Age-depended selection of chloroquine-sensitive *Plasmodium falciparum* in some part of Central Region of Ghana

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Abstract

Introduction

The return of chloroquine-sensitive *P. falciparum* in sub-Saharan Africa countries offers the opportunity for reintroduction of chloroquine either in combination with other drugs or as a single therapy for the management of malaria. The reintroduction of chloroquine can serve as a stopgap to salvage the impending danger of complete failure of malaria treatment due to artemisinin drug resistance. Further, chloroquine reintroduction requires the understanding of the underlying factors that influence the reemergence of chloroquine-sensitive *P. falciparum* in the endemic areas. This study assesses the effects of age on the pattern for selection of CQ sensitive *P. falciparum* markers in the Central Region of Ghana

Methodology

Genomic DNA was extracted from an archived filter paper blood blot from Cape Coast, Elmina, Assin Foso and Twifo Praso using Chelex DNA extraction method. The age information to each extracted sample was collected. The prevalence of chloroquine-sensitive genotyping of Pfcr77 and Pfm1 N86 was assessed using nested PCR and RFLP.

Results

The overall prevalence of CQ sensitive *P. falciparum* marker (Pfcr77) at Central Region of Ghana was 66.36%, whereas the prevalence of Pfcr77 at Cape Coast, Assin Foso, Twifo Praso and Elmina were 71.74%, 65.22%, 66.67% and 61.54% respectively. The prevalence of Pfcr77 among the age
categories showed that 0-5 years category predominantly selects CQ sensitive Pfcrt K76 marker at Cape Coast (34.76%), Assin Foso (37.68%) and Twifo Praso (39.98%). In the case of Pfmdr1 N86, the total prevalence was 84.11% with Cape Coast having 64%, Elmina with 92.26%, Assin Foso with 88.39% and Twifo Praso with 89.91% There was strong correlation of reemergence of chloroquine-sensitive malaria parasites between Cape Coast and Assin Foso, $(r=0.8568, p=0.0318)$ Cape Coast and Twifo Praso $(r=0.8671, p=0.0285)$ and Assin Foso and Twifo Praso, $(r=0.9913, p=0.0005)$.

Conclusion

The study showed that the selection and expansion of chloroquine-sensitive $P. falciparum$ are influenced by age and geographical area. This finding has a significant implication for the future treatment, management and control of $P. falciparum$ malaria.

Keywords: $P. falciparum$, chloroquine resistance parasites, chloroquine sensitive parasites, Pfcrt, Pfmdr1, Central Region, Ghana

Introduction

The return of chloroquine (CQ) sensitive Plasmodium falciparum in sub-Saharan Africa is associated with increased prevalence of wildtype genetic phenotypes [1, 2]. CQ had the most suitable therapeutic properties and excellent efficacy for malaria treatment [3]. Until the emergence of $P. falciparum$ resistance to CQ and subsequent withdrawal, CQ was widely used for self-malaria treatment [4]. This hampered the malaria control and treatment leading to CQ resistance and its associated high mortality especially among children in sub-Saharan Africa [5, 6]. The emergence of CQ sensitive $P. falciparum$ parasites offers the opportunity to introduce this vetoed drug either in combination therapy or as a single antimalaria drug [7, 8]. However, there is a major concern for the rapid reappearance of CQ resistant parasite in sub-Saharan African countries upon reintroduction [9]. Although the reappearance of CQ sensitive parasites have been observed in all countries, the rate of re-emergence varies from place to place [10]. The central region of Ghana has experienced a slow appearance of CQ sensitive $P. falciparum$ compared to other parts of the country [11, 12].

The $P. falciparum$ Pfcrt K76T and Pfmdr1 N86Y resistant markers are well established to be responsible for chloroquine resistance globally [13]. In Ghana, the prevalence of Pfcrt K76T and Pfmdr1 N86Y vary from one place to another [12, 15]. In Ghana, 11.6% and 8.1% of Pfcrt K76T and Pfmdr1 N86Y respectively has been reported as the least prevalence of the chloroquine-resistant parasite [16]. This is good news as the most successful deployed antimalarial drug could be used as stopgap to salvage the current challenges involved in the emerging artemisinin resistance [17, 18]. There are also no new antimalarial drugs, and lack of effective and efficient malaria vaccines for the treatment of malaria [19, 20]. Chloroquine in a combination with other effective antimalarial drugs could help in biding time for the search of new effective and efficient antimalarial drugs and vaccines [21].
Artesunate-amodiaquine (ASAQ), artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DHAP) serves as a first-line antimalarial drug for uncomplicated malaria in Ghana [22]. However, the emergence of artemisinin resistance in *P. falciparum* in South-East Asia and some parts of Africa has led to treatment failure of ACTs [23, 24]. A current survey from 2007-2016 archived samples from Ghana had shown that K13 mutations responsible for both Asian and African artemisinin resistance were prevalent in the Ghanaian *P. falciparum* parasites [25]. The study also frequently identified N599Y, K607E and V637G non-synonymous mutations in the samples [25]. This gives an indication that the Ghanaian *P. falciparum* parasites have the capacity to develop resistance to artemisinin [25, 26].

It is important to arm ourself against the pending danger that may be associated with a complete failure of artemisinin combination therapy. As such a failure could be catastrophic for malaria morbidity and mortality especially in the sub-Saharan region. Therefore, understanding the factors that influence the selection of chloroquine-sensitive *P. falciparum* would provide us with the necessary tools for early detection and containment of reemergence of chloroquine-resistant strains after the reintroduction of chloroquine for malaria treatment. This study analyzes the effects of age on the pattern for selection of CQ sensitive *P. falciparum* markers in the Central Region of Ghana.

**Methodology**

**Study sites**

The study was conducted in four districts in the Central Region of Ghana. The samples were selected from two ecological zones with different malaria endemicity. Assin Foso and Twifo Praso in the forest zone has a high *P. falciparum* malaria prevalence compared to Cape Coast and Elmina in the coastal zone which has lower malaria prevalence in Central Region of Ghana [15].

**Sample collection**

An archived *Plasmodium falciparum* infected blood blot samples from Cape Coast, Elmina, Twifo Praso and Assin Foso were used for this study [27]. The air-dried blood spots in zip-locked plastic envelopes containing silica gel stored at -20°C in the Department of Biomedical Sciences, University of Cape Coast were extracted using chelex extraction method. The excel data which contains the sample numbers and patient information at the department were obtained for the analysis.

**Genomic DNA extraction**

The Chelex-saponin DNA extraction method was used. Briefly, discs punched of about 2.5 mm (2x) were made from dried blood spot then transferred into a 1.5 ml Eppendorf tube. 50 µL of 10% saponin and 1 mL of 1x PBS were added to the blood spots in 1.5 ml tubes. The sample and saponin mixture was vortexed and frozen at 4 °C overnight. The filter was washed 3 x with 1 mL of 1 x PBS, followed with 30 µL of 20% chelex and 70 µL of DNase/RNase water. The sample was incubated at 95 °C for 10 min with an intermittent vortex. The gDNA samples were eluted from the filter by centrifuging at
13,000 rpm for 6 min and the supernatant transferred into a sterile 0.5 mL microfuge tube. The extracted gDNA were stored at -20 °C.

Amplification of PfCRT and Pfmdr1 by Nested Polymerase Chain Reaction (PCR)

The genome DNA of *P. falciparum* was amplified using primer pairs (P10-1 forw: TTGTCGACCTAACAGATGGCTCAC / P10-1 rev: AATTTCCTTTTATTTTCCAAATAAGGA for PfCRT and P1-1 forw: TTAAATGTTCCTGCCAACAATAGAAAAT / P1-1 rev: CTCCACATATACTTCTTGCAACAGTCTTTA for mdr1, primary reaction and P10 forw: CTTGTCTTGGATAATGTGCTC / P10 rev: GAACATAATCATAACAAATAAAGT for PfCRT and P1 forw: TGATATGTGCTGTATTATCAGGA / P1 rev: CTCTCCTATAATGGACATGGTA for secondary reaction). The primary PCR reaction mixture contained 0.2 µM of the primary primer pair, 5 µl DNA template, 1X PCR buffer, 200 µM dNTPs, 1.5 mM MgCl2 and 1.25 U of *Taq* DNA Polymerase in a 25 µl mixture. The PCR cycling conditions (95 °C for the 30s, [95 °C for 15 s, 53 °C for 1 min, 68 °C for 1 min], 68 °C for 5 min final extension. The secondary PCR was performed using 0.5 µL of the primary PCR reaction and 133.33 nM each of the second primer pairs. The PCR cycling condition was the same except the annealing temperature which was 60 °C for the second PCR. The 1 µl of the secondary PCR products were run on a 2% agarose gel and visualized using a CSL gel documentation system (CSL, UK).

Restriction fragment length polymorphism (RFLP)

The nested PCR products of PfCRT were digested with Apo I whereas that of Pfmdr1 were digested with Apo I and Afl III. The digested products (5 µl) were separated on 2% electrophoresis gel, stained with ethidium bromide and viewed under ultraviolet light. The PfCRT PCR product contains single Apo I site if the codon 76 of the PfCRT gene encodes for lysine (K76). For Pfmdr1 codon 86 was digested with both Apo I for asparagine and Afl III to confirm the tyrosine mutation at position 86 in all the Pfmdr1 samples that were not digested by Apo I. The digested samples were run on 2% agarose gel and visualized using CSL gel documentation system.

Data analysis

The data from RFLP analysis, patient ages and sample collection sites were organized using Microsoft Office Excel 2010 (Microsoft Corporation) and the statistical analyses were performed with GraphPad Prism software, version 8.4.3 (GraphPad Software). All mixed infection of wild type and resistant strains of PfCRT K76T and Pfmdr1 N86Y were excluded from the analysis. The ages of the patients were categorized into 0-5 years, 6-15 years, 16-30 years, 31-45 years and above 45 years before using it for further analysis. The data was translated into the prevalence and statistical significance were determined using paired t-test statistics.
Results

The overall prevalence of CQ sensitive *P. falciparum* markers Pfcr K76 and Pfmdr1 N86 at Central Region of Ghana were 66.36% and 84.11% respectively. The predominance of Pfcr K76 and Pfmdr1 N86 from *P. falciparum* parasites varies slightly among the study sites (Table 1).

The pattern of selection for chloroquine-sensitive markers Pfcr K76 gene among *P. falciparum* isolates showed age dependent selections at each of the study sites. The prevalence of Pfcr K76 among 0-5 years, 6-15 years, 16-30 years, 31-45 years and greater than 45 years of age were 34.76%, 15.21%, 23.94%, 19.55% and 6.53% respectively for Cape Coast. A similar pattern was observed for Assin Foso and Twifo Praso except at Elmina which showed a different pattern in the Pfcr K76 prevalence among the age categories with 5.14%, 7.69%, 43.58%, 23.07% and 20.52% for 0-5 years, 6-15 years, 16-30 years, 31-45 years and greater than 45 years respectively (Figure 1A and 1B).

The Pfcr K76 prevalence was compared between study sites. The results showed a significant difference in Pfcr K76 prevalence among the age categories between Cape Coast and Assin Foso, p<0.05; Cape Coast and Twifo Praso, p<0.01 and Assin Foso and Twifo Praso, p<0.0001 (Figure 2A). The correlation analysis between the mean difference for the selection of wildtype Pfcr K76 gene showed a significant positive association among Cape Coast vs. Assin Foso (r= 0.8568, p= 0.0318), Cape Coast vs. Twifo Praso (r= 0.8671, p= 0.0285) and Assin Foso vs. Twifo Praso (r= 0.9913, p= 0.0005) (Table 2). The individual mean prevalence of Pfcr K76 among the age categories showed that the pattern of distribution varies among the study sites with Cape Coast and Assin Foso having minimum and maximum mean distribution respectively. The mean ± S.E of the Pfcr K76 at Cape Coast (20±4.68, p=0.013) and Elmina (23.24±6.452, p=0.043) showed a significant difference in the mean distributions of Pfcr K76 among the age categories (Figure 2B, Table 3).

The Pfmdr1 N86Y mutation is well characterized to augment the Pfcr K76T mutation to confer high chloroquine resistance. The emergence of chloroquine-sensitive *P. falciparum* is associated with the return of Pfcr K76 and Pfmdr1 N86. The selection of Pfcr K76 was therefore confirmed with the prevalence of Pfmdr1 among the age categories at the individual study sites. The Pfmdr1 N86 showed a similar pattern of selection of Pfcr K76. The prevalence of Pfmdr1 N86 were 34.78%, 10.59%, 23.91%, 19.57% and 6.52% for 0-5 years, 6-15 years, 16-30 years, 31-45 years and >45 years respectively at Cape Coast. Cape Coast, Assin Foso and Twifo Praso showed an identical pattern of Pfmdr1 N86 distribution except for Elmina which exhibited a different pattern of Pfmdr1 N86 distribution among the age categories (Figure 3A).

The distribution showed a difference in the means at the individual levels. The mean±S.E of Pfmdr1 N86 among the age categories at individual study sites showed significant difference at Cape Coast (13.92±3.259, p=0.0129) and Elmina (12.30±4.25, p=0.0433) (Figure 3B, Table 3). The restoration of chloroquine-sensitive gene Pfmdr1 N86 among the study sites showed a positive correlation for Cape Coast vs. Assin Foso (r=0.8561, p=0.0321), Cape Coast vs. Twifo Praso (r=0.8664, p=0.0287) and Assin Foso vs. Twifo Praso (r=0.9914, p=0.0005) (Table 2). The results indicate a strong influence between study sites on the restoration of chloroquine-sensitive *P. falciparum* parasites in the Central Region of Ghana.

Discussion
The recovery of the CQ sensitive *P. falciparum* has come at a time when the treatment and management of malaria are constrained with development of resistance to almost all the most effective antimalarial drugs [28, 29]. Although the current advances and knowledge on malaria biology have the potential to identify drug targets for the development of safe, effective and efficient antimalarial drugs and vaccines, they are yet to produce an effective means to tackle the challenges facing the malaria treatment and management [30, 31]. As exploration for the new treatment strategies, identification of novel drug targets and the design of new antimalarial compounds are on-going, it is important to understand the dynamism and factors that influence the reemergence of CQ sensitive *P. falciparum* in the malaria-endemic areas [32, 33]. This could help in the proper way for the reintroduction of CQ as a temporal strategy to control malaria as we wait for the new effective and safe antimalarial drugs and vaccines [34]. This study focuses on the dynamic effects of age and how it influences the selection of CQ sensitive *P. falciparum* in selected areas in the Central Region of Ghana.

The study showed that CQ resistance markers have reduced to a significant level compared to the previous report from the region [35]. Although the steady decline for PfcrT76 and Pfmdr1 Y86 has been very slow especially at regional capital (Cape Coast) of Central Region of Ghana yet the expansion of the CQ sensitive *P. falciparum* strains have increased appreciably [12, 15, 27]. Similar findings on the reappearance of CQ sensitive parasites have been reported in Malawi, Cameroon, Ethiopia, Nigeria, Tanzania and Mozambique [9, 10]. The decrease in CQ resistant markers and subsequent parasite sensitivity to CQ treatment have been demonstrated clinically [36]. The slow appearance of Pfcr K76 and Pfmdr1 N86 is an indication that the maintenance of the integrity of CQ resistant phenotypes have fitness cost in the absent of CQ drug pressure [37, 38]. Hence, the reversion of mutant alleles to the wildtype alleles that are sensitive to CQ treatment [38]. Interestingly, a study has reported that in Anopheles arabiensis vectors, there is a selective picking and infection with wildtype *P. falciparum* harbouring Pfcr K76 and Pfmdr1 N86 genes in human population which harbours Pfcr T76 close to 90% among malaria parasites isolates [39, 40].

The host-parasite relationship is essential for the selection of traits which determines the fitness of parasites [41]. The factors such as virulence, resistance, the complexity of the parasite life-cycle and the demographic characteristics of the host influence the parasite selection, adaptation and survival [42, 43]. The study showed that the selection of Pfcr K76 and Pfmdr1 N86 alleles among *P. falciparum* isolates in Central region of Ghana is predominantly found in the age category 0-5 years and 16-30 years at Cape Coast, Assin Foso and Twifo Praso except in Elmina where the selection was low among 0-5 years old. The result takes a similar pattern of higher *P. falciparum* infection incidence among children compared to adults [44]. A higher infection prevalence among age < 16 years is associated with the age-dependent acquisition of malaria immunity [45]. Thus, the higher selection of CQ sensitive parasites with Pfcr K76 and Pfmdr1 N86 phenotypes by age 0-5 years is important for the parasites to avoid immune clearance and enhance propagation. Again, it has been shown that adults with age > 35 years harbour significantly lower proportion of gametocytes carriage compared to children with age < 17 years whiles 18-30 years form intermediate gametocyte carriage [46]. A higher gametocyte carriage in young children is an indication of transmission dynamics. The result of this study provides a link between the higher gametocyte carriage among young children and selective infection of CQ sensitive parasites in Anopheles mosquito vectors within an area endemic with CQ.
resistant parasites [44-46]. This link explains why reemergence and expansion CQ sensitive parasites are high among 0-5 years after CQ withdrawal.

Also, the recovery of CQ sensitive parasites is determined by eco-epidemiological factors such as migration and vector competence within an area. Thus, the expansion of Pfcrt K76 and Pfmdr1 N86 observed among the study sites could be as a result of migration. Cape Coast showed a positive correlation between Assin Foso and Twifo Praso for selection CQ sensitive \textit{P. falciparum} whereas Assin Foso showed a strong positive influence on Twifo Praso. A previous report indicated the possibility of Pfcrt sensitive parasite either upsurged from low levels in the indigenous parasite populations or migrated from bordering countries with persistent CQ drug pressure [47]. This observation supports the idea of migration of CQ sensitive phenotype from a place with persistent CQ pressure [1]. Such migration of Pfcrt K76 and Pfmdr1 N86 alleles could be due to rapid movements of goods and service as well as human traffics among Cape Coast (the regional capital), Assin Foso (Assin north municipal capital) and Twifo Praso (the district capital of Twifo Heman Lower Denkyira) in a cyclical manner. It has also been shown that transiting from malaria-endemic area to hypoendemic endemic areas exposes people to the changing risks of malaria infections [48, 49]. Thus, the pattern of malaria infection within a community is significantly influenced by the movement of people [49]. The positive correlations for the prevalence of Pfcrt K76 and Pfmdr1 N86 among three of the study areas take a similar pattern of the spread of drug resistance lineage parasites [50].

However, Elmina showed a different pattern for the selection of chloroquine-sensitive parasites among the age categories compared to the other study sites. Even though Elmina has close proximity to Cape Coast, there is geographical variation in the distribution CQ sensitive genotype between the two areas. A study conducted at the border of China and Myanmar reported divergent in drug resistance genes and in vitro drug sensitivity [51]. This observation could be explained by factors including the behaviours, the used ITNs and protection of young children in Elmina.

In conclusion, the study showed for the first time that the selection and expansion of CQ sensitive parasite are influenced by age and geographical area. It also establishes a link between two previous studies that reported selective infection of wildtype CQ parasite in Anopheles vector and high gametocytes carriage among young children. These findings have significant implication for the future treatment, management and control of \textit{P. falciparum} malaria.

\textbf{Declarations}

\textit{Ethical clearance and consent to participate}

Not applicable

\textit{Consent for publication}

Not applicable
Availability of data and materials

Not applicable

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None declared

Human and Animal Rights

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Author contributions statement

All authors contributed equally

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Figure legends

Figure 1: Chloroquine sensitive Pfcrt marker K76 selection is influenced by age categories
A. A box and whiskers plot showing the prevalence of chloroquine resistant markers for Pfcrt K76T and Pfmdr1 N86Y among the age categories at the individual study sites.
B. Age dependent selection of Pfcrt K76 marker for chloroquine sensitive parasites. The data is presented in part of whole using a donut chart.

Figure 2: Differential selection of Pfcrt K76 chloroquine sensitive marker at the study sites
A. Comparison of Pfcrt K76 among the study sites. The data is presented with box and whiskers
using minimum and maximum values. The statistical significance was tested using paired t-test statistics. NS = Not significant; * = p<0.05; ** = p<0.01; *** = p<0.0001. The prefix C-, E-, T- and A- stands for Cape Coast, Elmina, Twifo Praso and Assin Foso respectively.

B. The distribution of PfcrT K76 per age categories at the individual study sites. The data is presented in mean with standard error of the mean using the interleaved scatter diagram.

Figure 3: **Age and site selection of chloroquine sensitive P. falciparum with Pfmdr1 N86 genes.**

A. Pfmdr1 N86 gene selection is influence by age categories at individual study sites. The data is presented in part of whole using a donut chart.

B. Mean distribution of Pfmdr1 N86 among age categories across the study sites. The data is presented in mean with standard error of the mean across the study sites.

C. The comparison of prevalence of Pfmdr1 N86 between study sites. The data is presented with box and whiskers using minimum and maximum values with individual prevalence. The statistical significance was tested using paired t-test statistics. NS = Not significant; * = p<0.05; ** = p<0.01; *** = p<0.0001. The prefix C-, E-, T- and A- stands for Cape Coast, Elmina, Twifo Praso and Assin Foso respectively.

Table 1: Prevalence of PfcrT K76T and Pfmdr1 N86Y at the study sites

Table 2: Variation in the selection of PfcrT K76 and Pfmdr1 N86 among the study sites

Table 3: Differential selection of chloroquine sensitive PfcrT K76 and Pfmdr1 N86 among the age categories at the individual study sites
| Study sites      | Pfcr76 (%) | Pfmmdr1 86 (%) |
|-----------------|------------|----------------|
|                 | K          | K/T            | T              | N    | Y    |
| Cape Coast      | 71.74      | 13.04          | 15.22          | 64   | 36   |
| Assin Foso      | 65.22      | 11.59          | 23.19          | 88.39| 11.61|
| Twifo Praso     | 66.67      | 13.33          | 20             | 89.91| 10.09|
| Elmina          | 61.53      | 20.51          | 12.82          | 92.26| 7.74 |
| Study site                        | PfCrt K76          |       |       | PfMdr1 N86        |       |       |
|----------------------------------|--------------------|-------|-------|------------------|-------|-------|
|                                  | Mean diff ±S.E (95% CI) | r     | p     | Mean diff ±S.E (95% CI) | r     | p     |
| Cape Coast vs Elmina             | 0.322 ± 1.807 (-4.674 - 5.338) | -0.1183 | 0.4249 | -1.62 ± 5.629 (-17.25 - 14.01) | -0.1208 | 0.4236 |
| Cape Coast vs Assin Foso         | 6.082 ± 2.873 (-1.895 - 14.06) | 0.8568 | **0.0318*** | -2.00 ± 2.271 (-8.305 - 4.305) | 0.8561 | **0.0321*** |
| Cape Coast vs Twifo Praso        | 0.904 ± 1.072 (-2.072 - 3.88) | 0.8671 | **0.0285*** | -0.960 ± 2.518 (-7.950 - 6.03) | 0.8664 | **0.0287*** |
| Elmina vs Assin Foso             | 5.76 ± 3.73 (-4.596 - 16.12) | 0.1233 | 0.4217 | -0.38 ± 5.649 (-16.06 - 17.48) | 0.1226 | 0.4222 |
| Elmina vs Twifo Praso            | -10.94 ± 5.527 (-26.28 - 4.409) | 0.09033 | 0.4426 | 0.66 ± 6.058 (-16.16 - 17.48) | 0.0893 | 0.443 |
| Assin Foso vs Twifo Praso        | -5.178 ± 1.885 (-10.41 - 0.055) | 0.9913 | **0.0005*** | 1.04 ± 0.734 (-0.998 - 3.078) | 0.9914 | **0.0005*** |

Table 2
Table 3

| Study site    | PfCrt K76 Mean ± S.E (95% CI) | t    | p   | PfMdr1 N86 Mean ± S.E (95% CI) | t    | p   |
|---------------|--------------------------------|------|-----|--------------------------------|------|-----|
| Cape coast    | 20.0 ± 4.68 (7.01 – 32.99)    | 4.274| 0.013* | 18.00 ± 4.692 (4.871 - 22.97) | 4.271| 0.0129* |
| Elmina        | 23.24 ± 6.452 (0.99 – 39.01)  | 2.92 | 0.043* | 16.30 ± 4.25 (3.599 - 54.00) | 2.918| 0.0433* |
| Assin Foso    | 24.00 ± 7.619 (-0.049 - 40.05)| 2.763| 0.051 | 11.92 ± 4.313 (-0.055 - 23.90) | 2.764| 0.0507 |
| Twifo Praso   | 26.822 ± 6.764 (-0.077 - 33.721)| 2.721 | 0.052 | 16.96 ± 4.745 (-0.213 - 26.13) | 2.732| 0.0524 |
Figure 1
Figure 2
Figure 3

A) PfMdr1-N86

B) Distribution of PfMdr1-N86/Age (yr)

C) Prevalence(%)

NS

### Distribution of PfMdr1-N86/Age (yr)

- **C-PfMdr1 N86**
- **E-PfMdr1 N86**
- **A-PfMdr1 N86**
- **T-PfMdr1 N86**

### Prevalence(%)

- **C-PfMdr1 N86**
- **E-PfMdr1 N86**
- **A-PfMdr1 N86**
- **T-PfMdr1 N86**

**NS**

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