



ASYMPTOMATIC MALARIA IN AGULERI COMMUNITY, ANAMBRA STATE.

Public Health

Onwuachusi G.L.*	Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka., ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine, University of Lagos (CMUL), Idiaraaba, , Lagos, Nigeria. *Corresponding Author
Ekwunife C.A.	Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka. Anambra, Nigeria.
Oyibo W. A.	ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine, University of Lagos (CMUL), Idiaraaba, Lagos, Nigeria.

ABSTRACT

Till date Nigeria still carries the highest burden of malaria globally. The sustenance of the disease in the country could be due to the fact that there are asymptomatic individuals that act as reservoir host. This study determined the prevalence of asymptomatic malaria in Aguleri community during the low transmission season. Random sampling technique was applied in this study. This study was carried out during early January which was during the dry season. All individuals of both sexes, residing in the study location who presented themselves were consented and included in the study. Venous blood was collected in EDTA bottles after administration of informed consent. Rapid Diagnostic test (RDT) and malaria microscopy was done on the samples collected. Thick and thin films were prepared on the same slide and stained with 3% Giemsa working solution. The study included 195 participants which consisted a total of 105(54.1%) males and 89 (45.9%) females from community who consented to the study. The malaria prevalence for the study was 9.8%. Microscopy prevalence among different age groups included 0-5 years, 2(10.5%); >5-10 years, 9(47.4%); >10-15, 7(36.8%); >15-20 years, 0(0%); >20 years, 1 (5.3%). Infected individuals were all single species infection of *Plasmodium falciparum* alone with parasitaemia ranging from 63 – 13,084 with a gross mean parasite density (GMPD) of 953. The stages of the parasite found were 4(21.1%) for gametocytes only, 14(73.7%) for trophozoites only and 1(5.3%) for trophozoites and gametocyte stages. This study confirms asymptomatic malaria cases even during low transmission period in Aguleri community, Anambra state.

KEYWORDS

Asymptomatic Malaria, Prevalence, *Plasmodium falciparum*, Parasitaemia

INTRODUCTION

Human Malaria is a disease of public health importance in Sub Saharan Africa including Nigeria (Agboola *et al.*, 2010; Onyido *et al.*, 2014; WHO, 2021). Malaria control programmes mostly focus on the symptomatic cases; however, there are asymptomatic cases that are being overlooked which could be the reason why Nigeria is still endemic for malaria representing the largest bulk of the disease prevalence globally (WHO, 2021). It is a known fact that the long term goal of any malaria control programme is to successfully eliminate malaria. To achieve this, there is need to understand the parasite transmission processes, epidemiology and pathology of asymptomatic malaria.

Malaria disease presentation could be symptomatic or asymptomatic. Asymptomatic malaria which is the presence of asexual parasites in blood without the symptoms of illness such as fever, headache and chills (Baum *et al.*, 2015; Bousema *et al.*, 2014; Chen *et al.*, 2016; Shimizu *et al.* 2020) are more common in individuals with low parasitaemia and could be associated with chronic infections and adverse disease outcomes (Newton *et al.*, 1997; Worku *et al.*, 2014; Nankabirwa *et al.*, 2013).

Furthermore, there are reports that asymptomatic individuals also contribute to malaria transmission (Alves *et al.*, 2005; Vantaux *et al.*, 2018) this is because, infected individuals acts as a basic reservoir of parasites which in the long run they may become gametocyte carriers contributing to the persistent spread of the disease. Malaria control programme aims to move from a control phase to an elimination phase (The malERA Consultative Group on Basic Science and Enabling Technologies, 2011). With this shift in focus, there are the possibilities of the rise in other issues like the increase in asymptomatic cases, malaria susceptibility with a reduced infection rate which could give rise to severe or complicated cases of malaria hence the need for more grounded preparation during this phase. For the attainment of a malaria free nation it is vital to outline the malaria situation by taking cognizance of the role of both symptomatic and asymptomatic malaria.

Objective:

To determine the presence of asymptomatic malaria among individuals residing in Aguleri community, Anambra State.

Methodology:

Ethical Approval: Ethical approval was obtained from ethics board of

Nnamdi Azikiwe University Teaching Hospital (NAUTH) (NAUTH/CS/66/VOL8/24) and Anambra State Ministry of Health (MH/PHD/140/20).

Study Site and Sample Collection: The study was carried out in Aguleri community which is located in Anambra North Local government area of Anambra state. Sampling was done in early January of 2017 within the premises of St. Gabriel Anglican Church Aguleri after approval was gotten from the Priest in charge. The Priest communicated the date to the prospective participants and those who were interested in taking part in the study turned up for sampling. Random sampling technique was applied in this study.

All individuals of both sexes, residing in the study location who presented themselves were consented and included in the study. Questionnaires were filled on site and 2ml of venous blood collected in EDTA bottles after administration of informed consent. Participants with positive test result were referred to the nearest clinic for treatment. The study included a total of 195 participants.

Rapid Diagnostic Test

The blood samples collected were assayed using CareStart Malaria HRP2 Antigen (LOT NO: MO15H61) test that detects the presence of *Plasmodium falciparum* Histidine rich Protein 2 Antigen. The test is performed using 5µL of blood and the result was read at 20 minutes and recorded following the manufacturer's instructions (Access Bio).

Slide Preparation And Microscopy

The slides were prepared and stained following the standard protocol for malaria Diagnosis in research settings (WHO, 2015). Malaria microscopy was carried out following the WHO standard and there were two blinded independent reading of the slides by expert microscopist.

Data Analysis: Data we entered on Excel and analyzed using Excel Data Analysis Package and GraphPad Prism 5.

RESULT:

A total of 194 individuals were consented from Aguleri community, 28(14.4) and 19 (9.8%) was positive for malaria using RDT and Microscopy respectively (Table 1). A total of 105(54.1%) males, 89 (45.9%) females from Aguleri community who consented to the study were screened for malaria parasite. The age in years of the study

participants ranged from 3-65 years. Microscopy prevalence among different age groups included 0-5 years, 2(10.5%); >5-10 years, 9(47.4%); >10-15, 7(36.8%); >15-20 years, 0(0%), >20 years, 1 (5.3%) which was statistically significant (P<0.001).

Malaria RDT prevalence for the different age groups was also analysed and this was also statistically significant (P<0.001) (Table 1). Prevalence by sex using microscopy shows 10.5% in male and 9% in female. Using RDT prevalence by sex was 14.3% in male and 14.6% in female (Table 1). Infected individuals were all single specie infection of *Plasmodium falciparum* alone with parasitaemia ranging from 63 – 13,084 with a gross mean parasite density (GMPD) of 953. Grouped parasitaemia distribution by sex and age was not statistically significant (P>0.05) (Table 2)

The parasite stages identified during microscopy included gametocytes and trophozoites of *Plasmodium falciparum*. Distribution of the stages included 4(21.1%) for gametocytes only, 14(73.7%) for trophozoites only and 1(5.3%) for throphozoites and gametocyte stages (Figure 1).

Table 1: Grouped Asymptomatic Malaria Prevalence

	Age (Years)	Frequency (%)	RDT (%)		Microscopy (%)	
			Positive	Negative	Positive	Negative
Age Groups (Years)	0-5	7(3.6)	4(57.1)	3(42.9)	2 (28.6)	5(71.4)
	6-10	22(11.3)	11(50)	11(50)	9 (40.9)	13(59.1)
	11-15	130(67.0)	11 (8.5)	119(91.5)	7 (5.4)	123(94.6)
	16-20	6(3.1)	0(0)	6(100)	0 (0.0)	6(100)
	>20	29(14.9)	2(6.9)	27(93.1)	1 (3.4)	28(96.6)
	Total	194	28(14.4)	166(85.6)	19 (9.8)	175(90.2)
Sex	Male	105(54.1)	15(14.3)	90(85.7)	11(10.5)	94(89.5)
	Female	89(45.8)	13(14.6)	76(85.4)	8(9.0)	81(91.0)
	Total	194	28(14.4)	166(85.6)	19(9.8)	175(90.2)

Microscopy P<0.001; RDT P<0.001; Sex P=0.2754

Table 2: Grouped Parasitaemia By Sex And Age

Parameter		Parasite Density Ranges Parasite/ µl (%)				
		<100	101-500	501-1000	>1000	Total
Age Groups (Years)	0-5	2(40)	0 (0%)	0(0%)	0(0%)	2(10.5%)
	6-10	1(20)	1(33.3%)	1(50%)	6(66.7)	9(47.4%)
	11-15	1(20)	2(66.7%)	1(50%)	3(33.3)	7(36.8)
	16-20	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	>20	1(20%)	0(0%)	0(0%)	0(0%)	1(5.3%)
	Total	5(26.3%)	3(15.8%)	2(10.5%)	9(47.4)	19
Sex	Male	2(40)	2(66.7)	0(0%)	7(77.8)	11(57.9)
	Female	3(60)	1(33.3)	2(100)	2(22.2)	8(42.1)
	Total	5(26.3)	3(15.8)	2(10.5)	9(47.4)	19

Age: P=0.0059; Sex: P=0.2883

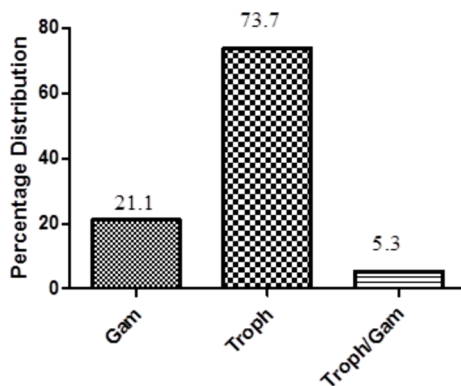


Figure1: Parasite Stages Distribution (gam-gametocytes Only; Troph-trophozoites Only; Troph/gam-trophozoites And Gametocytes)

DISCUSSION

This study was able to show the presence of asymptomatic malaria in Aguleri community during the low transmission season, irrespective

of the dry environmental condition and reduction in mosquito breeding grounds that is associated with dry weather conditions (Weli and Efe, 2015). In these cases, asymptomatic individuals may act as reservoir host (Kamanga *et al.*, 2010). This is possibly an adaptation of the parasite to ensure its transmission when condition becomes favourable (Andrade *et al.*, 2020). Hence, during this season, asymptomatic malaria is critical since it aids the preservation of the parasite.

Presence of malaria parasite was detected using microscopy and malaria RDT. This indicates that both test can be used for screening process to identify the infected individuals and treat as part of the malaria control strategies in the community. Though there were more positive cases using RDT than microscopy, however, this could be attributed to the persistence of HRP2 antigen (Michael *et al.*, 2021).

This study also showed that asymptomatic malaria is associated with low parasite density which was seen to range from 63-13,084 with a GMPD of 953. The presence of low grade parasitama among the infected individuals could be an adaptation for survival to ensure transmission during a more favourable season. This correlates with what has been reported by other studies that showed that asymptomatic malaria cases are more common in individuals with low parasitaemia or sub-microscopic Plasmodium infection and could be associated with chronic infections and and possibly give rise to the progression to symptomatic disease (Worku *et al.*, 2014; Nankabirwa *et al.*, 2013; Oyetunji *et al.*, 2020).

This study identified that asymptomatic malaria infection was seen to cut across the different age groups with the highest prevalence among children that are 0-5years followed by those >5-10years. Infection rate and age was seen to be statistically significant (P<0.05). Indicating that age has a role to play in the development of asymptomatic malaria as was reported by another study in Iwo, Nigeria (Igbeneghu *et al.*, 2011). Also infection was seen to be more in male than female study participant but the difference was not statistically significant. Also, the grouped parasite densities shows that there are different asymptomatic parasitaemia ranges by age and sex though this distribution was not statistically significant.

The different parasite stages encountered in this study included trophozoites, and gametocytes. The presence of gametocytes is another mechanism adopted by the parasite to ensure its propagation when conditions for transmission becomes favourable, usually during the rainy season. Gametocyte formation has been associated with response to environmental stressors (Liu *et al.*, 2011) and with changes in environmental conditions using cultured *Plasmodium falciparum* (Carter and Miller, 1979).

CONCLUSION

This study has identified the phenomena of asymptomatic malaria in Aguleri community during low transmission season. With malaria still an issue of concern in Nigeria, there is the need to look into the asymptomatic malaria cases which could be a contributing factor to the persistence of malaria in the country. The knowledge on the parasite sustenance strategies plays pivotal role in disease control. This could be applied in the implementation of intervention strategies for Malaria which in the long run will play key roles in the better control of the disease and possible elimination.

REFERENCES

- Alves, F.P., Gil, L.H.S., Marrelli, M.T., Ribolla, P.E.M, Camargo, E.P. and Da Silva, L.H.P.(2005). Asymptomatic carriers of *Plasmodium spp.* as infection source for malaria vector mosquitoes in the Brazilian Amazon. *Journal of Medical Entomology*, **42** (5):777–779.
- Andrade, C.M., Fleckenstein, H., Thomson-Luque, R., Doumbo, S., Lima, N.F., Anderson, C., Hibbert, J., Hopp, C.S., Tran, T.M., Li, S., Niangaly, M., Cisse, H., Doumtabe, D., Skinner, J., Sturdevant, D., Ricklefs, S., Virtaneva, K., Asghar, M., Homann, M.V., Turner, L., Martins, J., Allman, E.L., N'Dri, M-E, Winkler, V., Linás, M., Lavazze, C., Martens, C., Färnert, A., Kayentao, K., Ongoiba, A. Lavstsen, T., Osório, N.S., Otto, T.D., Recker, M., Traore, B., Crompton, P.D. and Portugal, S. (2020). Increased circulation time of *Plasmodium falciparum* Underlies Persistent Asymptomatic Infection in the Dry Season. *Nature Medicine* **26**: 1929-1940.
- Bousema, T., Okell, L., Felger, I. and Drakeley, C. (2014). Asymptomatic malaria infections: detectability, transmissibility and public health relevance. *Nat Rev Microbiol*, **12**(12): 833–40.
- Carter and Miller (1979). Recent Developments in Production and Purification of Malaria Antigens: Evidence for Environmental Modulation of Gametocytogenesis in *Plasmodium Falciparum* in Continuous Culture. *Bull World Health Organ.*, **57**(Suppl): 37–52.
- Chen, I., Clarke, S.E., Gosling, R., Hamainza, B., Killeen, G., Magill, A., O'Meara W., Price, R.N. and Riley E.M. (2016). "Asymptomatic" malaria: a chronic and debilitating infection that should be treated. *PLoS Med* **13**(1):e1001942.
- Igbeneghu, C., Odaibo, A.B., Olayeye, D.O. (2011). Impact of Asymptomatic Malaria on Some Hematological Parameters in the Iwo Community in Southwestern Nigeria.

- Medical Principles and Practice* **20**: 459–463.
7. Liu, Z., Miao, J., Cui, L., (2011). Gametocytogenesis in Malaria Parasite: Commitment, Development and Regulation. *Future Microbiol.* **6**(11): 1351-1369.
 8. Nankabirwa, J., Wandera, B., Kiwanuka, N., Staedke, S.G., Kanya, M.R. and Brooker, S.J.(2013). Asymptomatic Plasmodium Infection and Cognition among Primary School Children in a High Malaria Transmission Setting in Uganda. *American Journal of Tropical Medicine and Hygiene*, **88** (6): 1102–1108.
 9. Kamanga, A. Moono, P., Stresman, G., Mharakurwa, S. and Shiff, C. (2010). Rural HealthCentres, Communities and Malaria Case Detection in Zambia Using Mobile Telephones:A Means To Detect Potential Reservoirs of Infection in Unstable TransmissionConditions. *MalariaJournal*:9(1):96.
 10. Michael, O.S , Orimadegun, A.E. and Falade, C.O. (2021). Persistence of Plasmodium Falciparum Hrp2 Antigen after Effective Antimalarial Therapy. *Annals of Ibadan Postgraduate Medicine* **19**(1): 15-21.
 11. Oyetunji, I.A., Amoo, A.J., Adeyemo, T.A. and Ojewunmi, O.O. (2020). Asymptomatic MalariaInfection among Children with Sickle Cell Anaemia: The role of IL-10 and PossiblePredisposing Factors. *PAMJ - One Health*.2(7).
 12. Shimizu, S., Chotirat, S., Dokkulab, N., Hongchad, I., Khowsroy, K., Kiattitubtr, K., Maneechai,N., Manopwisedjaroen, K., Petchvijit, P., Phumchuea, K., Rachaphaew, N., Sriporote,P., Suansomjit, C., Thongyod, W., Khamsiriwatchara, A., Lawpoolsri, S., Hanboonkunupakama, B., Sattabongkot, J. and Nguitragool, W. (2020). Malaria cross-sectional surveys identified asymptomatic infections of Plasmodium falciparum, Plasmodium vivax and Plasmodium knowlesi in Surat Thani, a southern province of Thailand. *International Journal of Infectious Diseases* **96** (2020): 445–451
 13. The malERA Consultative Group on Basic Science and Enabling Technologies (2011) A Research Agenda for Malaria Eradication: Basic Science and Enabling Technologies. *PLoSMed*, **8**(1): e1000399.
 14. Vantaux A, Samreth R, Piv E, Khim N, Kim S, Berne L, Chy, S., Lek, D., Siv, S., Taylor, W.R and Menard, D. (2018). Contribution to malaria transmission of symptomatic and asymptomatic parasite carriers in Cambodia. *The Journal of Infectious Disease*, **217** (10):1561–1568.
 15. Weli, V.E. and Efe, S.I. (2015). Climate and Epidemiology of Malaria in Port Harcourt Region, Nigeria. *American Journal of Climate Change*, **4**: 40-47.
 16. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases (2015). Methods Manual Microscopy for the Detection, Identification and Quantification of Malaria Parasites on Stained Thick and Thin Blood Films in Research Settings. Procedure for Research on Diseases of Poverty UNICEF/UNDP/World Bank/WHO, Geneva, Switzerland.
 17. WHO (2020) World malaria report 2020: 20 years of global progress and challenges. World Health Organization, Geneva.
 18. Worku, L., Damtie, D., Endris, M., Getie, S. and Aemero, M. (2014). Asymptomatic Malaria and Associated Risk Factors among School Children in Sanja Town, Northwest Ethiopia. *Hindawi Publishing Corporation International Scholarly Research Notices* **2014**: 303269