

Volunteer Perception of Mosquito Bite Nodules and Malaria Parasite Density in Abraka, Southern Nigeria

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This study aims to provide baseline data of the indices of malaria prevalence in the study population as well as other malarionometric indices namely use of mosquito nets, subjective observation of mosquito bite nodules, parasite density. The objectives include an assessment of frequency of use of mosquito-net among students of Delta State University, Abraka using the study sample. Frequency of observation of mosquito bite nodules among the volunteers, frequency of use of anti-malarial medication in the last three months and malaria parasite density associated with level of use of mosquito nets. This study will help assess the impact of efforts to roll back malaria in the study population.

Key words: Mosquito Bite, Malarial Parasite, Abraka, Nigeria.

Malaria parasite density is also called parasite count and is a malarionometric index which focuses on assessing the severity of the burden of malaria infection (Kakkilaya, 2004).

This measure of parasitaemia is used in the study of fever threshold and estimation of fever risk, assessing the efficacy of the therapeutic interventions and in stratifying resistance to antimalaria treatment (Trape *et. al.*, 1985; Ejezie and Ezedinachi, 1992; Genton *et. al.*, 1994; Rogier *et. al.*, 1996; Delley *et. al.*, 2000; Montari *et. al.*, 2001; O'meara *et. al.*, 2005; Cheesbrough, 2006).

Calculating parasite density is also important for estimating herd immunity to malaria and for determining end point for interventions such as malaria vaccines, impregnated bed nets and chemosuppression (Schellenberg *et. al.*, 1994; Petersen *et. al.*, 1996; Montanari *et. al.*, 2001; O'meara *et. al.*, 2005).

Visual inspection of blood smears by light microscopy is the method available for quantifying malaria parasite density, doing so by comparing the ratio of counted parasites within a given number of microscopic fields (hpf) against either counted white blood cells (WBC) or counted red blood cells (RBC) within these same fields and then multiplying the ratio by either the measured or estimated density of WBCs or RBCs in the patients' blood (O'meara *et. al.*, 2005).

It is possible that total parasite quantity present, largely determines pathological and clinical manifestation of malaria, yet peripheral measurement of parasites density show a subset and not total parasite load (Armstrong-Schelenberg *et. al.*, 1994; Delley *et. al.*, 2000).

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Above observations suggest that consistency of technique and time of blood collections around noon (12.00-13.00 hours) should give a fairly high and reliable estimation of malaria parasite burden (David *et. al.*, 1983; Grovenor *et. al.*, 1998).

Relating malaria parasite density to fever onset appears to be a major clinical implication of studies on malaria parasite density. Malaria parasite density thresholds have been proposed for the development of fever due to malaria and it appeared to vary from study to study (Miller, 1985; Troupe *et. al.*, 1985; Velema *et. al.*, 1991; Rogier *et. al.*, 1996).

It is important to note that very high parasite levels >100000/uL can exist in persons with no symptoms at all, this is apparently a result of premonition (Hogh, *et. al.*, 1983; Hogh, 1996). Parasite density needed to trigger fever strongly varies from person to person, reflecting the possible role of immune factors on fever threshold (Rogier *et. al.* 1996; Delley *et. al.*, 2000)

An age dependent threshold effect of parasite density on occurrence of fever was described; the threshold varied from a maximum of 2.45 trophozoites /leucocytes at 1 year of age and a minimum of 0.5 trophozoites /leucocytes at 60 years of age. When an individual parasite density crossed the threshold level corresponding to his/her age, the risk of fever multiplied by 44 (Rogier *et. al.*, 1996).

Irrespective of appearance or not of symptoms, asymptomatic carriers of malaria parasites constitute a significant reservoir indispensable in any program to eradicate this pernicious disease. (Aguas *et al*, 2008)

The vector of malaria is the female *Anopheles* mosquito and factors favoring them their breeding and contact with humans include garbage heaps, swamps and stagnant pools of water that are close to houses as well as type of house (cement bulk are more protective than wood-plank houses) and state of mosquito nets (Nkwo-Akenji *et. al.*, 2006). According to MARA (Mapping Malaria Risk in Africa) sponsored project which based its findings on the Garki project (Molinuex and Gramicia, 1980) the only environmental co-variant from the water significantly related to transmission intensity was distance from the water indicating high

transmission in areas within 4 kilometers of water source (Gemperli *et. al.*, 2006).

Control measures in families in Nigeria have been studied. Children in families who used insecticides with coils as preventive measure against malaria, has the highest infection rate 50% followed by those using insecticides alone (37.2%), nets (35.1%), insecticides with nets (33.8%) coils alone 31.6%. Those who used none of the above measures had a rate of 31.6%. Those who used none of the above measures had a rate of 30.7% and those who used all had a rate of 0% (Adeoye *et. al.*, 2001). The importance of environmental factors can again be highlighted by the Zambian experience, where multiple control interventions including environmental management against anopheles larval stages and improvement in hygiene and sanitation reduced the overall malaria incidence and mortality rates by about 50% (Utzingen *et. al.*, 2001). However I did not find any studies on control measures and entomological inoculation among students in Delta state of Nigeria.

The public health impact of malaria control interventions such as the use of mosquito net/ insecticide treated net is estimated drawing from areas of stable malaria transmission (Lengeler C, 2004; Phillips-Howard *et al*, 2003). Such estimations are used to assess the impact of use of nets on disease burden.

In an area of stable malaria transmission, residents often observe mosquito bite nodules in the mornings at waking up. In such an area like Abraka, southern Nigeria, residents have taken to installation of mosquito nets on all shutters including the doors and this is usual practice in several privately owned hostels in the university village. In this study perception of mosquito bite nodules on waking was used as a measure of entomological inoculation and this was expected to be inversely proportional to level of use of the control nets and parasite density.

Frequency of use of anti-malaria medication is an indication of how often an individual develops symptoms suspected to be malaria. The usual perspective in areas of stable transmission in the absence of laboratory confirmation is that any fever is considered to be malaria unless there is another obvious cause, so individuals tend to purchase and take anti-malaria

medication on their own (Diallo *et. al.*, 2006).

Abraka is a semi-urban town located in the Niger-Delta region of Nigeria between latitude 5 degrees, 30 minutes and latitude 6 degrees north of the equator and longitude 6 degrees and 6 degrees, 30 minutes from the Greenwich meridian. The height above sea level is between 50-200 metres. Abraka is covered by rainforest vegetation and receives not less than 4000mm of rain annually with the rainy season starting as early as late January-early February and dry season between late November and early December. The indigenes are Urhobo by tribe and it is host to the main campus of the multi campus state university; Delta State University. The fresh water River Ethiope runs through the town very close to the university hostels. Being a university town and yet semi urban, it is host also to students from different parts of the country who live predominantly in privately owned hostels most of which fall grossly below expected housing standards. Several beach resorts line the course of the river with attendant diversion of water to create pools for recreation. Poor housing and proximity to stagnant water body are known factors in the transmission and prevalence of malaria (Nkuo-Akenji *et. al.*, 2006). This study was carried out in July-August 2008.

MATERIAL AND METHODS

Sampling

A random sample of students of College of Health Sciences Delta State University, Abraka and students presenting for health check and treatment at the university health centre were studied. Sample size calculation using an estimated prevalence rate among students at Eku, Delta State (Nmor and Egwunyenga, 2005) of 11.76% and with a maximum sampling error of 5%. Estimated minimum sample size called for was 159 students. However due to difficulty in obtaining informed consent from asymptomatic students willing to have their blood taken I was just able to obtain samples from 103 students comprising 60 females and 43 males. 93 were asymptomatic (apparently healthy) and 10 were symptomatic. 66 of the subjects lived in off-campus accommodation and 37 within the campuses but all lived within four kilometres of the River Ethiope.

Criteria for Inclusion

Volunteers with no history of fever in the previous two weeks, not on any anti-malaria treatment and not on any antibiotic nor having any objection to being a part of this research having being informed of the purpose, procedure, risks and benefits (informed consent) were recruited for the study. Only asymptomatic subjects were used for this aspect of the study. Ethical permission was sought and obtained from the Director of Health Services, Delta State University, Abraka.

Procedure

A questionnaire was administered at first contact, the first page of which is for informed consent.

Test period for subjects was at contact(week 0), after informed consent. Using a 20 microlitre capillary tubes, capillary blood was obtained from a finger prick between 12.00 noon and 13.00 hours on week 0. A drop of blood was used to make a thin film and three drops of blood was used to make a thick film of diameter about 2.0cm. A slide card was used to standardize the area to be covered by the thick film. Both the thick and thin film was made on the same slide. The thin blood film was air dried and fixed using absolute methanol and stained with Giemsa stain. The thick film shall be air dried and stained using Giemsa stain.

Stained slides were examined using x100 objective, x10 eyepiece and oil immersion. The thick film was used for counting malaria parasites against 200 white blood cells. The thin film was used to identify the species. I identified only *Plasmodium falciparum* species. The proportion of samples having malaria parasites were calculated as parasite rate. Parasite rate is really an index, of prevalence and is defined mathematically as the proportion of persons sampled who has a positive malaria parasite result divided by the number of persons who provided blood samples, expressed as a percentage (Delley *et. al.*, 2000; Schiff, 2002; Eisele *et. al.*, 2006; Hay *et. al.*, 2008). Using an estimated white blood cell count of 7000/ μ l, I calculated parasite density.

Data Analysis

Data was manually analysed and Microsoft excel was used to create the illustrative diagrams.

Table 1. Age Distribution of 103 Subjects investigated

Age Group (years)	Male		Female		Overall	
	No	%	No	%	No	%
15 – 19	17	16.51	13	12.62	30	29.13
20 – 24	21	20.39	35	33.98	56	54.37
25 – 29	3	2.91	9	8.74	12	11.65
30 – 34	2	1.94	2	1.94	4	3.88
>35	-	-	1	0.97	1	0.97
Total	43	41.75	60	58.25	103	100

Table 2. Distribution of average frequency of observation of mosquito bite nodules and use of mosquito nets among male and female asymptomatic subjects.

Average Frequency of Mosquito Bite	Male			Female		
	No	Net Use		No	Net Use	
		Yes	No		Yes	No
<1/year	6	-	6	12	7	5
1/year	3	-	3	6	-	6
>1/year	1	-	1	7	-	7
1/month	4	-	4	6	1	5
>1/month	5	1	4	5	-	5
1/week	7	2	5	12	2	10
>1/week	6	3	3	6	1	5
Daily				3	-	3
Total	36	6	30	57	11	46

RESULTS

Female subjects used mosquito nets more often than males (19.3% :16.7%) and male subjects had more frequent mosquito bites than males (72.2%:56.1%). The level of coverage of mosquito net use is 18.3% . 81.7% of the subjects had no mosquito net coverage and out of the, 56.1% see mosquito bite nodules at least each month. 62.4% of the subjects had frequent ($\geq 1/\text{month}$) mosquito bites and of these 44.8% were male and 55.2% were female, this proportion was not significantly different from a 50:50 distribution. (χ^2 at $p < 0.05$) Interestingly no subject reported negative

observation of mosquito bite nodules in the past 2 years. Several subjects who had mosquito net coverage still had frequent bite nodules , especially from $\geq 1/\text{month}$.

DISCUSSION

The preponderance of young adults aged 15-24 years in this study focuses our research on individuals who are at the peak of their homeostatic reserve and whose immune system are yet to begin to undergo homeostenosis (Resnick, 2001).

Volunteer perception of frequency of observation of mosquito bite nodules is a

Table 3. Distribution of ABO blood group, genotype and use of anti-malaria drugs (within three months prior to study) among male and female asymptomatic subjects.

ABO Blood Group	Male			Female			Total	
	AA	AS	A/M use	AA	AS	A/M use	No	%A/M use
A+	4	-	4	19	-	16	23	87.0
B+	7	2	7	4	1	4	14	78.6
AB+	-	-	-	4	-	3	4	75.0
O+	24	-	18	22	4	14	50	66.7
A-	-	-	-	-	-	-	-	-
B-	-	-	-	-	-	-	-	-
AB-	-	-	-	2	-	-	2	0.0
O-	-	-	-	-	-	-	-	-
Total	35	2	29	51	5	37	93	79.93

Parasite rate (slide positivity rate) among asymptomatic subjects at week 0 is 92.5%. Genotype AS harbored higher parasite densities than genotype AA, at density >1000/mm³, they however used anti-malaria drugs less frequently than genotype AA.

subjective indication of how often an individual is exposed to mosquito bites. Arguably this is expected to increase with absence of mosquito nets. It however appears that increase in the number of subjects having mosquito netted shutters did not significantly reduce or the frequency of mosquito bites. Oral discussion with several subjects revealed that most noticed the presence of nocturnal insects (usually found in the rainforest regions) inside their rooms despite the netted shutters. On inspection of some such residences, it was noticed that there several gaping spaces between the “mosquito net” panel and the windows. With the relative high atmospheric temperature (mostly 30°C and above) and the very, very poor power supply, many students in a bid to combat the heat, resort to leaving their windows ajar while keeping the anti-burglary and “mosquito netted” panels in place. This is coupled with the fact that malaria parasitaemia increases attractiveness of humans to mosquitoes (Lacroix *et. al.*, 2005) and this is dependent on human sweat components; hot humid environments. Poor infrastructure and climate in this case are drivers for transmission especially where most subjects (92.5%) harbor malaria parasites. This reflects ineffectual mosquito net protection and can

Table 4. Distribution of genotype and parasite density at week 0 among asymptomatic subjects.

Parasite Density Range (/mm ³)	Male		Female		Total	
	AA	AS	AA	AS	No	%
0 – 1	1	-	6	-	7	7.5
1 – 100	-	-	2	-	2	2.2
101 – 1000	16	-	11	-	27	29.0
1001 – 10000	17	2	31	4	54	58.1
>10000	-	-	1	2	3	3.2
Total	34	2	51	6	93	100

account for this observed inconsistency between use of mosquito nets and frequency of mosquito bites. It could also be due to a discrepancy of reporting.

The low coverage of nets indicates the level of this aspect of malaria control activities in a University town in 10 years of the roll back malaria initiative. The Nigeria malaria control program delivered about 17 million insecticide treated mosquito nets during 2005 - 2007 and this was estimated to cover only 23% of the population at risk (WHO, 2008). Moreover even the supplied nets were hardly distributed as they lay stored at local

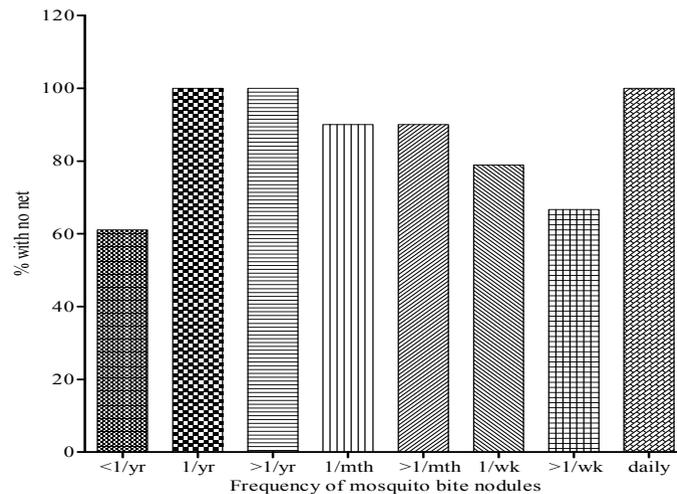


Fig. 1. Proportion of subjects without mosquito net against frequency of bite nodules

government stores. In an endemic rainforest region, net coverage should be high and effective if there is to be any hope of interrupting transmission and control the intensity of malaria in the Niger Delta. Of note here is the widespread scarcity of insecticide treated mosquito nets for beds; in-fact there are no known pharmacies or markets where this can be purchased in the state at this time.

However, given the protective premunition maintained by the presence of parasites in the blood and the penchant of Nigerian government policies to swing from end to end, the goal to control malaria should be such that still maintains some parasite in the population so that premunition is not lost since that would make previously semi-immune persons to become non-immune. This is because the attendant severe morbidity and mortality associated with *falciparum* malaria in non-immune persons is best avoided.

Whereas 70.97% of subjects had used anti-malaria drugs within the last three months, 81.7% had no mosquito net coverage. This leaves a small percentage-10.73% who had no mosquito coverage yet had no malaria attacks in the last three months. This 10.73% represents the effects of anti-malaria immunity.

The infrequency of use of anti-malaria drugs by subjects who are HBAS heterozygote (sickle cell trait) concurs with what is known, that

HBAS carriers have a sixfold reduction in the risk of severe falciparum malaria (White and Breman, 2001).

The parasite rate (slide positivity rate) at the first contact with respondents (week 0) is 92.5%. This agrees with the study in northern Nigeria (Engelbrecht *et al.*, 2000) which found a prevalence rate of 94.2%, and comparable to the studies in pregnant women in southwestern Nigeria like Osogbo—88.2% (Adefioye *et al.*, 2007) and Ibadan—73% (Onyenekwe *et al.*, 2005). It is however higher than the value obtained at the nearby town of Eku over an eight month period (Nmor and Egunyenga, 2005) which found an overall prevalence rate of 69.4% and a rate of 11.76% for students. Prevalence of malaria parasitaemia have been shown to fluctuate with season and to be highest at the height of the rainy season at which time the present study was carried out (Rogers and Randolph, 2000; Delley *et al.*, 2000; Feachem and Sabot, 2007; Hay *et al.*, 2008). The parasite density at week 0 ranged from 84/mm³ in a female respondent who was protected by mosquito nets to 63142/mm³ in a female respondent who was not protected by mosquito nets.

CONCLUSION

There is low mosquito net coverage in this university village in southern Nigeria ten years

into roll back malaria. This is associated with high and frequent use of anti-malaria medication and frequent observation of mosquito bite nodules. Even where there are mosquito netted shutters, coverage was noted to be often ineffectual leading to high reports of entomological inoculation. This study documents impacts of efforts to control malaria in an area of stable transmission in southern Nigeria.

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