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 ($X^2 = 6.62, P < 0.05$). The mosquitoes were segregated into different species and dissected to unveil a few microfilaria in the thoracic and abdominal, and mouth part regions. Out of 1213 mosquitoes dissected, 24 (1.9%) had developed stages of $L_1$, $L_2$ and $L_3$ of $W. bancrofti$, 8 (0.6%) had $L_2$ 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 ($X^2 = 8.28, P > 0.05$). 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 ($L_1, L_2$ and $L_3$) 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 $L_3$ with 17 (70.8%), followed by $L_1$ with 5 (20.8%) and lastly by $L_2$ 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 (Ottesen 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).
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/Ikunm, 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 OUBURA 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 capturing 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 search 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 Anophele 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 was caught during the two seasons Etono/Ikun had the highest number of mosquitoes caught both in the dry and rainy season 198(15.5%) followed by Adim185 (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 species342 (26.4%), Aedes species102(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

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

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

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

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%).

Infection Rate: Total number of mosquitoes infected
Total number of mosquitoes dissected

$= 24 = 0.02$

1213

Infectivity rate: Total number of mosquitoes with L3
Total number of mosquitoes dissected

$= 8 = 0.006$

1213

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 parethrum 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 spray. 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**


