

Full Length Research Paper

Wolbachia infected anthropophilic mosquitoes in The Gambia are not infected with filarial nematode

S. Sanneh^{1,2}, A. Kargbo^{1,3*}, M. E. Entonu¹, Y. K. E. Ibrahim¹ and S. Nasiru¹

¹Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria, Nigeria.

²School of Education, Gambia College, The Gambia.

³Department of Physical and Natural Science, School of Arts and Sciences, University of The Gambia, The Gambia.

Received 3 July, 2020; Accepted 27 July, 2020

***Wuchereria bancrofti* has been reported to cause 90% of all cases of lymphatic filariasis in Africa. wolbachia endosymbiot infect a wide range of insects and nematodes. This study was conducted in 48 settlements (8 from each division). A total of 2003 mosquitoes was pooled into 120 clusters from the 48 settlements in The Gambia. Polymerase chain reaction (PCR) was used to detect the presence of Wolbachia and filarial nematode and further differentiate Wolbachia into super groups among the positive samples. The level of association between Wolbachia and the genera of anthropophilic mosquitoes in The Gambia was also determined. Microscopic results showed 64.9% Anopheles, 32.0% Culex, 3.0% Aedes. PCR showed that, 34.17% of Wolbachia in the mosquito obtained varied among the three mosquito genera, with highest being among *Anopheles*. Only Wolbachia super group B was identified in *Culex* and *Aedes*. The purpose of this study carried out in The Gambia was to describe the most common mosquito species and to identify, by means of PCR, their association with the Wolbachia bacteria and the presence of nematodes responsible for filariasis, in order to understand the role that this bacterium plays in the chain of filarial transmission.**

Key words: *Wuchereria bancrofti*, Filariasis, *Anopheles gambiae*, *Culex quinquefasciatus*, elephantiasis.

INTRODUCTION

Lymphatic filariasis, typically known as elephantiasis and it is one of the neglected tropical disease caused by a microscopic parasite which can affect man (WHO, 2020). It can disfigure and damage the lymphatic system and can cause abnormal enlargement of body parts, thereby causing grave pain, severe disability and stigma. About 893 million people in 49 countries worldwide are at risk of contracting this disease (WHO, 2020). It is caused by a

thread- like filarial worms (microfilariae): *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori* (Lambert, 2005). The adult worms which could measure up to 4 -12 cm long, house and mature as adults in the body's lymph nodes and lymph vessels for a period of 4 to 8 years (Bockarie et al., 2009; Pfarr et al., 2009). Three species that causes lymphatic filariasis, *W. bancrofti* is most widely spread and is responsible for more than 90% of

*Corresponding author. E-mail: akargbo@utg.edu.gm. Tel: +2203854579.

infection (Rebollo et al., 2015a). Microfilariae of *W. bancrofti* exhibit nocturnal periodicity (Nwoke et al., 2010); during the day, they are found in the deep veins and during the night between 10 pm and 4 am, they migrate to the peripheral circulation. This behaviour gives them the chances of being picked up by the night biting vectors during their blood meals (Nwoke et al., 2010). The species of mosquitoes capable of transmitting these filarial nematodes are many but varies between geographical regions. In most urban and semi urban areas, *W. bancrofti* is vectored mainly by *Culex* mosquito while in the rural areas, transmission from one person to another is mainly by *Anopheles* mosquito (Singh et al., 2013). The main vectors in The Gambia are *Anopheles gambiae* in the rural areas while in the urban areas transmission is by *Culex quinquefasciatus*. Studies have revealed the presence of an endosymbiont, Wolbachia in arthropods and nematodes (Bouchery et al., 2012). Wolbachia is a Gram-negative intracellular α -proteobacterium belonging to the Order *Rickettsiales* (Bouchery et al., 2012). The specie *Wolbachia pipientis* was first identified in *Culex* mosquito (Hertig and Wolbach, 1924; Porennan et al., 2008). It is classified into sixteen sub groups (A-Q) (Guan et al., 2016). Super group G have been removed because its status is currently not clear (Baldo and Werren, 2007). Of the sixteen sub groups, C and D are commonly found in filarial nematodes (Werren et al., 2008), while A and B are found in mosquitoes (Ravikumar et al., 2011). According to Dyab et al., (2016), Wolbachia are maternally inherited and infect a wide range of insects and nematodes and play an important role in the development and pathogenesis of the filariae *Onchocerca volvulus*, *Brugia malayi* and *W. bancrofti*. Wolbachia as a parasite, relates with its host in a number of ways, some of which are parasitism while others are mutualism. The parasitic relationship includes feminization of genetic males, pathenogenetic induction resulting in the development of unfertilized eggs, the killing of male progeny from infected females and sperm-egg incompatibility (Werren et al., 2008). Wolbachia appear to contribute to the inflammation process which is a major pathological symptom of filarial infections (Dyab et al., 2016). The most common effect of Wolbachia infection in mosquitoes is cytoplasmic incompatibility. Mating of infected male with the uninfected female mosquitoes of the same species, does not result into fertilization (Sinkins, 2004). Wolbachia has the potential to be used as biocontrol in both pest and in biomedical applications (Zabalou et al., 2004; Engelstadter and Hurst, 2009). In 2013, the World Health Organization listed The Gambia as among 73 countries considered endemic for lymphatic filariasis (WHO, 2013) and according to Cano et al. (2014), Global Atlas of Helminth Infection, The Gambia still had a high probability of transmission. Lymphatic filariasis is mainly characterized by the occurrence of inflammatory pathogenesis, and

Wolbachia bacterium which is an endosymbiont of filarial nematode appear to contribute to this inflammation (Barton et al., 2010; Bouchery et al., 2013). The endosymbiont Wolbachia could be genetically transformed to modify the disease transmitting abilities of mosquitoes (Yeap et al., 2011; Fraser et al., 2017). It is crucial to know which Wolbachia strains are present in population before releasing engineered infected individuals because pre-existing natural infections can interact with and alter the dynamics of introduced strains (Duron et al., 2010; Atyame et al., 2011). Understanding the role of Wolbachia in filarial transmission can provide a template for breaking the transmission chain of the disease. Effective and efficient surveillance systems need to be put in place for most arthropod-borne diseases across The Gambia (Kargbo and Kuye, 2020). The purpose of this study carried out in The Gambia was to describe the most common mosquito species and to identify, by means of polymerase chain reaction (PCR), their association with the Wolbachia bacteria and the presence of nematodes responsible for filariasis, in order to understand the role that this bacterium plays in the chain of filarial transmission.

MATERIALS AND METHODS

Study area

The Gambia is a West African country that lay between latitude 13° and 14° and longitude 13° and 17°. The country is almost completely surrounded by Senegal except on the western side where it bordered with the Atlantic Ocean. The Gambia is divided into eight Local Government Areas namely: Banjul, Kanifing, Brikama, Mansa Konko, Kerewan, Kuntaur, Janjanbureh and Basse. For the purpose of this research, the former divisions as shown in Figure 1 was used; Banjul, Western, North Bank, Lower River, Central River and Upper River division.

Sample size

The sample size was determined by using the formula

$$N = Z^2 pq / d^2$$

as described by Thrusfield (2007) based on 95% confidence interval and prevalence of 50%.

Where;

N is the sample size of pools of mosquito (to be calculated)

Z = 1.96 for 95% confidence level

p = 0.5 (Prevalence)

q = Complementary probability (1-0.5)

d = 0.05

$N = 1.96 \times 1.96 \times 0.5 \times 0.5 / 0.05 \times 0.05$

$N = 0.9604 / 0.0025$

$N = 384$ pools of mosquitoes

Sampling techniques

Multi stage sampling technique was employed. For each division, 8 settlements were selected through balloting, and for each settlement, 8 compounds were selected through balloting. The

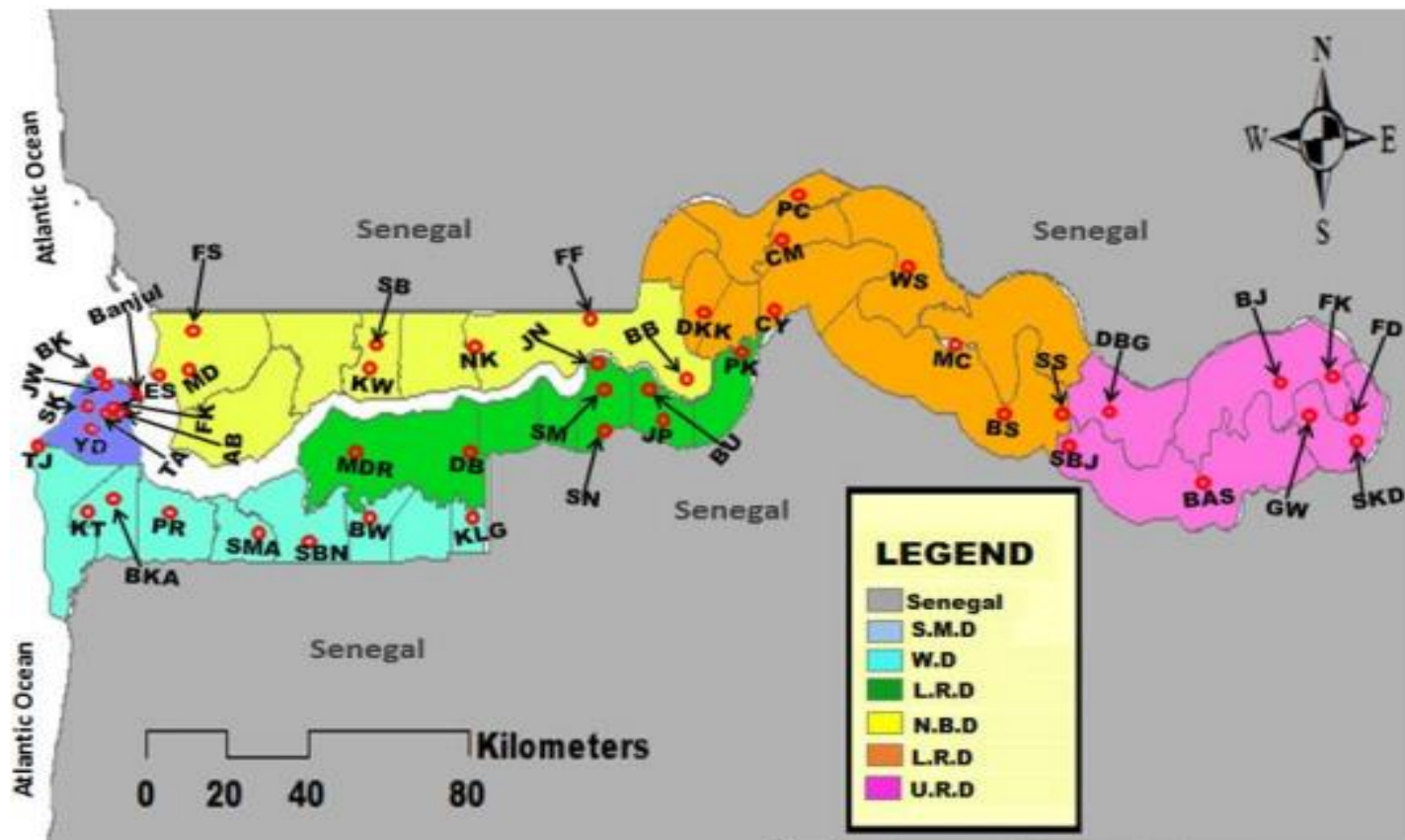


Figure 1. Map of The Gambia showing the sample site. SMD= Saint Mary's Division, WD= Western Division, NBD= North Bank Division LRD= Lower River Region, CRD= Central River Region; FK = Fagi Kunda, TJ = Tanji, ES = Essau, SM = Soma, MC= Macaty; TA = Tallinding, PR = Pirang, KW= Kerewan, MDR = Manduar, CY – Choya; SK = Sukuta, BW = Bwiam, NK= No Kunda, JN = Jinoi, DKK=Dankunku; BK = Bakau, SMA = Somita, FS = Fass, PK = Pakaliba, CM= Chamen; JW = Jeswang, SBN = Sibonor, SB = Saaba, SN = Senoba, PC= Panchang; AB = Abuko, KT = Kiti, FF = Farafenni, DB = Dumbuto, WS= Wassu; YD = Yundum, BKA = Brikama, BB = Bambali, BU = Buiba, SS = Sare Sofi; Banjul = Banjul, KLG = Kanglagi, MD = Madina, JP = Japineh, BS= Bansang; BAS= Basse, SKD = Song Kunda, FT= Fatoto, GW= Garawol, BJ= Baja, DBG = Diabugu, SBJ = Sare Bojo; FD = Foday Kunda.

compounds selected are the sampling points/pools. This equates to 64 sampling points/pools for each division giving a total of 384 sampling points/pools for the entire country (Table 1).

Collection of mosquito samples

G'rumba is a name of a container in Jola language made of clay. Jola is a tribe in the Senegambia region and some parts of Guinea Bissau deeply rooted in herbal medicine. The container is mostly used by herbalists in the preparation and storage of concoctions. It has been observed that while in storage, a scourge of mosquitoes seeks shelter and a source of water for drinking in such containers. The container could also be a breeding ground if kept in that position for weeks. It is from this concept; this study formulated a trap for collecting mosquitoes for purpose of this research. The materials used here are buckets and transparent piece of clothes sawed into sacs with a control valve made of rope. In the setup, water is filled into the buckets to one quarter full. Small branches with leaves of shrubs were then put inside to create a bushy environment that can be a temporary home for mosquitoes and other flies within that environment. The harvesting processes involved carefully capping the mouth of the bucket with the mouth

of the designed sac such that when the bucket is shaken the mosquitoes will run into the sacs. The sac can then be removed carefully and the valve closes to prevent any trapped mosquito from escaping. The activities of mosquitoes are time bound. Some genera are active during the night while others are active in some parts of the day. At around midday, mosquitoes are generally not active. During such periods, they could be found hanging on walls in dark areas or any other surfaces nearby. In this method of collection, the traps were set to provide such resting platforms for the mosquitoes during such inactive periods of their time. The traps were positioned in baths rooms, verandas, sitting rooms and other immediate environments. The compound owners and the community were sensitized on the whole operation. The selected area for the traps was cleared of any other material that could provide a resting platform for the mosquitoes. The traps were laid from 8 am in the mornings to 2 pm the next day (a period of 30 h). Harvests were made at 2 pm as described above. The live mosquitoes were left in the sacs for 48 h to weaken and then transferred into tubes containing silica in sachets (for preservation) and labelled to indicate division, pool number and date of harvest. Once harvested, the small branches with leaves were discarded and the water poured to avoid the setup being used by mosquitoes for breeding (Sankung et al., unpublished method of trapping

Table 1. The sampling sites for each division in The Gambia.

Division	Sampling sites
SMD	Fagikunda , Banjul, Tallinding, Sukuta, Bakau, Jeswang, Abuko and Yundum
WD	Tanji, Pirang, Bwiam, Somita, Sibanor, Kitti, Brikama and Kalagi
NBD	Essau, Kerewan, No kunda, Fass , Saaba, Farafenni, Bambali and medina
LRD	Soma, Manduarr, Jinoi, Pakaliba, Senoba, Dumbuto, Buiba and Japineh
CRD	Macaty, Choya, Dankunku, Chamen, Panchang, Wassu, Sare- Sofi and Bansang
URD	Basse, Song-Kunda, Fatoto, Garawol, Baja, Diabuqu, Sarebojo and Foday kunda

SMD= St Mary's Division, WD= Western Division, NBD= North Bank Division, LRD= Lower River Division, CRD= Central River Division, URD Upper River Division.



Figure 2. (a) G'ruumba (b) G'ruumba Trap (Modified mosquito trap).

mosquitoes). The mosquitoes were then transported to Department of Biochemistry Ahmadu Bello University, Zaria Kaduna State, Nigeria for laboratory analysis (Figure 2).

Morphological identification of the mosquitoes

The mosquitoes were identified per pool into genera level according to Stone et al. (1959) using microscopy at the Department of Veterinary Parasitology and Entomology Laboratory Ahmadu Bello University Zaria. The main taxonomic features used were; body colour, spotted and unspotted wings, length of palps in comparison with their proboscis and presence of black strips on body and legs. The number of each genera of mosquito for each pool were established and placed in tubes and labelled. The sex of the mosquitoes was also determined for every pool based on the presence of bushy feathers around the antenna. For improving the sensitivity and cost effectiveness in the DNA analysis by Polymerase Chain Reaction (PCR), each genera of mosquitoes in the sampling points/pools of each settlement/cluster were pooled together. This translates into 48 clusters of Anopheles genera mosquitoes, 48 clusters of Culex genera mosquitoes and 24 clusters of Aedes genera of mosquitoes. DNA was extracted from 120 clusters of mosquitoes.

Identification of Wolbachia and microfilariae

DNA extraction

Dry mosquito samples by cluster were homogenized by grinding into powder in eppendorf tubes (one for each cluster) using fresh micro pestle for each cluster. 100 µl of distilled water was added to each of the samples with less than 5 mosquitoes while 200 µl of distilled water was added to samples with more than 5 mosquitoes. The samples were briefly vortexed to distribute the particles evenly. Quick-DNA™ miniprep plus kit (Zymo) was used with strict adherence to manufacturer instructions (Vanek et al., 2011).

DNA quantification

The DNA samples were removed from the freezer, thawed and briefly vortexed. The lower and the upper pedestals of the Nano drop Spectrophotometer (Denovix DS- 11+) were cleaned and blanked with 2 µl of deionized water. The samples were then run one after the other with intermittent cleaning of the lower and the upper pedestals with tissue paper after every sample run. The concentration of the DNA and the absorbance of 260/280 ratio (for purity) were recorded.

Identification of Wolbachia DNA using 16SrDNA and wsp gene

Detection using 16S rDNA

To determine the presence of Wolbachia DNA, PCR technique was employed using 16S Wolb F and 16S Wolb R (forward and reverse primers) specific primers: 16S rDNA (W-Specf 5'-CATACCTATTCGAAGGGATAG-3', W-Specr 5'-AGCTTCGAGTGAAACCAATTC-3') (Baldini et al., 2014). The amplification were carried out with a Gradient Thermo Cycler and the reaction mixture for each of the 120 cluster sample consisted of 4 µl of DNA template, 2.5 µl of 10X buffer, 2.5 µl of 2.5 mM (each) dNTPs, 0.35 µl of 20 µM each of forward and reverse primers (16SWolbF/16SWolbR), 0.25 µl of 5U/µl Taq DNA polymerase and a volume of 15.05 µl DNase free water to make a final reaction volume of 25 µl. The amplification reaction protocol used was as follows initially of 2 min at 95°C then 40 cycles of 30 s at 95°C, annealing at 55°C for 30 s and extension at 72°C for 30 s and a final extension of 3 min at 72°C with an expected band size of 438 bp (Baldini et al., 2014; Dyab et al., 2016).

Detection of Wolbachia super group A using wsp136F and wsp691R

All positive samples from 16SrDNA screening were subjected to wsp136F/wsp691R screening. The primer sequence are wsp136F 5'TGAAATTTTACCTCTTTTC 3' and wsp691R 5'AAAAATTAACGCTACTCCA 3'. These primers are specific to Wolbachia super group A. PCR amplification were carried out for all the positive samples for 16SrDNA. A total reaction volume for a single reaction used was 25 µl. The mix contained 4 µl of DNA template, 2.5 µl of 10x reaction buffer, 2.5 µl of dNTPs mix, 0.35 µl each of forward (wsp136f) and reverse (wsp691r) primer, 0.127µl of 5U/µl Taq polymerase and 15.175 µl of DNase free water. The PCR reaction conditions were as follows; 1 cycle of initial denaturing for 1 min at 94°C followed by 35 cycles of 15 s at 94°C of denaturing, 30 s at 55°C of annealing, extension at 72°C for 1 min and a final extension for 7 min at 72°C with an expected amplicon size of 556 bp (Nugapola et al., 2017).

Detection of Wolbachia super group B using wsp81F and wsp522R

PCR amplification was carried out for all the positive samples for 16srDNA using Forward primer wsp81F 5'TGGTCCAATAAGTGATGAAGAAC 3'and a reverse primer wsp522R 5' ACCAGCTTTTGCTTGATA 3'. These primers are specific to Wolbachia super group B. A total reaction volume for a single reaction used was 25µl. The mixture contained 4µl of DNA template, 2.5 µl of 10x reaction buffer, 2.5 µl of dNTPs mix, 0.35 µl each of forward (wsp136f) and reverse (wsp691r) primer, 0.127 µl of 5 U/µl Taq polymerase and 15.175 µl of DNase free water. The PCR reaction conditions were as follows; initial denaturing of 1 cycle of 1 min at 94°C followed by 35 cycles of denaturing at 94°C for 15 s, 30 s at 55°C of annealing, extension at 72°C for 1 min and a final extension for 7 min at 72°C with an expected amplicon size of 442 bp (Nugapola et al., 2017).

Identification of microfilariae specie

Wuchereria bancrofti

The sspl gene is a repeat sequence which is a signature for *W. bancrofti*. PCR with specific primers for the Sspl gene were used to detect the presence of *W. bancrofti* in the sample. The forward

primer for the Sspl gene of *W. bancrofti* was *sspl* F 5'-CGT GAT GGC ATC AAAGTA GGG-3', and the reverse primer for the Sspl gene was *Sspl*R 5'-CCC TCA CTT ACC ATA AGA CAAC-3'. The PCR amplification for 48 cluster samples were carried out in a gradient thermal cycler. A total reaction volume of a single reaction of 25 µl, contained 4 µl of DNA template, 2.5 µl of 10x reaction buffer, 2.5µl of dNTPs mix, 0.35 µl each of forward and reverse primer, 0.127 µl of 5 U/µl Taq polymerase and 15.175 µl of DNase free water. The PCR reaction conditions were as follows: initial denaturing at 95°C for 5 min followed by 38 cycles of 1min at 94°C denaturing, 1 min at 56°C of annealing, 1 min at 72°C of extension and a final extension at 72°C for 10 min (Nugapola et al., 2017).

Brugia malayi

The *Hhal* gene in *Brugia malayi* is a tandem repeat sequence of about 320 bp. The *Hhal* gene is the target in the detection of *Brugia malayi*. The forward primer used was *Hhal* F 5'-GCG CAT AAA TTT ATC AGC-3'and the reverse primer was *Hhal* R 5'- GCG CAA AAC TTA ATTACA AAA GC-3'. The amplification of the gene was carried out using a Gradient Thermal cycler (Rebollo et al., 2015b). A total reaction volume of each of the 73 cluster samples used was 25 µl. This contained 2 µl of DNA sample, 2.5 µl of 10 x reaction buffers, 2.5 µl of dNTPs mix, 0.35 µl of each of forward and reverse primers, 0.125 µl of 5 U/µl of Taq polymerase and 15.175 µl of DNase free water. The reaction protocol was as follows; 95°C for 5 min, 38 cycles of 30 s at 95°C, 30 s at 56°C 30 s at 72°C, 5 min at 72°C, with an expected product size of 320 bp. The products from the nested PCR were subjected to gel electrophoresis (35 min at 100V) in 1% agarose gel stained with Ethidium and visualized using Chem-Doc Imaging System (BIO-RAD).

Statistical analysis

The association between Wolbachia and different genera of mosquitoes were analysed using bivariate fit of quadratic regression of Statistical Analysis System (SAS 9.4). Relationship between variables was determined using correlation analysis. Charts were fitted in Microsoft Excel. Significant association was set at $p < 0.05$.

RESULTS

Distribution of Anthropophilic Mosquito Genera in The Gambia. A total of 2003 anthropophilic mosquitoes from six divisions in The Gambia were classified into genera by microscopy method (magnification X10). Three genera of anthropophilic mosquitoes were identified as Anopheles, Culex and Aedes. *Mansonia* genera of mosquito was not detected in the total anthropophilic mosquitoes collected (Table 2). A total of 65.0% of the anthropophilic mosquitoes in The Gambia are Anopheles genera, 31.9% are Culex genera, while 3.1% belong to Aedes genera. None of the anthropophilic mosquitoes belongs to the *Mansonia* mosquito genera. The distribution pattern of the genera was similar across the six divisions. Figure 3 shows the pooled samples of anthropophilic mosquitoes in different divisions in The Gambia. There was significant differences ($p < 0.05$) among the mosquito genera across the division. SMD and CRD had the highest population of mosquitoes which was highly significant ($p < 0.05$) different from those caught in the other regions.

Table 2. Population of anthropophilic mosquitoes by genera in different divisions.

Division	Frequency (%)		
	Anopheles	Culex	Aedes
SMD	216 52.6	165 40.1	30 7.3
WD	265 74.7	84 23.9	5 1.4
NBD	207 70.4	81 27.6	6 2
LRD	176 69.3	68 26.8	10 3.9
CRD	255 65.5	129 33.1	6 1.5
URD	180 60	114 38	5 2

SMD = St Mary's Division; WD = Western Division; NBD = North Bank Division;
LRD = Lower River division; CRD = Central River division; URD = Upper River
Division.

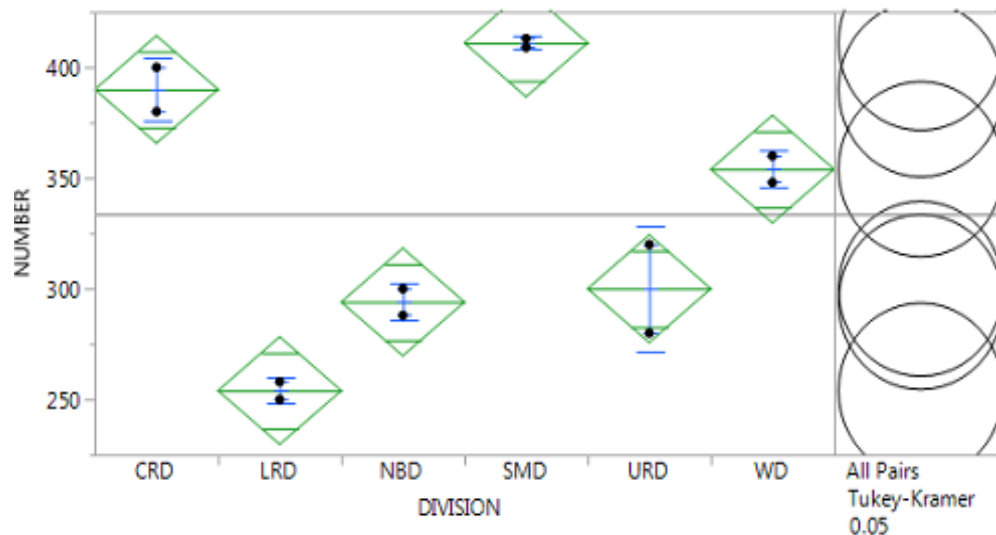


Figure 3. Pooled samples of anthropophilic mosquitoes in different divisions in The Gambia. CRD = Central River Division; LRD = Lower River Division; NBD = North Bank Division; SMD = St Marys Division; URD = Upper River Division.

Sexual dimorphism of anthropophilic mosquito genera

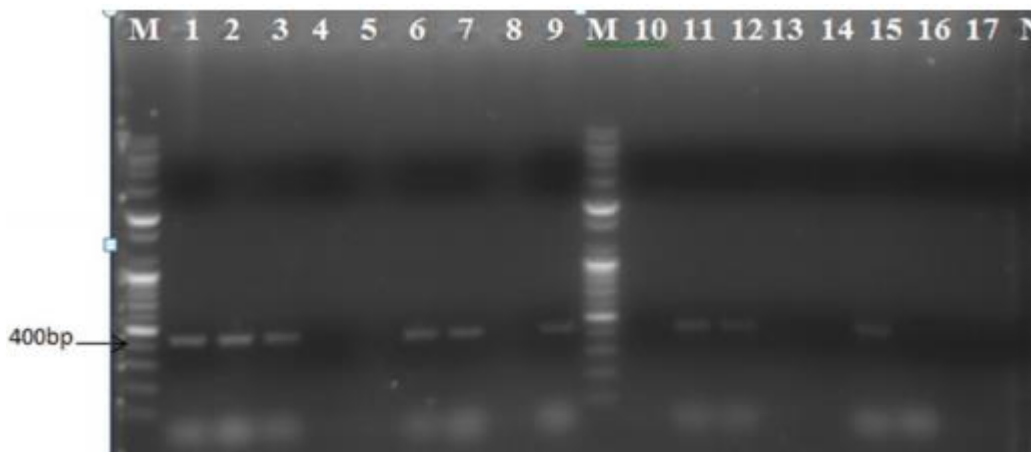
Sexual dimorphism of 2003 anthropophilic mosquito from

the six divisions in The Gambia were determined by microscopic technique, of which 82.1% were female while 13.0% were males. (Table 3) There is a significant difference ($p < 0.05$) in sexual dimorphism among

Table 3. Sexual dimorphism of anthropophilic mosquitoes in different divisions in The Gambia

Division	Frequency %		
	Female	Male	Aedes
SMD	386	25	30
	93.9	6.1	7.3
WD	299	55	5
	84.4	15.5%	1.4
NBD	232	64	6
	78.9	21.8	2
LRD	206	48	10
	81.1	18.9	3.9
CRD	352	38	6
	90.3	9.7	1.5
URD	269	31	5
	89.7	10.3	2

SMD = St Marys Division, WD = Western Division; NBD = North Bank Division; LRD = Lower River Division; CRD = Central River Division; URD = Upper River Division.

**Figure 4.** Molecular detection of Wolbachia in anthropophilic mosquitoes.

anthropophilic mosquitoes in The Gambia.

Detection of Wolbachia and classification into super group

Results of the DNA analysis from 120 clusters of mosquitoes for presence of Wolbachia and super group using 16SrDNA and wsp primers are presented in Figure

4 and 5. The results indicated a 34.17% incidence (Table 4) of Wolbachia in anthropophilic mosquitoes in The Gambia. All the three genera of mosquitoes were infected with Wolbachia (Figure 4). A higher proportion of the Anopheles harboured Wolbachia (50%), while Aedes had a significantly lower rate. The Wolbachia detected in Culex and Aedes belong to Super group B (Figure 5). Though Wolbachia was detected in Anopheles genera using 16SrDNA, the super group could not be determined

Table 4. Pooled samples of Wolbachia prevalence in anthropophilic mosquito genera.

Mosquito genera	Frequency		
	Cluster total	No. positive	No. negative
Anopheles	48	24 50%	24 50%
Culex	48	15 31.3%	33 68.8%
Aedes	24	2 8.3%	22 91.7%

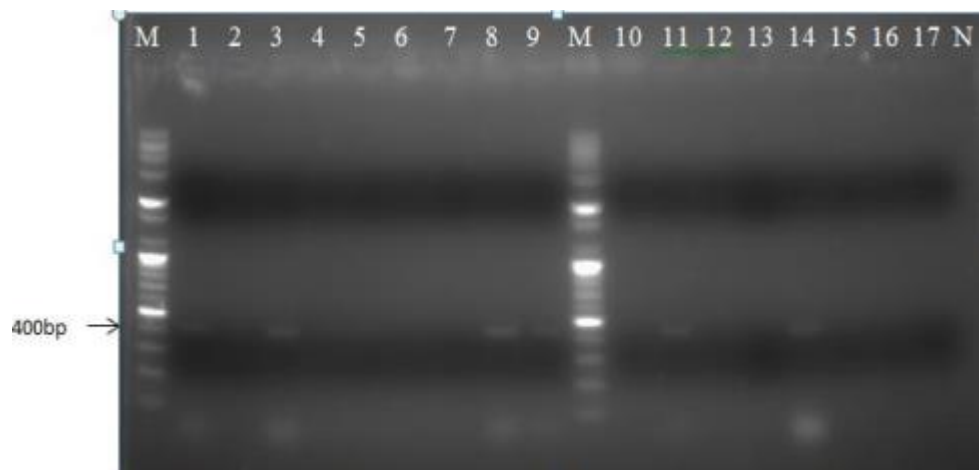


Figure 5. Molecular detection of Wolbachia super group B in Aedes samples.

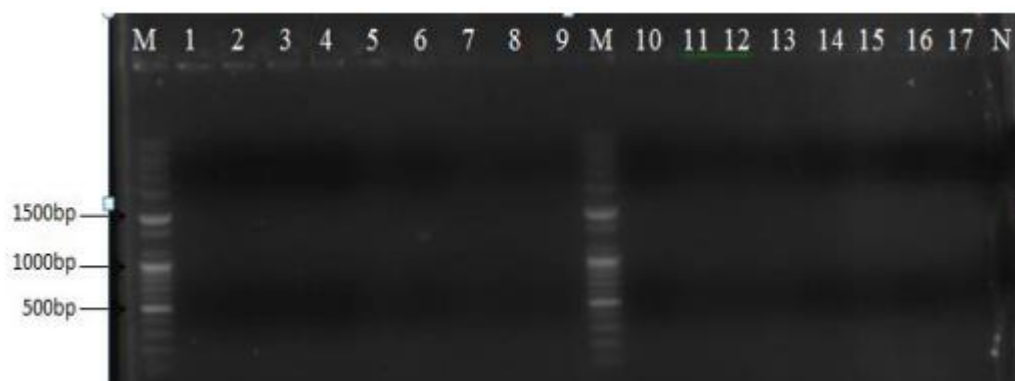


Figure 6. Molecular detection of *W. bancrofti* and *B. malayi* in anthropophilic mosquitoes.

using wsp primers. This was due to the insensitivity of wsp primers in detecting Wolbachia in Anopheles. However, no filarial nematode was detected in mosquitoes as seen Figure 6.

Wolbachia in anthropophilic mosquitoes in The Gambia. Lane M is the DNA marker. Lane N was the negative controls, that is: PCR reaction without gDNA. Samples from mosquitoes are represented in the

Table 5. Distribution of Wolbachia super groups among anthropophilic mosquito genera in the Gambia

Genera	Mosquito		% Frequency	
	No. Cluster	No. positive	Wolbachia A	Wolbachia B
<i>Anopheles</i>	48	0	0	0
<i>Culex</i>	48	15	0	31.3
<i>Aedes</i>	24	2	0	8.3
	120	17	0.0	14.17

Table 6. Bivariate fit of anthropophilic mosquito genera and Wolbachia

Genera	RMSE	R ²	r	P value
<i>Culex</i>	36.4	0.63	0.79	0.05*
<i>Anopheles</i>	84.35	0.36	0.60	0.29
<i>Aedes</i>	7.63	0.37	0.61	0.25

RMSE – Root mean square error; R²- Coefficient of determination; r - Correlation
p- Probability of regression coefficient I

following lane. PCR amplification of the 16SrDNA (438 bp) of Wolbachia in anthropophilic mosquitoes Lanes 1, 2, 3, 6 and 7 were positive for *Anopheles* samples. Lanes 9, 11, 12 and 15 was positive for *Culex* samples (Baldini et al., 2014; Dyab et al., 2016) (Figure 5). Wolbachia in anthropophilic mosquitoes in The Gambia. Lane M is the DNA marker. Lane N was the negative controls, that is: PCR reaction without gDNA. Samples from mosquitoes are represented in the following lane. Lane M is the DNA marker. Lane N was the negative controls, that is: PCR reaction without gDNA. Samples from mosquitoes are represented in the following lane. Lanes 1, 3, 6, 7, 8, 9, 11, 12 was positive for Wolbachia super group B in *Culex* samples Lane 14 was positive for Wolbachia super group B in *Aedes* samples. PCR amplification of the wsp (442 bp) for Wolbachia Super group B in anthropophilic Mosquitoes. This size of the signal were calculated by comparing its mobility to that of the standards' bands in the marker lane as demonstrated by (Nugapola et al., 2017) (Table 5).

Wolbachia – mosquito association

The association of Wolbachia and anthropophilic mosquito's genera in The Gambia was analysed using bivariate fit of quadratic regression of statistical analysis system (SAS 9.4). The analysis in Table 6 shows that Wolbachia has high and significant association ($p < 0.05$) with *Culex*. The association between Wolbachia and *Culex* was strong and positive ($r = 0.79$) with coefficient of determination of 63%. Association of both *Anopheles* and *Aedes* with Wolbachia is not significant ($p > 0.05$), though the correlation was high between Wolbachia and *Anopheles* ($r = 0.6$), the correlation between Wolbachia and *Aedes* is low (0.61).

Screening for the presence of *W. Bancrofti* and *B. Malayi* DNA in anthropophilic mosquitoes

Results of the screening of the DNA of the mosquitoes for filarial nematode using *ssp1* and *Hhal* gene primers (Figure 6) indicated that there were no nematode in the sampled mosquitoes Both *W. bancrofti* and *B. malayi* were absent in the mosquitoes in the six divisions of The Gambia.

Wolbachia in anthropophilic mosquitoes in The Gambia. Lane M is the DNA marker. Lane N was the negative controls, that is: PCR reaction without gDNA. Samples from mosquitoes are represented in the following lane. Lanes 1, 2, 3,4,5,6,7,8,9 were all negative for *Anopheles* samples, Lanes 10,11,12,13 and 14 were negative for *Culex* samples Lanes 15, 16 and 17 were all negative for *Aedes* samples. PCR amplification of the *sspl* (188 bp) and *hhal* (320 bp) of *W. bancrofti* and *B. malayi* in anthropophilic mosquitoes Nugapola et al., (2017).

DISCUSSION

Various genera of mosquitoes in The Gambia are distributed across all the divisions of the country, with the exception of *Mansonia* genera which was not detected among the anthropophilic family of mosquitoes sampled. Availability of fresh, clean or turbid water plays an important role in the distribution of mosquitoes. Fresh water body from rivers or ponds constitutes the highest environments for mosquito breeding sites. Although SMD and WD lacked with fresh river waters, urbanization has played a major role in providing mosquito breeding grounds through numerous sewages and waste water bodies produced daily by human activities (M'koumfa et

al., 2018). In CRD and URD, the high population could be attributed to the existence of fresh water bodies from the river which could provide a good breeding grounds for the mosquitoes. NBD and LRD registered the lowest in anthropophilic mosquito population compare to the other divisions. These two divisions characterized by sparsely distributed populations produces less sewages and waste water bodies couple with salty river waters reduces the chances of mosquito breeding compared to other divisions. The mosquito population in these two divisions are thus hampered by lack of adequate fresh water bodies all year round. The high proportion of Anopheles genera among mosquitoes in The Gambia is not unexpected. Anopheles is not restricted to clearly defined habitats for breeding. The genera have the potentials to breed in all open water bodies (Laporta et al., 2011). The lower proportion of Anopheles in SM when compared to other divisions, could be attributed to the fact that SMD is the most highly dense in human population with the highest industrial activities. SMD has also been designated as the most polluted divisions. Anopheles mosquitoes generally prefer unpolluted water bodies for breeding (Geissbühler et al., 2007; Laporta et al., 2011). It has been reported that pollution as a result of urbanization has eliminated certain species of Anopheles from urban centres (Geissbühler et al., 2007; Laporta et al., 2011). The least populated among the three existing genera in anthropophilic mosquitoes is the Aedes genera. Aedes genera generally breeds in artificial containers such as pots, tyres, open barrels containing clean water mainly of rain or natural containers of rain water such as holes in trees (Paupy et al., 2009; Nazri et al., 2013). The increased awareness in the fight against mosquito borne diseases which have resulted in the cleaning of the environment, by collecting discarded pots, tyres or any container which could hold water for mosquito breeding and the decline in amount of rain water received in recent times might have contributed to the low occurrence of Aedes mosquito genera in The Gambia. Difference in sexual dimorphism among anthropophilic mosquitoes in The Gambia is significantly higher in SMD, WD, CRD and URD where Wolbachia infection was evident in this study. The relationship between these two factors (mosquito sex and Wolbachia) has been explained by several researchers. Wolbachia induces cytoplasmic incompatibility in arthropods and distort the sex ratio (Bordenstein et al., 2001; Charlat et al., 2006; Telschow et al., 2007). Through cytoplasmic incompatibility Wolbachia modifies the spermatozoa such that male mosquitoes dies early in embryogenesis and also Wolbachia was also reported to have the potentials to feminize male mosquitoes during early embryogenesis. The significant differences in sexual dimorphism observed in this study may be attributed to the presence of Wolbachia in anthropophilic mosquito populations. The three genera of mosquitoes; Anopheles, Culex and Aedes among anthropophilic mosquitoes in The Gambia

are infected with Wolbachia. The incidence of infection is greater in Anopheles than Culex and Aedes. Although the mosquito-Wolbachia association is significant only in Culex, only the super group B of Wolbachia was identified in Culex and Aedes anthropophilic mosquitoes in The Gambia. The super group in Anopheles could not be detected by wsp primers in this study as also reported by (Kittayapong et al., 2000; Wiwatanaratanaaburt, 2013; Nugapola-nalaka et al., 2017). The insensitivity of wsp primers in detecting Wolbachia in Anopheles mosquitoes could be attributed to some form of mutation in the surface protein gene in Wolbachia. The inability of wsp primers in detecting Wolbachia has previously led to the conclusion that Anopheles genera are not naturally infected with Wolbachia (Nugapola et al., 2017). This conclusion spurred scientists in the World to attempt introducing Wolbachia artificially into natural population of Anopheles. However, screening with 16srDNA has now revealed that Anopheles mosquito can also harbour Wolbachia. The super group of the Wolbachia in Anopheles will be better determined by sequencing. None of the 120 clusters of anthropophilic mosquitoes (comprising 2003 mosquitoes) was positive for *W. bancrofti* and *B. malayi*, suggesting absence transmission of lymphatic filariasis in The Gambia at the sampling time. This finding is in agreement with the conclusion of Rebollo et al. (2015a) who stated that there is no longer active filarial transmission in the country as at the time of their study. It is probable that the high incidence of Wolbachia infection in anthropophilic mosquitoes could have contributed to this. This assumption is further strengthened by the very high dimorphic difference among the mosquito sexes.

Conclusion

Anopheles mosquitoes constitute the predominant anthropophilic mosquito genera in The Gambia. A high proportion of anthropophilic mosquitoes in the country are infected with Wolbachia, super group B, particularly in Culex and Aedes. *W. bancrofti* and *B. malayi* DNA were not detected in anthropophilic mosquitoes in The Gambia and hence no active transmission of lymphatic filariasis in the Gambia exist at the time of this study in The Gambia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was funded by the Africa Centre of Excellence for Neglected Tropical disease and forensic Biotechnology, Ahmadu Bello University, Zaria, Nigeria

and The Africa Centre of Excellence Project Team, The Gambia. Finally, we thank Dr. Idowu Aimola and the Late Dr. T.T Gbem for their helpful comments and assistance in the course of this work.

REFERENCES

- Atyame CM, Duron O, Tortosa P, Pasteur N, Fort P, Well M (2011). Multiple Wolbachia determinant control the evolution of cytoplasmic incompatibilities in *Culex pipiens* mosquito populations. *Molecular Ecology* 20(2):286-298.
- Baldini FN, Jullen P, Perrine MW, Robert S, Roch KD, Abdoulaye D, Elena A, Levashina A, Flaminia C (2014). Evidence of natural Wolbachia infections in field populations of *Anopheles gambiae*. *Nature Communication* 6(5):3985.
- Baldo L, Werren H (2007). Revising Wolbachia super group typing based on wsp: Spurious Lineages and discordance with MLST. *Current Microbiology* 55:367-373.
- Barton ES, Mark JT, Jeremy MF (2010). The Wolbachia endosymbiont as anti-filarial nematode target. *Journal of Symbiosis* 51:55-65.
- Bockarie MJ, Taylor MJ, Gyapong JO (2009). Current practices in the management of lymphatic filariasis. *Expert Review Anti-infection* 7:595-605
- Bordenstein SR, Patrick O, Werren JH (2001). Wolbachia-induced incompatibility precedes other hybrid incompatibilities in *Masonia*. *Nature* 409(6821):675-677.
- Bouchery T, Lefoulon E, Karadjian G, Nieguitsila A, Martin C (2013). The symbiotic role of Wolbachia in Onchocercidae and its impact on filariasis. *Elsevier* 9(2):131-140.
- Bouchery T, Lefoulo E, Karadjia G, Nieguitsila A, Martin C (2012). The symbiotic role of Wolbachia in Onchocercidae and its impact on filariasis. *Clinical Microbiology and Infection* 19(2):131-40.
- Cano J, Maria P, Golding N, Pullan RL, Crellen T, Soler A (2014). The global distribution and transmission limits of lymphatic filariasis: Past and present. *Parasites and Vectors* 7:466.
- Charlat S, Reuter M, Dyson EA, Hornett EA, Duploux AMR, Davies N, Roderick G, Wedell N, Hurst GDD (2006). Male-killing bacteria trigger a cycle of increasing male fatigue and female promiscuity. *Journal of Current Biology* 17(3):273-277.
- Duron O, Raymond M, Weill M (2010). Many compatible Wolbachia strains coexist within natural populations of *Culex pipiens* mosquito. *Heredity* 106(6):986-993.
- Dyab AK, Lamia AG, Abeer EM, Yasser M (2016). Finding Wolbachia in Filarial larvae and Culicidae Mosquitoes in Upper Egypt Governorate. *Korean Journal of Parasitology* 54(3):265-272.
- Engelstadter J, Hurst GDD (2009). The ecology and evolution of microbes that manipulates host reproduction. *Annual. Review of Ecology. Evolution and Systematic* 40:127-149.
- Fraser E, Bruyne T, Omaetxe I, Stepnell J, Burns L, Flores A, O'Neill L (2017). Novel Wolbachia Trans-infected *Aedes aegypti* mosquitoes possess diverse fitness and vector competence phenotype. *Journal of Arthropods* 5(1):211-216.
- Geissbühler Y, Chaki P, Emidi B, Govella NJ, Shirima R, Mayagaya V, Mtasiwa D, Mshinda H, Fillinger U, Lindsay SW, Kannady K, de Castro MC, Tanner M, Killeen GF (2007). Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malaria Journal* 6:126.
- Guan HW, Ling-Yi J, Jin H X, Da-Wei H (2016). Discovery of a new Wolbachia super group in cave spider species and the lateral transfer of phage WO among distant hosts. *Journal of Infection, Genetics and Evolution* 41:1-7.
- Hertig M, Wolbach SB (1924). Studies on Rickettsia-like microorganisms in insects. *Journal of Medical Research* 44:329-374.
- Kargbo A, Kuye RA (2020). Epidemiology of tsetse flies in the transmission of trypanosomiasis: Technical review of The Gambia experience. *International Journal of Biological Science and Chemical Science* 14(3):1093-1102.
- Kittayapong P, Baisley KJ, Baimai V, O'Neill SL (2000). Distribution and diversity of Wolbachia infection in southeast Asian mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology* 37(3):340-345.
- Lambert SWJ (2005). *Lymphatic filariasis: Transmission, Treatment and Elimination*. ISBN 10: 90-9019949-7.
- Laporta Z, Ramuo G, Riburo C, Sallum AM (2011). Habitat suitability of Anopheles vector species and association with human malaria in the Atlantic forest in South-eastern Brazil. *Memorias do Instituto Oswaldo Cruz* 106(1):111-119.
- M'koumfa B, Yaffa S, Bah A (2018). The impact of saline-water intrusion on the livelihoods of Gambian Rice Growing Farmers. *Research and Review: Journal of Ecology and Environmental Science* 6(1):January- March 2018.
- Nazri CD, Abu-Hassan A, Rodziah I (2013). Habitat characterization of *Aedes specie* breeding in Urban Hotspot Area. *Elsevier* 85:100-109.
- Nugapola-Nalaka NWP, Priyanka WA, De-Silva P, Parakrama-Karunaratne SHP (2017). Distribution and phylogeny of Wolbachia strains in wild mosquito population in Sri Lanka. *Parasites and Vectors* 10:230.
- Nwoke BEB, Nwoke EA, Nkaga CN, Nwachukwu MI (2010). Epidemiological characteristics of Bancroftian filariasis and the Nigerian environment. *Journal of Public Health and Epidemiology* 2(6):113-117.
- Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009). *Aedes albopictus*, an arbovirus vector from the darkness to light. *Elsevier* 11:1177-1185.
- Pfarr KM, Debrah AY, Specht S, Hoerauf A (2009). Filariasis and lymphoedema: Parasite Immunology 31:664-672.
- Porennan LT, Keddie BA, Braig HR, Harris HL (2008). The endosymbiont Wolbachia pipiens induces the expression of host antioxidant proteins in an *Aedes albopictus* cell line. *PLoS ONE* 3(5):e2083.
- Ravikumar H, Prakash BM, Sampathicumar S, Puttaraju HP (2011). Molecular subgrouping of Wolbachia and bacteriophage WO infection among some Indian Drosophila specie. *Journal of Genetic* 90:507-510.
- Rebollo MP, Sambou SM, Thomas B, Biritwum N-K, Jaye MC, Kelly-Hope L, Escalada AG, Molyneux DH, Bockarie MJ (2015a). Elimination of Lymphatic Filariasis in The Gambia. *PLOS Neglected Tropical Diseases* 9(3):e0003642.
- Rebollo MP, Sime H, Assefa A, Cano J, Deribe K, Gonzalez-Escalada A, Shafi O, Davey G, Brooker SJ, Kebede A, Bockarie MJ (2015b). Shrinking the lymphatic filariasis map of Ethiopia: reassessing the population at risk through nationwide mapping. *PLoS Neglected Tropical Disease* 9(11):e0004172.
- Singh G, Raksha A, Urhekar D (2013). Advanced Techniques for Detection of Filariasis: A Review. *Journal of Clinical and Diagnostic Research* 8(12):FC05-FC08.
- Sinkins SP (2004). Wolbachia and cytoplasmic incompatibility in mosquitoes. *Insect Biochemistry and Molecular Biology* 34(7):723-729.
- Stone A, Knight KL, Starcke H (1959). A synoptic catalog of the mosquitoes of the World (Diptera, Culicidae, Culicini). *Thomas Say Foundation* 6:1-358.
- Telschow A, Flor M, Kobayashi Y, Hammerstein P, Werren JH (2007). Rees, Mark, ed. "Wolbachia-Induced Unidirectional Cytoplasmic Incompatibility and Speciation. *PLoS ONE* 2(8):e701.
- Thrusfield MV (2007). *Veterinary epidemiology*. 3rd edition. Oxford Blackwell 610p.
- Vanek D, Silerova M, Urbanova V, Saskova L, Dusbska J, Beran M (2011). Genomic DNA extraction protocols for bone samples: The comparison of Qiagen and Zymo Research spin columns. *Forensic Science International: Genetics Supplement* 3(1):e397-e398.
- Werren JH, Baldo L, Clack M (2008). Wolbachia: Master manipulators of invertebrate biology. *Nature Review Microbiology* 6(10):51741-51744
- WHO (2013). *Lymphatic filariasis: Managing morbidity and preventing disability*. World Health Organization Global Programme to Eliminate Lymphatic Filariasis. SSBN: 978 92 4 150529 1. 1-20.
- WHO (2020). *Lymphatic filariasis, Key facts*, 2nd March, 2020. <https://www.who.int/news-room/factsheets/detail/lymphatic-filariasis>. Accessed 3/07/2020
- Wiwatanaratnaburt I (2013). Geographic distribution of Wolbachial

- infections in mosquitoes from Thailand. *Journal of Invertebrate Pathology* 114:337-340.
- Yeap HL, Mee P, Walker T, Weeks AR, Neill SL, Johnson P, Ritchie SA, Richardson KM, Doig C, Endersby NM, Hoffmann AA (2011). Dynamics of the popcorn 'Wolbachia infection in outbred *Aedes aegypti* informs prospects for mosquito vector control. *Journal of Genetics* 187:583-595.
- Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C, Bourtzis K (2004). Wolbachia induced cytoplasmic incompatibility as a means of insect pest population control. *Proceedings of National Academy of Science United States of America* 101(42):15042-15045.