Advances in Science and Technology, Vol. 1, No. 2 / 3, April - September 2007, pp92-98.

Experimental Transmission of *Wuchereria bancrofti* by Mosquitoes and Periodicity Pattern in Western Coast of Ghana

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(Received: July 14, 2007; Accepted: September 21, 2007)

Abstract

The transmission potential of *Wuchereria bancrofti* by four mosquitoes namely *Anopheles gambiae* s.l., *Anopheles funestus* group, *Culex* sp. and *Mansonia* sp. were investigated in the western coast of Ghana. Human volunteers that served as baits for the mosquitoes had varying levels of microfilaraemia ranging between 1-257mf/100¼l of blood with geometric mean intensity (GMI) of 265.59mf/ml of blood. Bimodal peaks of mf/ml of blood at 23:30 and 1:30 GMT were statistically not significant ($t = 0.491$, $P < 0.05$). The hourly collection of the 1,271 wild mosquitoes revealed a composition of *An. gambiae* s.l. (88.27%), *An. funestus* group (9.5%), *Culex* sp. (0.62%) and *Mansonia* sp. (2.21%). Infected mosquitoes were *An. gambiae* and *An. funestus* of which *An. gambiae* were more significantly susceptible to infection than *An. funestus* (OR = 1.738, CI = 0.411 - 7.349). Molecular studies showed that 93.54% of infected *An. gambiae* s.l. was *An. melas* and 6.48% were *An. gambiae* s.s. The *An. gambiae* s.s. were all M molecular form. The observed results therefore identified *An. melas* as the major vector of lymphatic filariasis.

Keywords: *Anopheles melas*, *Wuchereria bancrofti*, Periodicity pattern, Ghana.

1.0 Introduction

Lymphatic filariasis is caused by parasitic filarial worms, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, which live almost exclusively in human. Female mosquitoes of the *Anopheles, Culex, Aedes* or *Mansonia* species depending on the geographic region transmit the parasites. For instance, in Gambia and Tanzania, *An. melas* and *An. merus* respectively have been implicated to transmit the parasite (Southgate and Bryan, 1992). The parasite reported to cause lymphatic fiariasis in Ghana is *W. bancrofti* and the most important vectors of the disease were identified as *An. gambiae* and *An. funestus* which differ in vectorial role and capacity (Dzodzomenyo *et al.*, 1999; Gyapong *et al.*, 1994; Dunyo *et al.*, 1996).

Periodicity is a well-known phenomenon that occurs with many filarial worms, and various hypotheses put forward to explain periodicity have been reviewed (Oishi, 1959). According to the periodic pattern of microfilariae in the human host's peripheral blood, lymphatic filariasis caused by *W. bancrofti* may be separated into three forms: nocturnal periodic form, widely found in tropical and subtropical zones in Africa, Asia and Latin America, in which microfilarial densities peak close to midnight (Aikat and Das, 1977); a non-periodic or diurnal, sub-periodic form, prevalent in the islands of the South Pacific, in which maximum densities of mf occur around 16:30 hours (Dreyer *et al.*, 1996); and a nocturnal, sub-periodic form, with a focal distribution in Western Thailand, which is characterized by a peak in microfilarial density at around 20:30 hours (Harinasuta *et al.*, 1970 a, b; WHO, 1992). *Anopheles* spp. are mostly nocturnal in their activities, thus emergence from pupae, mating, blood feeding and oviposition normally occur in the evenings, at night or early in the morning around sunrise.

In line with the global programme to eliminate lymphatic filariasis (GPELF), proper identification of vectors

of the disease would help to appropriately strategize on vector control as the best method to achieve the GPELF goal that would be cost-effective (WHO, 2002). The aim of this study therefore was to identify the vector of lymphatic filariasis and also to observe the pattern of nocturnal periodicity along the western coast of Ghana.

2.0 Subjects and Methods

2.1 Study Area

The study was conducted in two Districts in the Western Region of Ghana. The Districts are Nzema East and Ahanta West. The communities in Nzema East where the study was carried out are Ampain, Azuleloanu, Adjan and Bobrama; while that for Ahanta West are Butre and Busua communities. The two Districts are located in the southern part of the Western Region of Ghana (Figure 1). Nzema East District lies between 04º.50'-05º.20'N and longitudes 02º.5'-02.55'W, while Ahanta West lies on latitude 4.45''N and between longitudes 1º.58'-2º.15'W. These areas lie within the tropical rain forest belt with coastal vegetation being largely mangrove swamp. The highest monthly mean temperatures is 34°C recorded between March and April, while the lowest mean temperature 20ºC, is observed in August. The average relative humidity is between 70%-80% in the dry season and 75% to 80% in the raining season with mean rainfall of over 1, 700 mm.

2.2 Subjects and Feeding Experiments

The 21 positive volunteers who participated in the experiment comprised of 14 males and 7 females. Each of the 21 consented subjects slept under a mosquito net with one side partly opened for mosquito entry. Using an aspirator, mosquitoes were collected hourly between 21:00-6:00 GMT (Subramanian *et al.*, 1998).

At the mid-point of each collection hour, a finger prick sample of blood was collected from each of the volunteers using 100ìl heparinized capillary tube. The blood was mixed with 900ìl 3% acetic acid and the number of microfilariae (mfs) determined by microscopy, using a Sedgwick Rafter counting chamber (McMahon et al., 1979). The Geometric Mean Intensity (GMI) is calculated as antilog [Ó log (x+1)/n], where x is the number of mf/ml of blood in the microfilaraemic individuals and in the number of people examined.

2.3 Morphological Identification

The mosquitoes were morphologically identified using the keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

2.4 Mosquito Maintenance and Dissection

The mosquito species collected were maintained approximately 26-28°C and 70-80% relative humidity and were fed on 10% sucrose solution until day 12 post collection when they were killed, dissected and examined for the microfilaria (mf) and the infective stage (L3) of the parasite. Also mosquitoes that died just after collection and before day 12 were dissected immediately using the dissecting microscope (OLYMPUS SZ 11, Japan). The legs and wings were removed and the head, thorax and abdomen were then separated and each part transferred into separate drops of saline on the same slide. The labium was separated from other parts of the proboscis and the infective larvae L3s emerged and dislodged in the saline. The remaining part of the head, thorax, and abdomen were teased apart under the high power of the

dissecting microscope and examined for microfilariae. Careful examination of the slides at 100X magnification was done under a compound microscope (OLYMPUS B50, Japan) to rule out negativity for parasite. The mosquitoes positive for filarial infection were recorded, and all positive slides were kept at -4ºC until they were ready to use for molecular identification of mosquito species.

2.5 Molecular Studies

The mosquitoes collected were subjected to Polymerase Chain Reaction (PCR) method for the identification of the member species (Scott *et al.*, 1993) and Collins *et al.* (1987) with slight modifications. Our primers abbreviated UN, GA, ME, AR, QD, and 10 x PCR buffer were supplied by Sigma, USA. Amplification was carried out using a thermal cycler (MJ Research Inc. USA). The *Hha* I enzyme digestions were carried out using the recommended protocol of the manufacturers (Sigma-Aldrich, USA and Promega, USA).

2.6 Statistical Analysis

Data were subjected to statistical analysis, namely, t - test and multivariate logistic regression using Microsoft Excel and Instat statistical packages respectively.

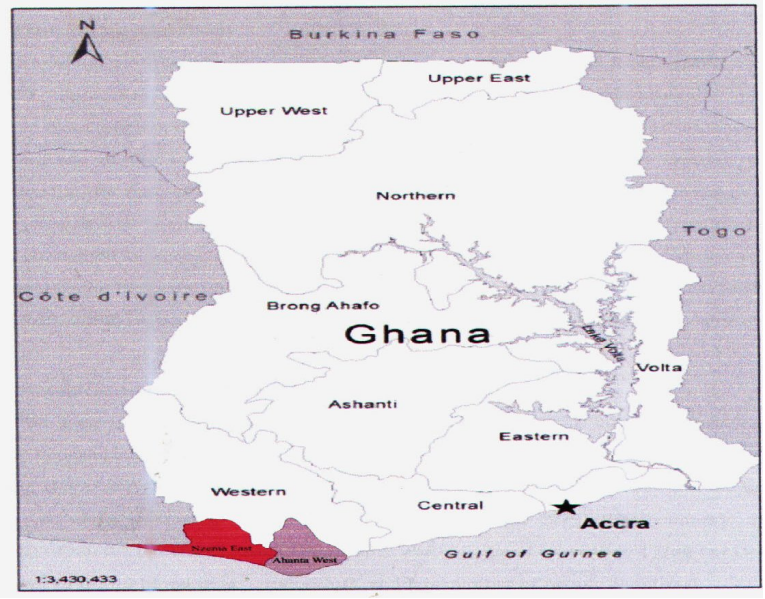

Figure 1: Map of Ghana showing the study sites (shaded).

3.0 Results

The relative abundance of mosquitoes collected and their parasitic load are presented in Table 1. Of the 1,271 mosquitoes collected 1,118 (88.27%) were *An.gambiae* s.l., 120 (9.5%) were *An. funestus* group, 4 (0.62%) *Culex* species and 29 (2.29%) were *Mansonia* species. In all, 34 mosquitoes showed positivity for microfilaraemia as 32 (2.86%) in *An. gambiae* s.l., 2 (1.66%) *An. funestus* group while no microfilariae were recovered from *Culex* species and *Mansonia* species. Also a total of 49mfs and 29L3s were found in *An. gambiae* s.l., 3mfs and 3L3s were observed in *An. funestus* group. In all, the relationship between the proportion of mosquitoes infected and the level of infection in *An. gambiae* and *An. funestus* showed that *An. gambiae* is significantly more susceptible to infection than *An. funestus* (OR = 1.738, CI = 0.411 - 7.349).

The pattern of nocturnal periodicity of *W. bancrofti* in the human blood as expressed in geometric mean

Table 1: The number of mosquitoes collected and parasite load.

Mosquitoes	Number caught and dissected	Number positive with the parasite Number (%)	Number of mfs recovered	Number of L_3s Recovered
Anopheles gambiae s.l.	1,118	32 (2.28)	49	29
Anopheles funestus	120	2 (1.66)	3	3
Culex sp.	4	0 (0)	0	0
Mansonia sp.	29	0 (0)	0	0
Total	1,271	34	52	32

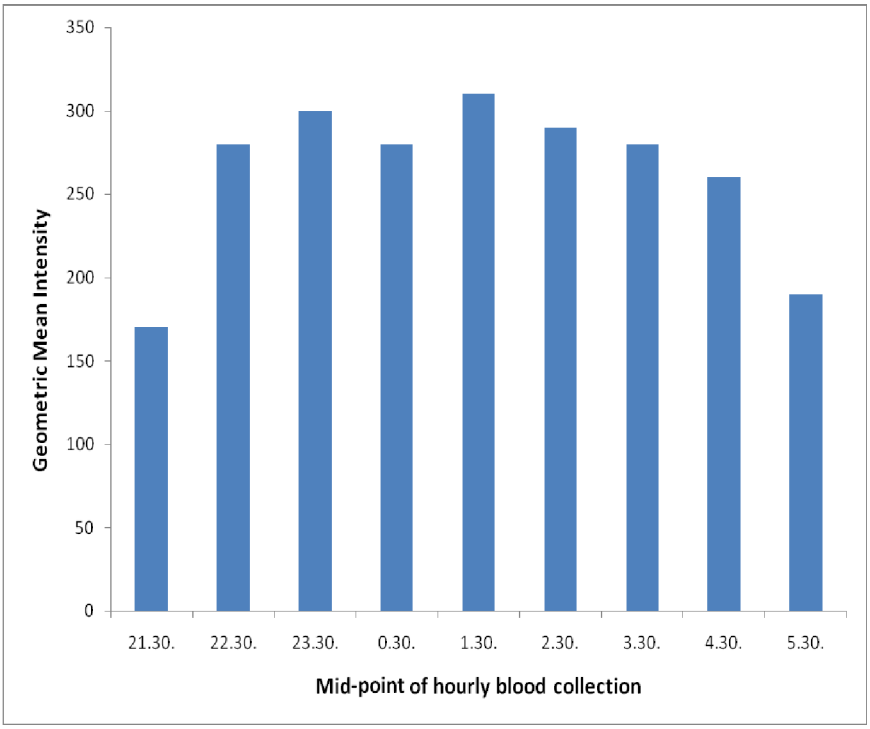

Figure 2: Geometric mean intensity of microfilaraemia per milliletre of blood observed at each hour interval.

intensity of microfilaraemia per millitres of blood at each hour interval is presented in Figure 1. Bimodal peaks observed at 23:30 and 1:30 were not statistically significant ($t = 0.491$, $P < 0.05$).

Anopheles. gambiae s.l. were identified using PCR. Out of the 1,127 *An. gambiae* s.l. collected, 131 (11.62%) were identified for which 126 were successful. Of these 126, 123 (97.61%) were *An. melas* and 3 (2.39%) were *An. gambiae* s.s. The number of infected *An. gambiae* s.l. was 32 of which 30 (93.75%) were *An. melas* and 2 (6.25%) were *An. gambiae* s.s. All *An. gambiae* s.s. were M molecular form.

4.0 Discussion

The data on the composition of mosquitoes in our study areas revealed the preponderance of *An. gambiae* s.l. as *An. melas* (93.75%) and *An. gambiae* s.s. (6.25%) while few *Culex and Mansonia* sp. were reported. This pattern of distribution is related to the ecology of our study area situated in or near lagoon with mangrove swamps. This observation accords the reports of (Appawu *et al.*, 1994; Yawson *et al.*, 2004). For instance Taylor *et al.* (2000) observed that salinity among other ecological factors were the determinants of the composition of the flies in their mangrove environment.

Lack of microfilaraemia in *Culex* and *Mansonia* sp. delineated them as improbable vectors of lymphatic

filariasis in our study areas in Ghana. This information corroborates the information in WHO (1989), where it was documented that urban *Culex quinquefasciatus* cannot transmit some strains of *W. bancrofti*. Conversely the reverse situation applies in other areas such as India and Malaysia where the *Culex*-adapted strains of *W. bancrofti* cannot be transmitted by anophelines (WHO, 1989).

The examination of 21 volunteers for nine hours showed that microfilarial concentration in peripheral blood peaked at 23:30 and 1:30 GMT. The observed periodicity is similar to those reported from other parts of Africa (Tanaka, 1981; Gatika *et al.*, 1994). This pattern is probably because night biting species of mosquitoes serve as vectors in our study areas and indeed throughout the continent. In conclusion, the results on periodicity observed in this present study therefore delineate the most appropriate time when transmission is highest. High priority for vector controls should be given at this period to reduce man vector-contact and indeed the transmission of lymphatic filariasis in our study areas.

Since it has been documented that vector controls have led to the elimination of lymphatic filariasis from large parts of China, Malaysia, Korea and several island of the pacific (Ottesen, 2000), there is need to institute the same in our locality. While we await possible intensive control measures as advocated by Esterre *et al*. (2001) and Rajagopalan *et al*. (1988), there is urgent need to put in place and strengthen existing health education on control of the vector using indoor residual spraying and insecticide treated material.

Aknowledgements

The authors thank Mr Joseph Ochere, Mr Sampson Otoo and Mr Isiae Sibomana for their technical assistance. This research was supported by Deutcher Akademischer Austauschdienst e.v. (DAAD) Germany. The project also received additional support from WHO/TDR Research grant to Prof. Daniel Adjei Boakye (Identification number: A00638and was undertaken as part of a five year WHO/TDR LF II project entitled "Trends levels in the transmission of lymphatic filariasis before and after mass drug administration of ivermectin and albendazole".

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