Predictive markers of transmission in areas with different malaria endemicity in north-eastern Tanzania based on seroprevalence of antibodies against *Plasmodium falciparum*

Robert Diotrephes Kaaya (robertkaaya@yahoo.com)
Kilimanjaro Christian Medical University College https://orcid.org/0000-0001-6100-0205
Debora C Kajeguka
Kilimanjaro Christian Medical College: Kilimanjaro Christian Medical University College
Johnson J Matowo
Kilimanjaro Christian Medical College: Kilimanjaro Christian Medical University College
Arnold J Ndaro
Kilimanjaro Christian Medical Centre
Franklin W Mosha
Kilimanjaro Christian Medical College: Kilimanjaro Christian Medical University College
Jaffu O Chilongola
Kilimanjaro Christian Medical College: Kilimanjaro Christian Medical University College
Reginald A Kavishe
Kilimanjaro Christian Medical College: Kilimanjaro Christian Medical University College

Research note

Keywords: Malaria, Plasmodium falciparum, Seroprevalence, transmission, Tanzania

Posted Date: July 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-586890/v2

Abstract

Objective The objective of this study was to assess *Plasmodium falciparum* exposure in areas with different malaria endemicity in north-eastern Tanzania using serological markers; *PfAMA-1* and *PfMSP-1*$_{19.}$

Results Bondo had a higher seroprevalence 36.6% (188) for *PfAMA-1* as compared to Hai 13.8% (33), χ^2=34.66, p<0.01. Likewise, Bondo had a higher seroprevalence 201(36.6%) for *PfMSP-1* as compared to Hai 41 (17.2%), χ^2 =29.62, p<0.01. Anti-*PfAMA-1* titters were higher in malaria positive individuals (n=47) than in malaria negative individuals (n=741) (p=0.07). Anti-*PfMSP*-1 antibody concentrations were significantly higher in malaria-positive individuals (n=47) than in malaria-negative individuals (n=741) (p=0.003).

Antibody response against *PfAMA-1* was significantly different between the three age groups; <5 years, 5 to 15 years and >15 years in both sites of Bondo and Hai. Likewise, antibody response against *PfMSP-1*$_{19}$ was significantly different between the three-age groups in the two sites. (p<0.001). We also found significant differences in the anti-*PfAMA-1*and anti-*PfMSP-1*$_{19}$ antibody concentrations among the three age groups in the two sites (p=0.004 and 0.005) respectively. Immunological indicators of *Plasmodium falciparum* exposure have proven to be useful in explaining long-term changes in the transmission dynamics especially in low transmission settings.

Introduction

Africa carries the highest burden of malaria with more than 70% of all malaria cases and deaths[1]. Each year, 10 to 12 million people contract malaria and more than 80,000 dies[2,3]. *Plasmodium falciparum* is mainly responsible for 99.7% of estimated malaria cases. [4].

In many countries, local malaria transmission has decreased due to the extensive efforts being devoted to malaria control and elimination [5]. Despite Tanzania's large populations at risk for malaria, transmission varies between its regions significantly, with *Plasmodium falciparum* accounting for 96 percent of cases [6], malaria prevalence varies from <1 percent in the highlands of Arusha to as high as 15 percent in the Southern Zone and 24 percent along the Lake and Western Zones. Immunity to *P. falciparum* malaria is poorly understood, however, evidence shows that antibody-dependent cellular mechanisms play a key role in immunity against *P. falciparum* malaria parasite [7,8]. The rate of its development is believed to be associated with transmission intensity which is stage-specific and is rarely sterile[6]. In many epidemiological studies, the determination of malaria transmission has been based on the antibody levels against *P. falciparum* antigens [9]. Recent immunological studies revealed that antibodies against merozoite antigens act as biomarkers of malaria exposure and that, with increasing exposure and responses of higher levels, antibodies may act as biomarkers of protective immunity [10].

Apical membrane antigen 1 (AMA-1) is expressed on merozoites and sporozoites of *P. falciparum* as a type I integral membrane protein [11]while Merozoite surface protein1 (MSP-1), is a highly conserved protein among *Plasmodium* species as well as the most abundant protein expressed on the surface of merozoites[12]. Antibodies against MSP-1 and AMA-1 antigens are potential markers of both exposure to *P. falciparum* and protection against the disease[7,13] and have proven to be informative, in areas where transmission has dropped to low sustained levels, for monitoring the timing and magnitude of transmission reduction[13] as well as in obtaining epidemiological information in malaria control programmes[14].

In areas with low malaria transmission, it has become extremely difficult to detect changes in transmission intensity using conventional methods such as the entomologic inoculation rate (EIR) or malaria prevalence rates. Low transmission areas (low endemicity) sometimes have low mosquito density, below the detection limits of common mosquito trapping methods [15, 16] and the parasite prevalence also becomes less reliable [17-19]. Malaria serological markers may aid in estimating malaria transmission intensity [20–22]. Seroconversion rates may provide insight into recent changes in malaria transmission [23]. Due to the fact antibodies can persist for months or years after infection, seroconversion rates are less affected by the effects of unstable or seasonal transmission [20, 21]. We investigated the antibody response to recombinant AMA-1 and MSP-1 in individuals living in two regionally distinct malaria-endemic zones.

Materials And Methods

Study area

The study was conducted during April and December 2014 in two different areas of the Tanzanian mainland. The first site was Bondo in the Tanga region, inhabited by 7970 people [24]. The second study site was Hai in Kilimanjaro region, located at the foot of Mount Kilimanjaro [14]. Participant recruitment procedures and study design have been previously described [25], (Additional file 1: Figure S1).

Sample collection

A blood sample was obtained by finger prick, a blood spot was prepared from each participant, then dried and stored for further analysis.

Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Indirect immunosorbent Assay (ELISA)was performed using two *Plasmodium falciparum* surface antigens, *Plasmodium falciparum MSP* 1_{19} (Pf*MSP* 1_{19}) and *Plasmodium falciparum AMA-1*(*PfAMA-1*) [26].

Malaria parasite detection by polymerase chain reaction (PCR)

Parasite DNA was extracted using the simple Chelex-Saponin method, followed by *Plasmodium falciparum* identification using previously used [27,28].

Data analysis

All data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism8 software (San Diego, CA).

Results

Population characteristics and Malaria prevalence

The study enrolled a total of 788 participants, 239 (30.3%) from Hai and 549 (69.7%) from Bondo. Males were 283 (35.9%) and females were 505 (64.1%). About 405 (51.4%) participants had more than 15 years of age, 212 (26.9%) were between 5-15 years and 171(21.7%) were below 5 years. The malaria prevalence by mRDT was 8.6%

(47) in Bondo and 0% in Hai (Fisher exact test *p<0.001). By PCR, malaria prevalence was 20.4% (161), with Bondo having a higher prevalence 28.1% (n= 154) than Hai 2.9%, (n= 7), χ^2=64.64, p<0.01(Additional file 2: Table S1).

Seroprevalence of Anti- *PfAMA-1* and *PfMSP-1*$_{19}$ antibodies

Bondo had a higher seroprevalence 36.6% (188) for *PfAMA-1* as compared to Hai 13.8% (33), χ^2=34.66, p<0.01. Likewise, Bondo had a higher seroprevalence 201(36.6%) for *PfMSP-1* as compared to Hai 41 (17.2%) (χ^2=29.62, p<0.01). In Bondo, participants with more than 15 years had a significantly higher seroprevalence of *PfAMA-1* 61.7% (116) (χ^2 =58.69, p<0.001) and *PfMSP-1*$_{19}$ 63.7 (128) (χ^2 =65.36, p<0.001) as compared to other age groups. Likewise, participants with 5-15 years and <5 years had a higher prevalence of malaria as measured by mDRT (χ^2 = 30.76, p<0.001) (Table 1).

Table 1: Age-specific Prevalence of Malaria by serology, mRDT, Microscopy and PCR

Study site	Age group	PfAMA-1 % (n)		PfMSP-1$_{19}$ % (n)		mRDT % (n)		PCR % (n)	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Bondo	<5 years	9.0 (17)	32.7 (118)	14.4 (29)	30.5 (106)	46.8 (22)	22.5 (113)	18.8 (29)	26.8 (106)
	5-15 years	29.3 (55)	36.6 (132)	21.9 (44)	41.1 (143)	48.9 (23)	32.7 (164)	36.4 (56)	33.2 (131)
	>15 years	61.7 (116)	30.7 (111)	63.7 (128)	28.4 (99)	4.3 (2)	44.8 (225)	44.8 (69)	40.0(158)
		χ^2 = 58.69, p<0.001		**χ^2 =65.36, p<0.001**		**χ^2 = 30.76, p<0.001**		**χ^2 = 3.8, p=0.1**	
Hai	<5 years	18.2 (6)	14.6 (30)	9.8 (4)	16.2 (32)	0.0 (0)	15.1 (36)	14.3 (1)	15.1 (35)
	5-15 years	6.1 (2)	11.2 (23)	4.9 (2)	11.6 (23)	0.0 (0)	10.5 (25)	28.6 (2)	9.9 (23)
	>15 years	75.8 (25)	74.3 (153)	85.4 (35)	72.2 (143)	0.0 (0)	74.5 (178)	57.1 (4)	75.0 (174)
		***p=0.6**		***p=0.2**		-		***p=0.2**	

*Computed by Fisher exact test.

Anti- *PfAMA-1* and *PfMSP-1*$_{19}$ antibody concentrations

Anti-*PfAMA-1* titters were higher in malaria positive individuals (n=47) than in malaria negative individuals (n= 741) (Mann-Whitney U test, p=0.07) (Additional file 3: **Figure S2 A**). Anti-*PfMSP*-1 antibody concentrations were significantly higher in malaria-positive individuals (n=47) than in malaria-negative individuals (n=741) (Mann-Whitney U test, p=0.003) **(**Additional file 3: **Figure S2 B).**

We determined whether the two sites differed in antibody concentration.

and found that anti-*PfAMA-1* antibody concentrations, were higher among participants in Bondo (n=549) as compared in Hai (n=239), (Mann-Whitney U test, p<0.001) (Additional file 3: Figure S2 A). Anti-*PfMSP-1* antibody concentrations were higher among participants in Bondo (n=549) than those of Hai (n=239), (Mann-Whitney U test, p=0.01) (Additional file 3: Figure S2 B).

In assessing whether these differences were influenced by age, we calculated the differences among <5 years, 5 to 15 years and >15 years per site. Antibody response against *PfAMA-1* was significantly different between the three age groups in both sites. (Kruskal–Wallis test, p< 0.001) (Table 1). Likewise, antibody response against *PfMSP-1*$_{19}$ was significantly different between the three-age groups in the two sites (Kruskal–Wallis test, p<0.001) (Table 1). We also found significant differences in the anti-*PfAMA-1*antibody concentrations among the groups (Kruskal–Wallis test, p=0.004), as indicated in Figure 2A and 2B. Lastly, we also noted significant differences in the anti-*PfMSP-1*$_{19}$ antibody concentrations among the age groups (Kruskal–Wallis test, p=0.005) (Figure 2C and 2D).

Discussion

The purpose of this study was to use immunological markers to investigate malaria transmission patterns in areas with diverse malaria endemicities.

In this study, malaria prevalence by PCR in Bondo was 28.1%. Since Bondo is a malaria-endemic area, malaria transmission occurs nearly all year long with a peak period from April to June. In 2011 a study conducted in Tanga suggested a widening of the age group at risk for malaria infection to older children of 5-15 years [29]. No significant difference was observed in malaria prevalence among all age groups in the present study. A previous study conducted in two villages in the same region about 70 kilometres from the current study found a re-emergence of malaria despite previous reports of a decline in malaria [33] It is estimated that parasite prevalence at that time was 25% and it stayed there throughout 2016 [34]. PCR analysis of Hai found 2.9% parasite prevalence, thus remaining an area of low transmission and The mRDT test was negative, which suggests low-density parasite circulating in the population, similar to earlier findings [35]. There is, however, some evidence that individuals harbouring sub-microscopic parasites could be sources of new infections since mosquitoes can carry parasites with very low density. (<5parasites/µl) [27,31,32], and hence, the use of a more sensitive diagnostic tool like PCR in clinical malaria diagnosis is necessary. Consequently, scientific evidence from these findings is consistent with the notion of mass drug therapy for individuals with microscopic parasites considering efforts to eliminate malaria.

Antibodies to malaria antigens can explain long-term changes in malaria transmission dynamics [26]. To understand malaria transmission patterns immunological markers were used in parallel with the parasitological indicators. In Hai, the overall seroprevalence was 13.8% for *PfAMA-1* and 17.2% for *PfMSP-1*$_{19}$.

In 2009 a survey conducted in Moshi found low seroprevalence in children suggesting very low exposure to malaria parasite [36] In our study, Interestingly, when the age-dependent analysis was done, older children (5-15 years) had a relatively low seroprevalence to *PfAMA-1* antigens only as compared to younger children and Adults. This phenomenon is only seen when not is because older children experienced repeated malarial exposure as compared to younger ones most of whom were protected by treated bed nets. In populations with low immunity,

such as young children, antibodies to MSP-1 act as a significant biomarker of malaria exposure and with increasing exposure the antibodies may contribute to protective immunity[10].

Seroprevalence in moderate malaria transmission setting such as Bondo can play a small role in determining malaria transmission patterns although seroprevalence is almost two folds higher than Hai with the seroprevalence of 34.2% and 36.6% for *PfAMA-1* and *PfMSP-1*$_{19}$ respectively. A slight decline in seroprevalence was observed in the study area when compared with previous studies [26,35], indicating a long-term reduction in malaria parasite exposure**,** which may be attributed to intense malaria interventions in Tanzania [37,38]. The malaria burden in Africa can only be determined using current information about different malaria transmission areas. We analysed the results by stratifying age and relate with antibody concentrations against both *PfAMA-1* and *PfMSP-1*$_{19}$antigens. In both sites, the results show that overall antibody levels were low in children, compared to adults.

Study results showed that the overall concentration of *PfMSP-1*$_{19}$ (by PCR) was significantly higher in participants with positive malaria tests than in non-positive participants. As expected Bondo had significantly higher antibody concentrations against both antigens as compared to Hai. In Bondo, median OD was increasing with age as observed for both antigens. The results demonstrated that anti-*pfAMA-1* and anti-*pfMSP-1*$_{19}$ antibodies are reliable biomarkers for parasite exposure as well as vector activity and disease transmission in the study areas. Children with <5 years present with low antibody titters suggesting a lack of recent malaria exposure and this makes the group vulnerable to the symptomatic manifestation of the disease. Earlier findings revealed that more than half of the participants reported being symptomatic and 14.1% were malaria positive by mRDT [21]. There is evidence of malaria transmission in low malaria-endemic areas, where traditional malaria indicators like prevalence and sporozoite levels may underestimate the burden of the disease.

Conclusion

The immunological indicators of Plasmodium falciparum exposure have been useful for explaining long-term changes in the dynamics of transmission particularly in areas of low transmission like Hai. Malaria prevalence in Bondo continues to be high, despite intervention and transmission at the sub microscopic level has been observed.

Study limitation

This study might not explain the recent changes in malaria transmission since it was a cross-sectional survey. A longitudinal study would have been appropriate in explaining seasonal variations in malaria infection rates across the study areas. Findings from this study, however, provide enough evidence that immunological markers can suggest the possibility of ongoing malaria transmission particularly in low-endemic areas.

Abbreviations

CRERC: College Research and Ethics Review Committee

OD: Optical Density

AMA-1: Apical membrane antigen1

PCR: Polymerase Chain Reaction

ELISA: Enzyme-Linked Immuno-Sorbent Assay

ssurRNA: small sub-unit ribosomal RNA

PfMSP 1_{19}: Plasmodium falciparum Merozoite Surface Protein 1

PfAMA-1: Plasmodium falciparum Apical Membrane Antigen 1

Declarations

Patient consent for publication

Not required.

Patients and Public Involvement

Participants were not involved in the design of this study. Community leaders were involved during participant's recruitment. There is a plan to disseminate results to the participating sites.

Ethics approval and consent to participate.

Ethical approval was obtained from the Kilimanjaro Christian Medical University College Research and Ethics Review Committee (CRERC) with certificate number 658. Permission to conduct the study was sought from Handeni/Bondo and Hai district authorities. Written informed consent was obtained from all participants and from parents or guardians for children under 18 years of age who agreed to participate in the study.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was partly supported by DANIDA through DFC in the Building strong Universities (BSU) project. Also, RDK is supported by DELTAS Africa Initiative grant #DEL-15-011 to THRiVE-2. The funding sources had no role in the study design, data collection, analysis, and interpretation of results or in the decision to submit the manuscript for publication.

Author's contributions

RDK: Conceptualization of the study, data analysis, and writing the original draft of the manuscript; DCK: Funding acquisition, investigation, data analysis and review of the manuscript; JJM, AJN, FWM and JOC: Interpretation of data and critical review of the manuscript; RAK: Overall study design and review of the manuscript

All the authors have read and approved the final version of the manuscript.

Acknowledgments

The authors would like to thank all participants and Community leaders in Bondo and Hai for their cooperation. We also like to thank KCMUCo-PAMVERC for the research facilities and space to conduct our laboratory experiments.

References

1. WHO. World Malaria Report-2019 [Internet]. Vol. WHO/HTM/GM, World Health Organisation. 2019 [cited 2020 May 20]. p. 238. Available from: https://www.who.int/malaria/publications/world-malaria-report-2019/World-Malaria-Report-2019-briefing-kit-eng.pdf?ua=1

2. MalariaSpot. Malaria in Tanzania [Internet]. MalariaSpot. 2016 [cited 2020 May 25]. Available from: https://malariaspot.org/en/eduspot/malaria-in-tanzania/

3. Kilonzi M, Minzi O, Mutagonda R, Sasi P, Kamuhabwa A, Aklillu E. Comparison of malaria treatment outcome of generic and innovator's anti-malarial drugs containing artemether-lumefantrine combination in the management of uncomplicated malaria amongst Tanzanian children. Malar J [Internet]. 2019;18(1):1–8. Available from: https://doi.org/10.1186/s12936-019-2769-z

4. WHO. Malaria Fact Sheet [Internet]. World Health Organisation. 2020 [cited 2020 May 20]. Available from: https://www.who.int/news-room/fact-sheets/detail/malaria

5. Dhiman S. Are malaria elimination efforts on right track? An analysis of gains achieved and challenges ahead. Infect Dis Poverty. 2019;8(14):1–19

6. MoHCDGEC (Tanzania Mainland), MoH (Zanzibar), NBS, OCGS, and ICF.2017. Tanzania Malaria Indicator Survey (TMIS) 2017. Dar es Salaam, Tanzania, and Rockville, Maryland, USA:.

7. Greenhouse B, Ho B, Hubbard A, Njama-Meya D, Narum DL, Lanar DE, et al. Antibodies to Plasmodium falciparum antigens predict a higher risk of malaria but protection from symptoms once parasitemic. J Infect Dis. 2011;204(1):19–26.

8. Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P, et al. Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg. 1991;45:297–308.

9. Roy A, Adak T. Evaluation of malaria endemicity by peptide ELISA. J Commun Dis. 2005 Sep;37(3):183-9. PMID: 17080701.

10. Stanisic DI, Fowkes FJI, Koinari M, Javati S, Lin E, Kiniboro B, et al. Acquisition of antibodies against Plasmodium falciparum merozoites and malaria immunity in young children and the influence of age, force of infection, and magnitude of response. Infect Immun. 2015;83(2):646–60.

11. Yap A, Azevedo MF, Gilson PR, Weiss GE, O'Neill MT, Wilson DW, et al. Conditional expression of apical membrane antigen 1 in Plasmodium falciparum shows it is required for erythrocyte invasion by merozoites. Cell Microbiol. 2014;16(5):642–56.

12.Kale S, Yadav CP, Rao PN, Shalini S, Eapen A, Srivasatava HC, et al. Antibody responses within two leading Plasmodium vivax vaccine candidate antigens in three geographically diverse malaria-endemic regions of India. Malar J [Internet]. 2019;18(1):1–13. Available from: https://doi.org/10.1186/s12936-019-3066-6

13. Wong J, Hamel MJ, Drakeley CJ, Kariuki S, Shi YP, Lal AA, et al. Serological markers for monitoring historical changes in malaria transmission intensity in a highly endemic region of Western Kenya, 1994-2009. Malar J. 2014;13(1).

14. Kerkhof K, Sluydts V, Willen L, Kim S, Canier L, Heng S, et al. Serological markers to measure recent changes in malaria at population level in Cambodia. Malar J. 2016;15(1):1–18

15. Oesterholt MJ, Bousema JT, Mwerinde OK, Harris C, Lushino P, Masokoto A, et al. Spatial and temporal variation in malaria trans[1]mission in a low endemicity area in northern Tanzania. Malar J. 2006;5:98. DOI: 10.1186/1475-2875-5-98

16. Smith T, Charlwood JD, Takken W, Tanner M, Spiegelhalter DJ. Mapping the densities of malaria vectors within a single village. Acta Trop. 1995;59:1–18. DOI: 10.1016/0001-706X(94)00082-C

17. Hay SI, Smith DL, Snow RW. Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis. 2008;8:369– 78. DOI: 10.1016/S1473-3099(08)70069-0

18. Beier JC, Killeen GF, Githure JI. Short report: Entomologic inocula[1]tion rates and Plasmodium falciparum malaria prevalence in Africa. Am J Trop Med Hyg. 1999;61:109–13.

19. Yekutiel P. Problems of epidemiology in malaria eradication. Bull World Health Organ. 190; 22:669–83. (9, 18)

20. Coran P, Coleman P, Riley E, Drakeley C. Serology: a robust indicator of malaria transmission intensity? Trends Parasitol. 2007;23:575–82. DOI: .1016/j.pt.2007.08.023.

21. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria expo[1]sure. Proc Natl Acad Sci U S A. 2005;102:5108–13. DOI: 10.1073/ PNAS.0408725102.

22. Mwanziva C, Shekalaghe S, Ndaro A, Mengerink B, Megiroo S, Mosha F, et al. Overuse of artemisinin-combination therapy in Mto wa Mbu (river of mosquitoes), an area misinterpreted as high en[1]demic for malaria. Malar J. 2008;7:232. DOI: 10.1186/1475-2875- 7-232.

23. Stewart L, Gosling R, Griffi n J, Gesase S, Campo J, Hashim R, et al. Rapid assessment of malaria transmission using age-specifi c sero[1]conversion rates. PLoS One. 2009;4:e6083. DOI: 10.1371/journal. pone.0006083

24. PHC. Population and Housing Census-Tanzania (2012) [Internet]. National Bureau Of Statistics: United Republic of Tanzania. 2013 [cited 2018 Jun 20]. Available from: www.nbs.go.tz/

25. Kajeguka DC, Kaaya RD, Mwakalinga S, Ndossi R, Ndaro A, Chilongola JO, et al. Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. BMC Infect Dis. 2016 Dec 26;16(183):1–9.

26. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SLR, Carneiro I, et al. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A. 2005;102:5108–13.

27. Miguel RB, Coura JR, Samudio F, Suárez-Mutis MC. Evaluation of three different DNA Extraction Methods from Blood sample colected in dried blood filter papers in Plasmodium Subpatent Infection from the Amazon Region Brazil. Rev Inst Med Trop Sao Paulo. 2013;55(3):205–8.

28. Haanshuus CG, Mohn SC, Mørch K, Langeland N, Blomberg B, Hanevik K. A novel, single-amplification PCR targeting mitochondrial genome highly sensitive and specific in diagnosing malaria among returned travellers in Bergen, Norway. Malar J. 2013;12(26):1–8.

29. Winskill P, Rowland M, Mtove G, Malima RC, Kirby MJ. Malaria risk factors in north-east Tanzania. Malar J. 2011;10(98):1–7.

30. Karl S, Gurarie D, Zimmerman PA, King CH, St. Pierre TG, Davis TME. A sub-microscopic gametocyte reservoir can sustain malaria transmission. PLoS One. 2011;6(6): e20805:1–9.

31. Schneider P, Bousema JT, Gouagna LC, Otieno S, Van De Vegte-Bolmer M, Omar SA, et al. Submicroscopic Plasmodium falciparum gametocyte densities frequently result in mosquito infection. Am J Trop Med Hyg. 2007;76:470–4.

32. Okell LC, Bousema T, Griffin JT, Ouédraogo AL, Ghani AC, Drakeley CJ. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nat Commun. 2012;3(1237).

33. Ishengoma DS, Mmbando BP, Segeja MD, Alifrangis M, Lemnge MM, Bygbjerg IC. Declining burden of malaria over two decades in a rural community of Muheza district, north-eastern Tanzania. Malar J. 2013 Jan;12(1):338.

34. Ishengoma DS, Mmbando BP, Mandara CI, Chiduo MG, Francis F, Timiza W, et al. Trends of Plasmodium falciparum prevalence in two communities of Muheza district North-eastern Tanzania: Correlation between parasite prevalence, malaria interventions and rainfall in the context of re-emergence of malaria after two decades of progressive. Malar J [Internet]. 2018;17(252):1–10. Available from: https://doi.org/10.1186/s12936-018-2395-1

35.Shekalaghe SA, Bousema JT, Kunei KK, Lushino P, Masokoto A, Wolters LR, et al. Submicroscopic Plasmodium falciparum gametocyte carriage is common in an area of low and seasonal transmission in Tanzania. Trop Med Int Heal. 2007;12:547–53.

36. Stewart L, Gosling R, Griffin J, Gesase S, Campo J, Hashim R, et al. Rapid Assessment of Malaria Transmission Using Age- Specific Sero-Conversion Rates. PLoS One. 2009;4(6).

37. WHO. World Malaria Report 2012 [Internet]. World Health Organization. 2013 [cited 2019 Jun 12]. p. 1–288. Available from: https://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_full_report.pdf

38. PMI. President's Malaria Initiative: Fighting Malaria and Saving Lives: Tanzania country profile. USAID. 2016.

Figures

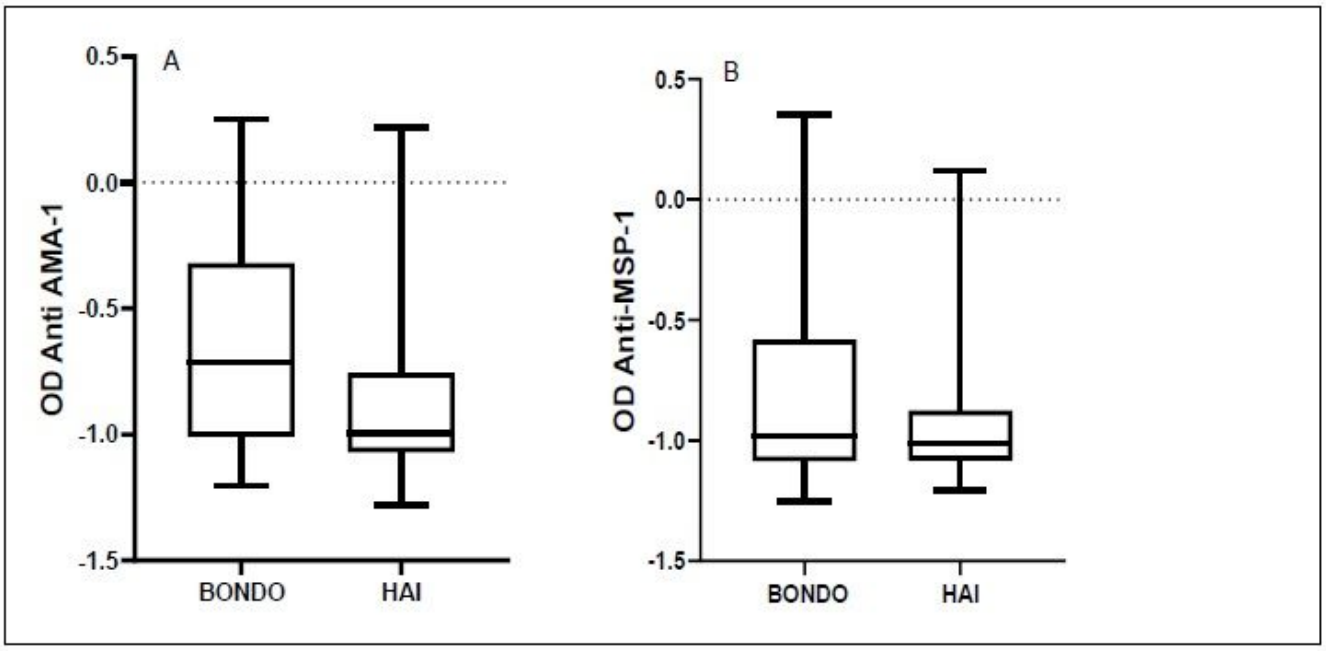

Figure 1

A graph showing mean OD values for PfAMA-1 (Figure 1A) and PfMSP-119 (Figure 1B) at Bondo and Hai sites. Presented in the Y-axis is the Log10 transformed OD values in two sites (X-axis).

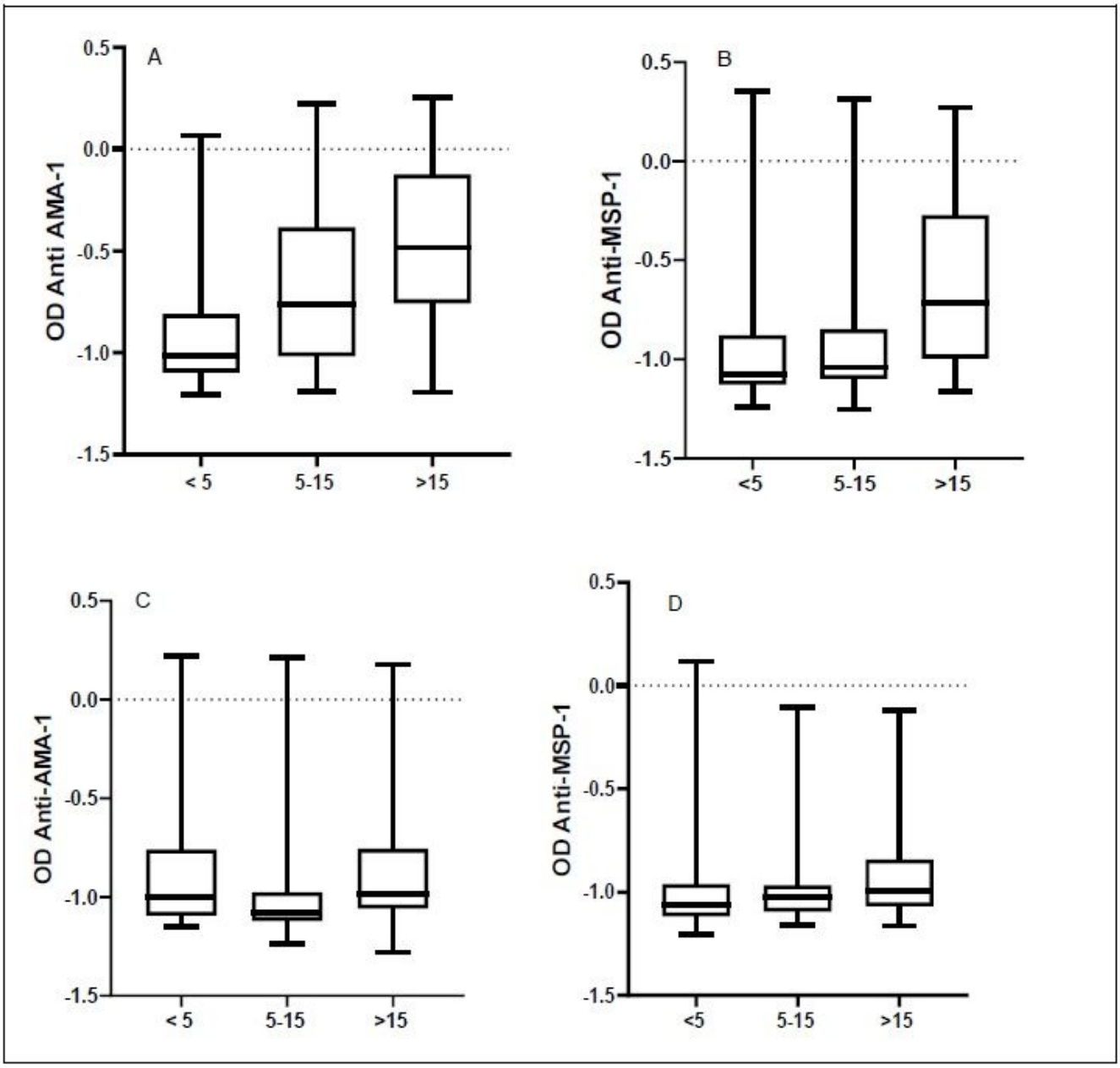

Figure 2

A graph showing mean OD values for anti-PfAMA-1 antibodies and anti-PfMSP-119 antibodies. Figure 2A and figure 2B show OD values for anti-PfAMA-1 and anti-PfMSP-119 in Bondo respectively. Figure 2C and figure 2D show OD values for anti-PfAMA-1 ad anti-PfMSP-119 antibodies in Hai respectively. Presented in the Y-axis is the Log10 transformed mean OD values in different age groups (X-axis).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1figureS1.pdf
- Additionalfile2TableS1.docx

- Additionalfile3figureS2.pdf