A comparative study of the risk factors of malaria within urban and rural settings in the Sahelian region of Cameroon and the role of insecticide resistance in mosquitoes.

ABSTRACT

Background: Cameroon is among the 11 countries that account for 92 % of malaria infection in sub-Saharan-Africa in 2018, and Maroua III Health District and her environs witnessed a malaria outbreak in 2013 with hundredths of deaths.

Aim: To determine the risk factors of malaria in the urban and rural population and to investigate the level of mosquito's resistance to Deltamethrin and Permethrin.

Methods: It was a cross-sectional community-based study carried out in August, September and October 2019, in which questionnaires were administered to 500 participants, to obtain information on demographics, socioeconomics, behavioral, and environmental factors thought to be associated with malaria infection in both rural and urban settings. Blood samples were collected for diagnosis of malaria and bivariate and multivariate regression analysis were used to identify risk factors of malaria. Mosquito resistance to Deltamethrin and Permethrin were determined using the CDC Bottle Bioassay test.

Results: Malaria prevalence was 52.2 % which was significantly higher (P = 0.016) in rural areas (57.6%) than urban areas (46.8%). The prevalence of asymptomatic malaria was 43.4% and the geometric mean parasite density was 6333.60 parasites/µL of blood. Malaria infection was significantly (P<0.001) associated with children (64.1%) and teenagers (58.1%). Likewise, the infection was significantly associated with the presence of crops around homes (P=0.031), usage of old LLINs for more than three years and in urban settings, with primary education level

(*P*=0.023). The overall mortality of *Anopheles species* was 93.57% (91.19% in rural and 95.83% in urban areas) for deltamethrin which was more sensitive than 83.85% (85.24% in rural and 82.46% in urban areas) for permethrin.

Conclusion: Relevant data for malaria control in Maroua III health district, a typical Sahelian environment has been generated, and indicates that most of the burden of malaria is borne by children and teenagers.

Key words: Malaria, Risk factors, insecticide resistance, Mosquitoes, Maroua, Cameroon.

ABBREVIATIONS

LLINs: long-lasting insecticidal Nets

HD: health district

GMPD: geometry mean parasite density

BACKGROUND

Malaria remains one of the major public health problems in Africa and in 2019, the WHO African Region accounted for 94% of malaria cases globally [1] while in Cameroon all of its inhabitants live in malaria endemic areas with 71% living in high transmission areas [2, 3]. Cameroon is one of the 15 countries that accounts for nearly 80 % of malaria deaths globally [4] and this infection is endemic in Cameroon with the degree of prevalence varying from one ecological zone to another [5]. The proportion of deaths due to malaria is highest in the Northern Regions (26% in Far North and 27% in the North Region) where the malaria season is shortest [2]. In 2013, the Far North Region of Cameroon witnessed an upsurge of malaria infection, where more than 10,000 people were treated for malaria, within a period of one month in Maroua town alone, and more than 600 people lost their lives to malaria, within that period [6]. In an unpublished data from Far North Regional Delegation of Public Health which covers Maroua III health district, 51776 cases of malaria were recorded in the first quarter of 2019, with an infant mortality rate of 37.25% **[**7**]**. The current study is aimed at determining the prevalence, risk factors and roll of insecticide resistance in Maroua III health district.

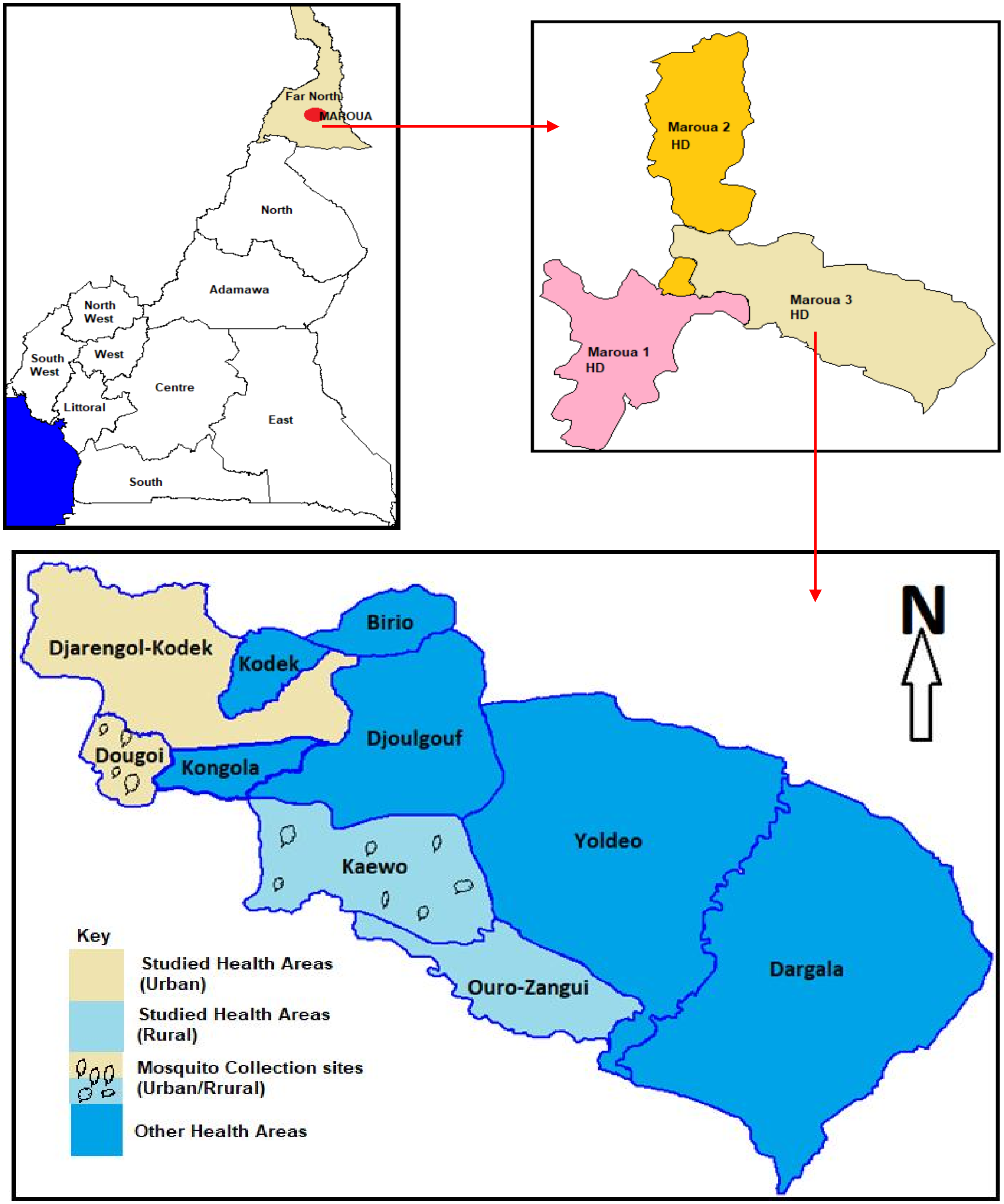
Maroua III health district comprise of urban and rural settlements and is part of the Sahel region of Cameroon with hot semi-arid climate and a lowland topography, with poor drainage pattern, causing stagnant water in most neighborhoods during the raining season. In urban areas, it is common practice to drain sewage from homes into the read, which creates breeding sites for mosquitoes, while in rural areas, poorly constructed houses allow easy movement of mosquitoes in and out of homes and the abundant vegetation around house, may serve as mosquito habitat. It is there for necessary to investigate if these factors are associated with the risk of malaria infection in this health district.

Since 2000, progress in malaria control has resulted primarily from expanded access to vector control interventions particularly in sub-Saharan Africa where long-lasting insecticidal nets (LLINs) usage is the mainstay of malaria prevention strategies and in Cameroon 50% of the population had accessed to LLINs [8]. Mass distribution of LLINs took place in 2011 [9], in 2015 and in 2019 in Cameroon, which includes Maroua III health district [1]. However, insecticide resistance continues to expand due to lack of monitoring for resistance in the local mosquito vector population and resistance to pyrethriods used in LLINs have been reported in the WHO regions of Africa and elsewhere [10]. Inhabitants of Maroua III health district, still complain of mosquito bites during LLINs usage. This may result from resistance of local mosquito vectors to pyrethriods, which permits mosquitoes to land on LLINs and bite users, through the pores of the bed nets. Thus, the need to determine the insecticide resistance profile of local mosquito vectors in Maroua III health district to the pyrethriods; Delthametrin and Permethrin.

METHODS

Study Area

Maroua is the capital of the Far North Region of Cameroon, located on longitude 14.3210° E and latitude 10.5925° N. This study was conducted in localities within Maroua III health district which consist of rural and urban settlements and is divided into ten (10) health areas Figure 1. The health areas are Kodek, Birio, Dargala, Djarengol-kodek, Djoulgouf, Dougoi, Kaewo, Kongola, Ouro Zangui and Yoldeo. Amongst these ten health areas, Djarengol-kodek and Dougoi are urban cities while the rest are villages. This area is part of the Sahel region of Cameroon with hot climate and is characterized by heavy rainfall and strong winds during the raining season which last from June to October and high temperatures during the dry season in the months of November to May. The area is occupied mostly by Maroua city dwellers who work within the city and peasant farmers in the various villages who are mostly Fulani Muslims. The common language spoken in both urban and rural areas is Fulfulde.



Far North
MAROUA
North
Adamawa
North West
West
South West
Littoral
Centre
East
South
Maroua 2 HD
Maroua 1 HD
Maroua 3 HD
N
Djarengol-Kodek
Kodek
Birio
Dougoi
Kongola
Djoulgouf
Yoldeo
Kaewo
Dargala
Ouro-Zangui
Key
Studied Health Areas (Urban)
Studied Health Areas (Rural)
Mosquito Collection sites (Urban/Rrural)
Other Health Areas

Figure 1: Map of Maroua III health district showing the study sites and where mosquitoes were collected

Study Design

A cross-sectional study was carried out involving in-depth interviews of participants from house to house in both rural and urban communities of Maruoa III health district using a structured questionnaire, in the months of August, September and October of 2019. Pretesting of the questionnaire was carried out in Meskine health area which is also found in the Far North Region. Two villages, Kaewo and Ouro-Zangui were randomly selected from balloting, using the names of the eight villages in folded and twisted pieces of paper, and the two urban areas Djarengol-kodek and Dougoi were included in the study for sample collection and questionnaire administration as shown in Figure 1.

The questionnaire captured demographic information, which included age, sex, occupation, level of education, marital status and religion, as well as socio-economic status which include house type, house hold size, and toilet type. Environmental and behavioral characteristics also obtained using the questionnaire included presence of ceiling in houses, if windows are screened with nets, if participant often stays out late into the night, presence of stagnant waters around residence, crop cultivation around residences, presence of bushes around residence, LLINs usage, use of insecticide sprays, and living with animals in homes. People who had taken anti-malaria treatment less than two weeks before the survey were excluded from the study. A systematic sampling technique was use to select households for data collection and in recruiting participants in both rural and urban settlements of the Health District, a household was skip after visiting the nearby house, until a total of 250 samples was achieved for each setting. Body temperature of the participants were measured using an infrared thermometer (Manufactured by Medifriend, RoHS model, England-United Kingdom,) and a finger prick was done using a sterile disposable lancet, to obtain a blood sample for laboratory analysis.

Laboratory Analysis

Diagnosis of malaria was done by microscopy. Thick blood films were made from participants blood samples, air-dried and transported to Kaewo integrated health center laboratory where they were stained with 5 % Geimsa for 25 minutes, rinsed, air dried and stored for onward transportation to the University of Buea life science laboratory for observation. The samples were observed under the light microscope at X100 objective (oil immersion). A smear was declared negative, after observing 100 high power fields and no malaria parasite was seen. Positive slides were quantified by counting the number of parasites against 200 white blood cells and the parasites/µl blood calculated by assuming a leucocyte count of 8000 per microliter as described elsewhere [11].

Ethical considerations

Participants were informed on the potential benefit and aim of the study before obtaining their consent. Parents or guardians gave consent for minors (0- 18 years) by filling out and signing the consent form and assent was also obtained from the minors who took part in the study. Ethical approval was obtained from the Faculty of Health Science Institutional Review Board of the

University of Buea reference number: 2019/979-05/UB/SG/IRB/FHS. Administrative authorization was obtained from the Far North Regional Delegation of Public Health reference number: 374/ar/19/MINSANTE/SG/DRSP/EN/YT/MRA. Administrative authorizations were sought from village heads (Lawanats), quarter heads and community leaders of concerned localities. Written consent was obtained from each studied participant. For most of the participants who were unable to read or write French or English, the information was read and explained to them in Fulfulde language, which they best understand and consent was indicated by thumb printing the consent form. Participants were given full right to participate or refuse participation in the study.

Mosquito collection

Mosquitoes larvae and pupae were collected between August 2019 and October 2019 in Kaewo (rural area) and Dougoi (urban area), which were selected randomly, from rural and urban sites where questionnaire administration and blood sample collection took place (fig 1). In each locality where there was stagnant water, breeding sites were identified and larvae were collected and reared locally by storing the stagnant water in buckets covered with a net, until adults emerged. The adults were fed with 10% glucose solution for 2- 4 days before bioassay was conducted. Morphological identification was done using the identification criteria by Gillies *et al* 1987 [12] and Anopheles species were found to be dominant (>90%).

Insecticide susceptibility bioassay

Mosquito’s resistance to LLINs in both rural and urban settlements of the health district were investigated using the CDC (Centers for Disease Control and Prevention) Bottle Bioassay test technique. The assay determines if the active chemical substance (insecticide used in LLINs-Pyrethroid) is able to kill mosquitoes from a specific location (rural and urban area of Maroua III health district) at a given time (30 minutes).

The CDC Bottle Bioassay test kit comprising of 250ml Wheaton bottles, micropipettes, mouth aspirator, timer, titration flasks, and necessary insecticides were provided by CDC, 1600 Clifton Road. NE, Atlanta, GA, USA. The CDC bottle bioassay is an essential tool for detecting resistance to insecticides, during which five 250-ml Wheaton bottles with screw lids were washed with warm soapy water, rinsed thoroughly with water at least three times and air dried. After drying, the bottles and caps were marked with permanent stickers with one of the bottles marked as control and the rest as test bottles. Using a pipette, 1ml of acetone was added into the control bottle. Using another pipette, 1 ml of the freshly prepared Deltamethrin stock insecticide solution (12.5 µg/mL in acetone solution) was added into the four test bottles. The bottles were caped and swirled until the interior of the bottles were completely coated. The bottles were then uncapped and allow for 4 hours to completely dry in a horizontal position and protected from light, before the introduction of mosquitoes for the experiment.

Mosquitoes that were collected from the different health areas in the Health District (Fig. 1) were used. The mosquitoes were first grown and fed with sugar solution for 3 days before the experiment. Using a mouth filter aspirator, between 10-40 mosquitoes (total of 96 mosquitoes) were gently blown into each bottle (control and test bottles). After filling the 5 bottles with the mosquitoes, the timer was started and at Time 0, the number of dead and/or live mosquitoes were counted and recorded in an appropriate recording form. Dead and/or live mosquitoes were counted and recorded after every 15 minutes for up to 2 hours which marked the end of the experiment. Mosquitoes were considered dead when they can no longer stand to fly. Graphing of the total percentage mortality (Y axis) against time (X axis) was done on a linear scale. During the investigation the diagnostic time (30 minutes) was the most critical value because it represents the threshold between susceptibility and resistance. The procedure was repeated using 21.5 µg/mL of permethrin, dissolved in acetone solution.

Reference diagnostic doses and diagnostic time for the insecticides used were 12.5ug/ml in 30mins for Deltamethrin and 21.5 ug/ml in 30min for permethrin to achieve100% mortality against which results were compared. Resistance was assumed to be present if a portion of the test population survived the diagnostic dose at the diagnostic time (30 minutes). If test mosquitoes survived beyond this threshold, these survivors represent a proportion of the population that is resistant to the insecticide. All mosquitoes that died before the diagnostic time, after exposure to the insecticide-coated bottles were considered as susceptible.

Statistical Analysis

Analysis was done by using Epi Info 7.2.3 and Statistical Package for the Social Sciences (SPSS) version 25 and a p-value < 0.05 was considered significant. The prevalence of malaria in both rural and urban areas of the Health District was computed using the formula;

$$\text{Prevalence} = \frac{number\ of\ positive\ cases\ \text{by}\ microscopy}{Sampled\ size}\ x\ 100$$

Logistic regression analysis was used to identify risk factors associated with malaria by comparing demographic factors, socioeconomic status, behavioral factors and environmental factors with the presence or absence of malaria infection as dependent variable. Bivariate logistic retrogression was used initially to identify significant risk factors at *P*-value <0.05, which were confirmed using multivariate regression analysis. Crude and adjusted Odds Ratios (OR) as well as their 95% confidence intervals (CI) were computed for comparative analysis of rural and urban settlement in the Health District. The QtiPlot was used to compute a graph of resistance analysis, in which percentage mortality in mosquitoes were plotted against time, to determine the mean mortality rate and the result was compared with WHO standard for resistance monitoring which states that a mortality of 98 to 100% at the recommended diagnostic time indicate susceptibility.

RESULTS

Prevalence of malaria in Maroua III Health District

Of the 500 blood samples examined using microscopy, the prevalence of malaria in Maroua III health district was 52.2% Fig 2. The prevalence was significantly higher (*P*=0.016) in rural areas (57.6%) as compare to urban areas (46.8%). Asymptomatic malaria parasitemia in rural areas (49.6%) was more than quadruple symptomatic malaria (8%) and this was similar in urban areas, where asymptomatic malaria infection (37.2%) was also higher than symptomatic malaria infection (9.6%). The prevalence of asymptomatic malaria was significantly higher (*P* = 0.029) in rural areas than in urban settings. In the entire district, the prevalence of asymptomatic malaria was 43.4% as compare to symptomatic malaria which was 8.8 % and 3.2% (16) of the participants who were not infected with malaria had fever with temperature greater than 37.6^0C. The geometry mean parasite density (GMPD) for rural settlement was 6333.60 parasites/µL of blood, that of urban setting was 4333.28 parasites/µL of blood and for the entire health district it was 5333.44 parasites/µL blood.

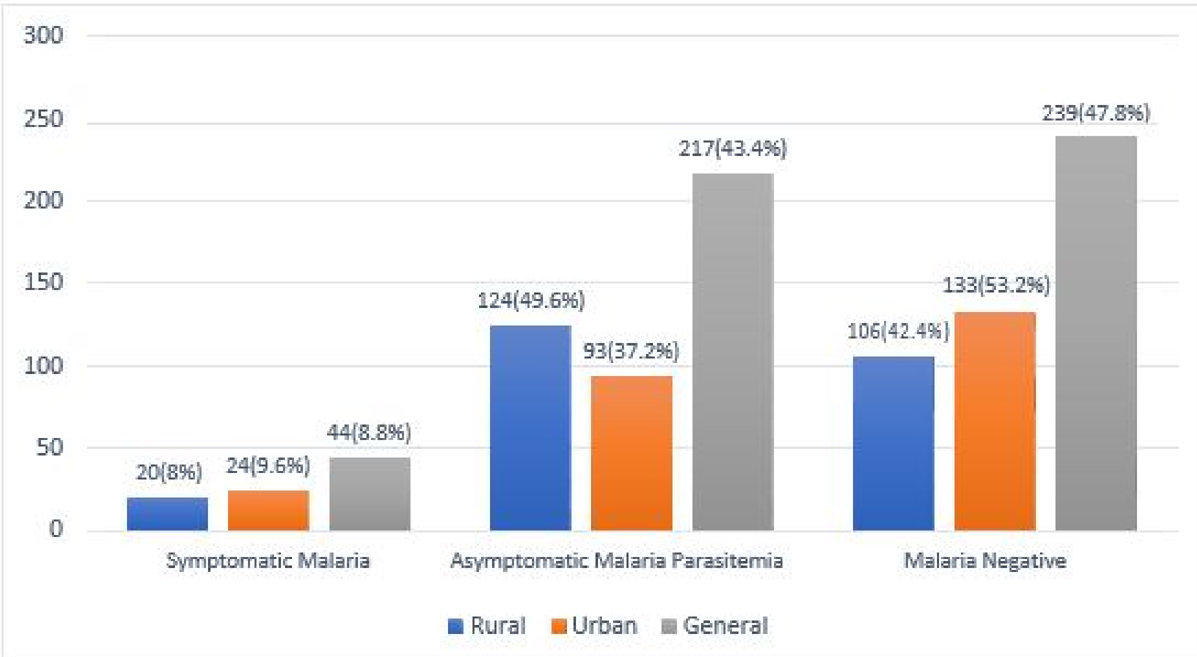


Figure 2: Prevalence of malaria in Maroua III Health District

Association of malaria infection with demographic factors in Maroua III Health District

Bivariate analysis showed that children (64.1%) and teenagers (58.1%) were significantly (P<0.001) more associated with malaria infection, than adults (36.8%) Table 1. Likewise, malaria was more associated with participants who had no formal education (50.3%) than with those with university education (18.2%). Malaria infection was significantly more associated with participants who live in houses build with mud block and aluminum roof (57.5%, P=0.013) and in houses build with mud block and grass roof (57.9%, P=0.016) than in participants who live in cement block houses (44.4%). Also, malaria infection was significantly (P=0.028) more associated with participants who use pit toilets (56.9%) than with those who use water system toilets (47.1%). Multivariate analysis showed that malaria infection was significantly (*P*<0.001) more associated with children and teenagers.

Table 1. Bivariate and multivariate analysis of demographic factors associated with malaria infection

Characteristics	Frequency (%)	Prevalence (%)	P-value chi square	Bivariate analysis		Multivariate analysis	
				COR (95% CL)	P value	AOR (95% CI)	P value
Gender							
Female	145(29.0)	81(55.9)	**0.294**	1	**0.295**	-	
Male	355(71.0)	180(50.7)		0.81 (0.55 - 1.20)			
Total	500(100)	$X^2 = 1.100$					
Age							
> 18 years (Adults)	190(38.0)	70(36.8)		1			
< 2 years(Infants)	11(2.2)	7(63.6)	**<0.001***	3.00 (0.85 - 10.61)	**0.088**		
2 – 10yrs (Children)	170(34.0)	109(64.1)		3.06(1.20 - 4.71)	**<0.001***	2.75(1.79 – 4.23)	**<0.001***
11-18yrs(Teenagers)	129(25.8)	75(58.1)		2.38 (1.51 - 3.76)	**<0.001***	2.26(1.43 – 3.56)	**<0.001***
	500(100)	$X^2 = 30.347$					
Educational level							
No school	157(31.4)	79(50.3)		1			
Primary	268(53.6)	153(57.1)	**0.003***	1.31 (0.89-1.95)	**0.176**	-	
Secondary	53(10.6)	25(47.2)		0.88 (0.47-1.64)	**0.692**		
University	22(4.8)	4(18.2)		0.22 (0.07-0.68)	**0.008**		
	500(100)	$X^2 = 14.251$					
Occupation							
Unemployed	37(7.4)	18(48.6)		1			
Business	46(9.2)	18(39.1)		0.68 (0.28 – 1.63)	**0.385**	-	-
Driver	24(2.0)	8 (33.3)		0.53 (0.18-1.53)	**0.240**		
Farmer	46(9.2)	18(39.1)	**0.001***	0.68 (0.28 – 1.63)	**0.385**	-	-
Health Worker	38(7.6)	14(36.8)		0.62 (0.25 - 1.55)	**0.303**		
Pupil	208(41.6)	127(61.1)		1.66 (0.82 - 3.34)	**0.160**		
Student	92(18.4)	57(62)		1.72 (0.80 - 3.71)	**0.168**		
Teacher	9(1.8)	1(11.1)		0.13 (0.02 - 1.16)	**0.068**		

		X^2 =39.590					
Marital Status							
Married	132(26.4)	40(30.3)	**<0.001***	1			
Single	368(73.6)	121(32.9)		3.46 (2.26 **-** 5.29)	**<0.001***	**-**	**-**
	500(100)	X^2 = 35.065					
Religion							
Christian	76(15.2)	47(61.8)	**0.066**	**-**	**-**	**-**	**-**
Muslim	424(84.8)	214(50)					
	500(100)	X2 = 3.373					
House type/roof							
Cement block	207(41.4)	92(44.4)		1			
Mud with Al sheet roof	160(32.0)	92(57.5)		1.69 (1.12 -2.57)	**0.013***	1.54 (0.87 – 2.72)	**0.136**
Mud with grass roof	133(26.6)	77(57.9)	**0.014***	1.72 (1.11 – 2. 67)	**0.016***	1.68 (0.82 – 3.43)	**0.156**
	500(100)	X^2 = 8.536					
Household size							**-**
1 – 10	311(62.2)	88(28.3)	**0.390**	1			
> 10	188(37.8)	56(29.8)		0.85(0.59 -1.23)	**0.390**	**-**	
		X^2 = 0.739					
Toilet type							
Pit	260(52.0)	148(56.9)	**0.028***	1			
Water System	240(48.0)	113(47.1)		0.67 (0.47 – 0.96)	**0.028***	1.00 (0.55 – 1.81)	**0.995**

COR: Crude Odds Ratio **AOR: Adjusted Odds Ratio.** *** Statistically significant association, p < 0.05** **X^2 = Pearson's Chi square test.**

1 = Reference group **Al = Aluminum**

Demographic factors associated with malaria infection in rural and urban settings

Bivariate analysis (Table 2) revealed that in rural settings, malaria infection was significantly (*P*=0.004) associated with children (68.1%), when compared to adults (46.5%), while in urban settings, malaria infection was significantly associated with children (59.2%, *P*<0.001), infants (75%, *P*=0.018) and teenagers (58.1%, *P*<0.001), when compared to adults (28.8%). In urban settings, malaria infection was significantly (*P*=0.027) associated with participants with a primary level of education (56.1%), when compared to participants who had no formal education (40%), this was not the case in rural settings. In rural settings, malaria infection was significantly associated with unemployed participants (58.3%) when compared to farmers (31.3%), contrary to what obtains in urban setting, where malaria infection was significantly (*P*=0.043) associated with students (63.3%), when compared to unemployed participants (30.8%). In rural settings, malaria infection was significantly (*P*=0.003) associated with singles (62.7%), when compared to married participants (40.4%), likewise in urban settings, malaria infection was significantly (*P*<0.001) associated with singles (57.1%), when compared to married participants (22.7%). Multivariate analysis showed that malaria infection was associated with participants who had a primary level of education (*P*=0.005) and with participants who were single (*P*=0.046) in urban settings, but no risk factor was associated with malaria in rural settings.

Table 2: Bivariate and multivariate analysis of demographic factors associated with malaria infection in Rural and Urban settings

Characteristics	Rural							Urban						
	Frequency (%)	Prevalence (%)	P-value chi square	Bivariate analysis		Multivariate analysis		Frequency (%)	Prevalence (%)	P-value chi square	Bivariate analysis		Multivariate analysis	
				COR (95% CL)	P value	AOR (95% CI)	P value				COR (95% CL)	P value	AOR (95% CL)	P value
Gender														
Female	77(30.8)	47(61.0)	**0.463**	1	**0.463**	-		68(27.2)	34(50.0)	**0.536**	1	**0.536**		
Male	173(69.2)	97(56.1)		0.82 (0.47 - 1.41)				182(72.8)	83(45.6)		0.84 (0.48 - 1.46)			
Total	250(100)	X^2 = 0.539						250(100)	X^2 = 0.384					
Age														
> 18 years (Adults)	86(34.4)	40(46.5)		1				104(41.6)	30(28.8)		1			
< 2 years(Infants)	3(1.2)	1(33.3)	**0.025***	0.58 (0.05 - 6.58)	**0.656**			8(3.2)	6(75.0)	**<0.001***	7.40(1.41- 38.75)	**0.018***	5.74(0.96-34.82)	**0.055**
2 – 10yrs (Children)	94(37.6)	64(68.1)		2.45 (1.34 - 4.50)	**0.004***	1.48(0.82 – 2.68)	**0.196**	76(30.4)	45(59.2)		3.58 (1.92 - 6.68)	**<0.001**	1.66(0.67 - 4.11)	**0.276**
11-18yrs(Teenagers)	67(26.8)	39(58.2)		1.60 (0.84 - 3.05)	**0.152**			62(24.8)	36(58.1)		3.42 (1.77- 6.60)	**<0.001**	0.98(0.37 - 2.57)	**0.967**
		X^2 = 9.356							X^2 =24.497					
Educational level														
No school	82(32.8)	49(59.8)		1				75(30.0)	30(40.0)		1			
Primary	136(54.4)	79(58.1)	**0.625**	2.97 (0.51-17.16)	**0.224**	-		132(52.8)	74(56.1)	**0.002***	1.91 (1.08 - 3.40)	**0.027***	2.34(1.30 - 4.21)	**0.005***
Secondary	26(10.4)	14(53.8))		1.77 (0.49-15.66)	**0.248**			27(10.8)	11(40.7)		1.03 (0.42 - 2.53)	**0.946**		
University	6(2.4)	2(33.3)		2.33 (0.36-15.05)	**0.373**			16(6.4)	2(12.5)		0.21 (0.05 - 1.01)	**0.052**		
		X^2 = 1.752							X^2 =14.995					
Occupation														
Unemployed	24(9.6)	14(58.3)		1				13(5.2)	4(30.8)		1			
Business	13(5.2)	7(53.8)		0.83 (0.21 - 3.24)	**0.793**			33(13.2)	11(33.3)		1.13 (0.28 - 4.48)	**0.867**		
Driver	5(2.0)	4 (80.0)		2.86 (0.28-29.56)	**0.379**			19(7.6)	4(21.1)		0.60 (0.12 - 3.01)	**0.535**		
Farmer	32(12.8)	10(31.3)	**0.041***	0.33 (0.12 - 0.98)	**0.046***	0.43(0.17 – 1.07)	**0.068**	14(5.6)	8(57.1)	**<0.001***	3.00 (0.62-14.62)	**0.174**		
Health Worker	18(7.2)	10(55.6)		0.89 (0.26 - 3.07)	**0.857**			20(8.0)	4(20.0)		0.56 (0.11 - 2.81)	**0.483**		
Pupil	111(44.4)	72(64.9)		1.32 (0.54 - 3.24)	**0.547**			97(38.8)	55(56.7)		2.95 (0.85-10.23)	**0.089**		
Student	43(17.2)	26(60.5)		1.09 (0.40 - 3.02)	**0.865**			49(19.6)	31(63.3)		3.88 (1.04-14.41)	**0.043***	1.94(0.89 – 4.26)	**0.098**
Teacher	4(1.6)	1(25.0)		0.24 (0.02 - 2.64)	**0.242**			5(2.0)	0(0.0)		-	-		
		X^2 =14.615							X^2 =31.635					
Marital Status														
Married	57(22.8)	23(40.4)	**0.003***	1				75(30.0)	17(22.7)	**<0.001***	1			
Single	193(77.2)	121(62.7)		2.48 (1.36 - 4.55)	**0.003***	1.50(0.72 – 3.12)	**0.279**	175(70.0)	100(57.1)		4.55 (2.45 - 8.45)	**<0.001**	2.67(1.02 – 7.01)	**0.046***
		X^2 = 8.915							X^2 =26.248					
Religion														
Christian	19(7.6)	11(57.9)	**0.978**	-	-	-	-	57(22.8)	36(63.2)	**0.005***	1		-	-
Muslim	231(92.4)	133(57.6)						193(77.2)	81(42.0)		0.42 (0.23 - 0.78)	**0.006**		

Characteristics	Rural							Urban						
	Frequency (%)	Prevalence (%)	P-value chi square	Bivariate analysis		Multivariate analysis		Frequency (%)	Prevalence (%)	P-value chi square	Bivariate analysis		Multivariate analysis	
				COR (95% CL)	P value	AOR (95% CI)	P value				COR (95% CL)	P value	AOR (95% CL)	P value
		X2 = 0.001							X^2 = 7.970					
House type														
Cement block	11(4.4)	6(54.5)		1				196(78.4)	86(43.9)		1			
Mud with Al sheet roof	106(42.4)	61(57.5)	**0.977**	1.13 (0.32 -3.93)	**0.848**	-		54(21.6)	31(57.4)	**0.078**	1.72 (0.94 - 3.17)	**0.079**	-	-
Mud with grass roof	133(53.2)	77(57.9)		1.15 (0.33 - 3.94)	**0.829**									
		X^2 = 0.047						X^2 =3.111						
Household size							-							
1 – 10	148(59.2)	88(59.5)	**0.474**	1		-		163(65.2)	79(48.5)	**0.469**	1		-	-
> 10	102(40.8)	56(54.9)		0.83(0.50 -1.38)	**0.474**			87(34.8)	38(43.7)		0.83 (0.49 - 1.40)	**0.470**		
		X^2 = 0.513							X^2 = 0.523					
Toilet type							-							
Pit	250	144(57.6)	-	-	-	-		10(4.6)	4(40.0)	**0659**	1			
Water System	0	0(0.0)						240(96.0)	113(47.1)		1.34(0.37 – 4.85)	**0.661**		
									X^2 = 0.195					

COR: Crude Odds Ratio **AOR: Adjusted Odds Ratio.** *** Statistically significant association, $p < 0.05$** **X^2 = Pearson's Chi square test.**

1 = Reference group **Al = Aluminum**

Environmental and behavioral factors associated with malaria infection

From bivariate analysis (Table 3), malaria infection was significantly (*P*=0.038) associated with participants who live in houses with no ceiling (54.4%), compared to those who live in houses that have ceiling (42.6%). Likewise, malaria infection was significantly (*P*=0.005) associated with participants who live in houses with no window net (53.6%), compared to those who live in houses with window nets (25%). Participants who had crops around their houses (58.1%) were significantly (*P*<0.001) more associated with malaria infection than those who did not have crops around their homes (37.3%). Also, participants who had used their LLINs for more than three years (58.8%) were significantly (*P*<0.001) more associated with malaria infection, than those who had use their LLINs for less than three years (37.4%). Multivariate analysis showed that participants who had crops around their homes (*P*=0.031) and those who had used their LLINs for more than three years (*P*<0.001) were significantly associated with malaria infection in Maroua III health district.

Table 3: Bivariate and multivariate analysis of environmental and behavioral factors associated with malaria infection

Variable	Frequency (%)	Infected (%)	P value chi square	Bivariate analysis		Multivariate analysis	
				COR (95% CL)	P value	AOR (95% CL)	P value
Does house have ceiling							
Yes	94(18.8)	40(42.6)		1			
No	406(81.2)	221(54.4)	**0.038***	1.61(`1.03-2.54)	**0.039***	0.96 (0.56 – 1.62)	**0.867**
Windows have nets							
Yes	24(4.8)	6(25.0)		1			
No	476(95.2)	255(53.6)	**0.005***	3.46(1.35-8.87)	**0.010***	1.86 (0.66 – 5.21)	**0.239**
Often stay out at night							
No	231(46.2)	110(47.6)		1			
Yes	269(53.8)	151(56.1)	**0.057**	1.41(0.99-2.00)	**0.058**	-	
Presence of stagnant H_2O 10-20 m							
No	247(49.4)	120(48.6)		1			
Yes	253(50.6)	141(55.7)	**0.109**	1.33(0.94-1.89)	**0.110**	-	
Crops around house							
No	142(28.4)	53(37.3)		1			
Yes	358(71.6)	208(58.1)	**<0.001***	2.34(1.56-3.47)	**<0.001***	1.82 (1.06 – 3.13)	**0.031***
Bushes around house							
No	89(17.8)	30(33.7)	**<0.001***	1	**1**		
Yes	411(82.2)	231(56.2)		2.52(1.56 – 4.08)	**<0.001***	1.75 (0.90 – 3.42)	**0.101**
Use of LLINs							
No	64(12.8)	34(53.1)		1			
Yes	436(87.2)	227(52.1)	**0.874**	0.96(0.57- 1.62)	**0.874**	1	
Age of LLINs							
Less than 3 years	155(31.0)	58(37.4)		1			
More than 3 years	345(69.0)	203(58.8)	**<0.001***	2.39(1.62-3.53)	**<0.001***	2.42(1.61- 3.64)	**<0.001***
LLINs have holes							
No	275(55.0)	142(51.6)		1			
Yes	225(45.0)	119(52.9)	**0.780**	1.05(0.74-1.50)	**0.780**		
Use Insecticide Sprays							
No	348(69.6)	187(53.7)		1			
Yes	152(30.4)	74(48.7)	**0.298**	0.82(0.56-1.20)	**0.299**	-	
Source of water							
Pipe borne	52(10.4)	19(36.5)		1			
Built wells	419(83.8)	220(52.5)	**0.002***	1.92(1.06 – 3.49)	**0.032***	0.64 (0.29 – 1.41)	**0.271**
Opened-wells	29(5.8)	22(75.9)		5.46(1.97-15.15)	**0.001***	1.59(0.48 - 5.18)	**0.446**
Water storage method							

Animals rearing in homes						
No	17(6.8)	12(70.6)		1		
Yes	233(93.2)	132(56.7)	**0.262**	0.55(0.19-1.60)	0.268	-

Environmental and behavioral factors associated with malaria infection in rural and urban areas

In urban areas, malaria infection was significantly (*P*=0.034) associated with participants who live in houses with no window nets (48.9%) compared to those whose windows have nets (23.8%), base on bivariate analysis (Table 4). In urban areas, malaria infection was also significantly (*P*=0.012) associated with participants who often stay outdoors late into the night (54.8%) compared to those who do not (38.9%). Those who had crops around their homes (58%) were significantly (*P*=0.001) associated with malaria infection in urban areas than those who do not (37.7%). Likewise, in urban areas, participants who live in houses that were close to bushes (54%) were significantly (*P*=0.002) associated with malaria infection than those who did not (33.7%). I rural areas, participants who had used their LLINs for more than three years (61.7%) were significantly (*P*=0.012) associated with malaria infection, compared to those who used it for less than three years (42.6%). Similar results were obtained in urban areas where those who had used their LLINs for more than three years (55%) were significantly (*P*=0.002) associated with malaria infection, than those who had used it for less than three years (34.7%). Multivariate analysis showed that LLINs usage for more than three years was significantly associated with malaria infection in rural (*P*=0.006) and urban (*P*=0.001) areas and the presence of crops around homes in urban areas (*P*=0.017) was significantly associated with malaria infection.

Table 4: Bivariate and multivariate analysis of environmental and behavioral factors associated with malaria infection in rural and urban areas

Variable	Rural							Urban						
	Frequency (%)	Infected (%)	P value chi square	Bivariate analysis		Multivariate analysis		Frequency (%)	Infected (%)	P value chi square	Bivariate analysis		Multivariate analysis	
				COR (95% CL)	P value	AOR (95% CL)	P value				COR (95% CL)	P value	AOR (95% CL)	P value
Does house have ceiling														
Yes	4(1.6)	2(50.0)		1				90(36.0)	38(42.2)		1			
No	246(98.4)	142(57.7)	**0.758**	1.37(0.19-9.85)	**0.757**	-		160(64.0)	79(49.4)	**0.276**	1.34(0.79-2.25)	**0.277**	-	
Windows have nets														
Yes	3(1.2)	1(33.3)		1				21(8.4)	5(23.8)		1			
No	247(98.8)	143(57.9)	**0.394**	2.75(0.25-30.73)	**0.411**	-		229(91.6)	112(48.9)	**0.023***	3.06(1.09-8.64)	**0.034***	1.55(0.50-4.78)	**0.445**
Often stay out at night														
No	105(42.0)	61(58.1)		1				126(50.4)	49(38.9)	**0.011***	1			
Yes	145(58.0)	83(57.2)	**0.893**	0.97(0.58-1.61)	**0.893**	-		124(49.6)	68(54.8)		1.91(1.15-3.16)	**0.012***	1.40(0.82-2.41)	**0.222**
Presence of stagnant H_2O 10-20 m														
No	127(50.80)	69(54.3)		1				120(48.0)	51(42.5)	**0.190**	1			
Yes	123(49.2)	75(61.0)	**0.288**	1.31(0.79-2.17)	**0.288**	-		130(52.0)	66(50.8)		1.40(0.85-2.30)	**0.191**		
Crops around house														
No	4(1.6)	1(25.0)		1				138(55.2)	52(37.7)	**0.001***	1			
Yes	246(98.4)	143(58.1)	**0.182**	4.17(0.43-40.61)	**0.219**	-		112(44.8)	65 (58.0)		2.29(1.37-3.81)	**0.001***	2.08(1.14-3.80)	**0.017***
Bushes around house														
No	0(0.0)	-						89(35.6)	30(33.7)	**0.002***	1			
Yes	250(100)	144(57.6)						161(64.4)	87(54.0)		2.31(1.35-3.96)	**0.002***	1.62(0.87-3.10)	**0.126**
Use of LLINs														
No	24(9.6)	12(50.0)		1				40(16.0)	22(55.0)	**0.257**	1			
Yes	226(90.4)	132(58.4)	**0.431**	1.40(0.61-3.26)	**0.430**	1		210(84.0)	95(45.2)		0.68(0.4-1.33)	**0.259**	-	
Age of LLINs														
Less than 3 years	54(21.6)	23(42.6)		1				101(40.4)	35(34.7)	**0.001***	1			
More than 3 years	196(78.4)	121(61.7)	**0.012**	2.17(1.18-4.00)	**0.013***	2.45(1.30-4.61)	**0.006***	149(59.6)	82(55.0)		2.31(1.37-3.89)	**0.002***	2.70(1.52-4.78)	**0.001***
LLINs have holes														
No	143(57.2)	86(60.1)		1				132(52.8)	56(42.4)	**0.142**	1			
Yes	107(42.8)	58(54.2)	**0.294**	0.76(0.46-1.27)	**0.294**			118(47.2)	61(51.7)		1.45(0.88-2.39)	**0.143**		
Use Insecticide Sprays														
No	156(62.4)	93(59.6)		1				192(76.8)	94(49.0)	**0.212**	1			
Yes	94(37.6)	51(54.3)	**0.407**	0.80(0.48-1.35)	0.406	-		58(23.2)	23(39.7)		0.69(0.38-1.25)	**0.215**	-	

Source of water													
Pipe borne	-	-						52(20.8)	19(36.5)	**0.094**	1		
Built wells	221(88.4)	122(55.2)	**0.029***	1		1		198(79.2)	97(49.5)		1.70(0.91-3.19)	**0.098**	
Opened-wells	29(11.6)	22(75.9)		1.55(1.05-6.22)	**0.039***	3.04(1.21-7.64)	**0.018***						
Water storage method													
Opened Containers	0(0.0)	-						1(0.4)	0(0.00)		-		-
Closed Containers	250(100)	144(57.6)		-	-	-	-	249(99.6)	117(47)				
Animals rearing in homes													
No	101(20.2)	47(46.5)		1				84(33.6)	35(41.7)	**0.246**	1		
Yes	399(79.8)	214(53.6)	**0.202**	1.33(0.86-2.06)	**0.203**	-		166(66.4)	82(49.4)		1.37(0.81-2.32)	**0.248**	-

1 = Reference **COR: Crude Odds Ratio** **AOR: Adjusted Odds Ratio** ***Statistically significant association p < 0.05** **H20 = Water**

Effect of Deltamethrin on *Anopheles* mosquitoes obtained from Maroua III Health District

In Kaewo (rural area), 91.19% of the Anopheles species were susceptible to Deltamethrin insecticide and 8.81% were resistant, after the diagnostic time of 30 minutes fig 3. Contrary to what obtains in Kaewo, a greater percentage of mosquitoes susceptible to Deltamethrin (95.83%) was observed in Dougoi (urban area) and 4.18% (6 mosquitoes out of 96) were resistant after the diagnostic time of 30 minutes. On a whole, 93.51% of mosquito in the entire health district were susceptibility to Deltametrin. The percentage of mosquito from Kaewo, and Dougoi susceptible to Deltamethrin were within the WHO range of 80 to 97% mortality which is interpreted as 'possibility of resistance that needs to be confirmed'.

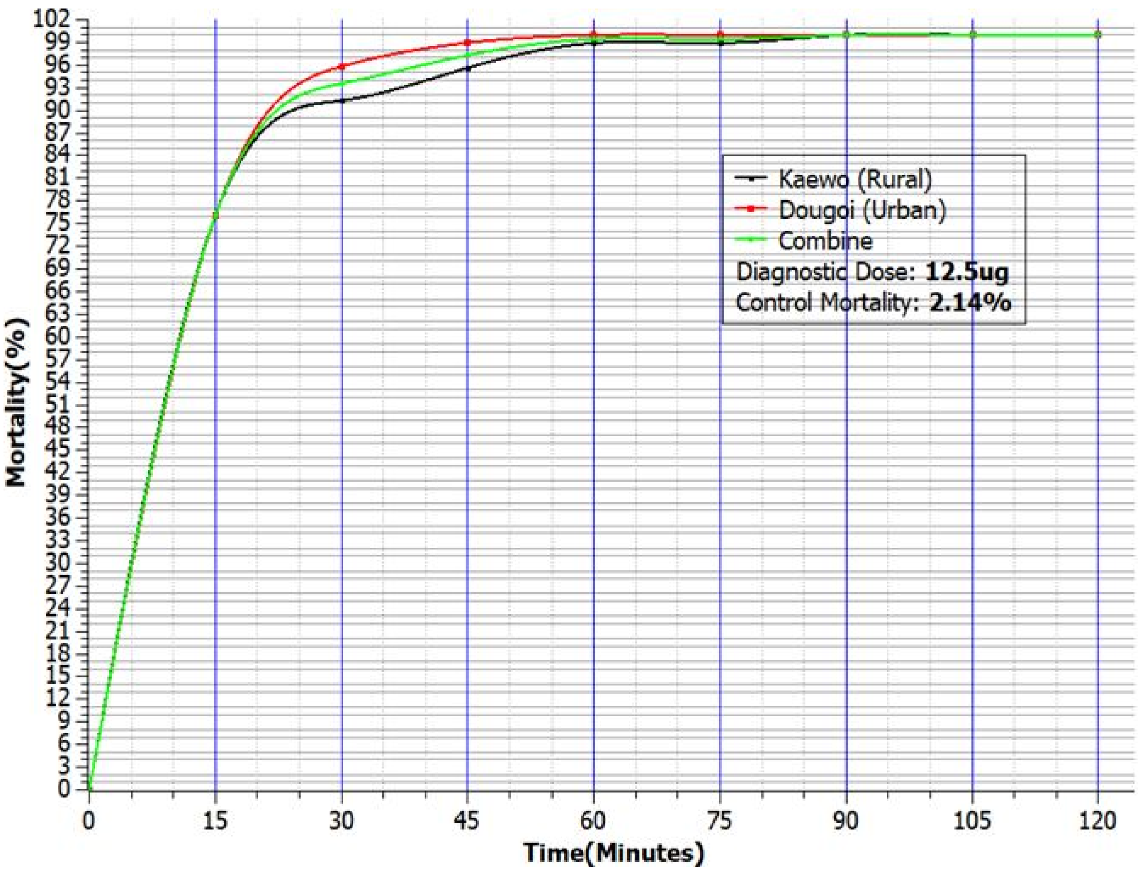


Figure 3: Mortality of *Anopheles* species mosquitoes observed after two hours of

exposure to CDC bioassay bottles treated with Deltamethrine in Kaewo and Dougoi health areas of Maroua III HD.

Effect of Permethrin on mosquitoes obtained from Maroua III Health District

Mosquitoes obtained from Kaewo showed 85.24% susceptibility to permethrin and 14.76% resistance, after the diagnostic time of 30 minutes Fig 4. However, a lower susceptibility of mosquitoes (82.46%) was observed in Dougoi and 17.54% of the mosquitoes were resistant to permethrin at the diagnostic time of 30 minutes. On a whole, a percentage susceptibility of 83.85 was obtained for the Maroua III health district. These percentages of susceptibility are within the WHO range of 80 to 97% mortality which is interpreted as 'possibility of resistance that needs to be confirmed'.

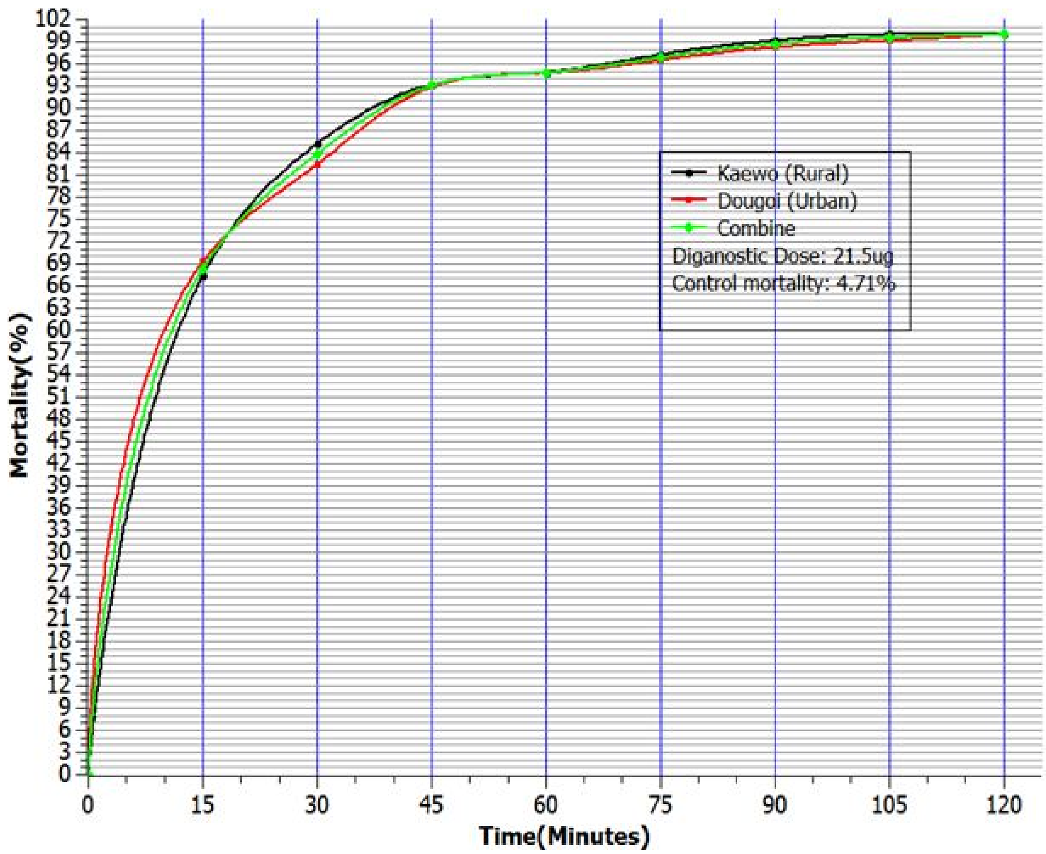


Figure 4: Mortality of *Anopheles* species mosquitoes observed after two hours of

exposure to CDC bioassay bottles treated with Permethrin in Kaewo and Dougoi health areas of Maroua III HD.

DISCUSSION

The present investigation, which was carried out in a Sahel area, with seasonal malaria revealed an overall malaria infection prevalence of 52.2 % which was higher than that reported in Douala (45.47%)**,** the economic capital of Cameroon [13], despite ongoing control measures. The prevalence of malaria was found to be significantly higher in rural areas (57.6%) than in urban settings (46.8%). This is in agreement with the observation that, the level of malaria transmission in any area is generally higher in rural than in urban settings due to environmental factors [14, 15]. Generally, it is considered that suitable breeding sites are scarce in highly populated urban areas, and this leads to a reduction in the frequency and transmission dynamics of malaria. However, evidence of the adaptation of malaria vectors to the African urban environment has been reported in the past [16]**.** This work was carried out in the rainy season, which is a high transmission season with a high number of breeding sites in these areas, leading to an increase in vector density with a high inoculation rates and consequently higher prevalence of malaria infection. Past studies have also reported seasonal variation in malaria prevalence which was higher during the rainy season than in the dry season [17, 18].

Bivariate analysis revealed that malaria infection was significantly associated with the age group 2 – 10 years amongst the rural population, and this age group accounted for 20.4% of the malaria cases in the entire health district. A study carried out in Yagoua and Maga, in the Far North Region of Cameroon, as far back as 1985, also showed that children between 5 to 9 years old, had the highest prevalence of malaria infection [19]. Several studies have shown that parasite prevalence rates in children aged 2–10 years are reliable indicators of malaria endemicity [20, 21]. Base on the classification scheme of malaria endemicity reported elsewhere [22], the infection in rural and urban areas of Maroua III health district can be classified as meso-endemic.

The distribution of the malaria infection in Maroua III health district is heterogeneous and vary greatly between rural and urban settlements. Malaria infection was found to decrease with increasing level of education in urban communities and the infection was significantly associated with those of primary and nursery education level. This may reflect the inept knowledge and poor practices of preventive strategies against malaria and invariably suggest that, sensitization campaigns may have an effect on the burden of malaria. Base on age, malaria was significantly associated with children and teenagers, in the entire health district, following multivariate analysis. In fact, children and teenagers make up 70.5% of the infected population in Maroua III health district. The periodic screening and treatment of children and teenagers in schools can mitigate the burden of malaria. Malaria infection was significantly associated with occupants of houses with walls made of mud. Such houses usually have open eaves which allows entry of Mosquitoes [23]. The absence of ceiling was also associated with malaria infection. Which suggest open eaves and no ceiling greatly facilitate mosquitoes' access to homes, as has been reported in East and West Africa [23, 24,25]. Malaria infection was significantly associated with users of pit toilets. These toilets may be serving as breeding sites for mosquitoes which are released through the open mouth of the pit toilets. Thus, the presence of standard houses in a community, are of great benefit to its occupants and the inhabitants.

Malaria infection was significantly associated with participants who stayed out of their homes late into the night. It is common practice amongst inhabitants of Maroua III health district to sleep in the open air, during hot weather and participants also cited heat as the primary barrier for non-usage of LLINs at night, thus exposing themselves to mosquito bites. Occupants of houses whose windows were screened with nets, enjoyed a significantly better protection from malaria and LLINs users had lower risk of malaria infection, which was not significant. This implies sleeping under LLINs is good, but screening of windows with nets is more beneficial in this study area. This is in agreement with findings in Yaounde, Cameroon where screens on windows were significantly associated with fewer mosquitoes collected indoors [26]. The presence of crops around homes was significantly associated with malaria infection, which corroborates with similar findings in Bolifamba, South West Region of Cameroon, where malaria infection was associated with the presence of bushes around homes [27]. In this study, most of the parameters under investigation were uniform in rural settings, which explains why risk factors could not be identified, unlike in urban settings where practices vary. Living in the same house with farmed animals was not associated with protection from malaria infection, contrary to observations in a neighboring region where 27.2% of the mosquitoes captured were engorged with sheep and cow blood [28].

The effectiveness of insecticide-based malaria vector control interventions in Africa is threatened by the spread and intensification of pyrethroid resistance in targeted mosquito populations. These results suggest the possibility of wide spread resistance of mosquitoes to permethrin and deltamethrin throughout Maroua III Health District. Les campagnes experimentales d'eradication du paludisme dans le Nord de la Republique du Cameroun also reported mosquitoes' resistance to pyrethroids in the 1950s [29]. A review of the evolution of insecticide resistance to the malaria vectors in Cameroon from 1990 to 2017 showed an increase in mosquito's population resistance to insecticides due to an increased use of treated bed nets, insecticide sprays and the use of insecticides in agriculture [30] and this suggest that insecticide resistance can be recognized as a serious threat for control interventions, implemented to fight against malaria. On a whole, mosquitoes in Maroua III Health District showed higher resistance to permethrin than deltamethrin, with values of 83.85% and 93.57% percent susceptibility, respectively. It was also observed that the mortality rate of *Anopheles* in urban areas of the district was higher (95.83%) as compare to mortality in rural areas (91.39%) for the insecticide deltamethrin. This may be due to the high usage of insecticides in agriculture within rural areas, which can promote resistance. Studies conducted on *Anopheles gambiae* distribution and insecticide resistance in Douala and Yaoundé showed a high prevalence of insecticide resistance in mosquitoes originating from agricultural-cultivated sites compared to other sites [31]. Also, resistance was higher against permethrin as compare to deltamethrin suggesting deltamethrin may be more effective than permethrin. This may be due to the fact that the first LLINs distributed in Cameroon, before 2016 were impregnated with permethrin and consequently mosquitoes may have developed resistance to this insecticide over time. A higher mortality of *Anopheles coluzzii* from deltamethrin than permethrin has been reported in the Guinea savanna of Cameroon [32], and similar trend of greater resistance to permethrin over deltamethrin has also been reported in northern Benin [33]. Although studies on multiple insecticide resistance mechanisms in *Anopheles* g*ambiae* populations from Cameroon showed that *Anopheles arabiensis* population sampled in Pitoa health area were more susceptible to permethrin than deltamethrin [34].

CONCLUSION

Malaria is of significant public health importance in Maroua III health district, with a prevalence of 52.2% and children and teenagers bear the greatest part of the burden. Risk factors associated with the infection include; staying out of the house late into the night, usage of LLINs older than three years and presence of crops around homes. Also, mosquitoes in Maroua III health district showed greater sensitivity to Deltamethrin than Permethrin.

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COMPETING INTERESTS

The authors declare that they no competing interest over this work.

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AUTHORS' CONTRIBUTIONS

“RBN: Conceived the topic, participated in writing the proposal, data analysis and interpretation and wrote the first draft of the manuscript. SFN: Collected the data, participated in writing the proposal, data analysis and interpretation and contributed in the write-up of the final manuscript. SNE: Assisted in data analysis and interpretation and contributed in the write-up of the final manuscript. VPKT: Took part in data analysis and interpretation and in the write-up of the final manuscript. All the authors read and approved the final manuscript.”

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