at home"? Also, were participants visited after identifying the head, or happened both at the same time, visiting the household and then identifying the household head?
6. Confusing use of household head – parent - caregivers, are these refer all to the same (i.e. are household heads always parents?)
7. The authors cite the Nigerian national treatment policies for parasite co-infections, which however is not the main source which remains unclear, is there an actual study that could be cited?
8. *"five to 10 years"* write out numbers below twenty, or use numbers consistently.
9. Possible gender bias in writing 'he' for the pediatrician "He performed a physical examination for each participant and graded splenomegaly according to Hackett's criteria".
10. *"Concomitantly, during the same session..."* consider removing duplication?
11. *"The study numbers"* is not explained before; do the authors mean participant identifiers or study participant numbers?
12. Unclear sentence: *"The tail segment of the thin films was viewed to identify the species of malaria parasite, using the typical description of parasite species, having been trained on parasite identification"*, who has been trained?

13. Unclear sentence: "We determined the prevalence of malaria by parasitaemia and RDT by determining proportions." Maybe the authors could add formula pos mRDT/tested by mRDT; pos BS/ tested by BS to be clearer?
14. Consider reducing the black background in figures and tables and not using 3D plots, see some general rules on scientific figures in ³.
15. Be consistent with 'malaria' or 'clinical malaria' to distinguish malaria infection from clinical malaria, i.e. Fig 2 caption and title, and text description.
16. Confusing Fig 2, as it reads as if 'no parasite can also cause malaria'? Would a barchart be more appropriate? Or consider rephrasing the figure title.
17. Reference number 36 is missing or not needed (sample procedures line 11), reference list goes only up to 33.
18. 'The finding of *P. falciparum* mono-parasitaemia supports the fact that *P. falciparum* is the dominant species of Plasmodium in Sub-Saharan Africa' Consider rephrasing like 'confirms/ support the other findings of mono-parasitaemia' to avoid the term 'fact'.
19. The discussion structure might need some revision for better understanding, for example, the last paragraph on severe malaria in the discussion might fit better after discussing uncomplicated malaria and before discussing monoparasitaemia in other studies.

References

1. Nankabirwa J, Wandera B, Kiwanuka N, Staedke SG, et al.: Asymptomatic Plasmodium infection and cognition among primary schoolchildren in a high malaria transmission setting in Uganda. *Am J Trop Med Hyg.* 2013; **88** (6): 1102-1108 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Griffin JT, Ferguson NM, Ghani AC: Estimates of the changing age-burden of Plasmodium falciparum malaria disease in sub-Saharan Africa. *Nat Commun.* 2014; **5**: 3136 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Rougier N, Droettboom M, Bourne P: Ten Simple Rules for Better Figures. *PLoS Computational Biology.* 2014; **10** (9). [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: malaria epidemiology in school children, malaria surveillance, mathematical modelling of malaria

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 16 Sep 2020

Usman Nasir Nakakana, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria

1. Please elaborate on your methods.
 - If cluster sampling proportionate to size was used, list all the wards and settlement in a table with their population sizes. This can be included in the supplement.
 - How was cluster selection done? Did you stratify by ward and settlement sizes before random sampling? Be explicit about what was done and how it was done
 - **This has been done, there were two strata, selected independently. The first stratum was proportional to size, i.e at the ward level.**
2. Please use the discussion section to discuss the implications of your study for treatment and control and the wider scientific community. What are the study limitations? The bulk of this section should not be spent comparing your findings with other studies. We would refer the authors to this [link](#) on how to write a discussion section. There are other resources online as well.
I have added sections and modified the discussion as per your recommendations. Implications to the wider scientific community are included.
3. In the discussion, studies are cited that report monoparasitaemia, which seems circular/contradictory to the title of '*unusual*' monoparasitaemia, as the discussion indicates that this could have been expected? The authors may consider rephrasing the title or to add a stronger argumentation in the introduction and discussion (see major comment 1).

It has been explained why the results are unusual, given the population studied i.e community-based. this is reflected in the introduction and conclusions as suggested

4. The conclusions are missing in the main text, but included in the abstract.

They have now been included in the main text.

5. The potential reasons for the disappearance "*either due to faltering control of P. falciparum or more efficient control of other species*" (last part of the abstract conclusion) could be discussed more in the discussion as one would expect from the title.

This has been included in the discussion.

6. Severe versus clinical malaria. The study aim states a focus on uncomplicated malaria, however, Table 4 only shows severe malaria, which should also show uncomplicated malaria (or instead). The WHO criteria for severe malaria were used, does that include criteria for uncomplicated malaria if not which criteria were used? (Paragraph on Procedures, line 9). How were severe cases treated in the study did they also receive ALu at home or were referred to the next hospital or health facility? Moreover, it would be relevant to show uncomplicated malaria per age in addition to parasitaemia in Table 3.

Table 3 has been modified to include other parameters such as the age-specific prevalence of uncomplicated and severe, complicated malaria. Figure 2 has also been modified for more clarity

Minor comments

1. Previous studies reported the highest parasitaemia among school-aged children ¹ and prevalence curves that peak among school children while incidence curves peak in children under the age of five ². The article here found that parasitaemia is highest at age two and then decreases by age. A sentence for clarification on this would be helpful that explain the decrease over age?

This has been explained as the steady-state assumption.

2. The authors found 65% to be non-malaria fevers, this is interesting and the authors may consider highlighting it as a finding and adding it to the discussion (Fig 2).

The figure has been changed for clarity. What is reported was that 34.8% of all children had malaria parasitaemia and the nature of parasitaemia i.e symptomatic and asymptomatic has been clarified.

3. "*Scientists have modelled the anopheles mosquito vector extensively based on its characteristics, but not so much the parasite*" the reference to this study and to niche models of vectors is confusing, as the author's study is not doing niche modelling or geographic mapping of parasites. It would seem more appropriate to have a description of the geographical occurrence of parasites leading over to the next sentence.

The sentence has been rephrased.

4. Missing reference for the Duffy antigen sentence line 6 to 9 in the first paragraph (*"An absence of the Duffy antigen on red blood cells of West Africans has long been postulated to be responsible for the absence of P. vivax in these areas, but cases of P. falciparum, P. malariae and P. ovale, in order of decreasing occurrence, have been found"*).

Reference provided.

5. *"Participants were visited at their homes and while in the household"* Perhaps should be "while being at home"? Also, were participants visited after identifying the head, or happened both at the same time, visiting the household and then identifying the household head?

Clarified that it happened in the house.

6. Confusing use of household head – parent - caregivers, are these refer all to the same (i.e. are household heads always parents?)

Clarified, the household head gives permission and it could be a parent or other person.

7. The authors cite the Nigerian national treatment policies for parasite co-infections, which however is not the main source which remains unclear, is there an actual study that could be cited?

The treatment guidelines are the ones cited.

8. *"five to 10 years"* write out numbers below twenty, or use numbers consistently.

I thought this was one to nine, it has been edited.

9. Possible gender bias in writing 'he' for the pediatrician "He performed a physical examination for each participant and graded splenomegaly according to Hackett's criteria".

Clarified, the lead investigator is the corresponding author and is male.

10. *"Concomitantly, during the same session..."* consider removing duplication?

Done.

11. *"The study numbers"* is not explained before; do the authors mean participant identifiers or study participant numbers?

Study number is explained.

12. Unclear sentence: *"The tail segment of the thin films was viewed to identify the species of malaria parasite, using the typical description of parasite species, having been trained on parasite identification"*, who has been trained?

Clarified.

13. Unclear sentence: *"We determined the prevalence of malaria by parasitaemia and RDT by determining proportions."* Maybe the authors could add formula $\text{pos mRDT}/\text{tested by mRDT}$; $\text{pos BS}/\text{tested by BS}$ to be clearer?

Formula provided for each instance.

14. Consider reducing the black background in figures and tables and not using 3D plots, see some general rules on scientific figures in ³.

Figure changed.

15. Be consistent with 'malaria' or 'clinical malaria' to distinguish malaria infection from clinical malaria, i.e. Fig 2 caption and title, and text description.

Figure 2 has been modified.

16. Confusing Fig 2, as it reads as if 'no parasite can also cause malaria'? Would a bar chart be more appropriate? Or consider rephrasing the figure title.

Figure 2 changed.

17. Reference number 36 is missing or not needed (sample procedures line 11), reference list goes only up to 33.

References updated.

18. *'The finding of P. falciparum mono-parasitaemia supports the fact that P. falciparum is the dominant species of Plasmodium in Sub-Saharan Africa'* Consider rephrasing like 'confirms/ support the other findings of mono-parasitaemia' to avoid the term 'fact'.

Fact has been deleted

19. The discussion structure might need some revision for better understanding, for example, the last paragraph on severe malaria in the discussion might fit better after discussing uncomplicated malaria and before discussing monoparasitaemia in other studies

20. **Some modification has been done**

Competing Interests: Nothing to declare

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