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High Prevalence of Asymptomatic Plasmodium falciparum Infection in Primary School Children in the Volta Region of Ghana

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Abstract

Asymptomatic Plasmodium falciparum infections are very common in older children and can pose a great problem for malaria control programs. This study was a cross sectional study-design that looked at the prevalence of Plasmodium falciparum infection among school aged children in five primary schools in 3 districts in the Volta Region of Ghana. Questionnaires were administered and blood samples were collected for malaria detection using RDT and microscopy. Pearson chi square test was used to evaluate the association between P. falciparum infection and other variables in the study. A total of 550 primary school children were enrolled in this study. Three hundred and five children (55.45%) were positive for malaria with RDT and 249(45.27%) were malaria positive with microscopy. Children from Evangelical Presbyterian (EP) primary school in Afegame and Davanu primary schools, both in remote, rural and farming communities had the highest prevalence of P falciparum (RDT, 92, 74.80%, p<0.001; Microscopy, 73, 59.35%, p=0.007) and (RDT, 57, 72.15%, p<0.001; Microscopy, 36, 45.57% , p=0.007), respectively. There was a significant higher prevalence of RDT positive boys than girls seen in this study (152, 61.40%, p=0.023). A significant higher prevalence of P. falciparum infection was seen with RDT, in children whose mothers were farmers (109, 69.87%, p=0.001). There was a high prevalence of asymptomatic P. falciparum infection in the three districts, especially in rural areas. As a matter of urgency, malaria control programs should intensify efforts in these areas to reduce the burden of detrimental asymptomatic infections in school going children.

Introduction

Malaria remains a serious public health issue, especially in endemic areas where pregnant women and children are worst affected [1]. Malaria morbidity and mortality are more pronounced in children in endemic areas, particularly in younger aged children under 5years of age [2].

Malaria can present as clinical infection or subclinical asymptomatic infections [3] [4]. Symptomatic or clinical infections result in more hospital visits and admissions, and drugs utilization. Clinical complications like anaemia, hypoglycemia, coma and death can arise if cases are not adequately and properly managed [5]. Clinical symptoms of *Plasmodium falciparum* usually occur with the less immune, such as children below 5 years of age [5]. Asymptomatic malaria is a result of more developed immunity seen in adults and older children in endemic areas [5]. It has also been argued that asymptomatic infections are needed to build the immunity in children, protecting them from severe diseases [6]. However, asymptomatic infections have been implicated in impacting negatively on the wellbeing of children; causing anaemia, malnutrition and poor intellectual development [7] [8] [9]. More importantly, asymptomatic infections especially in older children create a constant source of infection, with asymptomatic persons acting as reservoir for infection in the population [5] [10]. This has grave consequences for malaria control programs [10].

Malaria control program in endemic areas like Ghana, focus on younger children less than 5 years of age. A major threat to this program is the asymptomatic infections especially in older children, a group that is not targeted by the control programs. Patients with asymptomatic infections do not report to the hospital, hence are not treated. However, the mosquito vectors are capable of picking up the plasmodium from these patients, [11]. Thus there is a strong likelihood that asymptomatic infections might be prevalent among school children in Ghana who are not part of the target group of the national malaria control program making them reservoir of malaria infection. Hence, this study tends to look at the prevalence of asymptomatic *Plasmodium falciparum* among primary school children in three districts in the Volta Region of Ghana.

Methodology

Study area

This study was carried out in three districts of the Volta region of Ghana. The Volta region is one of the ten regions in Ghana located between latitudes 50º45"N and 80º45"N in the southeastern part of the country bounded by Togo on the East and Lake Volta on the West. The districts include, Ho municipal area (which is the administrative capital of the region), Adaklu, and Agotime-Ziope districts. These districts are close to each other and share boundaries. The rainfall pattern is characterized by two rainy seasons referred to as major (from March to June) and the minor (from July to November).

Study population

School children aged 6 to 14 years were selected from primary schools in the three districts. Primary schools in rural or densely populated areas were particularly selected for this study. A total of five primary schools were selected for this study; Freetown primary school in Ho municipal, Dave and Davanu Primary schools in Adaklu district and Evangelical Presbyterian (EP) Primary schools in Afegame and Kpetoe both in Agotime Ziope district. Children, from primary one to six, who signed the assent form and whose parent agreed and signed the informed consent form were recruited into the study.

Ethical clearance

This study was carried out after due ethical clearance and approval was obtained from the Ghana Health Service Ethical Review Committee. (ID NO: GHS-ERC: 29/11/15). Written informed consent was obtained from the parents of children who participated in the study and assent forms were signed by the children before they were enrolled onto the study.

Data collection

This was a cross sectional study involving administration of questionnaires and blood sample collection. These took place from March 14th to April 14th 2016, between 9:30am and 3pm each day.

Questionnaire administration

Children enrolled for this study were asked about their demographic and socio economic status vis-á-vis occupation of both parents, the type of housing, family size, who child stays with and the use of mosquito net.

Blood sample collection

Three mL of blood was collected from each participant by a trained phlebotomist and transferred into ethylenediamin etetraacetic acid (EDTA) anticoagulant tubes. These samples were then transported in an ice chest to the laboratory and on the same day; malaria investigation was conducted using Rapid Diagnostic Test (RDT) and microscopy.

Rapid diagnostic test

Rapid Diagnostic Test (RDT) was done using Bioline SD kit for the detection of histidine rich protein 2 (HRP-2) of *Plasmodium falciparum* (Standard Diagnostics, INC., South Korea). All tests were done with strict observance of the manufacturer instructions. In summary using a sample micro pipette 5 micro litre of the stored blood sample in EDTA tube was added into the small well of the testing kit. Two drops of assay buffer were added into the buffer well and the test result was read in 20 minutes. The presence of two color band indicated a positive result. The presence of only one band within the result window indicates a negative result.

Microscopy

A drop of blood was placed on a labeled slide and without delay a cover slip was used to make a thin smear of blood evenly on the slide making a thin film which was later fixed with methanol for 5 minutes and then stained with 10% Geimsa for 10 minutes and left to air dry. Thick film was made by gently spreading the drop of blood on a slide. It was later stained with 10% Geimsa for 10 minutes. The slide was then drained vertically and left to air dry. When the thick and thin films were completely dry, a drop of immersion oil was applied on the stained slide and later viewed with ×100 high- power microcopy. Two microscopists read the slides independently and a third microscopist was called upon to resolve discordant results. The presence of asexual forms of the parasite in the blood smear was considered positive.

Definition of asymptomatic malaria

Asymptomatic infection was defined as positive RDT or microscopy result with no associated clinical symptoms.

Haemoglobin estimation and anaemia classification

Some of the blood sample collected was used for hemoglobin estimation. Hemoglobin estimation was performed using, automated haematology analyzer (Sysmex, Kakogawa, Japan). In this study, anaemia was classified into severe (Hb<7g/dl), moderate (7-9.9g/dl), and mild anaemia (<11g/dl) [13].

Statistical analyses

A frequency distribution was performed for all the socioeconomic and demographic characteristics of the primary school children in the study (age, occupation of parents, education of parents etc.,), and other characteristics like malaria, anaemia. These characteristics of the children were further analyzed using Pearson χ^2 tests and ANOVA for the comparison of means.

Pearson χ^2 tests analysis was used to investigate the association between *Plasmodium falciparum* and other variables in this study. Analyses were done with 95% confidence interval (CI) and a p value of 0.05 or below (i.e. $p \leq 0.05$) was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

A total of five schools were sampled from Adaklu and Agotime-Ziope districts and the Ho municipality. Figure 1 shows a flow chart depicting the distribution of the primary school children in the three districts. Dave and Davanu primary schools from Adaklu district had 120 and 107 primary school children enrolled with 105 and 90 participating in the study respectively. In Agotime-Ziope district, EP primary school in Afegame had 185 children enrolled and 155 participating, while 270 children enrolled and 238 participated in EP primary school Kpetoe. Freetown primary school from in Ho municipality had 249 children enrolled while 182 participated in the study. A total of 550 primary school children with complete data were sampled for this study. This included 84 primary school children from Dave, 79 from Davanu, 123 from Afegame, 139 from Kpetoe and 125 children from Freetown primary school. Table 1 shows the baseline characteristics of these children. There were more female (301, 54.73%) than male (249, 45.27%) in the study population. Majority of the children's parents were traders (mothers, 334, 60.73%; fathers, 254, 46.18%). The children predominantly live in compound houses (196, 35.64%). Children in this study live with their fathers only (25, 4.55%), mothers only (80, 14.55%), both parents (318, 57.82%), grandparents (70, 12.73%) or with persons other than their parents or grandparents (57, 10.36%).

The prevalence of *Plasmodium falciparum* infection among the primary school children was 55.45% (305/550) with rapid diagnostic test (RDT) and 45.27% (249/550) for microscopy. Table 2 stratified the primary school children according to their RDT and microscopy status. There was a significant higher prevalence of RDT positive boys than girls seen in this study (152, 61.40%, p=0.023). There was also significant heterogeneity in the prevalence of *P. falciparum* infection between the children in the five schools of the three districts in this study (RDT, p<0.001; Microscopy, p=0.007).). EP primary school in Afegame had the highest prevalence of *P. falciparum* infection with both RDT (92, 74.80%)) and microscopy (73, 59.35%), followed by Davanu primary school RDT (57, 72.15%) and microscopy (36, 45.57%). However there was no significant difference in the prevalence of *P. falciparum* among the children at the district level (p=0.12) (Figure 2). Occupation of the mother was significantly associated with RDT positive *P. falciparum* infections, with a higher proportion of infection seen in children whose mothers were farmers (109, 69.87%, p=0.001). Anaemia was significantly associated with *P. falciparum with* a significant proportion of moderately (11, 78.57%) and mildly anaemic children being RDT positive for *Plasmodium falciparum* infections.

Flow chart

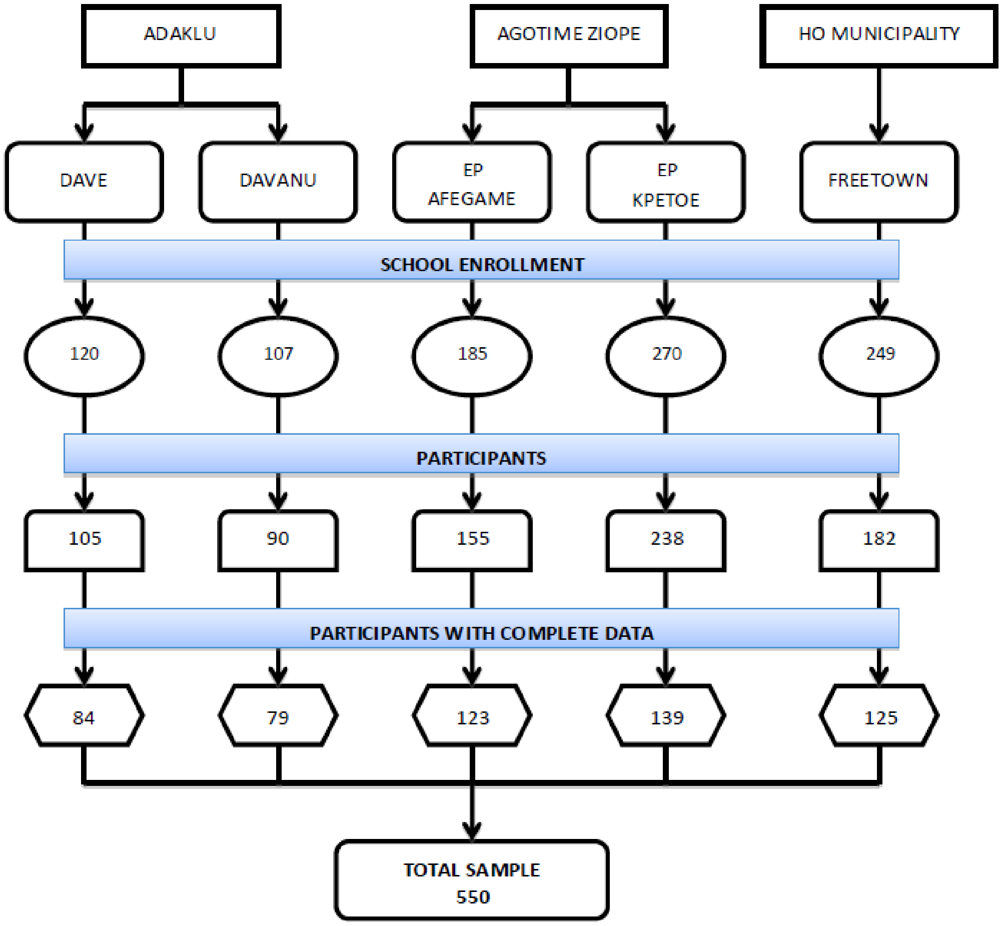

Figure 1

Table 1. General baseline characteristics of primary school children in 3 districts of the Volta region of Ghana.

Parameter	Characteristics	Frequency (n = 550)	Percentage (%)
Sex	Male	249	45.27
	Female	301	54.73
Occupation of father	Trader	254	46.18
	Farmer	170	30.91
	Civil servant	103	18.73
	Unemployed	23	4.18
Occupation of mother	Trader	334	60.73
	Farmer	156	28.36
	Civil servant	34	6.18
	Unemployed	26	4.73
House Type	Family compound	196	35.64
	Single Room	165	30

	Semidetached house	189	34.36
Family size	2	69	12.55
	3	120	21.82
	4	106	19.27
	5	96	17.45
	6	65	11.82
	>6	94	17.09
Who child stays with	Father	25	4.55
	Mother	80	14.55
	Both parents	318	57.82
	Grant parents	70	12.73
	Others	57	10.36
Use of Mosquito Net	Yes	375	68.18
	No	175	31.82
Name of school	Dave	84	15.27
	Freetown	125	22.74
	Davanu	79	14.36
	Afegame	123	22,36
	Kpetoe	139	25.27

Data is presented as frequency and proportions

Table 2. Characteristics of primary school children in 3 districts of the Volta region of Ghana, stratified by Plasmodium falciparum infection status

CHARACTERISTICS		**Laboratory confirmed malaria diagnosis**					
		RDT			**Microscopy**		
		Malaria Positive N = 305 (%)	**Malaria Negative N 245= (%)**	**P value**	**Malaria Positive N = 249 (%)**	**Malaria Negative N 301= (%)**	**P value**
Age (yrs.) Mean ± SD		10.80± 2.7	11.02±2.6	0.601	10.55±2.7	11.18±2.6	0.006
Sex of child	Male	152(61.04)	97(38.96)	0.023	113(45.38)	136(54.62)	0.929
	Female	153(50.83)	148(49.17)		136(45.18)	165(54.82)	
Occupation of Father	Trader	132(51.97)	122(48.03)	0.096	114(44.88)	140(55.12)	0.058
	Farmer	110(64.71)	60(35.29)		88(51.76)	82(48.24)	
	Civil servant	53(51.46)	50(48.54)		4(39.81)	62(60.19)	
	Unemployed	10(43.48)	13(56.52)		6(26.09)	17(73.91)	
Occupation of Mother	Trader	172(51.50)	162(48.50)	0.001	144(43.11)	190(56.89)	0.252
	farmer	109(69.87)	47(30.13)		77(49.36)	79(56.64)	
	civil servant	17(50.00)	17(50.00)		19(55.88)	15(44.12)	

	unemployed	7(26.92)	19(73.08)		9(34.62)	17(65.38)	
House types	Family house	121(61.73)	75(38.27)	0.14	101(51.53)	95 (48.47)	0.137
	Single room	92(55.76)	73(44.24)		69 (41.82)	96(51.18)	
	Semi detached	91(48.15)	98(51.85)		80(42.32)	109(57.67)	
Family size	2	35(50.72)	34(49.27)	0.838	32(46.38)	37(53.62)	0.493
	3	72(60.00)	48(40.00)		59(49.17)	61(50.83)	
	4	58(54.72)	48(45.28)		48(45.28)	58(54.72)	
	5	51(53.12)	45(46.88)		46(47.92)	50(52.08)	
	6	37(56.92)	28(43.08)		22(33.85)	43(66.15)	
	>6	52(55.34)	42(44.68)		42(44.68)	52(55.32)	
Who child stays with	Father only	15(60.00)	10(40.00)	0.992	17(68.00)	8(32.00)	0.267
	Mother only	44(55.00)	36(45.00)		33(41.25)	47(58.75)	
	Both parents	177(55.66)	141(44.34)		139(43.71)	179(56.29)	
	Grant parents	40(57.14)	30(42.86)		34(48.57)	36(51.43)	
	Others	29(50.88)	28(49.12)		26(45.61)	31(54.39)	
Use of Mosquito Net	Yes	220(58.67)	155(41.33)	0.082	172(45.87)	203(54.13)	0.399
	No	85(48.57)	90(51.43)		77(44.00)	98(56.00)	
Name of school	Dave	38(45.24)	46(54.76)	<0.001	38(45.24)	46(54.76)	0.007
	Freetown	60(48.00)	65(52.00)		48(38.40)	77(61.60)	
	Davanu	57(72.15)	22(27.85)		36(45.57)	43(54.43)	
	Afegame	92(74.80)	31(25.20)		73(59.35)	50(40.65)	
	Kpetoe	58(41.73)	81(58.27)		54(38.85)	85(61.15)	
Haemoglobin	Severe anaemia	0(0.00)	1(100.00)	0.006	0(0.00)	1(100.00)	0.334
	Moderate	11(78.57)	3(21.43)		9(64.29)	5(35.71)	
	Mild	60(69.77)	26(30.23)		41(47.67)	45(52.33)	
	Normal	234(52.12)	215(47.88)		199(44.32)	250(55.68)	

Note: SD = standard deviation. P-values derived from Pearson chi-square test for categorical variables and ANOVA for the mean of continuous variables.

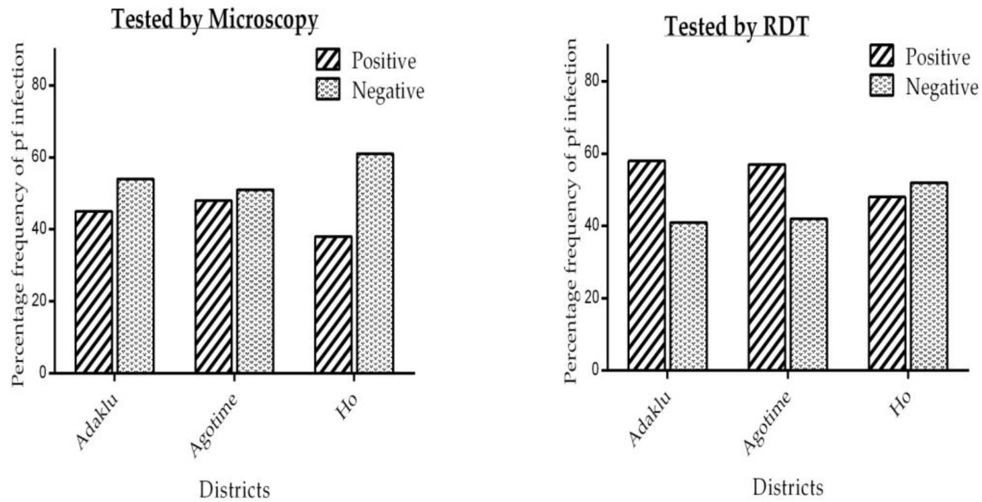

Figure 2. Prevalence of Plasmodium falciparum infections in 3 districts of the Volta region of Ghana.

Discussion

This was a cross sectional study that aimed at evaluating the prevalence of asymptomatic *Plasmodium falciparum* infection among primary school children in three districts in the Volta Region of Ghana. It involved five hundred and fifty primary school children from five primary schools.

There was a high prevalence of asymptomatic *P falciparum* infection in primary school children, as observed by this study, which usually constitutes majority of all infections in malaria endemic environment [13]. Older children especially school going children are usually asymptomatic with *P. falciparum* infection, serving as reservoirs of infection and transmission in malaria endemic populations [10]. So, while progress has been noted in malaria control programs especially for children under five years of age in malaria endemic Africa, increase parasite prevalence has been noted in asymptomatic school-aged children [14] [15]. School-aged children are mainly asymptomatic for *P. falciparum* infection because of the gradually developing partial immunity which reduces the acute clinical manifestation of the disease [13] [10]. This progressive build -up of partial immunity is known to start from the early ages of between 2-5 years, after repeated exposure to infection leading to progressive decline from clinical manifestation, to silent asymptomatic infection in adolescent and adult hood [16]. More so, it is established that in areas of high malaria transmission, peak *P. falciparum* infection is seen in younger age group such as children under the age of five years [17] [18]. Thus, as malaria control and intervention programs are recording decline in malaria transmission intensity in many countries including Ghana, there will be an expected shift of the burden of the disease to older children like school-aged children [19] [20].

The prevalence of *P. falciparum* with microscopy was observed to be 45.3% in this study. The prevalence was very much higher than a similar study conducted in South-Tongu district of the same Volta region of Ghana (9.2%) [21]. Several factors might have contributed to the discrepancy noted in the prevalence of *P. falciparum* infection in the two studies. The earlier study by Ayeh-Kumi et al. [21], was done in 2012 4 years before the present study, suggesting that seasonal changes and alteration in malaria dynamics might have contributed to the difference in prevalence. Again, the different socio-economic factors between South-Tongu district and the three districts considered in this particular study might have also played a role. However, a much older study conducted in a community in Adaklu district in 2010 by Egbi et al [22], showed a similar high prevalence of asymptomatic malaria (68.7%) in school-going children. Prevalence of asymptomatic malaria in children in Dave (RDT=45.24%; Microscopy=45.24%) and Davanu (RDT=72.15%; Microscopy=45.57%) primary schools both in the Adaklu district in this present study was also high. Which suggest that *P. falciparum* infections might have maintained a relatively high prevalence over the past years in the Adaklu district. Children from Afegame and Davanu primary schools had the highest prevalence of *P. falciparum* infection with 75% and 72% for RDT and 59% and 46% for microscopy respectively. The least prevalence was seen in children from EP primary school in Kpetoe. The differences in the urban and rural settings of these areas might explain this finding. The differences in the urban and rural settings of these areas might account for the variations in prevalence. Kpetoe EP primary school is located in the urban area of Agotime-Ziope district, whilst Afegame and Davanu primary schools are situated among the remote, rural farming communities of Agotime-Ziope and Adaklu districts; and therefore are usually the worst hit with *P. falciparum* infection [23] [24] [25] [26]. Children whose parents are farmers recorded the highest prevalence of *P. falciparum* infection. This finding further supports the association between differences in the urban and rural settings and malaria infections. Agricultural activities like farming have been linked to increase *P. falciparum* infection in rural populations in Africa [25] [26]. This is basically due to farming activities like crop irrigation which involve the use of streams or wells to get water in watering cans and sometimes cultivated crops are connected to parallel channels carrying water to irrigate them. All these activities increase the breeding of the anopheles mosquito [25] [26].

Plasmodium falciparum infection was also observed to be higher among boys than girls in this study. This can be attributed to the adventurous urge of boys to stay and sleep outdoors (especially when is dark), exposing their bodies to increase mosquitoes' contact and bites. Females, on the other hand, are less adventurous, are seldom naked or exposed and are mostly indoors engaged in domestic chores especially in the evenings [27]. In addition, some studies have reported that, females have more anti-parasitic immunity than males due to hormonal and genetic peculiarities or factors [30] [31] [32].

Plasmodium falciparum infection with RDT was higher than microscopy in this study. This discrepancy between RDT and microscopy seen in this study has been reported in other studies [33] [34] [35]. These discrepancies are due to the inherent strengths and weaknesses of the two diagnostic methods [33]. Although microscopy is the gold standard for diagnosis of malaria, it is plagued with logistics and technical challenges [33 [35]. RDT, on the other hand it is a very easy test to carry out, requiring little or no expertise to perform and interpret [33]. Though the parasite threshold required to generate adequate titre of antigens to be detected by RDT is about $\geq$200p/μl [33][36], most RDTs especially those specific for *P. falciparum* as used in this study have over 95% sensitivity [37] [38]. Besides, some studies have also shown that RDT is more accurate and sensitive compared to microscopy; with it only below PCR in sensitivity in detecting parasite in the blood [35] [39].

It is important to discuss salient limitations of this study. Firstly, the definition of asymptomatic malaria might have been flawed by the fact that some children might appear well during the period of the study but may become symptomatic later. Furthermore, some children might have recently taken anti-malarial treatment prior to the study but was however not captured. It is also important to note that sample collected might not fully represent the population of children in the various districts. However efforts were made in the selection of children in this study to reduce errors due to sampling bias.

Conclusion

This study reported a relatively high prevalence of asymptomatic *Plasmodium falciparum* infection in three districts in the Volta region of Ghana. Children from primary schools in rural farming communities, like Afegame and Davanu in the Agotime-Ziope and Adaklu districts respectively, had the highest prevalence of asymptomatic infections. There is therefore the urgent need for the regional and districts arms of the malaria control programs to intensify efforts in these areas to help reduce the burden of detrimental asymptomatic infections in school going children.

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