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DETERMINATION OF VECTORIAL POTENTIAL OF *MANSONIA* SPECIES IN THE TRANSMISSION OF *WUCHERERIA BANCROFT* IN TANA-DELTA DISTRICT, COAST-KENYA
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ABSTRACT

***Background*: *Wuchereria bancrofti* are parasites causing bancroftian filariasis which is transmitted by different species of mosquitoes. Mosquitoes of *Anopheles, Culex* and *Aedes* species are known to transmit *W. bancrofti* parasites.**
Objective: To determining the potential of *Mansonia* species; *Ma. africanus* and *Ma. uniformis* in the transmission of *W. bancrofti*.
***Design*: Cross sectional study.**
***Setting*: Villages in Tana Delta district, Kenya.**
***Subjects*: In-door collected mosquitoes.**
***Results*: A total of one thousand, six hundred and sixty two (1632) female mosquitoes were collected by gravid traps, CDC light traps and Pyrethrum spray methods. Of these, 236 representing 14.5% were identified as *Mansonia* species. Two filarial larvae of stage two (L_2) were obtained from the dissected mosquitoes. Polymerase chain reaction assays did not show any amplified *W. bancrofti* DNA from the obtained larvae or the mosquitoes.**
***Conclusion*: There was no evidence that *Mansonia* species play a role in the transmission of *W. bancrofti*.**

INTRODUCTION

Lymphatic filariasis (LF) is caused by three of the threadlike nematode parasites; *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* species (1). Lymphatic filariasis is targeted by the Global Programme for Elimination of Lymphatic Filariasis (GPELF) for elimination by 2020 in endemic areas (2). The approach is by use of mass drug administration (MDA) with the combination therapy of albendazole and diethylcarbamazine (DEC) or ivermectin (3) and integrated vector control. *Wuchereria bancrofti* globally accounts for approximately 90% of all LF infections (4) and is the most common cause of lymphatic filariasis (LF) in Africa (5). Mosquitoes are capable of transmitting disease-causing viruses, protozoans, and filarial nematodes including lymphatic filariasis. The intensity of LF transmission is dependent on factors such as the density of the parasites in the human population and the availability of suitable vectors. The major mosquito species involved in LF transmission at the Kenyan coast are *Culex quinquefaseiatus*, *Anopheles gambiae* and *An. funestus* (6, 7), *Anopheles gambiae* and *An. funestus* play a significant role in more rural areas of Africa (8). Culex *quinquefaseiatus* is important in both urban and rural areas (7). Several *Aedes* species, particularly *Ae, polynesiensis*, are the major vectors of LF in the South Pacific islands where diurnally sub periodic *W. bancrofti* is common. *Brugia malayi* is primarily transmitted by *Mansonia* and *Anopheles* species and some *Aedes* species (6). *Brugia timori* is transmitted by *An. barbirostris*. The importance of these vectors varies from place to place depending on the local ecological conditions (9). *Mansonia* species though present in the Kenyan coast, have not yet been incriminated as vectors for *W. bancrofti.*

The endemicity of filariasis can be assessed using clinical, parasitological, immunological and

entomological methods. Entomological methods are used as a diagnostic tool for assessing presence of filarial infection and for monitoring the progress of control programmes in endemic areas. Polymerase Chain Reaction (PCR) assays is highly sensitive and specific method in the detection of filarial infections in both human and vectors (10). Polymerase Chain Reaction assays on pool screening of mosquitoes are used in the estimation of levels of transmission of LF in terms of vector infection rates (11, 12). The determination of the incidence of *W. bancrofti* in mosquito vectors are important for the baseline information, during and post eradication period. Xenodiagnosis is diagnosis of infections through the vectors. It involves mosquito dissection to search for filarial larvae and PCR assays to amplify filarial DNA in the mosquitoes. It is important in surveillance for the eradication of LF in endemic areas and it allows timely calculation of entomological measures of transmission intensity as transmission changes in human can take years to be detected (12). Xenodiagnosis is more acceptable to communities as it does not involve human populations directly as the other invasive procedures for obtaining specimens (12).

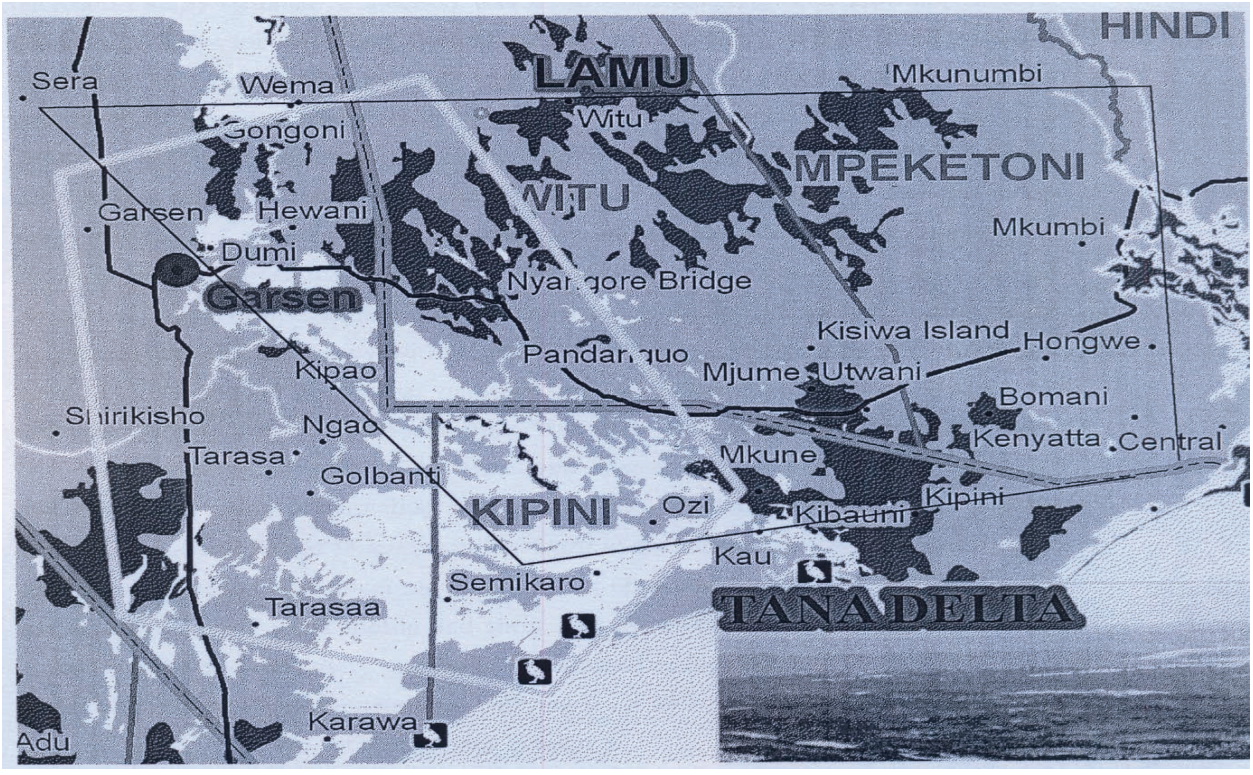
Entomological studies on the behaviour of vectors have shown that both biting and feeding behaviour of *Mansonia* species are compatible with a potential for lymphatic filariasis transmission. *Mansonia* species dissection to determine presence of infective stages (L_3) of *W. bancrofti* larvae and *W. bancrofti* DNA detection in the mosquito by PCR was used in this study to determine *W. bancrofti* transmission potential.

The current study presents a report on *W. bancrofti* infection rate in *Mansonia africanus* and *Ma. uniformis* in Tana Delta district, Kenya. The results add information on known LF mosquito vectors in the Kenyan coast. This information is of importance to National Programmes for Elimination of Lymphatic Filariasis in the control efforts.

MATERIALS AND METHODS

Study site. The study was carried out at Tana Delta district of Coast Province, Kenya (figure 1). The rainfall ranges between 220-900mm per year with two rain seasons. Rainfall in this district is erratic, long rains fall between March and May and short rains between October-December. Flooding occurs regularly due to the heavy rainfall in upstream areas of the Tana River. The altitude ranges between 0-200m above the see level. The average temperature is 30°C. The major ethnic groups are the Pokomo, who are farmers, and the Orma and Wardey, who are predominantly nomadic. Fishing activities are also carried out in the area. The presence of water bodies (swamps, dams and the Tana River) provide ideal habitant for mosquito breeding.

Figure 1
A map of Divisions and some villages in Tana River and Tana Delta Districts



Mosquito collection and identification: The purpose of the study was explained to the village chiefs and village headmen for verbal consent. The household heads also gave consent for their houses to be used. Indoor collection of mosquitoes was done using CDC light traps, gravid traps and pyrethrum spray catches. Collection was done from Ki1elengwani, Hewani, Idsowe, Chakamba, Kisiwani farm and Onindo villages for ten days in the month of May, 2009 after long rains which coincide with high transmission intensity of *W. bancrofti* due to high mosquito densities (13). Light traps and gravid traps were set from 7.00pm to 7. 00 am in selected houses. Pyrethrum spray catches were done on different days from seven. 00 pm-100. 00 pm but in the same houses used for the traps. The use of the three methods of collection was to maximise the catches and obtain mosquitoes of different abdominal status; blood fed, half gravid, and gravid which have a high chance of infectivity. Female mosquitoes were identified to species based on the morphological characteristics with a standard dissecting microscope and taxonomic keys (14). Dissection for filarial parasites was performed on the head, thorax and abdomen (15) of *Mansonia* mosquitoes. This was done within 6-12 hours from the time of mosquito collection. Fifty *Mansonia* species mosquitoes were dissected on saline solution using a dissecting microscope X10 to determine the present larvae stages (L_I, L_2 and L_3) of *Wuchereria bancrofti* in the mosquito. Parasite larvae stages and parasite species identification was done on observation (16). Stage 1 larvae (L_1) are sausage shaped, L_2 is motile and short and L_3 is very motile, long and infective. Infection rates and infectivity rates were determined as follows;

$$\text{Infectivity rate} = \frac{\text{No. of mosquitoes carrying } L_3 \times 100}{\text{No. of mosquitoes dissected}}$$

$$\text{Infection rate} = \frac{\text{No. of mosquitoes carrying } L_1 \text{ and } L_3 \times 100}{\text{No. of mosquitoes dissected}}$$

Filariae larvae obtained from dissection were identified morphologically and by PCR. The mosquitoes were preserved in silica gel in the field and frozen at -80^{0}C after transportation to the laboratory.

DNA isolation and Amplification by Polymerase Chain Reaction: Extraction of DNA from 100 mosquitoes and the obtained larvae was carried out by alkaline precipitation method as by Rarnzy, 2002 (10). Conventional PCR assays to amplify Ssp 1 repeat of *W. bancrofti* DNA in mosquitoes were performed using the method by Zhong *et al*., (17). The primers used were 5′ CAACCAGAATACCATTCATCC 3′ and 5′CGTGATGGCATAAAGTAGCG 3′ to amplify a 188-bp product in gDNA of *W. bancrofti* (17).

PCR product analysis and Scoring of the bands: Two percent (2% WN) of agarose gel was used to analyse PCR products. Deoxyribonucleic acid (DNA) amplified products were visualised as bands under ultraviolet (UV) light on a transilluminator and the results were recorded and photographed using a Polaroid camera. The positive bands in the gel electrophoresis were recorded as those appearing at the position equivalent to 188bp (non-coding DNA sequence in W banerofti Ssp1 repeat DNA sequence) of the DNA positive control marker (17).

Data management and analysis: Double entry of data in record books and Microsoft excel spreadsheet were done. The data were analysed using the statistical software, SPSS Version 10.0. Generalised linear model univariate analysis was used to test statistical differences in the abdominal status of collected mosquitoes by the three methods.

RESULTS

Mosquito collection and species identification: A total of 1632 mosquitoes were collected by CDC light traps, gravid traps and pyrethrum spray catches. Five mosquito genera were identified; *Culex* mosquito species were 1048 (64.2%), *Aedes* species 188 (11.5%), *Mansonia* species 236 (14.5%), *Anopheles* species 148 (9.1%) and *Ficalbia* species 12 (0.7%) (Figure 2). Mosquitoes of different abdominal status; unfed, blood fed, gravid and half gravid were obtained (Table 1). The abdominal status of the mosquitoes obtained by different methods were significantly different (df=3, f= 0.841, p=< 0.526).

Figure 2
Mosquito species identified from trapped mosquitoes in the study area during collection

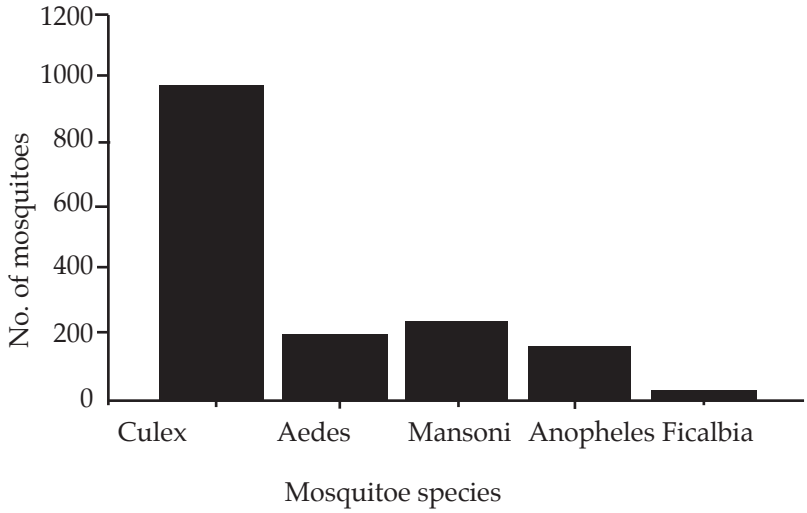


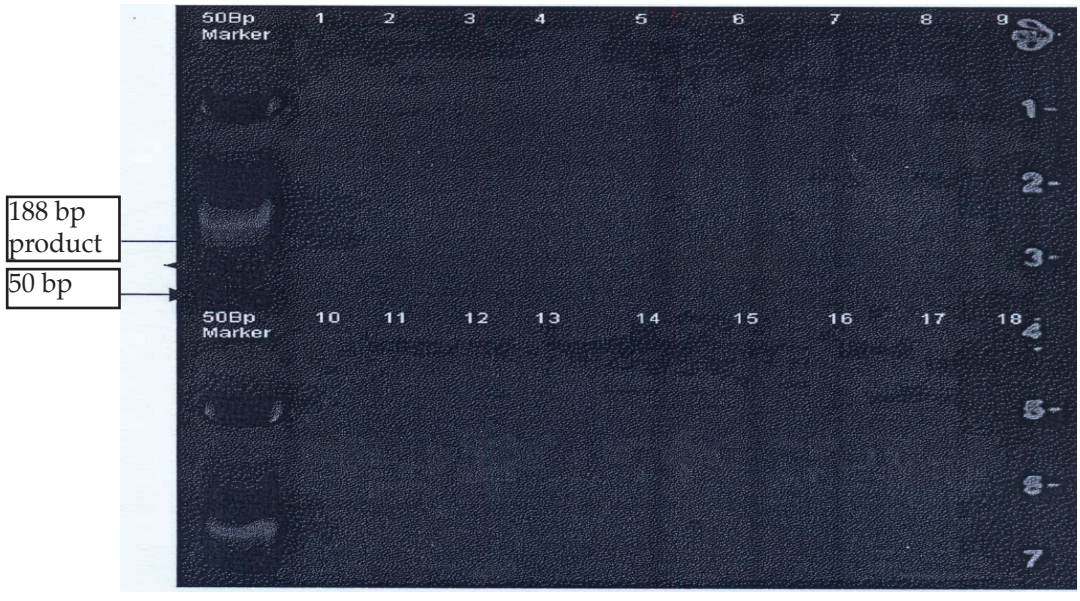
Table 1
Abdominal status of the mosquitoes collected by the different methods

Collection method	Blood fed				
	Fed	Unfed	Gravid	Half gravid	Total
Light traps	75	1163	13	14	1265
Gravid traps	1		54	1	56
Pyrethroid spray	6	297	1	7	311
Total	82	1460	68	22	1632

Larvae status of the dissected mosquitoes: Out of the 50 *Mansonia* mosquito species dissected, only two had stage II filarial larvae (L_2) as identified based on morphology which were not of *W. bancrofti* species.

Polymerase chain reaction and gel electrophoresis: None of the mosquitoes tested positive for *W. bancrofti* DNA by the conventional PCR method (plate 1). In addition, the two larvae obtained from the dissection and which had been identified as filarial species based on morphology did not give positive results with the *W. bancrof*ti PCR assay, indicating that they could not be confirmed as bancroftian filariasis species.

Plate 1
Agarose Gel electrophresis for PCR product analysis to detect the presence of W. bancrofti DNA in Mansoni mosquito species



Label: 50Bp = Molecular weight ladder, well 1 = positive control, well 2 = Negative control, wells 3-9 and wells 10-18 = Mosquito specimens.
NB; No band was seen on the negative control and on all the test specimen wells.
Label: Wells 1 and 11 = Molecular weight marker-100bp, well 2 = positive control, well 3 = Negative control, wells 4-10 and wells 12-20 = Mosquito specimens.
NB; No band was seen on the negative control and on all the test specimen wells.

DISCUSSION

Mansonia africanus and *Ma. uniformis* were not found to play any role in transmission of *W. bancrofti* in this study, The two larvae L_2 obtained from dissection were not of *W. bancrofti* species. In addition, *W. bancrofti* DNA could not be amplified from *Ma. africanus* and *Ma. uniformis* (Plate 1). These findings compare well with those by Kasili *et al*. (2009)(8) who found no *W. bancrofti* larvae in all the *Mansonia* species dissected in the studies of seasonal variation of filariasis transmission in vectors in Coastal Kenya. These results were also similar to those of Onapa *et al*. (18) who found that *Mansonia* uniformis had a limited potential to support development of *W. bancrofti* to the infective stages and they did not appear to play any role as vectors of *W. bancrofti* under natural conditions. These results suggest that there were no *W. bancrofti* larvae development in *Mansonia* species.

Experimental infections of *Mansonia* species of mosquitoes were found to show some larvae development (18). The larvae were seen to show microfilariae ex-sheath and first stage larvae (L_1) were seen to accumulate in the thorax and only few developed to second stage (L_2) and further to infective stages (L_3) (18). Other studies have found *Mansonia africanus* and *Ma. uniformis* containing infective larvae of animal origin (19). Previous records incriminating *Mansonia* species as vectors of *W. bancrofti* in Africa were probably due to misidentification (19).

In conclusion, there was no evidence that Mansonia species play any role in transmission of *W. bancrofti* in Tana Delta district, Kenya. The study provides relevant information to ongoing control efforts and support future campaigns aimed at eliminating filariasis.

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