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Attractiveness of human subjects to the malaria and lymphatic filariasis vectors in parts of Ebonyi state, Nigeria

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Abstract

Malaria and Lymphatic filariasis (LF) are African's most important vector-borne diseases transmitted by mosquitoes. Attractiveness of pregnant women and Alcohol drinkers was investigated in sentinel sites in Ebonyi State, Nigeria from March to May, 2017. Two cohorts were used; pregnant women and non-pregnant women households, Alcohol drinkers and non alcohol drinkers households. Mosquitoes were caught indoors once a week by Mechanical Aspirator (MA) and Pyrethrum knock down (PKD) techniques and identified using standard morphological keys. Attractiveness was determined by estimating the number of mosquitoes caught and number of blood fed collected and assessed for parity and physiological status. Of the 331 engorged mosquitoes, cohorts for pregnant women (70.78%) and Alcohol drinkers (68.49%) had more mosquitoes than cohorts for non-pregnant women (29.22%) and non alcohol drinkers (30.51%). Overall parity rates of the mosquitoes were 39.6% versus 20.13% for cohorts of pregnant women and non-pregnant women and 51.98% versus 20.90% for cohorts of alcohol drinkers and non alcohol drinkers respectively. Pregnant women and Alcohol drinkers attracted significantly more *Anopheles* (the main malaria and LF vectors in the area) than non-pregnant women and non alcohol drinkers (89.91% versus 22.02%, 83.74% versus 26.83%). However, the cohorts attracted similar proportions of culicine mosquitoes (24.44% versus 46.67% and 47.62% versus 52.38%). Pregnant women who consume alcohol could be at higher risk than non-pregnant women 79.09% versus 29.9%. This study supports the view that some individuals within a community are at risk from mosquito-borne diseases than others and need to be integrated into public health policies for control.

Keywords: Vector attractiveness, human subjects, malaria and filariasis vectors

Introduction

Attractiveness of biting insects is important in medical contexts mostly in the dynamics of transmission of pathogens by mosquitoes. It has been observed that when selecting a human host, mosquitoes have a preference for certain individuals [1]. *Anopheles* species and *Culex quinquefasciatus*, the primary malaria and lymphatic filariasis (LF) vectors have tremendous vectorial capacity which is usually assessed by their strong preferences for feeding on humans [2]. The rate of contact between host and mosquitoes has long been recognized as a crucial determinant of transmission³ and successful controls depends on understanding the interaction [4, 5]. Various factors are known to contribute to the differential attractiveness to biting insects. For example, it has been reported that heat and moisture, olfactory cue [6], distinct body odor [7], diet, general health condition, reproductive status [8, 9] are used by mosquito vectors to locate and orientate towards human hosts.

Studies have shown that pregnant women due to physiological changes are significantly more attracted to *Anopheles* mosquitoes than non-pregnant women [10, 11]. Beer consumption have equally been found to increase human attractiveness to *Anopheles* [2]. While people infected with malaria have been observed to be more attracted to *Anopheles* during transmissible stage in what seem like host-parasite manipulation [13], no unequivocal data exist on filarial parasite manipulation. Predictions of transmission usually assume that all individual are at equal risk from mosquito bites. However, there is evidence that humans vary in their attractiveness to mosquitoes [14, 1]. Thus, host-vector contact is far from random [15].

Despite the co-endemicity of malaria and LF and sharing of exclusively night biting endophagic vectors, human attractiveness to their vectors has been differential and limited to either experimental studies or cross-sectional field surveys.

Thus, in scientific literature there is a conspicuous lack of data on the attractiveness on human subjects to these vectors, a fact which suggest rarity of the attractiveness.

Despite also potential consequences on exposure to malaria and LF vectors, there is a serious lack of data on how diet and pregnancy affect human attractiveness to malaria and LF vectors from natural populations. This study was part of arthropod-borne pathogen research tailored to evaluate the threat that mosquito borne pathogens pose to human health.

Materials and Methods

Study Area

The study was conducted in two (2) sentinel villages; Orijiariafor and Ndiagu Obu in Ohaukwu LGA of Ebonyi State Nigeria (7°31'18"018"N and 5°36'16"015"E).

The areas are typical and represented the highest malaria and LF endemic villages in Nigeria. The sites were chosen based on preliminary immunochromatographic card test (ICT) survey and malaria indicating the presence of both diseases in the area. The ecology of the area has been described in details [16, 17]. The inhabitants are Igbos who mainly live in mud walls and thatched houses. Houses with block walls and or iron sheets roofing are extremely rare. They are peasants growing mainly maize, rice yam and cassava. Livestock kept include cattle and goats with some animals tethered inside human dwellings.

Ethical consideration/Study protocol

Ethical clearance and permission was approved by the Post Graduate Research Board of the Zoology Department of Imo State University, Owerri, Nigeria and Ebonyi State Ministry of Health. All participants were adult volunteers enrolled after the nature of study was explained. Informed verbal consent was obtained from residents of households on mosquito collections. Purpose, procedure and benefits were made known to them. Volunteers were given sufficient time to consider their involvements.

Subject/Volunteers

Two cohorts were used for the study; the households for pregnant women/non-pregnant women and households for alcohol/water drinkers. The cohorts were carefully selected to exclude those houses where animals were tethered inside. Ten pairs of women in 10 different houses each pair consisting of pregnant and non-pregnant women of roughly similar age, weight and parity were chosen. After the study protocol, the 20 women were invited to participate and all gave their informed consent.

The later cohort consisted of adults aged 20-45 years in good health and not using any medication. Twenty eight (28) participants were randomly selected and assigned to the palm wine (n=18) and the water group (n=10). The participants were excluded from smoke, drink or use of deodorants. The beverage used in this study was *Raffia* species (Palm wine) which had stayed till the following day (popularly called overnights). This is the commonest consumed alcoholic drink

in the Eastern part of Nigeria including Ebonyi State.

They are predominantly consumed by males during the evening at specific joints called "Bar Joints". For the study, we bought the kegs of palm wine from three different joints in the villages and had a long night discussion with the participants while the drink. Participants for the water group drank portable sachet water from known source (Dachi water).

Mosquito Sampling Techniques/Laboratory Processing

The density/abundance and parity status of the mosquitoes were assessed in households. Houses were visited twice monthly during the mornings and indoor resting mosquitoes were collected by pyrethrum spray catch (PSC) and mechanical aspirator [18]. The time and period of collection were chosen to catch fully engorge vectors and reflected the habit of vectors. Houses were of similar constructions to avoid the effect of variability. Indoor resting mosquitoes collected in these selected houses were taken to a temporary dissection center as time allowed. Visual identification (for morphology) was made using different keys and characteristics [19] and sorted by abdominal status (fed, unfed, gravid and half gravid). Blood fed mosquitoes were further assessed for parity status by observing the degree of ovarian trachioles [20]. Attractiveness was indicated by estimating the proportions caught and blood fed from each cohort.

Statistical Analysis

Data were analysed by Chi square using Epi Info 6 computer software statistical analysis programme (Version 2003) to compare the indices. Vector density and rates were calculated using percentages.

Results

Of 331 mosquitoes caught and assessed for density and parity, 46.53% (154/331) were from households for pregnant/non-pregnant women while 53.47% (177/331) were from palm wine/water drinkers households respectively. Cohorts for pregnant women (70.78%) and alcohol drinkers (68.49%) had significantly greater mosquitoes than cohorts for non-pregnant women (29.22%) and water drinkers (30.51%).

Overall, parity rates of the mosquitoes were 39.61% versus 20.13% for cohorts of pregnant women and non-pregnant women and 51.98% versus 20.90% for alcohol drinkers and water drinkers respectively (Tables 1 & 3). Furthermore, pregnant women (89.91%) and alcohol drinkers (83.74%) attracted significantly more *Anopheles* species than non-pregnant women (22.02%) and water drinkers (26.83%). However, the cohorts attracted similar proportions of culicine mosquitoes; (24.44% versus 46.67%) for pregnant and non-pregnant women and (47.62% versus 52.38%) for alcohol and water drinkers respectively (Tables 2&4).

Combination of mosquitoes collected from similar attraction households showed significant difference ($P<0.05$). While households for pregnant women and alcohol drinkers had 70.09%, households for non-pregnant women and water drinkers had 29.91% (Table 5).

Table 1: Overall vector densities and physiological status.

Classification of data	Households (%)		Total
	PW	NPW	
1. Species			
<i>An. gambiae</i> sl	71(65.14)	13(28.89)	84(54.55)
<i>An. funestus</i> sl	27(24.77)	11(24.44)	38(24.68)
<i>Cx. quinquefasciatus</i>	11(0.09)	18(46.00)	29(18.83)
<i>A. aegypti</i>	0(0.00)	3(6.67)	3(1.95)
2. Abdominal condition			
Overall parous	61(39.61)	31(20.13)	91(59.09)
Overall nulliparous	48(31.17)	18(8.97)	62(40.26)
3. Parity and Blood meal			
Gravid	49(44.95)	26(57.78)	75(82.42)
Not gravid	12(11.01)	5(11.11)	17(18.68)
Blood fed	58(53.21)	26(57.78)	84(94.31)
Unfed	3(2.25)	0(0.00)	3(3.30)
4. Rate (%)			
Parous	39.61	20.13	59.09

Legend: for table 1 and 2

PW = Pregnant women
 NPW = Non-pregnant women

Table 2: Relative attraction of the study groups for mosquito vectors

Periods	<i>Anopheles</i> (%)		<i>Culex</i> (%)	
	PW	NPW	PW	NPW
Week 1	13(59.09)	9(40.91)	4(57.14)	3(42.86)
Week 2	16(69.57)	7(30.43)	3(37.50)	5(62.50)
Week 3	17(85.00)	3(15.00)	0(0.00)	5(1.00)
Week 4	21(100.00)	0(0.00)	1(100.00)	0(0.00)
Week 5	31(86.11)	5(13.89)	3(27.27)	8(72.72)
Total	98(89.91)	24(22.02)	11(24.44)	21(46.67)

Table 3: Overall vector densities and physiological status

Classification of data	Alcohol consumption (%)		Total
	AD	NAD	
1. Species			
<i>An. gambiae</i> sl	66(53.66)	15(27.27)	81(45.51)
<i>An. funestus</i> sl	37(30.08)	17(30.91)	54(30.34)
<i>Cx. quinquefasciatus</i>	18(14.63)	22(40.00)	40(22.47)
<i>A. aegypti</i>	1(0.81)	0(0.00)	1(0.56)
<i>Mn. Africana</i>	1(0.81)	0(0.00)	1(0.56)
2. Abdominal condition			
Overall parous	92(51.98)	37(20.90)	129(72.88)
Overall nulliparous	31(17.51)	18(10.17)	49(27.53)
3. Parity and Blood meal:			
Gravid	71(57.72)	30(54.55)	101(78.30)
Not gravid	21(17.07)	07(12.73)	28(21.71)
Blood fed	75(60.98)	28(50.91)	103(79.84)
Unfed	17(13.82)	05(9.09)	22(17.05)
4. Rate (%)			
Parous	51.98	20.90	72.88

Legend: for table 3 and 4

AD = Alcohol drinkers
 NAD = Non-Alcohol drinkers/ water drinkers

Table 4: Relative attraction of the study group for mosquito

Periods	<i>Anopheles</i> (%)		<i>Culex</i> (%)	
	AD	NAD	AD	NAD
Week 1	7(70.00)	3(30.00)	5(62.50)	3(37.50)
Week 2	21(100.00)	0(0.00)	4(50.00)	4(50.00)
Week 3	17(70.83)	7(29.17)	2(25.00)	6(75.00)
Week 4	32(69.57)	14(30.43)	6(66.67)	3(33.33)
Week 5	26(74.29)	9(25.71)	3(33.33)	6(55.57)
Total	103(83.74)	33(26.83)	20(47.62)	22(53.38)

Table 5: Combination of mosquitoes collected from similar attraction households.

Species	No (%) collected		Total
	PW/AD	NPW/NAD	
<i>An. gambiae sl</i>	137(83.03)	28(16.67)	165(49.85)
<i>An. funestus sl</i>	64(69.57)	28(30.43)	92(27.97)
<i>Cx. quinquefasciatus</i>	29(42.03)	40(57.97)	69(20.85)
Others	2(40.00)	3(60.00)	3(1.51)
Total	232(70.09)	99(29.91)	331

Legend

PW/AD = household from pregnant women and Alcohol drinkers.

NPW/NAD = households from non-pregnant women and non alcohol/water drinkers.

Discussion

Host preferences has been studied in the laboratory and field with a range of tools. The principle of these devices is that mosquitoes are exposed to two or more host odours simultaneously in a choice situation and then express a positive response to a particular host by an upwind flight and landing [12].

In the laboratory elimination of certain genetic traits by stress, selective breeding and lack of exposure to natural environmental condition including loss of feeding preference are inherent limitations to this [21, 22]. However field survey is conducted by examination of blood meal origin [23] and observing mosquito behaviour in a choice situation [24]. Results of blood meal origin is often biased in favour of the most abundant host species which may not be the preferred host [25]. Observational could highlight the technical difficulties of getting an accurate estimation of the biting preferences of a population of vectors. Behavioral observations with modifications as ours provide a more objective tool for assisting host preferences.

We explored the effects of alcohol consumption and pregnancy on human attractiveness to natural population of malaria and LF vectors. The results demonstrated enough evidence of the enhanced proportion of mosquitoes attraction to pregnant women and alcohol drinkers against the other cohorts (non-pregnant women and water drinkers). Similar results have been differentially reported elsewhere in Gambia and Burkina Faso [10, 26]. This is a vivid reflection that mosquitoes caught engaged in odour-mediated flight (mosquito activation) and mosquito orientation that is odour-mediated anemotactic response. These processes in host seeking are important and facilitate vector-human contacts [27, 6]. Water consumption and non pregnancy affected these behaviour and probably suggested that the other cohorts were responsible for increased attractiveness. Thus strong evidence that alcoholism and pregnancy increased human attractiveness to *An.gambiae* and *Culex quinquefasciatus*, the principal vectors of malaria and LF in Africa.

The differential attractiveness to mosquitoes between human host is still incompletely understood. Increased attractiveness to pregnant women has been linked with increased body temperature and exhaled breath [28], release of pregnancy specific volatile substances in their breath or skin surface [29, 28]. Thus, they may be physiologically more attractive to mosquito and are therefore more exposed than non-pregnant women. This increased attractiveness combined with impaired immunity and the adherence factors in the placenta have been found to enhance susceptibility of pregnant women to malaria [30].

Alcohols are known to be quickly absorbed from the gastrointestinal track into the blood and metabolized [31]. Fifteen minutes have equally been found to be sufficient interval for

alcohol to be present in blood, breath, urine and sweat [32]. While it is clear that alcohol consumption leads to decrease body temperature that of carbon dioxide (CO₂) remains unclear. Though it is widely accepted that CO₂ attracts blood feeding insects, increased exhaled breath with CO₂ analyser could not account for attractiveness [26]. This could be because compounds called attractants also can act as repellents at high concentrations. Probably metabolism of alcohol induce changes in breath or odour markers. Mosquitoes may have also evolved preferences for alcoholics due to reduced host defensive behaviour or nutritious blood meal. Further inquest to eliminate the possibility that other ingredients in palm wine could be driving force apart from alcohol require future studies.

Consumption of alcohol is a widespread phenomenon and most pressing global health priorities [33]. Palm wine used herein is a very popular drink in South Eastern, Nigeria. It is more commonly savored by the Yorubas, Urhobos and Igbos at various joints in the evening after a day's work basically to relax [34]. Thus, increase attractiveness raises crucial issues on control of malaria and LF. It is a feat that local control of both malaria and LF can only be reached if people who are bitten the most by their vectors can be identified. By confirming alcoholics and pregnant women as risk factors, our study has showcased a potential underlying cause of heterogeneous biting and also provided insights into the feasibility of targeted interventions. This findings may be underestimation since alcohol contributes substantially to the global burden of diseases [35] and together with pregnancy both are known to compromise host immune defense against parasites. Nelson and Kolls [36] had observed that moderate or chronic alcohol intake can have strong immunosuppressive effects. By extension alcoholics are both at risk of exposure to mosquito vectors as well as being more vulnerable to the parasites. Interestingly, most men in this part of the country would drink palm wine or other alcohols preparatory to sexual activities with their partners including wives. Also the non fermented sap (called fresh) is proposed to pregnant women and lactating mothers as well as infants whose mothers can not produce the necessary milk for their nursing because of the nutritional wealth [37]. Given the importance of alcohol intake in the groups that are most at risk from malaria and LF, this is a possibility that require urgent attention.

As recommended in the Abuja Declaration [38], treated bed nets should be employed urgently in Ebonyi State, Nigeria and elsewhere to protect pregnant women and alcohol drinkers (including other high risk group especially during the riding season).

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Author's contributions

Conceived and designed the study: AAA, BEBN, JII

Analyzed the data: JII, MON, GU

Wrote the article: AAA, JII, BEBN

Obtained ethical approval: BEBN, GU

Recruited the volunteers for study: MNE, CAI, GU.

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