

ENTOMOLOGICAL SURVEY OF MOSQUITOES RESPONSIBLE FOR THE TRANSMISSION OF LYMPHATIC FILARIASIS IN Biase Cross River State, Nigeria

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This work was carried out in Biase, Cross River State, Nigeria from March to June, 2014. A total of 1296 mosquitoes made up of 795 (61.3%) *Culex* species, 342 (26.4%) *Anopheles* species, 102 (7.9%) *Aedes* species, and 57 (4.4%) of other Genera were caught using human bait and pyrethrum spray methods. Of the 1296 mosquitoes caught, 804 (62%) were caught in the rainy season while 492 (38%) were caught during dry season. The number of mosquitoes caught during dry and rainy seasons was statistically significant ($\chi^2 = 0.62$, $P < 0.05$). The mosquitoes were segregated into different species and dissected to unveil any microfilaria in the thoracic, abdominal, and mouth part regions. Out of 1213 mosquitoes dissected, 24 (1.9%) had developed stages of L₁, L₂ and L₃ of *W. bancrofti*, 8 (0.6%) had L₃ larvae. *Anopheles spp* had the highest number of mosquitoes infected 11/329 (3.3%), *Culex spp* had a 13/743(1.7%) while out of the 98 *Aedes* species dissected none had any filarial worm seen. Ten (41.6%) larva was found in the head of both *Anopheles* and *Culex*, while 8(33.3%) and 6 (25%) were found in the thorax and abdomen respectively. The two types of mosquitoes infected was statistically significant ($\chi^2=8.28$, $P>0.05$). There was a positive correlation between the infection rate among mosquitoes in the dry and rainy season ($r = 0.85$, $P<0.05$).The distribution of filarial larva (L1, L2 and L3) in the body of mosquitoes showed that Out of the 11*Anopheles* infected, 4 (1.2%) filarial worms were found in the head, 5 (1.4%) in the thorax and 2(0.5%) in the abdomen while out of the 13 *Culex* mosquitoes infected, 6 (0.7%) filarial worm were found in the head, 3(0.4%) in the thorax and 4 (0.5%) in the abdomen. The highest number of filarial worms seen was L3 with 17 (70.8%), followed by L1 with 5 (20.8%) and lastly by L2 with 2 (8.3%). This study has shown that *Anopheles* species and the *Culex* species are the vectors of lymphatic filariasis in the study area.

Keywords: Mosquitoes, Transmission, Lymphatic filariasis, Biase

BACKGROUND

Lymphatic filariasis is caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. *Wuchereria bancrofti* is responsible for Ninety percent of cases of lymphatic filariasis cases found in the tropics and sub-tropical area's worldwide (WHO, 2002). Lymphatic filariasis is transmitted by *Anopheles*, *Culex*, *Aedes*, *Ochlerotatus*, and *Mansonia*(Addis et al,2000). The vectors feeds at night and the microfilariae are present in the blood in the greatest number around midnight hence exhibit nocturnal periodicity. The global burden of lymphatic filariasis is not known and its endemicity and prevalence is ongoing. Lymphatic filariasis

(LF) is endemic in 83 countries with 120 million people infected (WHO, 2002). Lymphatic filariasis prevalence in Africa is striking and about 40 million people are affected in the sub-Saharan region alone (WHO, 2002). Worldwide, Africa account for 40% of all cases of lymphatic filariasis (Ottensen et al, 2000; WHO, 1999). In recent decades the epidemiology of lymphatic filariasis has varied tremendously. The disease was controlled or eliminated in many islands of the Pacific, and was reduced dramatically in China. India and Africa are still the most endemic areas with lymphatic filariasis worldwide and have witnessed few changes in recent decades (Dreyer et al, 1997).

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Therefore lymphatic filariasis control could be achieved only through different strategies of integrated vector control along with Mass Drug Administration (WHO,2002). Lymphatic filariasis elimination programme will be based on the studies of the mosquito vectors responsible for the transmission of the diseases in endemic communities (Molyneux et al, 2004). This research work intends to identify the species of mosquitoes responsible for Lymphatic filariasis transmission in the study area of Biase, LGA, CRS, Nigeria

MATERIAL AND METHODS

Biase local government is made up of 11 wards namely: Abayong, Akpet/Abini, Etono/lkum, Adim, Ehom, and Mbiakpan, ° Agwagune, Umon and Ekei. (Total population 89737 males and 79446 females, census 2007). Biase local government is bordered in the north east by Yarkur and OBUBRA local government, in the south by AKAMKPA and ODUKPANI local government and in the west by ABIA State. There are 19 health centres and 11 health posts in the whole Biase local government. It is a large local government with a population of 169183 (89737 males) and (79446 females) according to the census carried out in 2007. The major occupation of the population is agriculture and fishing for those living in riverine areas. The administration of Ivermectin for the control of Onchocerciasis is ongoing in the Local Government of Biase. The migration of inhabitants for employment in urban areas is not common.

Capturing and dissection of mosquitoes

Twenty houses in each ward were randomly selected for the catching of mosquitoes after proper explanation of the aim of the research to the head of each of the household selected. The Permission to enter each of the household was sought and they had the right to refuse or withdraw at any point of time of the study. Mosquitoes were captured for four months (March-June) using human landing catches (HLC), and pyrethrum spray catches (PSC).

Human Landing Catches

In each of the selected compounds, six people (in each ward) were recruited to catch night biting mosquitoes. Three people sat indoors and the other three outdoors between 7 p.m. to 10 p.m. During the exercise the team outdoor will rotate with the indoor team after one hour of collection to compensate for individual differences in attractiveness. The catching of mosquitoes as they were landing on the legs of residents was facilitated using the electric mosquito swatter. The captured mosquitoes were segregated in paper cups and labeled depending on the area of captivity whether indoor or outdoor.

PYRETHRUM SPRAY CATCHES

In each of the wards, randomly selected rooms were sprayed with pyrethrum insecticide formulation (Raid Insecticide) and allowed for 10 minutes. An insect collector searched and picked knock down mosquitoes and placed them on moist filter paper in labeled petri dishes. They spent at least 15 minutes in each room, searching for all the resting places of mosquitoes such as, walls, roof, hanging objects and beneath the surfaces of fixed objects.

Identification and Dissection of mosquitoes

The captured mosquitoes were maintained according to household number and were immediately transported to the laboratory and for identification and dissection on the same day. Both live and dead mosquitoes were stored in paper cups until dissection (up to 10 hours after collection). The identification of different species of mosquitoes was made visually and they were categorized as Anopheles species, Culex species, Aedes species and 'other' (those that were destroyed during the process of catching and could not be identified). The mosquitoes were anaesthetised and were segregated according to species. They were dissected individually to determine *W. bancrofti* infection status to include stage and location of the parasites in the body of the mosquito. Each mosquito was divided in three parts (head, thorax and abdomen) and were placed in three separate drops of normal saline for microscopic identification.

Each part was gently macerated with needles and was examined under a compound microscope for the presence of microfilaria. Each stage of filarial larva seen in each part of the body was recorded. The infection rate was the proportion of dissected mosquitoes positive for first (L₁) second (L₂) or third (L₃) stage larva, and the infectivity rate was the proportion of L₃ stage larva seen in the mosquitoes. The biting rate was the number of mosquitoes attempting to take a blood meal per person while the infective biting rate was the number of mosquitoes that will have at least one infective larva.

RESULTS

The mosquito survey responsible for the transmission of lymphatic filariasis was carried out in Biase LGA, CRS, Nigeria and the following results were obtained.

Table 1 shows the aggregate results of mosquitoes caught in the dry and rainy season by ward. A total of 1296 mosquitoes were caught during the two seasons. Etono/lkun had the highest number of mosquitoes caught both in the dry and rainy season 198 (15.5%), followed by Adim 185 (14.3%) and the least number of mosquitoes caught were in Ehom 102 (7.9%). The number of mosquitoes caught during the dry season dropped compared to that of the rainy season. Culex spp were more in numbers than Anopheles spp, Aedes spp and other genera. There was a statistically significant difference in the number of mosquitoes caught in the dry and rainy season ($X^2=0.62$, $P<0.05$).

The Aggregate results of mosquitoes caught in the dry and rainy season by type of mosquitoes are presented in Table 2. Of the 1296 mosquitoes caught, 804 (62%) were caught in the rainy season while 492 (38%) were caught in the dry season. Culex species 795 (61.3%) were highest in number, followed by Anopheles species 342 (26.4%), Aedes species 102 (7.9%) and lastly by Others 57() which denotes the species of mosquitoes that could not be identified because during the process of catching them, their body structures were destroyed including their wings and legs. The prevalence of infective stages of filarial worm in the body part of mosquitoes dissected to unveil the infected mosquitoes is presented in Table 3.

Of the 1213 mosquitoes dissected, only 24 (1.9%) were infected with L₁, L₂ and L₃ while 8 (0.6%) were infective (that is carried L₃). Fifteen (4.5%) and 9 (2.7%) were infected in the rainy and dry season respectively. The correlation analysis showed a positive correlation between the infection rate among mosquitoes in the dry and rainy season ($r=0.85$, $P<0.05$).

Table:1 Distribution of mosquitoes caught in the study area in the dry and rainy season by wards

Ward	March	April	May	June	Total
Abayong	26	19	33	42	120
Etono/lkun	49	32	62	55	198
Adim	32	41	58	54	185
Mbiakpan	27	33	45	62	167
Aguagune	17	21	37	40	115
Umon	31	38	56	44	169
Akpet/Abini	15	29	37	41	122
Erei	13	27	43	35	118
Ehom	24	18	27	33	102
Total	234	258	398	406	1296

Table : 2 Aggregate results of mosquitoes caught in the dry and rainy season by type of mosquitoes

Types of mosquitoes	Dry season (March-April)NO(%)	Rainy season (May-June)NO(%)	Total
Anopheles	132	210	342
Culex	294	501	795
Aedes	45	57	102
Others	21	36	57
Total	492(38)	804(62)	1296

There was a statistically significant difference in the infection rate between the two seasons ($X^2=0.87$, $P<0.05$). The prevalence of infective stages of filarial worm in mosquitoes dissected in the dry and rainy season is shown in Table 4. Twenty four (1.9%) had developing stages L₁, L₂ and L₃ of *W. bancrofti* larvae. Of the 24 mosquitoes found with infective stages, 8(0.6%) had L₃ larvae. *Anopheles spp* had the highest number of mosquitoes infected 11/329(3.3%), *Culex spp* had a 13/743 (1.7%) while out of the 98 *Aedes* species dissected none had any filarial worm seen. There was no statistically significant difference between the two types of mosquitoes infected ($X^2=8.28$, $P>0.05$).

The distribution of filarial larva (L₁, L₂ and L₃) in the body of mosquitoes in the dry and rainy season is presented in Table 4. Of the 24 infected mosquitoes, 10(41.6%) larva was found in the head of both *Anopheles* and *culex*, while 8 (33.3%) and 6 (25%) were found in the thorax and abdomen respectively. Out of the 11 *Anopheles* infected, 4 (1.2%) filarial worms were found in the head, 5 (1.4%) in the thorax and 2 (0.5%) in the abdomen while out of the 13 *culex* mosquitoes infected, 6(0.7%) filarial worm were found in the head, 3 (0.4%) in the thorax and 4 (0.5%) in the abdomen. The highest

number of filarial worm seen was L₃ with 17(70.8%) ,followed by L₁ with 5(20.8%) and lastly by L₂ with 2(8.3%).

$$\text{Infection Rate: } \frac{\text{Total number of mosquitoes infected}}{\text{The total number of mosquitoes dissected}} = \frac{24}{1213} = 0.02$$

$$\text{Infectivity rate: } \frac{\text{Total number of mosquitoes with L}_3}{\text{The total number of mosquitoes dissected}} = \frac{8}{1213} = 0.006$$

DISCUSSION

Filariasis is a major public health problem in Nigeria. With the continuous change in environmental factors, urbanization and availability of newer diagnostic tools (Chanteau, et al, 1994) the estimation of a 40% global burden due to filariasis in Nigeria (Michael et al, 1996) may be an understatement. The high prevalence of infection and infectivity recorded in the

mosquitoes indicates that previous annual mass treatment with ivermectin alone for the control of Onchocerciasis could not reduce or interrupt the transmission of *W. bancrofti* in the study area, where *Culex spp* and *Anopheles spp* appears to be the main vectors.

In a related study carry out in Burkina faso by kyelen *et al.*, (2003) , a 5years annual treatment with ivermectin alone (targeted at Onchocerciasis) could not reduce or interrupt the transmission of *W. bancrofti*. The results presented here describe the relative contribution of *Anopheles spp* and *Culex spp* to LF transmission in Biase local government. *Anopheles spp* (3.3%) appeared to harbor more developing stages of the larva than *Culex spp* (1.7%) . No developing stages of parasites were found in any of the *Aedes spp* and other genera of mosquitoes that were not identified. These findings differ with the one done in Central Nigeria by Audrey *et al* (2007) to determine the contribution of different mosquito species to transmission of lymphatic filariasis where only *Anopheles species* (2.9%) had developing stage L₁,L₂ and L₃ of *W. bancrofti* larvae.

In this study, the number of mosquitoes caught during the two seasons using human landing catches (51.9%) was higher than the pyrethrum spray catches (48.1%). This study agrees with the work done by Daniel *et al* (2007) in three villages in Ghana within the Winneba district where human landing catches accounted for 58% followed by Pyrethrum spray catches with 41% and light trap catches 0.3%. In the months of the dry seasons (March, April) the number of mosquitoes caught were smaller than the number caught in the months of the rainy season (May-June). There were seasonal fluctuations in abundance of mosquitoes in the two seasons as the total number of mosquitoes caught in the rainy seasons were more in number than those caught in the dry season. The highest number of infected mosquitoes recorded was also found during the month of June .

This also explained the reasons while the numbers of mosquitoes infected in the rainy season were more in number than those in the dry season. The number of infective stages (L₁-L₃) found in the body of mosquitoes reflect also the infection status of the exposed population. The number of larva (L₃) found in the heads of the mosquitoes reflects the infectivity status of the mosquitoes while the number of larva found in the head; thorax and abdomen reflects the infection status. The prevalence of mosquito infection with the presence of the third stage larvae found in the mosquitoes is an indication of the infectious status of the residents of the sampled compounds.

The oviparous mosquitoes usually search for suitable oviposition site before developing into L₃ and therefore these mosquitoes will leave the compound where they took the infective blood meal to other compounds where they will bite new people and the infection will continue to spread. As the infected females enter other compounds in search of blood meal , the infection becomes randomly distributed throughout the community since subjects live in a cluster setting in the ward and the possibility of one infected mosquito flying from one compound to another is possible.

It was also observed that because of power failure in the local government most participants remain outdoors in their compounds till late hours (between 11pm-12midnight) and most of the time men do stay half naked because of heat. These periods that participants remain outside coincides with the biting period of the vectors thus better transmission potentials. The mosquitoes bite mostly the legs and the hands around the fingers which are always exposed. Thus, this study

has shown that mosquito infectivity recorded is better indices of the community transmission and will likely mirror the human infection status in any particular settings.

In a study in Papua New Guinea, Bockarie *et al* (2002) showed that vectors control in the communities and mass drug administration aimed at reducing the microfilaraemia and mosquito infection had no influence in the abundance of human –biting mosquitoes and therefore transmission potentials remains unacceptably high. The different mode of controls employed in the local government by participants did not appear to be specific and accurate. The participants who appear to be using mosquito coils, bed nets, and insecticide only applied when they are about to go to bed meanwhile they had been exposed to mosquito bites before going to bed. Most of them lay outdoors because of heat till late hours in the night during which mosquitoes bite them at random making control measures cumbersome. So control of lymphatic filariasis in these areas will be effective only if integrated control are applied (Plaisier *et al*, 2000).

To achieve this aim, Lymphatic filariasis and Onchocerciasis need to be mapped out in areas where they are co-endemic. The benefits of integrated controlled programmes need to be articulated to the donor community, local programme managers and international technical committees (Molyneux *et al*,2004).

CONCLUSION AND RECOMMENDATION

While the global elimination program of lymphatic filariasis is ongoing, highly sensitive and specific diagnostic assays are necessary to monitor and control the program . The presence of the three vectors of lymphatic filariasis (*Culex*, *Anopheles* and *Aedes*) and with the proportion of *Culex* and *Anopheles* infected indicated that Lymphatic filariasis is an important public health problem in the study area.

REFERENCES

- Addis D, Dreyer G 2000. Treatment of lymphatic filariasis. In TB Nutman, Lymphatic Filariasis, Imperial College Press, London, chap. 5, p. 103-125.
- Audrey Lenhart, Abel Eigege, Alphonsus Kal, D Pam, Emmanuel S. Miri, George Gerlong, J. Oneyka, Y. Sambo, J. Danboyl, B. Ibrahim, Erca Dahl, D. Kumbak, A. Dakul, MY. Jinadu, John Umaru, Frank O. Richards and Tovi Lehmann. The Carter Center Atlanta, GA, USA and Jos, Nigeria. *Contribution of different mosquito species to the transmission of Lymphatic Filariasis in Central Nigeria: Implication for monitoring infection by PCR in mosquito pools. Filial Journal 2007, 6:14 doi: 10.1186/1475-2883-6-14*
- Bockarie MJ, Tisch DJ, Kastens W, Alexander ND, Dimber Z, Bockarie F, Ibam E, Alpers MP, Kazura JW (2002): Mass treatment to eliminate filariasis in Papua New Guinea. *England Journal of Medicine*, 347:1841-1848.
- Chanteau S, Moulia-Pelat JP, Glaziou NL (1994). Og4C3 circulating antigen: a marker of infection and adult worm burden in *Wuchereria bancrofti* filariasis. *Journal of Infectious Disease*; 170: 247-250.
- Daniel H, Duerr P, Dietz K, Eichner M (2007). Behavioural aspects of the control of parasitic diseases. *Bulletin of World Health Organization*; 57: 499-506.
- Dreyer G, Noroes J, Addiss D (1997). The silent burden of sexual disability associated with lymphatic filariasis. *Acta Tropical*, 63:57-60
- Kyelem, D., Sanou, S., Boatou, B., Medlock, J., Coulibaly, S. & Molyneux, D.H. (2003) Impact of long-term ivermectin (Mectizian) on *Wuchereria bancrofti* and *Mansonella perstans* infections in Burkina Faso: Strategic and Policy implications. *Annals of Tropical Medicine and Parasitology*. 97.827-838

- Michael E, Bundy DA, Grenfell BT (1996). Re.assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology*, 112:409-428.
- Molyneux, D. H. (2004), 'Neglected' disease but unrecognized success- challenges and opportunities for infectious disease control *Lancet*, 364, 380-383.
- OTTESEN EA.(2000) THE GLOBAL PROGRAMME TO ELIMINATE LYMPHATIC FILARIASIS. *TROP MED INT HEALTH*; 5: 591-4.
- Plaisier AP, Cao WC, van Ortmarsen GJ, & Habbema JD (2000): Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology*, 119:385-394.
- WHO (1999) :*Lymphatic Filariasis: Reasons for Hope* . Edited by: Dzenowagis J. Geneva, World Health Organization.
- World Health Organization (2002) *Lymphatic filariasis: the disease and its control*. Fifth report of the WHO expert committee on filariasis. Geneva.
- World Health Organization(2000). A training module on lymphaticfilariasis for drugdistributors in non-onchocerciasis co-endemic countries. Geneva; WHO.