

An Evolution From a Predominant K1 Allelic Variant to MAD20 of msp1 Gene Between 2015 to 2019 in Metehara, Southeast Ethiopia

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Abstract

Background: The complexity and quantity of parasite populations circulating in a specific location are reflected in the genetic diversity of malaria parasites (s). Between 2015 and 2019, this study in Metehara, South east, Ethiopia. set out to investigate the temporal dynamics of genetic diversity and multiplicity as a result of evolutionary change in the genes that contribute to *Plasmodium falciparum* infection elimination.

Method: Between 2015 and 2019, a cross-sectional study was carried out. from eighty-three dry blood spots from malaria patients who were screened for *P. falciparum* mono-infection by QPCR. From this seventy confirmed *P. falciparum* were genotyping to merozoite surface protein 1,2 and glutamate-rich protein using nested PCR.

Result: Between 2015 and 2019, seventy (84.3%) of the isolates were successfully genotyped for all three target genes in both years. In 2015 and 2019, the allelic distributions of the three genes differed significantly (P= 0.001). Overall, the most common allelic families for msp1 and msp2 were K1 and FC27 respectively. For glurp, eight distinct genotypes were identified. In 2015, the genotyping of msp1, msp2 and glurp was 25 (86.2%), 25 (86.2%) and 24 (82.2%) respectively. K1, MAD20 and RO33 all have 19(65.5%), 3(10.3%) and 3(10.3%) msp1 allelic families respectively. In 2019 the genes were 30 (73.2%), 39 (95.1%) and 30 (73.2%). K1, MAD20, and RO33 were genotyped for 6 (14.6 percent), 18 (43.9 percent) and 6 (14.6 percent) genotyping respectively. Over all the multiplicity of infection was 1.67 (95 percent CI 1.54-1.74) and the heterozygosity index for msp1, msp2, and glurp was 0.48, 0.70, and 0.55 respectively.

Conclusion: This study provides current information on the genetic diversity of *P. falciparum* populations in Metehara over five-year intervals, The progression of the dominant K1 variant from 2015 to MAD20 variant in 2019 was observed in this study.

Background

Malaria is one of the worst infections, claiming the lives of about 400,000 people worldwide. Despite its tremendous reduction in the previous two decades, malaria remains one of Ethiopia's biggest public health and socioeconomic challenges [1, 2]. Around 68 percent of Ethiopia's population lives in malaria-endemic areas [3]. Ethiopia has achieved a significant reduction in malaria cases and has entered the pre-elimination phase across the country. A change from high or moderate to low malaria transmission necessitates a better understanding of the current parasite population's distribution, dynamics and genetic structure, all of which are critical for achieving and maintaining eradication [2, 15].

Genotyping of a polymorphic region such as block 2 of the merozoite surface protein 1 (MSP1), block 3 of the merozoite surface protein 2 (MSP2) or the RII repeat region of the glutamate rich protein (GLURP) is generally used to determine *P. falciparum* genetic diversity [6–8]. The three polymorphic genes (msp1, msp2, and glurp) have been extensively researched [9, 11, 18, 20, 26] and are valuable for determining genetic diversity and infection multiplicity.

P. falciparum merozoite surface proteins 1 and 2 are important targets for blood-stage malaria vaccines [7] and can also be used to identify genetically diverse *P. falciparum* parasite sub-populations. MSP1 is a 190-kDa main surface protein that plays a key role in erythrocyte invasion [7, 8] as well as being a primary target of immune responses [6]. msp1 is divided into three allelic families: K1, MDA20 and RO33 type [14]. Block 2 comprises 17 sequence blocks that are separated by conserved portions. MSP2 on the other hand, is a five-block glycoprotein with the middle block

being the most polymorphic. Allelic groups FC27 and 3D7/IC1 both contain msp2 alleles. Glurp is a 220 kDa antigen that is produced throughout the malaria parasite's life cycle [6, 22, 31].

The collection of baseline data for these polymorphic biomarkers in the parasite population from various geographical regions and levels of malaria transmission is critical for current malaria control efforts in Sub-Saharan Africa, particularly Ethiopia [10, 11, 26]. As a result, the current study sought to investigate the genetic diversity of msp1, msp2 and glurp in natural *P. falciparum* populations in Metehara in order to gain a better understanding of the temporal changes in the diversity of these polymorphic markers following the implementation of massive intervention strategies in Ethiopia between 2015 and 2019.

Materials And Methods

Study site

Samples used for this study were collected from Metehara, south east Ethiopia, a sentinel site for monitoring of therapeutic efficacy to artemether-lumefantrine (Coartem®) in Ethiopia. (Fig. 1).

Metehara is located 8°33'N 39°16'E / 8.55°N 39.27°E / 8.55; 39.27 at an elevation of 947m (3,107ft), 134 km southeast of Addis Ababa. Metehara is the largest town in Fentale woreda. This study site is one of the settings carefully selected by the National Malaria Control Program (NMCP) as sentinel site to carry out epidemiological survey in order to obtain information paramount in guiding the national malaria management policy. The study was carried out between November to December 2019 during the peak transmission season at the Metehara health center. Metehara is situated in the rift valley area. Awash river and basin have a suitable habitat to support mosquitoes that transmit malaria. The sugar factory estate irrigation system depends on the nearby the Awash River for the cultivation of sugar-cane. The Beska river, provides breeding sites for the Anopheles mosquito. Currently *An. Stephani*, a new vector has been seen in Metehara and susceptibility for this new vector also done to support malaria elimination in Ethiopia [16, 17]. Malaria transmission in this area occurs perennially with peaks during the two rainy seasons (September – November and March - May). This area is endemic for both *P. falciparum* and *P. vivax*.

Study design

A cross-sectional study conducted in Metehara from November to December between 2015 and 2019.

Study population and blood sample collection

Plasmodium falciparum clinical samples were collected in 2015 during efficacy studies of artemisinin combination therapies conducted in Metehara and in 2019 from outpatients who had a history of fever within the previous 24 hours and symptoms consistent with clinical malaria during their visit to Metehara Health Center. Malaria quick detection kit (CareStart™ malaria Pf/Pv Combo test Lot. No. G40IR, expiration date 2021 June,13, Access Bio, Inc., New Jersey, USA) was used to draw finger-prick blood samples and evaluated them. Microscopy, Rapid diagnostic test and quantitative PCR (qPCR) were used for the detection of infections

Dried blood spots (DBS) were collected for molecular analysis using Whatman 903® filter paper (Schleicher & Schuell Bio Science, Keene, NH 03431, USA). The DBS were transferred to Ethiopian Public Health Institute's Malaria Research Laboratory and stored at 20°C until they were evaluated.

Exclusion criteria: Patients critically ill and unable to give blood, patients who have other serious chronic disease were excluded.

Inclusion criteria: Patients who do not have serious chronic disease were included in this study.

Genomic DNA extraction

A tiny section of the blood-blotted cards was cut and placed in a 1.5 ml microcentrifuge tube using a 3 mm Harris Micro Punch™ (Schleicher & Schuell Bio Science, Keene, NH 03431, USA).

The QIA amp DNA Blood Mini Kit (QIAGEN, DNeasy® Blood & Tissue Kit, Cat. no. 69506, USA) was used to extract DNA from the DBS according to the manufacturer's procedure.

PCR amplification and allele detection of msp1 gene

Genomic DNA was amplified using allelic specific primers by nested PCR (Additional files 1): (supplementary Table 1) Table S1. PCR amplification procedures were followed as previously described [13]. A thermal cycler was used to heat all of the PCR reaction mixtures (Perkin-Elmer Cetus PE 9600, Bio-Rad, Hercules, USA).

In a final volume of 20µl PCR reactions were carried out. 4µl gDNA, 10 µl GoTaq Green Master Mix (Promega), 0.5µl (0.5 M) of each primer, and 5µl nuclease free water were used in the primary round reaction. The secondary reaction was identical to the first reaction with the exception of 2µl of PCR amplicon.

Initial denaturation at 95°C for 3 min was followed by 35 cycles for primary and 30 cycles for secondary reactions of denaturation at 95°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 1:30 min, with a final extension at 72°C for 5 min. Each set of reactions comprised positives (3D7) and DNA-free water as negative controls.

Genotyping of msp2: Except for the family-specific primers, PCR reactions and master mix preparation were carried out similarly to msp1. The primers used to genotype the polymorphism areas of the msp2 gene in *P. falciparum* isolates are mentioned in: (Additional File 2) (Supplementary Table 2) Table S2.

Genotyping of glurp

PCR reactions were carried out in a final volume of 20 µl containing: 5 µl gDNA, 7.5 µl GoTaq Green Master Mix (Promega), 0.5 µl (0.5 M) of each primer, and 6.5 µl nuclease free water in initial rounds. In the secondary reaction, 2µl of PCR amplicon product was mixed with 9.5µl nuclease-free water in a 7.5µl GoTaq Green Master Mix and 0.5 µl (0.5 M) of each primer. The following were the cycling conditions for the primary and secondary PCR reactions: For the primary glurp PCR, the following conditions were used: 95°C for 3 minutes, 94°C for 1 minute, 45°C for 1 minute, 68°C for 3 minutes, 72°C for 3 minutes, followed by 30 cycles. Secondary PCR process at 94°C for 1 minute, 55°C for 2 minutes, 70°C for 2 minutes and 72°C for 3 minutes.

PCR products were resolved in 2 percent agarose gels (Caisson, Utah, USA), stained with ethidium bromide submerged in 0.5 TBE (Tris–borate EDTA) buffer electrophoresis at 120 volts, 400 ampere for 45 minutes, and visualized under UV trans-illumination and photographed at 302 nm on a gel documentation system (VersaDoc®, Bio-Rad, Hercules, USA). A 100 base pair (bp) DNA ladder marker was used to visually evaluate the size of DNA pieces (New England Biolabs. Inc, UK). A polyclonal infection was defined as the presence of more than one genotype, whereas a monoclonal infection was defined as the presence of only one allele. For the quality control of

alleles in each family, fragment sizes were calculated within a 20-bp interval for merozoite surface protein 1,2 and a 50-bp interval for glurp [25]. During the PCR cycle, both a positive control and a negative control were performed with the test for quality control purposes. The alleles were identified by comparing them to their genomic controls.

Ethical clearance

Ethical approval of the study was obtained from Institutional Ethical Review Board of Addis Ababa University (AAU), certificate reference number IRB/033/2018. In addition, written informed consent and/or assent were obtained from the parents and guardians of children and malaria positive individuals were treated according to national malaria guidelines in the health center [12].

Data analysis

The program IBM SPSS version 20 was used to conduct all statistical analyses (SPSS Inc. Chicago, IL, USA). The allelic frequency and mean MOI of the *msp1*, *msp2* and *glurp* genes were calculated using proportions of allele comparisons and Chi square tests. The MOI was compared using the student t test between 2015 and 2019. To assess the relationship between MOI, parasite densities and patient age groups the spearman's rank correlation coefficient was calculated. $P < 0.05$ was selected as a threshold for statistically significant differences. The expected heterogeneity (H_e) was calculated by the formula; $H_e = \left(\frac{n}{n-1} \right) \left(1 - \sum p^2 \right)$ where "n" stands for the number of the isolates analyzed and "p" represents the frequency of each different allele at a locus [14].

Results

Demographic and parasitological data

Microscopy, Rapid diagnostic test and quantitative PCR (qPCR) were used to diagnose a total of 83 screened from 2015 (N=33) and 2019 (N=50). (Additional files 2): (supplementary Table 2) Table S2. The study population's demographic and clinical characteristics are shown in (Table 1). In 2015, males accounted for 51.5 percent (17/33) of the total, while females accounted for 48.5 percent (16/33). In 2019 males accounted for 52.0 percent (26/50) while females accounted for 48.0 percent (24/50). For the samples collected in 2019, the mean average age of the participant was 24 (3–60) in 2015 and 25(4-60) in 2019 (Table 1). In 2015, asexual mean parasitaemia was 1593 (95 percent confidence interval: 992.1-2194.06), while in 2019, it was 3893.6. (95 percent CI 2286.05-5501.01).

Microscopy and RDT together analysis identified 81.9%. QPCR analysis did identify 84.3%. One case of mixed *P. falciparum*/*P. vivax* infections. This one misdiagnosed sample was not included in the genotyping ((Additional files 2): (supplementary Table 2) Table S2.

Allelic diversity of *msp1* genotyping: Between 2015 and 2019, a total of seventy isolates were successfully genotyped. In 2015, *msp1* accounted a total of 25 (86.2%). From this K1, MAD20 and RO33 were responsible for 19 (76.0%), 3 (10.3%) and 3 (10.3%) respectively (Figure 1). In 2019 of the *msp1* allelic family distribution (73.2 percent) was found in 30 isolates. The allelic families K1, MAD20 and RO33 each included 6 (14.6%), 18 (43.9%) and 6 (14.6%) members (Figure 2). The fragments sizes for *msp1* allelic families, K1, mad20 and Ro33 was (300-400bp), (300 to 400 bp) and (300-350) respectively (Additional files 3:) (supplementary Table 3) Table S3.

Allelic diversity of msp2 genotyping: Msp2 was effectively discovered in 25 of the 29 isolates collected in 2015 (86.2 percent). The allelic family FC27 was found in 13 (31.7%) of the samples, while IC/3D7 was found in 12 (41.4%). In 2019, msp2 accounted for 39 (95.1%) of the 41 samples obtained, with FC27 accounting for 26 (63.4%) and IC/3D7 accounting for 13 (31.7%) (Table 2). The following are the fragments sizes for msp2 allelic families. FC27 allelic families (500-900 bp) and IC/3D7 allelic families (400-800 bp) (Additional files 3:) (Additional Table 3) S3 Table S3.

Allelic diversity of glurp

Glurp was found in 24 (82.8 percent) of the 29 isolates in 2015 and in 30 of the 41 isolates in 2019 was (73.2 percent). Between 2015 and 2019, eight glurp RII region genotypes, coded I–VIII, with sizes ranging from 500 to 900bp were identified. In 2015 and 2019, genotype II (551-600bp) had the highest incidence of 5(17.2%) and 12(29.3%) respectively (Table 3).

MOI and expected heterozygosity

In 2015, the mean \pm SD MOI for the msp1 gene was (1.43 \pm 0.84). In 2019, the msp1 gene's MOI was determined to be 1.15 \pm 0.91. The difference in msp1 MOI between 2015(P=001) and 2019 isolates was statistically significant (P=0.011) (Table 4). In 2015, the mean \pm SD MOI of the msp2 gene was 1.18 \pm 0.67. In 2019, the mean \pm SD MOI of the msp2 gene was 1.39 \pm 0.59. The difference in msp2 MOI between 2015 and 2019 isolates was statistically significant (P=0.011) (Table 4).

In 2015, msp1 had a heterozygosity of 0.3, whereas in 2019, msp1 had a heterozygosity of 0.03 (Table 4). There is statically difference between 2015 heterozygosity and 2019 heterozygosity (P=001). Polyclonal infections with *msp1* genotypes in 2015 was 12(41.4%) and in 2019 msp1 genotypes polyclonal infection was 11(26.8%). *msp2* genotypes polyclonal infection in 2015 was 10(34.5%). *msp2* genotypes polyclonal in 2019 was 17(41.5.0%). There was no significant association between MOI and parasite density (Spearman rank correlation 0.162, P=0.560; Figure 2) or MOI and age (Spearman rank correlation 0.118, P=0.67; Figure 2).

Overall, the mean MOI of *P. falciparum* infection was 1.67 (95% CI 1.54-1.74). Overall, the heterozygosity was 0.48, 0.70, 0.58 for *msp1*, *msp2*, *glurp* respectively (Table 4).

Discussion

Malaria transmission and management tactics have been impacted by the genetic variety of *P. falciparum* parasites. In low-endemic locations, it's critical to figure out the genetic population structure of *P. falciparum* parasite isolates. Using three polymorphic antigen markers, we compared the genetic diversity of *P. falciparum* populations in Metehara, Oromia Regional State, South East, Ethiopia, between 2015 and 2019. Genotyping the two msp markers msp1 and msp2 revealed much more allele variations than genotyping glurp [11, 17, 18, 19], confirming previous findings [11, 17, 18, 19]. QPCR analysis did identify 84.3%. One case of mixed *P. falciparum*/ *P. vivax* infections. This one misdiagnosed sample was not included in the genotyping.

In the msp1 gene, K1 was the most prevalent allelic family in 2015, with 19 (65.5%) more than in 2019, in contrast to prior studies by others, in which Mad20 was the most prevalent allelic family 18 (43.9%) and this is comparable to what was observed in North West Ethiopia [39]. South West Ethiopia [28,40], Cameron [10], Senegal-Nigeria [30] and Madagascar [32] are among the countries represented. MOI calculations for each gene revealed that the mean MOI for msp1 was lower than msp2 and greater than glurp, which is consistent with the findings of [39, 10, 21].

FC27 type was the more common allelic family in *msp2* gene, which was similar to what had been reported from North West Ethiopia [21] However, differed from data from South West Ethiopia [28], North East Ethiopia [23], Burkina Faso [33] and Cameroon, where IC/3D7 was the more common allelic family. These discrepancies could be due to the rift valley's geographical location and reduced transmission intensity compared to South East Ethiopia's hot and humid climate.

The glurp genotyping between 2015 and 2019 both had the 551–600 base pair allele as the most frequent, whereas the 501–550 bp alleles were the most common ones from 2015. Limited genetic diversity of *P. falciparum* was observed in this study. Similar results have been reported in other areas like Northeast Ethiopia and Djibouti neighbor countries with low *P. falciparum* transmission [23, 29].

Expected heterozygote (H_e) diversity varied from 0.3 for *msp1* in 2015 to 0.03 for *msp1* in 2019, indicating that the parasite population in Metehara has intermediate to low heterozygosity, indicating decreased genetic diversity [29]. In our study low genetic diversity and high allelic frequencies seen a difference from previous study have been reported from other sites including South Western Nigeria [19], Burkina Faso [33], Cameroon [10] and from Ethiopia [40].

The declining malaria transmission, as a result of scaling up interventions, has been shown to affect the genetic diversity pattern and population structure of *P. falciparum* [29, 34]. Limited genetic diversity of *P. falciparum* was observed in this study. Similar results have been reported in other areas with low *P. falciparum* transmission [34]

As a result, the low MOI found in this study implies low malaria transmission intensity in Metehara. This is consistent with previous studies, which linked an increase in MOI to increased malaria endemicity and a low MOI for *msp1* and *msp2* to low malaria transmission intensity [11, 29, 34]. Like contrast, high endemicity settings in Cameroon [10] and Burkina Faso [33] have been observed to have a high amount of genetic diversity. The current study found that the *P. falciparum* parasite population in Metehara overall exhibited a low heterozygosity 0.48, 0.70, 0.55 for *msp1*, *msp2*, glurp, respectively consistent with that reported in Northeast Ethiopia for *msp2* ($H_e=0.5$) [33]. Heterozygosity in 2015 and in 2019 was 0.3 and 0.03 this is similar to the study report in East Asia Sabah which was $H_e =0.33$ (34).

In areas with declining local transmission, it is expected that lower parasite diversity (heterozygosity) will be present [34]. Declining diversity and transmission have been associated with improved malaria control interventions [29, 33, 34].

In this study, the total mean MOI ($MOI=1.67$) was low. Low malaria transmission areas are often associated with lower MOIs [33, 35]. This is in line with reports from Sudan and Djibouti's semi-desert environments [36, 29]. The majority of the research participants were over the age of 15, which is consistent with results from a malaria transmission area with lower intensity [33]. Age is thought to play a role in the development of *P. falciparum* immunity and may have an impact on MOI [38]. However, the most recent study found no link between age and MOI. Similar findings have been found in other countries [39]. As a limitation of this study, allele distinction should be improved in future studies by using more selective techniques like DNA sequencing or SNPs.

Conclusion

The majority of *P. falciparum* infections in Metehara, Oromia Regional State, South East Ethiopia, were monoclonal, according to this study. This is consistent with the low infection rate in this area. Further research in similar low-

transmission conditions with greater sample sizes is required. Further research into the dynamics of *P. falciparum* malaria variety in Ethiopian regions such as sequencing or SNPs is needed. In this study, there was an improvement in the reduction of MOI during a five-year period. The development of the K1 variant by the MAD20 variant was also seen. As a result of the changing epidemiology of malaria, this study supports the use of the *glurp*, *msp1*, and *msp2* genes in the characterization of *Plasmodium falciparum* infection, especially when the MOI is one of the primary parameters to be examined for malaria control strategies.

Abbreviations

He: Expected heterozygosity; MOI: Multiplicity of infection; *msp1*: merozoite surface protein 1; *msp2*: merozoite surface protein 2; GLURP: Glutamate rich protein; PCR: Polymerase chain reaction; TBE: Tris-borate EDTA.

Declarations

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Authors' contributions

AG was fully involved in all phases of the study, including in laboratory during Molecular analysis, data analysis, interpretation, and write-up of the manuscript; HI and LG were designed the study project critical revised the manuscript. AL was involved in statistical analysis of data HM, AS, were contributed to Data collection and critical revised the manuscript. All authors read and approved the final manuscript

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Availability of data and materials

All relevant data is included in manuscript, and the datasets analyzed in the study is available from the corresponding author on reasonable request. Additional data uploaded with main document

Competing interests

We declare that we have no competing interests.

Ethics approval and consent to participate

The research and ethical committee of Addis Ababa University Institutional Review Board (IRB) reviewed and approved the study protocol, as verified through certificate reference number IRB/033/2018 Addis Ababa University also approved the study protocol.

Consent for publication

Not applicable.

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Tables

Table 1
Demographic, parasitological and clinical features of study participants in Metehara, Ethiopia.

	Year 2015	Year 2019
Number of patients (males %)	17 (51.5)	24(48.0%)
Number of patients (female %)	16(48.5)	26(52.0%)
Age (yrs), median (range)	24 (3–60)	25(4-60)
<5 years, n (%)	2(6.9)	6(12.0)
6–15 years, n (%)	7(21.2)	9(18.0)
>15 years, n (%)	24(82.8)	35(70.0)
Feverish patients on day 0 (%) (axillary temperature >37.5°C)	27(81.8)	40(80.0)
Temperature (°C), mean (range)	38.03 (36.10–41.40)	37.7 (36.40–40.20)
Asexual parasite density, geometric mean (range)	1593. (95% CI 992.1-2194.06)	3893.6 (95% CI 2286.05-5501.01)
6–15 years, n (%)	1023 (672-4202)	1002(801-6490)
>15 years, n (%)	1001(900-7200)	2025(1245-8910)
Gametocytemic patients (%)	3.01	2.06
*n (%) is number of patients		

Table 2
Distribution of *msp1*, *msp2* and *glurp* alleles frequency between 2015 and 2019 in Metehara, southeast Ethiopia

Nested PCR positive results							
		msp1			msp2		glurp
Year	N	K1, n (%)	MAD20, n (%)	RO33, n (%)	FC27, n (%)	IC/3D7, n (%)	n (%)
2015	29	19(65.5)	3(10.3)	3(10.3)	13(44.8)	12(41.4)	24(82.8)
2019	41	6(14.6)	18 (43.9)	6(14.6)	26(63.4)	13(31.7)	30(73.2)
Total	70	25(35.7)	21(34.3)	9(12.9)	39(57.8)	25 (38.5)	54(77.1)
*n% is positive for nested PCR							

Table 3
Distribution of allelic variants of *glurp* RII repeat region of *P. falciparum* populations between 2015 and 2019 in Metehara, Southeast Ethiopia

Genotypes	Allelic size variant (bp)	2015 (N = 29), n/N (%)	2019 (N = 41, n/N (%))
I	501-550	4(13.8)	3(7.3)
II	551-600	5(17.2)	12(29.3)
III	601-650	3(10.3)	3(7.3)
IV	651-700	2(6.9)	3(7.3)
V	701-750	3(10.3)	2 (4.9)
VI	751-800	3(10.3)	2 (4.9)
VII	801-850	3(10.3)	3(7.3)
VIII	851-900	1(3.4)	2 (4.9)

***bp is base pair of RII variant, n/N (%) is percentage of positive P. Falciparum**

Table 4

Distribution of MOI, heterozygosity and polyclonal infection of *P. falciparum* *msp1*, *msp2*, and *glurp* polymorphism between 2015 and 2019 in Metehara, Southeast Ethiopia

Year	MOI (mean± SD)			He			P. value	PI n/N (%)	
	<i>msp1</i>	<i>msp2</i>	<i>glurp</i>	<i>msp1</i>	<i>msp2</i>	<i>glurp</i>		<i>msp1</i>	<i>msp2</i>
2015	1.43±0.84	1.18±0.67	0.20±0.42	0.3	0.56	055	P=0.001	12(41.4%)	10(34.5%)
2019	1.15±0.91	1.39±0.59	0.61±0.31	0.03	0.57	061	P=0.011	11(26.8%).	17(41.5%)
	Over all MOI	1.6* CI 1.54-1.74	Over all He	0.48	0.70	0.58			

CI: confidence interval, HE: heterozygosity index, MOI: multiplicity of infection, PI: polyclonal infection

Figures

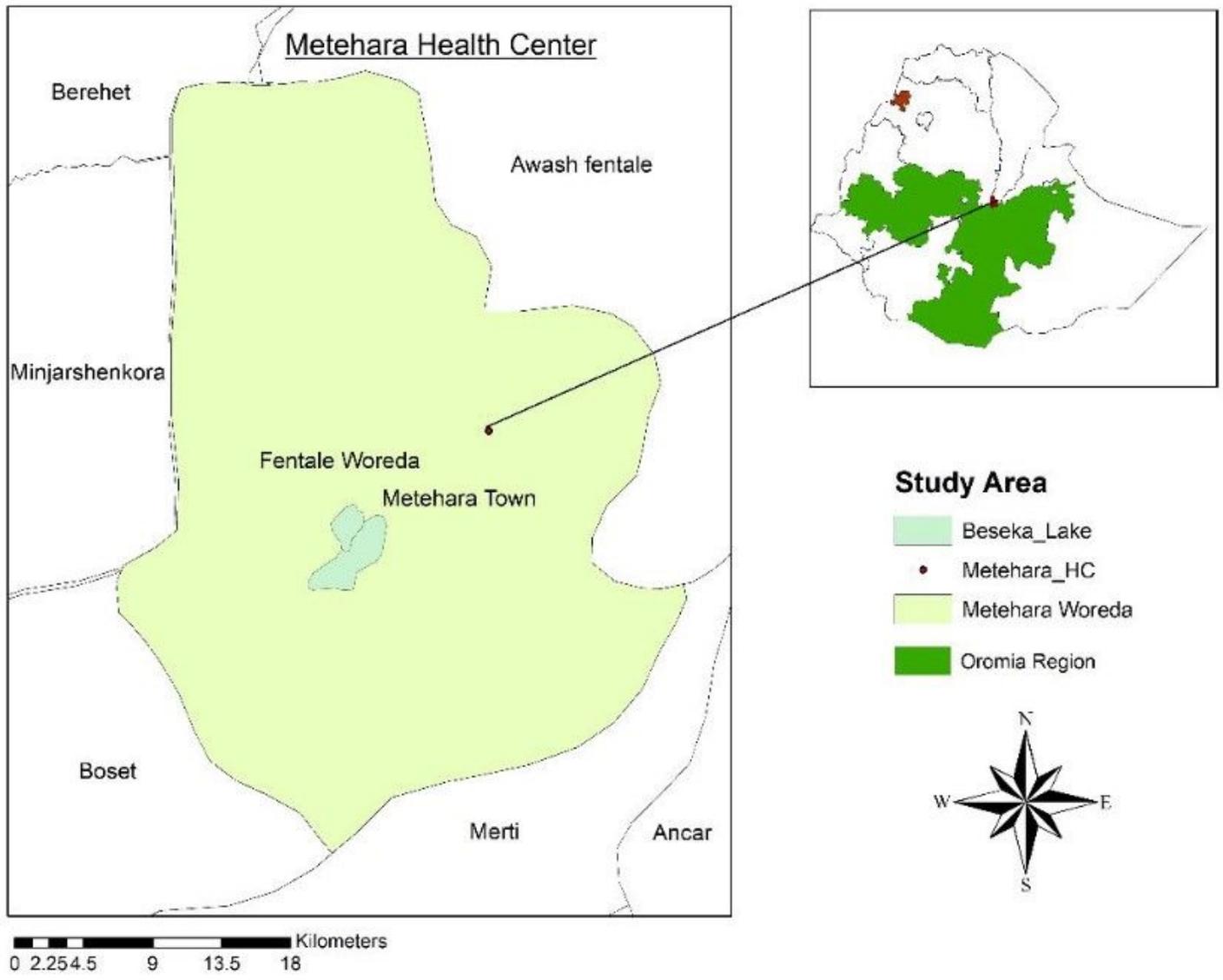


Figure 1

Map of Metehara the study area, showing sample collection sites.

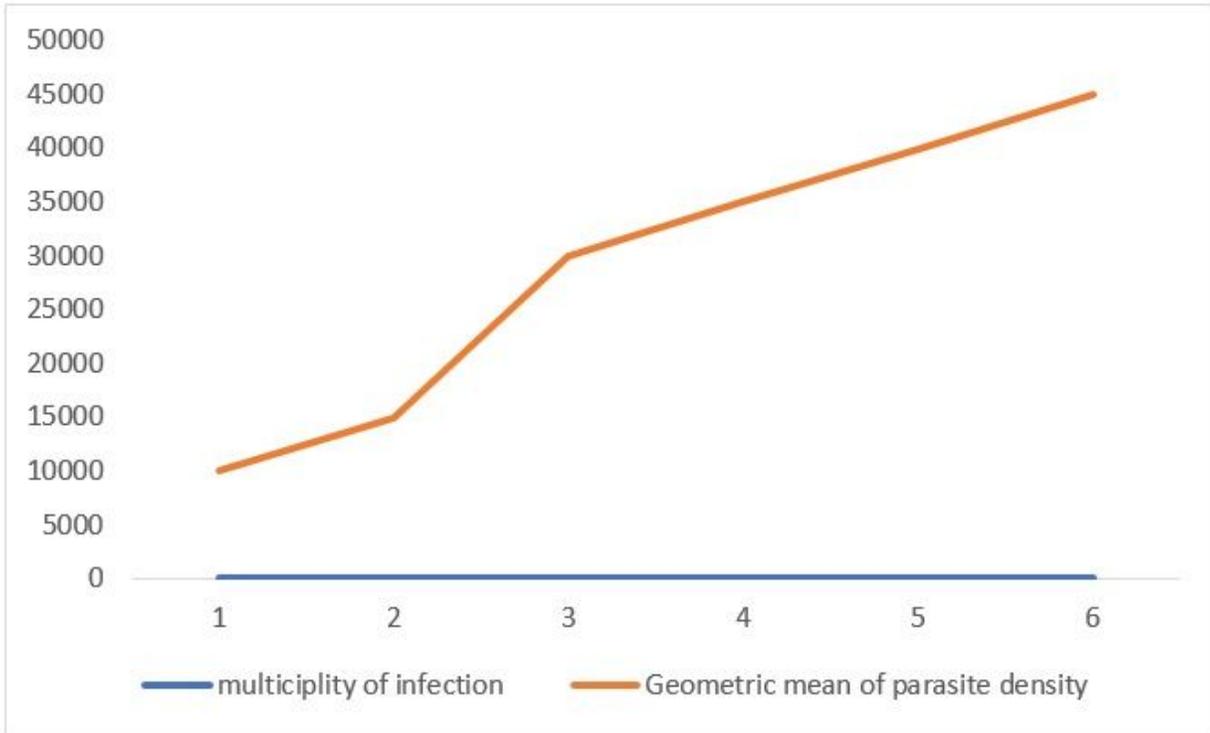


Figure 2

Relationship between geometric mean parasite density and multiplicity of *P. falciparum* infection

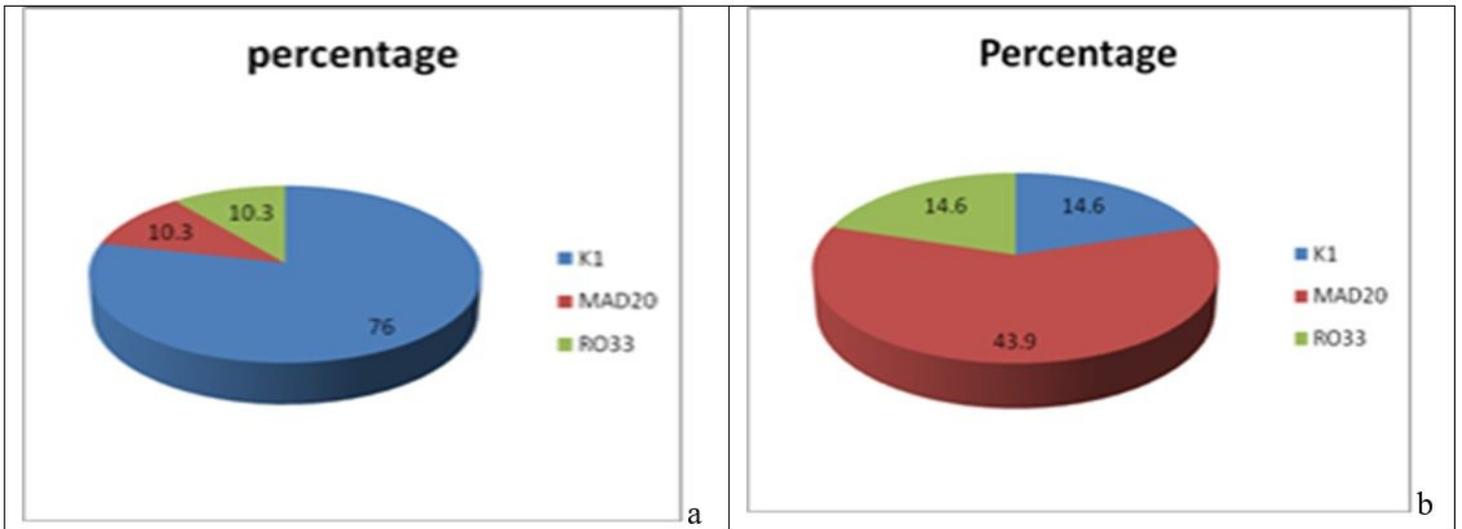


Figure 3

(a) Distribution of *msp1* allelic family in 2015 at Metehara, southeast Ethiopia
 (b) Distribution of *msp1* allelic family in 2019 at Metehara, southeast Ethiopia

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