Entomological assessment of lymphatic filariasis transmission in “hotspot” and control districts after several rounds of mass drug administration in Ghana

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ABR	annual biting rates
AGA	AngloGold Ashanti
CDD	community drug distributors
CFA	circulating filarial antigen
CHPS	community-based health planning and services
CVCs	community vector collectors
DDT	dichloro-diphenyl-trichloroethane
DEC	diethylcarbamazine
DNA	deoxyribonucleic acid
ESPEN	Expanded Special Project for Elimination of Neglected Tropical Diseases
EU	evaluation unit
FTS	Alere Filariasis Test Strip
GHS	Ghana Health Service
GPELF	Global Programme to Eliminate Lymphatic Filariasis
HLC	human landing collections
ICT	immunochromatographic test cards
IDA	ivermectin plus diethylcarbamazine plus albendazole
IEC	information, education and communication (for community engagement)
IRS	indoor residual spraying
IU	implementation unit
L_1, L_2, L_3	larval stage 1, 2 and 3
LF	lymphatic filariasis
LLNs	long-lasting insecticidal treated nets
MDA	mass drug administration
mf	microfilariae
MoFA	Ministry of Food and Agriculture
MX	molecular xenomonitoring
NMIMR	Noguchi Memorial Institute for Medical Research
NTD	neglected tropical disease
PCR	polymerase chain reaction
PSC	pyrethrum spray collections

RDT	rapid diagnostic test
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
s.l.	*senso lato*
s.s	*senso stricto*
Swiss TPH	Swiss Tropical and Public Health Institute
TAS	transmission assessment survey
VAL	validation
VC	vector control
WET	window exit trap
WHO	World Health Organization

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Summary

Background

Lymphatic filariasis (LF) is a major health problem, which mostly affects individuals in tropical and subtropical regions despite global efforts to control and interrupt its transmission in endemic countries. An estimated 120 million are infected, with about 40 million disfigured and incapacitated worldwide. The main strategy for the control of LF by the Global Programme to Eliminate Lymphatic Filariasis (GPELF) is through mass chemotherapy. In West Africa, specifically in Ghana, mass drug administration (MDA) commenced in the year 2000 with endemic districts receiving at least eight rounds of treatment. In principle, transmission of infections should have been interrupted in all areas after this long period of treatment with reported therapeutic coverage of more than 65%. However, recent information gathered from the Ghana Neglected Tropical Diseases Programme Unit has revealed ongoing transmission in some districts despite their involvement in at least eight rounds of MDA. The main aim of the GPELF is to eliminate this disease by year 2020. However, the current elimination status in Ghana poses a serious challenge in meeting this goal. It is therefore important to investigate driving factors that could possibly be responsible for the observed ongoing LF transmission in endemic districts in Ghana having undergone several rounds of MDA. This will provide information that will add on to existing evidence for appropriate intervention or approach specific to each district.

Aim and objectives

The main aim of this study was to explicitly look at entomological and sociological factors which might possibly be contributing to persistent LF transmission in “hotspot” districts, together with the development and validation of a community-based vector collection system. The specific objectives were (i) to establish a system for collecting large numbers of

mosquito samples for xenomonitoring, through the development of a community-based vector collection system; (ii) to determine the mosquito species composition in the various study districts; (iii) to determine the role of different species of mosquitoes in the transmission of lymphatic filariasis in the "hotspot" and control districts; (iv) to determine the role and variations in the cibarial armature of different mosquito species in the study communities; and (v) to undertake a questionnaire survey to determine compliance to MDA and possession and use of bednets and other vector control measures in the study districts.

Methods

This study was conducted in Ahanta West and Kassena Nankana West districts located in the Western and Upper East regions of Ghana, respectively. Both study areas were identified as "hotspot" districts in the country by the Ghana Neglected Tropical Disease Unit of the Ghana Health Service. This was due to high prevalence of LF in sentinel and cross check communities. Additionally, two control districts, Mpohor and Bongo, were also selected due to their zero microfilariae (mf) prevalence.

A 13-month (July 2015 - July 2016) collection of mosquitoes was concurrently conducted in all study districts. This involved the training of community vector collectors (CVCs) in the various mosquito collection methods, which included human landing catches, pyrethrum spray catches and window exit traps. Supervisors were further trained on how to package samples for shipment to the Noguchi research team. Sampled mosquitoes from the respective districts were later subjected to molecular analysis for the detection of *Wuchereria bancrofti* infections as well as determine the sibling species of the *Anopheles gambiae* complex. Mosquito dissections were also done to estimate various entomological transmission indices. Variations in cibarial armatures of various mosquito species were investigated by clearing of mosquito heads with chloral hydrate to make cibarial teeth visible for counting.

Questionnaires were administered in the various districts to obtain information on MDA compliance and vector control activities. Data were also obtained from the Ghana Neglected Tropical Disease Unit on the number of rounds and MDA coverage in the respective districts.

Results

A total number of 31,064 mosquitoes were collected from all the districts using human landing collections, pyrethrum spray catches and windows exit traps. Mosquitoes sampled were *Aedes, Anopheles coustani, An. gambiae, An. pharoensis, Culex* and *Mansonia* species. Molecular identification of *An. gambiae* complex showed *An. gambiae* s.s. in all districts. *An. arabiensis* and *An. melas* sibling species were identified from Kassena Nankana West/Bongo and Ahanta West districts, respectively. Furthermore, there was no difference in the shape and mean number of cibarial teeth of mosquitoes collected from hotspot and control districts in the Western and Upper East regions. In general, MDA coverage was ≥65% for all districts. However, MDA coverage in the Upper East region was <65% for Kassena Nankana West in 2003 and 2004/2005 in Bongo district.

Validation of mosquitoes sampled by CVCs showed no significant difference in the numbers sampled by CVCs and the research team in the dry ($P = 0.258$) and rainy ($P = 0.309$) season in southern Ghana. However, there was significant difference in the numbers sampled by research team and CVCs during the rainy ($P = 0.005$) and dry ($P = 0.033$) season in northern Ghana. Assessment of the cost-effectiveness of sampling mosquitoes for xenomonitoring activities using CVCs and research team was done. Results indicated that the cost of sampling mosquitoes was lower using CVCs compared to research team (USD 15.17 vs 53.74 USD). The highest recurrent and capital cost was personnel (USD 21,370.04) and transportation (USD 2,900.14) costs, respectively.

Furthermore, the assessment of *W. bancrofti* infection in mosquitoes as post-MDA surveillance tool using xenomonitoring was done. Results showed the sampling method human landing collections (27,739: 89.3%) recording the highest number of mosquitoes, followed by pyrethrum spray collections (2,687: 8.7%) and windows exit traps (638: 2.1%). Restriction fragment length polymorphism (RFLP) showed the high presence of *An. coluzzii* species in almost all districts. Dissections reported the presence of *W. bancrofti* in *An. melas* from Ahanta West district. Also, the annual transmission potential (ATP) for *An. melas* from the Ahanta West district was 7.4.

Conclusion/recommendations

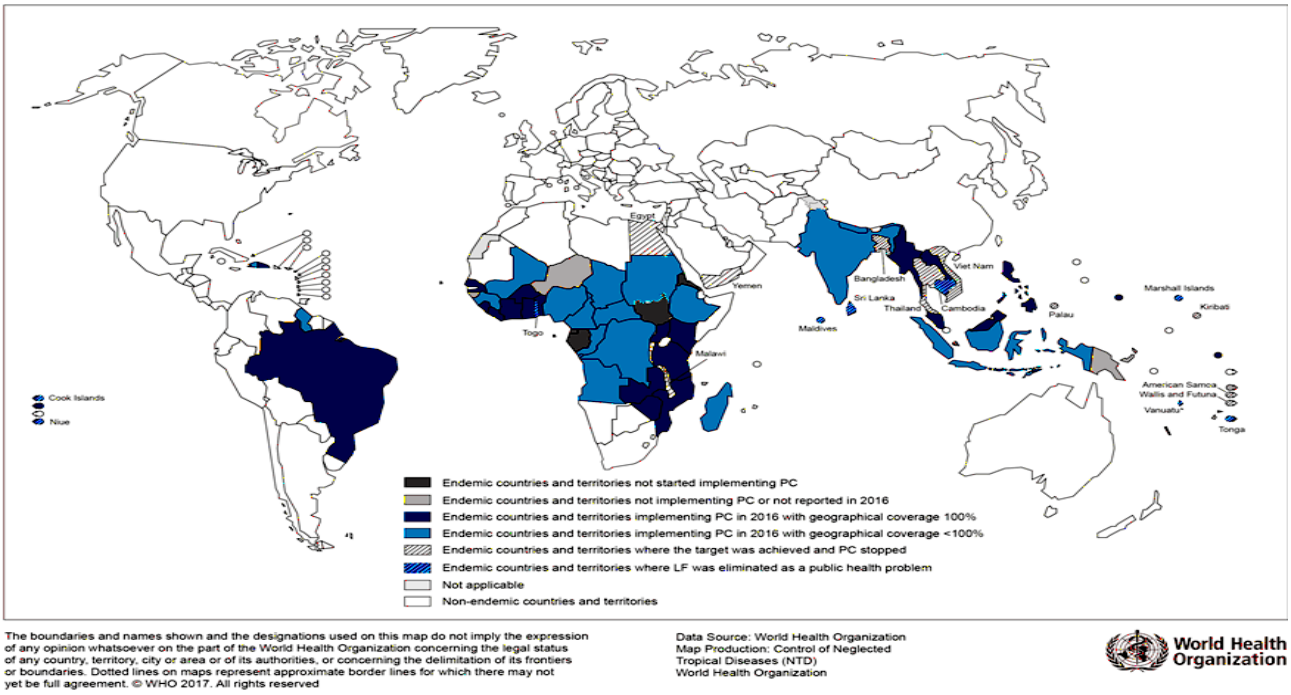
Persistent LF transmission in "hotspot" areas in this study presents information that shows the importance of local understanding of factors affecting elimination of LF. However, the study shows that it is feasible to use CVCs to sample large numbers of mosquitoes with minimal supervision. It is also cost-effective to use CVCs to collect mosquitoes for xenomonitoring compared to a dedicated research team. The inclusion of CVCs in xenomonitoring activities promotes active community participation and ownership of vector control activities. Additionally, *W. bancrofti* infections are found and sustained in Ahanta West district in *An. melas* that uses the phenomenon of limitation for lymphatic filariasis transmission. This study also showed the possibility of using xenomonitoring as a post-MDA surveillance tool. We recommend that LF interventions should consider spatial heterogeneities and best approach to use in all endemic foci. Moreover, xenomonitoring should be considered in the decision-making processes to stop or continue MDA by stakeholders and programme managers. Also, mosquito traps and sampling methods should be safe, practical and convenient for CVCs to use with less supervision and the inclusion of vector control activities by programme managers and stakeholders in planning intervention programmes.

Chapter 1: Introduction

1.1 Epidemiology and global distribution of lymphatic filariasis

Lymphatic filariasis (LF) is a leading cause of acute and chronic morbidity and disability in humans mostly located in the tropical and subtropical parts of the Americas, Asia, Africa and the Western Pacific (Bockarie and Molyneux, 2009; Owusu et al., 2015; Rebollo et al., 2015). LF which is endemic in 73 countries and affects 120 million people with about 1.46 billion people at risk of infection has been targeted as a public health problem for global elimination by 2020 (Rebollo et al., 2015). In achieving this goal of LF elimination as a public health problem globally, led to the formation of the Global Programme to Eliminate Lymphatic filariasis (GPELF) in 2000 after world health assembly adopted resolution WHA 50.29, passed in 1997 (Ottesen et al., 1997; Gyapong et al., 2018). The principal objective of GPELF was to interrupt LF transmission with preventive chemotherapy, together with managing morbidity and preventing disability (Ottesen, 2000; Ichimori et al., 2014).

Lymphatic filariasis parasites are harboured and transmitted by various mosquito species belonging to the genera *Anopheles*, *Aedes*, *Culex*, *Mansonia* and *Ochlerotatus* depending on the geographical location (Bockarie and Molyneux, 2009; Koudou et al., 2018). *Anopheles* and *Culex* species transmit LF in Africa (Ughasi et al., 2012). In West Africa, however, species belonging to the genera *Anopheles* act as principal vectors (Bockarie and Molyneux 2009; Aboagye-Antwi et al., 2015), with *Culex* species serving as main vectors in East Africa (Amuzu et al., 2010). About 90% of LF cases are transmitted by *Wuchereria bancrofti* worldwide, with *Brugia malayi* and *Brugia timori* accounting for the remaining infections (Taylor et al., 2010) which are mostly restricted to the Southeast Asian region (WHO, 2013a).



(Source: WHO Preventive Chemotherapy Joint Reporting Form. Annual country reports, 2016)

Figure 1.1 Distribution of lymphatic filariasis and status of preventive chemotherapy in endemic countries

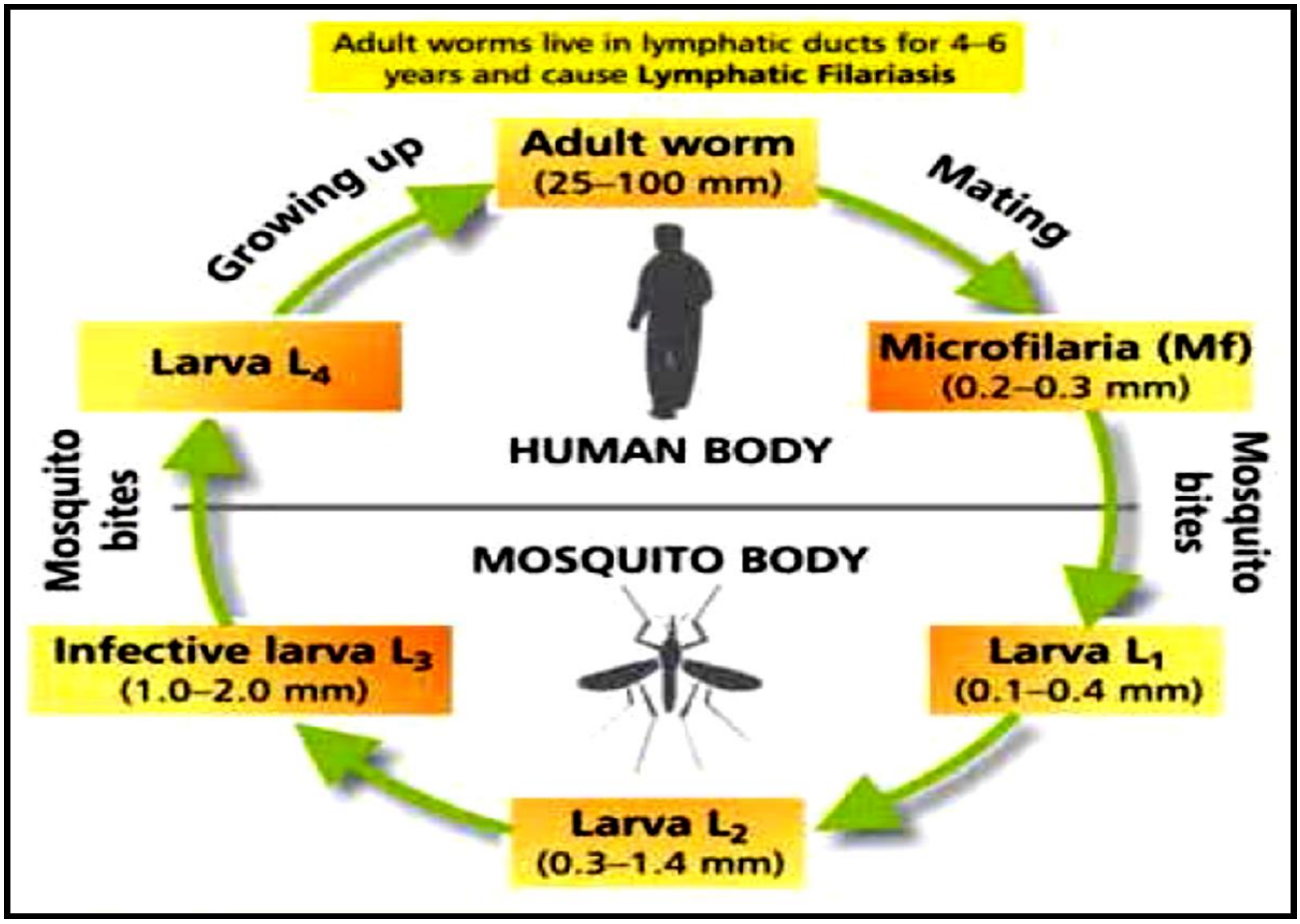
1.2 Transmission and life cycle of filarial parasites

The life cycle of parasites for both *Bancroftian* and *Brugian* filariasis are similar in mosquito and human hosts. Adult worms of filarial parasites are located in the nodules of the lymphatic system of humans where both male and female worms mate to produce microfilariae (mf) (Rebollo et al., 2015). With nocturnally periodic *W. bancrofti*, mf produced by adult female worms are able to circulate in the bloodstream to the peripheral blood vessels which most at times corresponds to the peak biting times of vectors (between 22:00 and 02:00 hours) (WHO, 2006). Female mosquitoes upon ingestion of blood meal ingest mf together with the blood. Microfilariae in the abdomen of mosquitoes move to the thoracic flight muscles where they transform into first stage larvae (L_1). The L_1 within a period of 12-14 days develops into the second (L_2) and infective third stage larvae (L_3), respectively (WHO, 2006). Female mosquitoes in an attempt to take a blood meal deposit L_3 located in the proboscis onto the skin. The L_3 larvae on the skin then move through the bite wound into the human body and in

the process develop into the adult worm L_4. Both male and female adult worms then migrate to the lymphatic vessels and lymph nodes where they mate to produce numerous mf into the bloodstream after about a year. The average life span of an adult worm is estimated to be between four to six years (Rebollo et al., 2015) Figure 1.2.

1.3 Factors affecting the transmission of filarial parasites

The intensity of LF transmission in an area is dependent on a number of factors. These factors could be environmental, behavioural, cellular, and biochemical (Beaty and Marquardt, 1996). Environmental factors like rainfall and temperature could influence the distribution and diversity of vectors indirectly affecting LF transmission (Bayoh et al., 2001; de Kelly-Hope et al., 2006; Souza et al., 2010). Additionally, there is a strong relationship between mf prevalence and intensity in humans, and mf intake and development in the mosquito vector (Koroma et al., 2013). This in turn means that lower mf intensity can lead to reduced LF transmission and vice versa (Southgate, 1992; Okorie and de Souza, 2016). Also, the vectorial capacity which mostly looks at the estimation of factors affecting the association between the vector and pathogen, together with the host to which the pathogen is transmitted is important in LF transmission (Okorie and de Souza, 2016). Exposure to infections is dependent on the vector density relative to man (vector abundance) and the human feeding behaviour (anthropophily) of the vector (Derua et al., 2012). Vector competence also necessary for transmission looks at how a vector is physiologically fit to maintain filarial parasites throughout their developmental stages (Boakye et al., 2004).



(Source: The pacELF way towards the elimination of lymphatic filariasis from the Pacific: 1999 – 2005).

Figure 1.2 The life cycle of *Wuchereria bancrofti* parasite

1.4 Density-dependent factors affecting lymphatic filariasis transmission

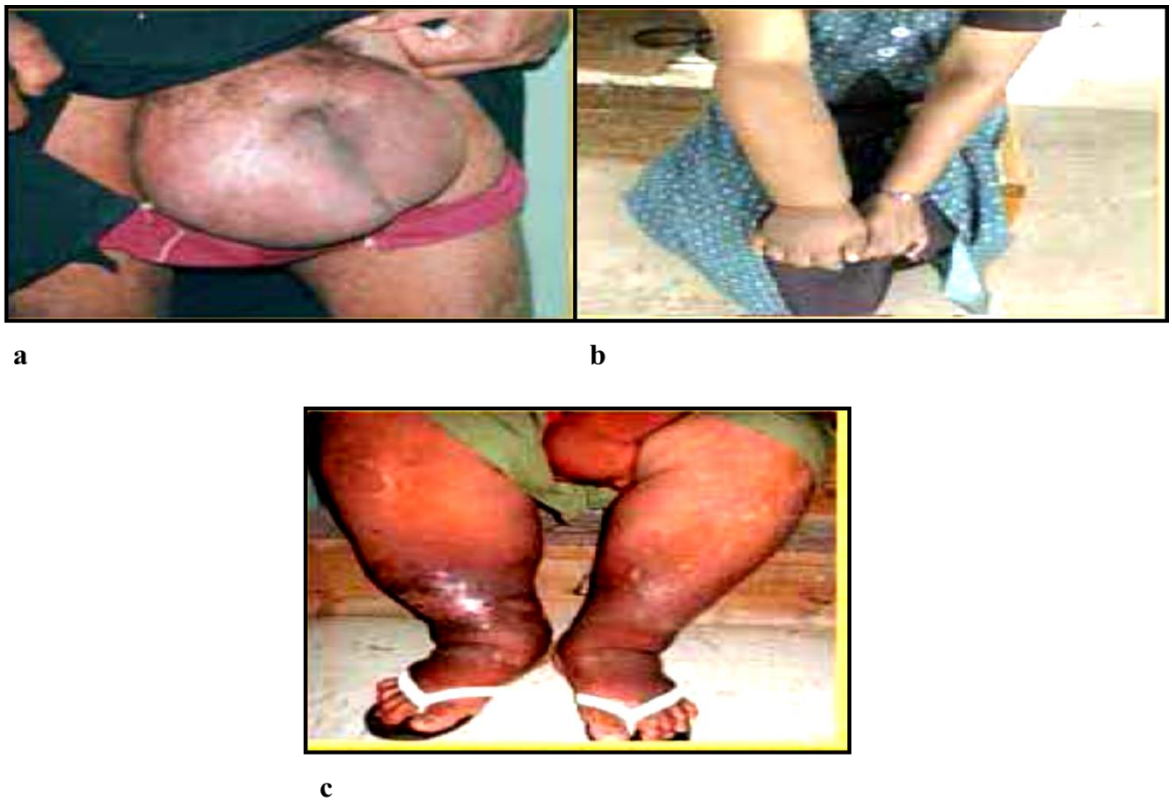
The GPELF strategy for the elimination of LF is based on mass chemotherapy for the reduction of circulating mf to threshold levels below which vectors cannot sustain transmission (de Souza et al., 2012). The competence of vectors to pick up mf at low filarial rates, support their development to the infective stage (L_3) and transmit to humans has to be understood for successful elimination of LF (Boakye et al., 2004). Vector-parasite combinations could also have an impact on transmission dynamics of LF based on the proportion of mf ingested which subsequently develop to L_3 (Southgate and Bryan, 1992; Pichon, 2002; de Souza et al., 2012). These vector-parasite combinations are described under

the density-dependent processes of “facilitation”, where mosquito species are unable to transmit parasites from humans at low mf rates, whereas with “limitation”, vectors can transmit at such low mf levels (Southgate and Bryan, 1992; Pichon, 2002; Boakye et al., 2004; de Souza et al., 2012). “Proportionality” on the other hand has a constant percentage of L_3 yield after ingestion of mf (Southgate and Bryan, 1992; de Souza et al., 2012). Therefore, in areas where vectors exhibit “facilitation”, MDA would be sufficient to interrupt transmission compared with areas where vectors exhibit “limitation” and therefore would require MDA being complemented with vector control (Boakye et al., 2004).

1.5 Clinical manifestations and pathogenesis of lymphatic filariasis

Clinical manifestations of lymphatic filariasis could be asymptomatic, acute or chronic (WHO, 2006). Asymptomatic infections present no signs of the disease for several years even though individuals may have circulating mf and also test positive for parasite antigen (Nutman and Kumaraswami, 2001; Gyapong et al., 2005). This type of infection normally results in altered immune system and damage to lymphatic vessels and kidneys (Gyapong et al., 2005). Acute infections on the other hand are mostly associated with filarial fevers due to inflammation of the lymph nodes, lymphatic vessels and connective tissues under the skin (WHO, 2006). Adult worms living in the lymphatics usually cause inflammation and dysfunction to the lymphatic system leading to chronic LF in affected individuals (Nutman and Kumaraswami, 2001). Some clinical manifestations associated with LF include hydrocoele, elephantiasis (lymphoedema), renal pathology resulting in chyluria, tropical pulmonary eosinophilia and acute dermatolymphangioadenitis (Gyapong et al., 1996; Koudou et al., 2018) Figure 1.5. The implications associated with physical manifestations of LF could present enormous personal and social effects on affected individuals. It can lead to divorce, sexual dysfunction and difficulty in having a marriage partner (Aboagye-Antwi et

al., 2015). Individuals are also normally subjected to scorn and stigmatization in their various communities leading to low self-esteem (Ahorlu et al., 2018). Furthermore, this could have serious socio-economic repercussions like unemployment for affected individuals and extra expenses incurred by relatives in caring for these patients (Aboagye-Antwi et al., 2015; Kouassi et al., 2018).



a **b**

c

(Source: The pacELF way towards the elimination of lymphatic filariasis from the Pacific, 1999 – 2005)

Figure 1.3 Physical manifestation and pathogenesis of lymphatic filariasis

a. hydrocoele, **b.** lymphoedema of the hand and **c.** lymphoedema of the leg (elephantiasis)

1.6 Programmatic steps of the GPELF in interrupting transmission

1.6.1 Mapping

The programmatic steps recommended by WHO (WHO, 2010) for interrupting transmission include mapping which is the first stage of the elimination programme. This step mostly

identifies implementation units (IU) that require mass drug administration (MDA) depending on the LF endemicity (Ichimori et al., 2014). The mapping process in order to identify an IU (mostly at the district level) eligible for MDA can review existing data by looking at both published and unpublished LF information, the existence of local names for LF, hospital information on hydrocelectomy as well as medical and health service reports (WHO, 2011). It should however be noted that the survey is not done in the entire IU but in very few areas (sentinel and spot check sites) within it (de Souza et al., 2015).

1.6.2 Mass drug administration

The main strategy adopted by the GPELF in the control of LF is mass drug administration (MDA) in endemic IU to reduce mf infection rates to levels that cannot sustain transmission (Biritwum et al., 2017b). About four to six rounds of MDA with effective minimum coverage (>65%) of the entire population is necessary in reducing mf in endemic communities (Ramaiah et al., 2002). However, the above decision was based on modelling good enough to roll out intervention programmes, as models may not have considered a confounding factor like spatial heterogeneities (Michael et al., 2017). This factor when considered in models may give predictions that might lengthen the timeline for LF elimination in an endemic area (Michael et al., 2017). MDA is mostly conducted using a community-based or directed approach in Africa as this has been proven to achieve high coverage levels (Koudou et al., 2018). Implementation of MDA is with albendazole in combination with either ivermectin or diethylcarbamazine (Gyapong et al., 2005). However, an approval was given by the WHO in 2017 for the use of a combination of the three drugs (IDA) in areas where onchocerciasis and loiasis are non-endemic (WHO, 2017a). As at 2015, the GPELF had provided a total of 6.7 billion treatments to endemic countries thereby causing a decline in endemicity to an estimated 36.6 million cases globally (WHO, 2017b). Since the inception of GPELF, 97

million LF cases have been prevented or cured which includes approximately 79.20, 18.73 and 5.49 million cases of mf carriers, hydrocoele and lymphoedema respectively (Gyapong et al., 2018). As at 2016, approximately 371.2 million persons in 32 countries from Africa required MDA (WHO, 2017b). However, Togo has been able to eliminate LF in Africa presenting one of the success stories of using MDA as intervention (Koudou et al., 2018). Furthermore, reported data also indicate MDA being stopped in Malawi, and scaled down in 9 other African countries (WHO, 2017b).

1.6.3 Post-MDA surveillance

According to WHO guidelines, mid-term progress evaluation is recommended after the third and fifth rounds of MDA in sentinel and spot check sites (WHO, 2011; Koroma et al., 2013). Additionally, the assessment of drug coverage after MDA is important to provide information on the level of participation of individuals in MDA within endemic regions (WHO, 2011). Guidelines and protocols have been provided by the WHO for successful monitoring and evaluation of LF infections post-MDA activities with diagnostic tests (Weil et al., 2013). These tests involve the detection of mf by examining stained blood using microscopy, or detecting circulating filarial antigen (CFA) in human blood by rapid diagnostic tests (RDTs) (Weil et al., 2013; Agbozo et al., 2018). RDTs recommended by the GPELF for use in LF endemic regions include BinaxNOW immunochromatographic (ICT) card (Weil et al., 1997) and the Alere Filariasis Test Strips (FTS) (Weil et al., 2013). CFA tests which are more sensitive than thick smear microscopy detect a 200 kDa parasite antigen, which is a sensitive and specific biomarker for the presence of adult *W. bancrofti* (Weil et al., 1997; Agbozo et al., 2018). They are also convenient to use because they require no electricity or skilled personnel, and can be used to test blood collected during the day or night in the field (Weil et al., 1997). Although BinaxNOW ICT cards were the first to be developed, challenges with

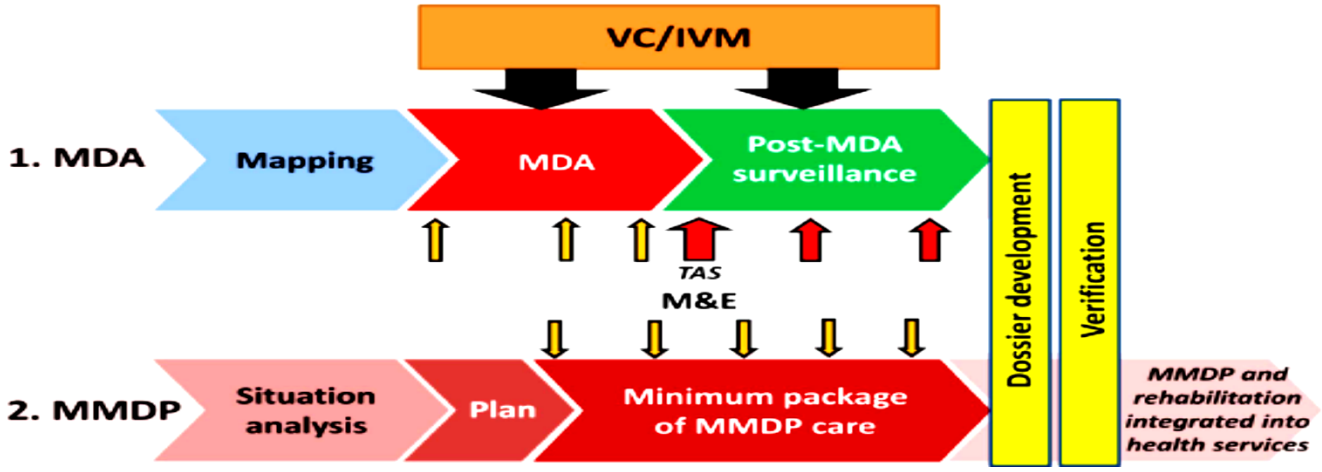
respect to its short shelf life of 3 months at ambient temperature, cost, narrow time window for reading test results and false-positive rates led to the development of FTS (Agbozo et al., 2018). Studies by (Weil et al., 2013) indicated that FTS has significant technical and practical advantages compared to BinaxNOW ICT cards, though more studies are needed to compare performance of both CFA tests in areas with low residual LF infection rates after multiple MDA rounds.

Furthermore, molecular xenomonitoring (MX) which is gaining recognition as one of the LF surveillance tools could be employed to complement CFA tests (de Souza et al., 2014; Schmaedick et al., 2014; Kouassi et al., 2015; Pilotte et al., 2016; Rao et al., 2016). MX can be used as proxy for the detection of *W. bancrofti* infections in humans using mosquito vectors (Schmaedick et al., 2014). Dorkenoo and colleagues (Dorkenoo et al., 2018) demonstrated the feasibility of using MX on a large-scale as post-validation tool to confirm the absence of infection in *An. gambiae* vectors of LF. It should however be noted that MX provides an indirect assessment of human infection (Schmaedick et al., 2014), and cannot provide direct measurement of ongoing transmission unless PCR targets the infective stage (L_3) of the parasite (Laney et al., 2008). Therefore, in order to increase the relevance of MX in programmatic decision-making process would require further development of efficient vector collection methods as well as improvement of understanding the relationship between prevalence of *W. bancrofti* DNA in mosquitoes, infection rates in humans and resulting transmission rates relative to critical thresholds (Schmaedick et al., 2014).

1.6.4 Transmission assessment survey (TAS)

The recommended post-MDA surveillance approach by the GPELF in making decisions to stop or continue MDA in an evaluation unit (EU) is by TAS (WHO, 2011; Chu et al., 2013; Ichimori et al., 2014; de Souza et al., 2015). TAS is used to determine if infections in endemic areas have been reduced to levels below which transmission cannot be sustained (de

Souza et al., 2015). The TAS target age group of 6-7 year old children is used since they have lived most or all their lives during MDA and therefore a filarial positive child would be indicative of recent LF infection (Chu et al., 2013; Ichimori et al., 2014). An implementation unit (IU) is considered eligible for TAS based on the criteria that at least five rounds of MDA has been conducted, MDA coverage for total population exceeds 65% and the mf and antigenaemia prevalence in sentinel sites or spot check sites is below 1% and 2%, respectively (WHO, 2011; Ichimori et al., 2014). The recommended diagnostic tools for the implantation of TAS in *W. bancrofti* and *Brugia* species endemic areas include immunochromatographic (ICT) test cards (filarial antigen) and Brugia rapid (BmR1 antibody test) respectively (WHO, 2011; Chu et al., 2013).



(Source: Lymphatic filariasis: a handbook of practical entomology for national lymphatic filariasis elimination programmes, WHO, 2016)

Figure 1.4 Strategy of the Global Programme to Eliminate Lymphatic Filariasis. Interrupting transmission through MDA and morbidity management and disability prevention (MMDP)

1.7 Vector control strategy for lymphatic filariasis elimination

Complementing lymphatic filariasis elimination programmes during MDA and post-MDA activities with vector control (VC) has been realised to play an important role in the interruption of LF in endemic areas (Bockarie et al., 2009; Ichimori et al., 2014). Implementation of VC reduces vector densities resulting in a decrease in vector-human

contact, thereby leading to lesser human exposure to filarial worms (WHO, 2013a). Vector control activities involving the use of long lasting insecticide-treated bed nets (LLNs) could greatly affect the transmission of LF (Koudou et al., 2018). An example can be seen in The Gambia where widespread use of LLNs for the control of malaria could have interrupted LF transmission (Rebollo et al., 2015). Furthermore, community-wide use of LLNs has been shown to have interrupted LF transmission in Nigeria (Richards et al., 2013) and Papua New Guinea (Reimer et al., 2013), respectively. Additionally, Solomon Island (Webber, 1979) and Togo (Brengues et al., 1969) are also known to have interrupted LF transmission by indoor residual spraying (IRS) using dichloro-diphenyl-trichloroethane (DDT).

1.8 Rationale

Lymphatic filariasis is a debilitating disease that mostly affects individuals in tropical and subtropical regions. The main strategy for the control of this disease is mass drug administration with a combination therapy of albendazole and ivermectin or diethylcarbamazine. However, in countries endemic for lymphatic filariasis but non-endemic for onchocerciasis and loiasis, a combination of the three drugs (IDA) has been proven to be effective. In West Africa, specifically in Ghana, mass drug administration commenced in year 2001 in ten districts, reaching national coverage by 2006. Therefore endemic districts would have received at least eight rounds of treatment. In principle, transmission of infection should have been interrupted in all areas after these numbers of years of treatment and reported therapeutic coverage of more than 65%. However, recent information gathered from the Ghana National Neglected Tropical Diseases (NTD) Programme has revealed ongoing persistent transmission in some districts despite their involvement in at least eight rounds of MDA. The main aim of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) is to eliminate LF by the year 2020. The current situation being observed in some districts in

Ghana poses a serious challenge in attaining this goal by the set time. There is therefore the need to investigate driving factors that might possibly be responsible for the current persistent ongoing transmission in the various endemic districts. This study was therefore designed to address these factors in the various districts as well as provide information on the appropriate intervention or approach specific to each district.

1.9 Objectives and aims

1.9.1 General objective

To investigate driving factors that could possibly be responsible for the present situation of ongoing lymphatic filariasis transmission in some districts in Ghana having undergone several years of mass drug administration.

1.9.2 Specific objectives

1. To establish a system for collecting large numbers of mosquito samples for xenomonitoring, through the development of a community-based vector collection system.
2. To determine the mosquito species composition in the various study districts.
3. To determine the role of different species of mosquitoes in the transmission of lymphatic filariasis in the “hotspot” and control districts.
4. To determine the role and variations in the cibarial armature of different mosquito species in the study communities.
5. To undertake a questionnaire survey to determine compliance to MDA and possession and use of bednets and other vector control measures in the study districts.

Chapter 2: An assessment of potential factors influencing lymphatic filariasis transmission in "hotspot" and "control" areas in Ghana: the importance of vectors

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2.1 Abstract

Background

Mass drug administration (MDA) programmes for the control of lymphatic filariasis in Ghana, has been ongoing in some endemic districts for 16 years. The study aimed to assess factors that could affect the success of MDA programmes for breaking transmission of lymphatic filariasis in Ghana.

Methods

The study was undertaken in two hotspots (Ahanta West and Kassena West) and two control districts (Mpohor and Bongo) in Ghana. Mosquitoes were collected and identified using morphological and molecular tools. A proportion of the cibarial armatures of each species was examined. Dissections were performed on *An. gambiae* for filarial worm detection. A questionnaire was administered to obtain information on MDA compliance and vector control activities. Data were compared between districts to determine factors that might explain persistent transmission of lymphatic filariasis.

Results

High numbers were sampled in Ahanta West district compared to Mpohor district (P = 0.002). There was no significant difference between the numbers of mosquitoes collected in Kassena Nankana West and Bongo districts (P = 0.185). *Mansonia* species were predominant in Ahanta West district. *An. coluzzii* mosquitoes were prevalent in all districts. *An. melas* with infected and infective filarial worms was found only in Ahanta West district. No differences were found in cibarial teeth numbers and shape for mosquito species in the surveyed districts. Reported treatment coverage was high in all districts. The average use of bednet and indoor residual spraying was 82.4% and 66.2%, respectively. There was high compliance in the five

preceding MDA treatments in Ahanta West and Kassena Nankana West districts, both considered hotspots of lymphatic filariasis transmission.

Conclusions

The study on persistent transmission of lymphatic filariasis in the two areas in Ghana present information that shows the importance of local understanding of factors affecting elimination of lymphatic filariasis. Unlike Kassena Nankana West district where transmission dynamics could be explained by initial infection prevalence and low vector densities, ongoing lymphatic filariasis transmission in Ahanta West district might be explained by high biting rates of *An. gambiae* and initial infection prevalence, coupled with high densities of *An. melas* and *Mansonia* vector species that have low or no teeth and exhibiting limitation.

Keywords: Lymphatic filariasis, microfilariae, mass drug administration, hotspots, vector control, systematic non-compliance

2.2 Background

Lymphatic filariasis is a debilitating disease affecting the health, productivity and wellbeing of infected individuals and communities (Gyapong et al., 2005; Krentel et al., 2013). Over 90% of infections worldwide is caused by *Wuchereria bancrofti* and the remaining by *Brugia* species (Bockarie and Molyneux, 2009). Mosquitoes belonging to the genera *Aedes*, *Anopheles*, *Coquillitedia*, *Culex*, *Mansonia*, and *Ochlerotatus* (depending on their geographical location) are involved in transmission (de Souza et al., 2012). In Ghana, the main vectors are *An*. *gambiae* and *An. funestus* senso lato (s.l.) and the minor are *An. pharoensis* (Dzodzomenyo et al., 1999) and *Mansonia* species (Ughasi et al., 2012).

It is assumed that in areas where the primary vectors are *Anopheles* species, about 5-6 rounds of mass drug administration (MDA) should be effective in breaking transmission of lymphatic filariasis (Snow et al., 2006). This assumption did not consider confounding factors such as spatial heterogeneities which when included in an intervention model may give predictions that could exceed the 5-6 rounds of MDA even with >65% MDA coverage for achieving lymphatic filariasis elimination in various endemic areas (Michael et al., 2017). A scenario modelled by Michael and colleagues (Michael et al., 2017) suggested that with the current MDA regimen, Ghana is likely to eliminate lymphatic filariasis by 2020. However, the authors indicated that lymphatic filariasis transmission is focal due to a number of factors including spatial heterogeneities (Michael et al., 2017). This therefore implies that interventions should at best consider these unique factors in each endemic foci. In Ghana, MDA commenced with five districts in the year 2000, and was scaled up to cover all endemic districts by 2006 (Biritwum et al., 2016). Hence, by 2014, each endemic district had received at least eight rounds of MDA, which was expected to have interrupted transmission. However, evaluations revealed that infections still persisted in 22 districts (‘hotspot’ districts) with microfilariae (mf) prevalence greater than 1% (Biritwum et al., 2017a).

The persistent transmission of lymphatic filariasis may be influenced by different factors (Kyelem et al., 2009; Amuzu et al., 2010; Ahorlu et al., 2018; Gyapong et al., 2018). These include pre-control lymphatic filariasis prevalence and infection intensity, population treatment coverage and compliance, vector competence and vectorial capacity and socio-cultural factors. *W. bancrofti* transmission in a vector population depends on the ability of mosquitoes to ingest and support the development of mf (Bryan et al., 1990). Importantly, mf ingested is affected by cibarial teeth, a physical barrier in the foregut of mosquitoes. This may influence the dynamics of filarial transmission and impact on control measures (McGreevy et al., 1978). Additionally, the initiation of infections for *W. bancrofti* depends on the availability of vector species and high vector biting rates (WHO, 2013a). The success of MDA also depends on the extent of the population treatment coverage. The recommended population treatment coverage by WHO should exceed 65% of the endemic population (WHO, 2011). Indeed, such MDA treatment coverage rates, coupled with effective compliance (i.e. willingness of individuals to ingest the drug), are necessary for a successful MDA programme.

In Ghana, lymphatic filariasis transmission persists in several districts, even after more than 10 rounds of MDA, despite reported average treatment coverage rates of >65%. Consequently, these districts are labelled as "hotspots" while others have passed the transmission assessment surveys (TAS) and have stopped MDA (Biritwum et al., 2016) are termed "control" for the current study. Our objective was to determine factors that influence the transmission of lymphatic filariasis, in selected hotspots and control districts in the Western and Upper East regions of Ghana.

2.3 Methods

2.3.1 Study sites

The study was conducted in eight communities from four districts in Ghana. There were four communities in two hotspot districts; namely, Asemkow (geographical coordinates 4°82' N, 1°88'W) and Antseambua (4°85' N, 1°93' W) in the Ahanta West district; and Badunu (10°96' N, 1°06' W) and Navio Central (10°96' N, 1°05' W) in the Kassena Nankana West district. Additionally, there were four communities in two control districts; namely, Balungo Nabiisi (10°93' N, 0°84' W) and Atampiisi Bongo (10°91' N, 0°82' W) in the Bongo district and Ampeasem (5°04' N, 1°94' W) and Obrayebona (5°00' N, 1°87' W) in the Mpohor district. The Ahanta West and Mpohor districts lie within the high rain forest vegetation climatic zone, whilst Kassena Nankana West and Bongo districts have sub-Sahelian climate (Figure 1).

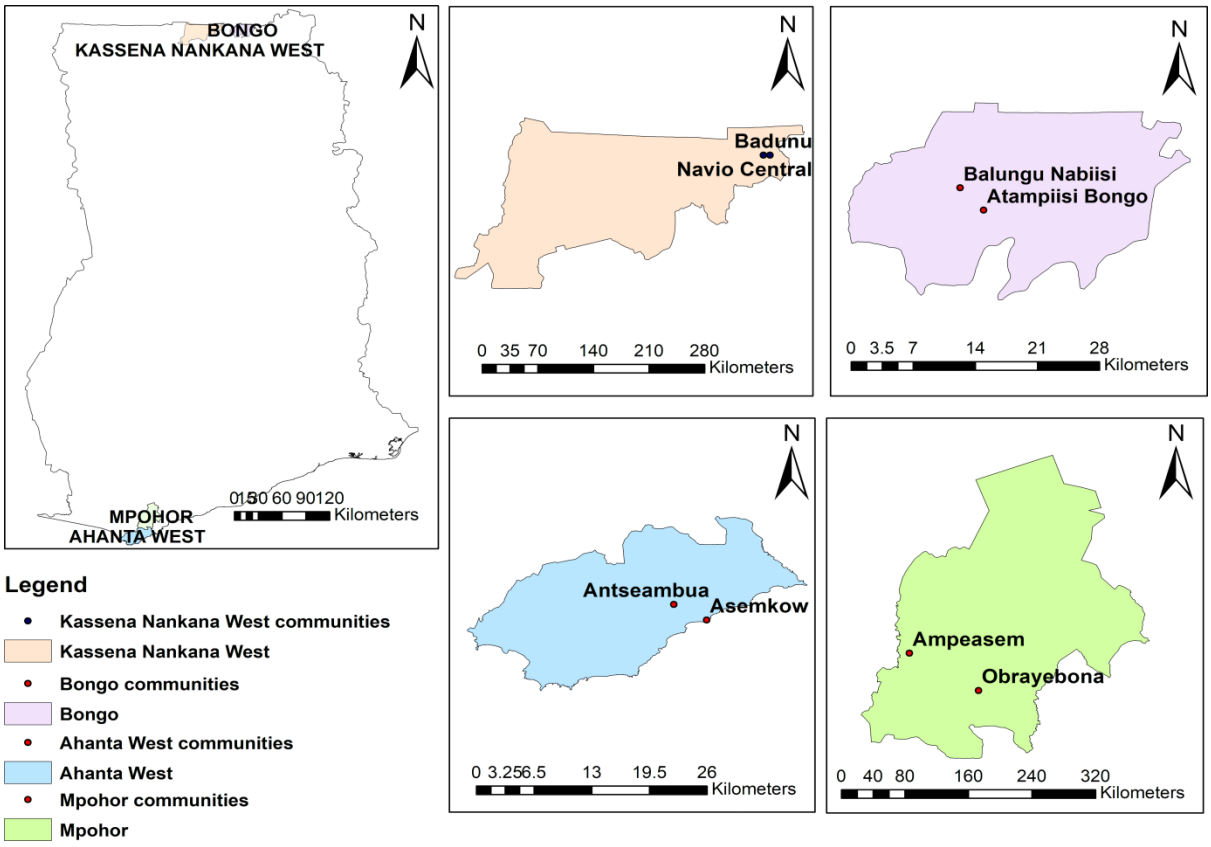

Figure 2. 1 Map showing lymphatic filariasis study districts from the Western and Upper East regions of Ghana

2.3.2 **Mosquito collection and processing**

Entomological surveys were conducted monthly in all the study communities. Mosquitoes were collected over a 13-month period from the beginning of July 2015 to the end of July 2016. Samples were collected using window exit traps, pyrethroid spray catches and human landing catches (WHO, 2013a). In each district, there were 16 community vector collectors (CVCs). Each district had two communities selected and the eight CVCs divided into two teams (4 per team). Human landing catches involved 2 CVCs sampling indoor, and the other 2 outdoor in 2 different households simultaneously for every sampling night. Mosquitoes were collected hourly from 21:00 to 5:00 hrs the next morning. Starting human landing catches earlier instead of the 21:00 hrs would not have had any significant impact on the results as relatively few *An. gambiae* s.l. bite before 21:00 in the Upper East region (Boakye et al., 2004). This time was therefore replicated in other districts to have a uniform setting. Pyrethrum spray collection was done by the CVCs from 5:00 to 8:00hrs in up to 10 different households. Before every sampling night, 2 window exit traps were fixed in 2 different households at 18:00 hrs, and removed after 8:00 hrs the next morning. Sampling was done twice a month in two different households every catch night in each community. All mosquitoes were identified at species level, using morphologic identification keys (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987).

Molecular identification was done by extracting DNA from mosquito legs using a standard protocol described by Xu and Xu (Xu and Xu, 1998). Sibling species of *An. gambiae* complex were identified using polymerase chain reaction (PCR), as described by Scott and colleagues (Scott et al., 1993). This was followed by restriction fragment length polymorphism (RFLP) to distinguish the species *An. coluzzii* and *An. gambiae* senso stricto (s.s.) (Fanello et al., 2002).

2.3.3. Assessment of infection and infectivity rates in *An. gambiae*

In general, the rationale for selecting mosquitoes was aimed at having proportional numbers of mosquitoes in the various districts dissected for the estimation of infection and infectivity. Samples collected with human landing catches were used to estimate infection, infectivity and annual biting rates. For estimation of infection and infectivity rates, *An. gambiae* samples were dissected and observed for the various stages of the parasites (WHO, 2013a).

2.3.4 **Cibarial armature characterisation**

The heads of 224 mosquitoes (anophelines and culicines) consisting of 14 mosquitoes per species for each district were selected with reference to similar studies (Chwatt and Major, 1945; Boza and Vargas, 2006; Amuzu et al., 2010). The mosquito heads were detached and placed in a 1.5 ml microcentrifuge tube containing clearing medium (consisting of equal volumes of chloral hydrate and phenol) (Amuzu et al., 2010). Tubes were kept in the dark for about a week to clear the mosquito heads (Amuzu et al., 2010). Clearing took longer for dark (highly melanised) mosquitoes, such as *Aedes* species (approximately one month). After clearing, the mosquito heads were placed on a clean glass slide and a drop of Puri's (mounting) medium was added before covering with a cover slip. The heads were mounted dorso-ventrally to enhance viewing and counting of the cibarial teeth. The cibarial armature was observed under a compound microscope at 1,000 X magnification. The mounted mosquito head was kept at room temperature for at least one week and the total number of cibarial teeth counted and recorded.

2.3.5 Questionnaire survey

Our study pursued a cross-sectional design with questionnaires randomly administered to individuals in the various districts. The questionnaire sought to obtain information about treatment compliance and involvement in vector control activities in the study districts.

2.3.6 Statistical analysis

Data were entered using Microsoft Excel (2013 version) and imported into STATA version 11 (Stata Corporation; College Station, TX, USA). We checked for significant differences of the cibarial teeth numbers according to mosquito species, and of mosquito abundance comparing hotspot and control sites using *F*-test. Data obtained from the National Neglected Tropical Diseases Control Programme pertaining to MDA coverage in the various communities within the various districts were entered in Excel and annual frequencies of MDA coverages calculated at the unit of the district. The frequencies for MDA compliance were analysed using EpiInfo version 7 (Centers for Disease Control and Prevention; Atlanta, CA, USA). Statistical significance was considered when *P* was below 0.05. Entomological parameters assessed included:

- Infection rate: proportion of mosquitoes found infected after dissection with any *W. bancrofti* larval stage -

  [Number of mosquitoes with (mf or L_1 or L_2 or L_3)]/[Number of mosquitoes dissected]*100
- Infectivity rate: proportion of mosquitoes found infected with one or more infective larvae. [Number of mosquitoes with L_3]/[Number of mosquitoes dissected]*100
- Annual biting rate: estimated number of mosquitoes biting a human per year – [(Number of mosquitoes caught)/(Number of catchers*number of catch night)]*365 days (McMahon et al., 1981; Appawu et al., 2001; WHO, 2013).

2.3.7 Ethics statement

This study was approved by the institutional review board of the Noguchi Memorial Institute for Medical Research (Accra, Ghana; CPN 077/13-14) and the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland; 122a). All CVCs consented verbally to participate in the study. Albendazole and ivermectin were

administered to CVCs before mosquito sampling commenced. Arrangement was also made with the nurses at the community-based health planning and services (CHPS) compound to provide treatment for CVCs who reported at their facility and tested positive for malaria.

2.4 Results

2.4.1 Mosquito species composition and abundance

A total of 31,064 mosquitoes were sampled from all the study areas. There was a significant difference in the number of mosquitoes collected from Ahanta West district compared to Mpohor district in the Western region (P = 0.002). No difference was observed between hotspot and control districts for the Upper East (P = 0.185). The mosquitoes collected in this study were *Aedes* species, *An. coustani*, *An. gambiae* s.l., *An. pharoensis*, *Culex* species and *Mansonia* species. *An. gambiae* s.l., which serves as the principal vector of lymphatic filariasis in Ghana, was the most abundant mosquito species sampled in hotspot and control districts in both the Western and Upper East regions. Relatively higher numbers were sampled from the Ahanta West district (Table 1). Figure 2 shows the total number of *An. gambiae* mosquitoes sampled for the various months from all the study areas. The ABRs for mosquitoes sampled by human landing catches in Ahanta West, Mpohor, Kassena Nankana West and Bongo districts were 15,987, 3,604.4, 376.3 and 306 bites per person respectively. There was a significant difference in ABR between Ahanta West and Mpohor districts (P = 0.002), but not between Kassena Nankana West and Bongo districts (P = 0.718). Mosquitoes belonging to the genus *Mansonia* were the second most abundant sampled in Ahanta West district (n = 2,434) compared to Mpohor (n = 80). The Upper East region, however, had *Culex* being the second most abundant species with relatively high numbers sampled from Kassena Nankana West district (n = 879) compared to Bongo (n = 626). In Ahanta West district, more *Culex* species collected compared to Mpohor district. Relatively low numbers

of *Aedes*, *An. pharoensis* and *An. coustani* were sampled from all study areas in the Western and Upper East regions.

Table 2. 1 Species composition and abundance of mosquitoes collected from the study sites

		Total number of mosquito species collected (2015-2016)								
District (hotspot/control)	**Region**	***An. gambiae***	***An. pharoensis***	***An. coustani***	***Culex* species**	***Ma. uniformis***	***Ma. africana***	***Aedes* species**	**Total number of mosquitoes collected (%)**	**Species identified molecularly**
Ahanta West (hotspot)	Western	18,880	36	4	1,221	774	1,660	9	22,584 (72.7)	*An.coluzzii/An. melas*
Mpohor (control)	Western	4,603	10	3	81	61	19	7	4,784 (15.4)	*An. coluzzii*
Kassena Nankana West (hotspot)	Upper East	1,239	4	13	879	9	3	44	2,191 (7.1)	*An.coluzzii/An. arabiensis*
Bongo (control)	Upper East	826	4	2	626	3	2	42	1,505 (4.9)	*An.coluzzii/An. arabiensis*
	Total								31,064 (100)	

Molecular identification of the *An. gambiae* complex showed that *An. gambiae* s.s., *An. melas* and *An. arabiensis* were the only species identified as sibling species. *An. arabiensis* were identified in both hotspot and control districts in the Upper East region, whilst *An. melas* were found only in Ahanta West district in the Western region. Further molecular analysis of *An. gambiae* s.s. indicated that *An. coluzzii* species (previously the M form of *An. gambiae* s.s.) (Coetzee et al., 2013) was the only species in the study areas.

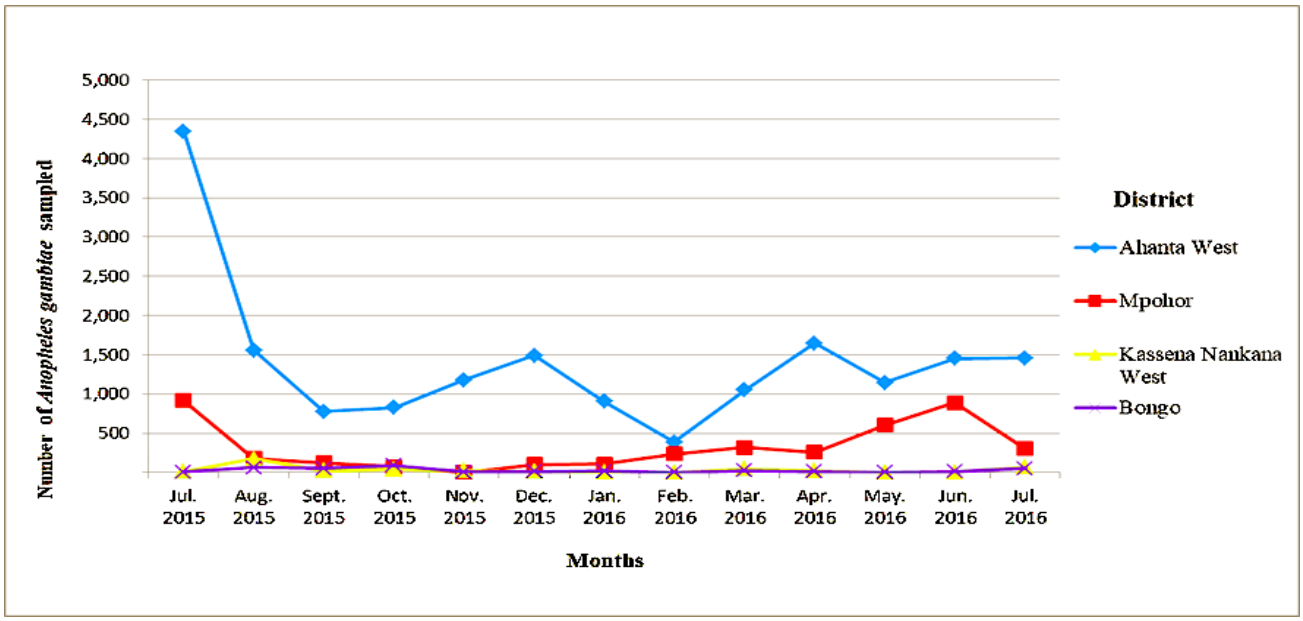

Figure 2. 2 *Anopheles gambiae* sampled from Western and Upper East regions, Ghana from July 2015 to July 2016

2.4.2 Infection and infectivity rate for *An. gambiae* complex

A total of 1,116 mosquitoes were selected for the 13 months spanning both wet and dry seasons in all districts. Ahanta West, Mpohor, Kassena Nankana West and Bongo districts had a total of 320, 368, 217 and 211 mosquitoes dissected respectively. A total of eight mosquitoes were found positive for the various stages of the filarial parasite (mf, L_1, L_2, L_3), with 2 samples being infective (L_3). All samples found positive were *An. melas* found only in the Ahanta West district. The average infection and infectivity rates were 0.025 (2.5%) (95% CI 0.8, 4.2) and 0.006 (0.6%) (95% CI 0.0, 1.5) respectively. Conventional PCR was used to

confirm the presence of *W. bancrofti* (Ramzy et al., 1997). Dissected samples from Mpohor, Kassena Nankana West and Bongo however tested negative for the filarial parasite.

Table 2. 2 The annual biting rates for lymphatic filariasis vectors in four districts, Ghana

Annual biting rate (ABR) (bites/person/year)				
Mosquito species	**Ahanta West**	**Mpohor**	**Kassena Nankana West**	**Bongo**
An. gambiae	15,987	3604.4	376.315	306.6
***Mansonia* species**	2093.5	63.2	9.7	4.4

The annual biting rates due to human landing catches for *An. gambiae* complex and *Mansonia* species, vectors for lymphatic filariasis transmission in four districts from Ghana.

2.4.3 Cibarial armature characterisation

Out of 224 mosquito heads processed, 140 samples properly cleared, and hence, were used for cibarial armature analysis. These samples were from both hotspot and control districts. The observation of the cibarial teeth of *An. gambiae* complex all showed that the teeth were sharp, pointed and long, but relatively fewer than that of *An. pharoensis*, which had pointed deep–rooted narrow based teeth. *Culex* species had the highest number of teeth, which were short, small sized and blunt. *Aedes*, *Ma. uniformis* and *Ma. africana* species had no cibarial teeth. The above description for the structure and shape of the cibarial teeth was similar for all mosquito species from hotspot and control districts in the two regions (Table 3). The structure of cibarial armatures of the various species are shown in Figure 3. The mosquito species with the highest mean number of teeth was observed among *Culex* mosquitoes for both hotspot and control sites in the Western and Upper East regions, and the lowest observed in *An. melas*, which was found only in Ahanta West district (Table 3). There were no significant differences in the mean number of teeth between *An. coluzzii* (F = 2.121, P = 0.243) from hotspot and control study areas in the Western region. This was same for *Culex*

(F = 3.000, P = 0.250) from this region. Results from Bongo and Kassena Nankana West also showed no significant differences in the mean number of teeth for *An. coluzzii* (F = 0.628, P = 0.277), *Culex* (F = 0.583, P = 0.231) and *An. pharoensis* (F = 0.571, P = 0.363).

Table 2. 3 Mosquito heads from the Western and Upper East regions, cleared and cibarial armature examined

District (hotspot/control)	Mosquito species	Mean no. of teeth/SD	Median (teeth range)	Description of teeth (shape)
Ahata West (hotspot)	*An. coluzzii*	16.0/ ± 1.0	16 (15-17)	Sharp/pointed/long
	Culex species	24.3/ ± 2.2	24.5 (21-27)	Small/blunt/short
	Mansonia species	0.0/ ± 0.0	0 (0)	Teeth absent
	Aedes species	0.0/ ± 0.0	0 (0)	Teeth absent
	An. melas	13.3/ ± 0.5	13 (13-14)	Sharp/pointed/long
Mpohor (control)	*An. coluzzii*	16.0/ ± 1.7	15 (15-18)	Sharp/pointed/long
	Culex species	25.2/ ± 1.4	25 (23–27)	Small/blunt/short
	Mansonia species	0.0/ ± 0.0	0 (0)	Teeth absent
	Aedes species	0.0/ ± 0.0	0 (0)	Teeth absent
Kassena Nankana West (hotspot)	*An. coluzzii*	15.8/ ± 1.8	15 (13–18)	Sharp/pointed/long
	An. pharoensis	21.3/ ± 1.5	21 (20–23)	Pointed/deep-rooted/narrow based
	Culex species	26.8/ ± 2.0	26 (25–30)	Small/blunt/short
	Mansonia species	0.0/ ± 0.0	0 (0)	Teeth absent
	Aedes species	0.0/ ± 0.0	0 (0)	Teeth absent
	An. arabiensis	16/ ± 0.0	16 (16)	Sharp/pointed/long
Bongo (control)	*An. coluzzii*	15.8/ ± 1.4	15 (14-18)	Sharp/pointed/long
	An. pharoensis	20.7/ ± 1.2	20 (20–22)	Pointed/deep-rooted/narrow based
	Culex species	25.8/ ± 2.7	24 (24-30)	Small/blunt/short
	Mansonia species	0.0/ ± 0.0	0 (0)	Teeth absent
	Aedes species	0.0/ ± 0.0	0 (0)	Teeth absent
	An. arabiensis	16/ ± 0.0	16 (16)	Sharp/pointed/long

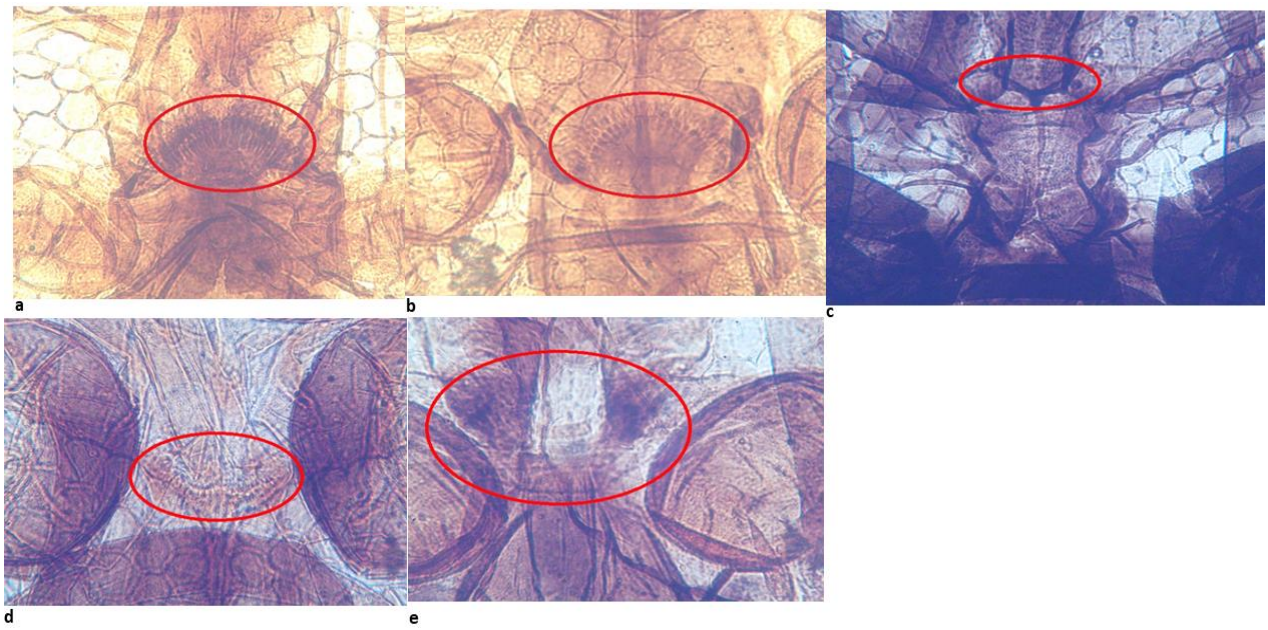

Figure 2. 3 Cibarial armatures of mosquitoes from Western and Upper East regions, Ghana, July 2015 to July 2016

a. *An. gambiae* complex b. *An. pharoensis* c. *Aedes* species d. *Culex* species and e. *Mansonia* species. The cibarial armatures of the mosquito species *Culex*, *An. gambiae* complex and *An. pharoensis* have cibarial teeth present. There are no cibarial teeth present for *Aedes* and *Mansonia* species.

2.4.4 MDA coverage and baseline (pre-intervention) mf and antigenaemia prevalence

Analysis of MDA coverage data showed the treatment coverage for the various years in both Ahanta West and Mpohor districts to be above 65%. However, in the Upper East region, Kassena Nankana West and Bongo districts had greater than 65% MDA coverage for all years indicated except in 2003 for Kassena Nankana West and 2004/5 for Bongo districts (Figure 4). By 2016, Ahanta West, Mpohor, Bongo and Kassena Nankana West districts had been involved in 16, 11, 13 and 15 rounds of MDA, respectively. However, there were no MDA data for some of the years (from 2000 to 2014) in all the districts. Data were absent for Mpohor, Kassena Nankana West and Bongo districts for 2001. Ahanta West/Mpohor and Bongo had no data for the years 2002 and 2010, respectively. All districts, however, had no data for 2007, 2008, 2009, 2011 and 2012.

A retrospective assessment of baseline mf and antigen prevalence for the various districts showed high baseline mf and antigenaemia prevalence for all districts, except Mpohor where zero prevalence was reported for both mf and antigen. The baseline mf and antigen prevalence for Ahanta West, Kassena Nankana West and Bongo districts were; 19.5% and 32.8%, 29.4% and 45.3%, and 16.7% and 21.2%, respectively (Table 3).

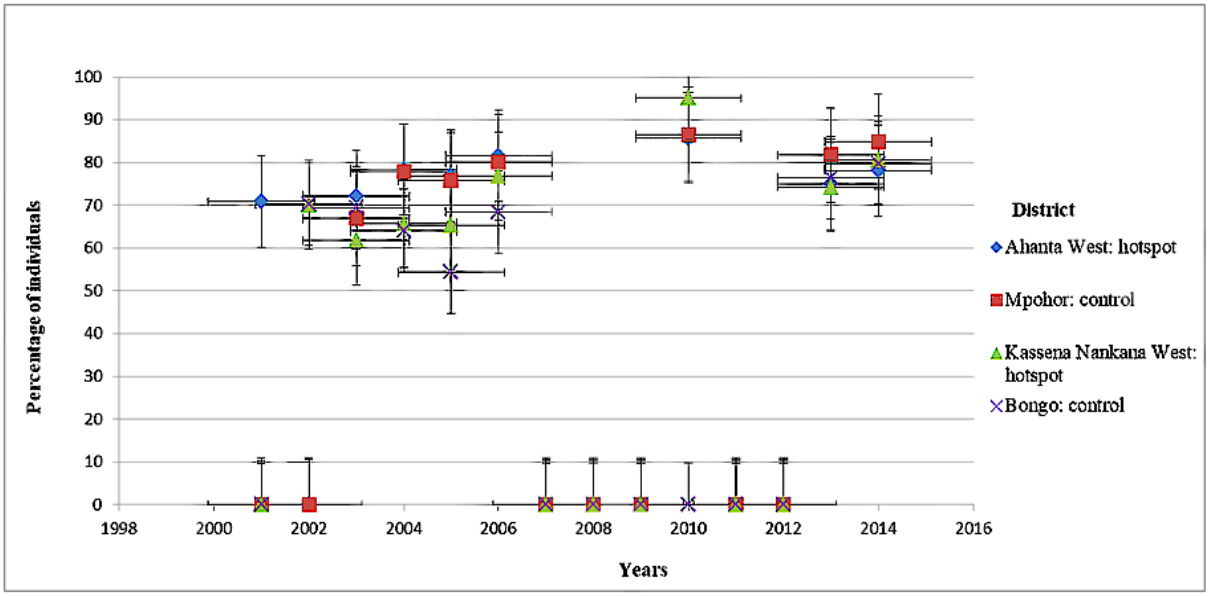

Figure 2. 4 MDA coverage for hotspot and control districts in the Western and Upper East regions, Ghana

Table 2. 4 Baseline microfilariae and antigenemia prevalence from the Ghana NTD Programme

District (hotspot/control)	Baseline mf prevalence (year)	Baseline antigen prevalence (year)
Ahanta West (hotspot)	19.5% (2000)	32.8% (2000)
Mpohor (control)	0 (2000)	0 (2000)
Kassena Nankana West (hotspot)	29.4% (2000)	45.3% (2000)
Bongo (control)	16.7% (2004)	21.2% (2004)

2.4.5 Demographic characteristics

Questionnaires from 438 individuals (229 females, 209 males) were analysed in the four districts from the Western and Upper East regions. The age distribution of the respondents

ranged from 15 to 92 years (mean = 37.4 years; median = 35 years). Half of the respondents were farmers (n = 220; 50.2%), 62 were fishermen (14.2%), while 26 were unemployed (5.9%) or involved in other occupations (n = 130; 29.7%).

2.4.6 MDA compliance

Questionnaire data showed that out of the 110, 108, 108 and 112 respondents from Ahanta West, Mpohor, Kassena Nankana West and Bongo districts, 90.0%, 53.7%, 87.0% and 89.3%, respectively, affirmed their participation in MDA activities. In relation to MDA compliance, the percentages of individuals shown to have complied with the previous five rounds of MDA were 47.3%, 3.7%, 31.5% and 9.8% for Ahanta West, Mpohor, Kassena Nankana West and Bongo districts, respectively. Our results revealed relatively high proportion of individuals from Mpohor district did not participate in MDA activities (Figure 5).

2.4.7 Vector control

Information on vector control activities from respondents in our four study districts indicated that bednet usage and indoor residual spraying were relatively high: 69.1-91.1% for bednet and 38.9-85.5% for indoor residual spraying.

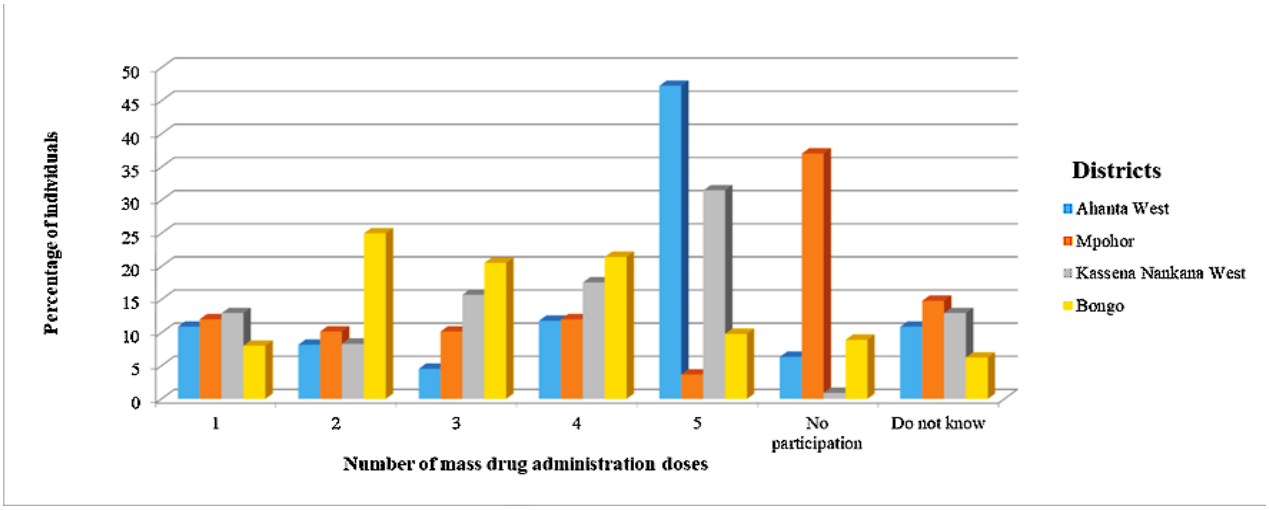

Figure 2. 5 Compliance to last five MDA doses in study districts, Western and Upper East regions, Ghana

2.5 Discussion

It is estimated that for the interruption of lymphatic filariasis transmission microfilariae prevalence should be less than 1% or antigen prevalence less than 2% (WHO, 2011). This criteria is used for the roll out of intervention programmes in all lymphatic filariasis endemic regions. In Ghana, control of lymphatic filariasis by means of MDA has been going on for almost two decades. At the time of the current study in 2016, most endemic communities should have interrupted transmission and began transmission assessment survey (TAS) or post-MDA surveillance. However, there are endemic foci still having transmission even after several rounds of MDA (Biritwum et al., 2017a). Mathematical model simulations suggest that different countries may have different mf breakpoints for interruption of lymphatic filariasis (Michael et al., 2017). There is therefore the need to have a critical look at the 1% microfilariae or 2% antigen thresholds used in various endemic regions for interruption of transmission. The reasons contributing to this persistent transmission are not clear. Vector species and abundance (WHO, 2013a), vector control activities (Koudou et al., 2018), vector competence, MDA compliance and therapeutic coverage (Kyelem et al., 2009), drug efficacy (Osei-Atweneboana et al., 2011) and possible genetic susceptibility of vectors (Kelly-Hope et al., 2006) are important factors that govern the transmission of lymphatic filariasis. However, in any particular situation either all or some of these factors may be important and need to be understood to resolve any ongoing transmission. Results derived from the current study showed that, with the exception of *An. melas*, mosquito species composition was similar in hotspot and control districts. However, higher numbers of mosquitoes were obtained from hotspots, compared to control districts in the same ecological zone. The transmission of lymphatic filariasis is significantly influenced by vector density (WHO, 2013a). The consistent high number of mosquitoes collected from Ahanta West compared to Mpohor district might be contributing to the persistence of lymphatic filariasis transmission in Ahanta

West district after several rounds of MDA. Additionally, on-going lymphatic filariasis transmission in Kassena Nankana West district might be explained by the relatively high number of mosquitoes collected in this district, compared to Bongo.

Vector-parasite density dependent relationships of limitation, stable transmission of lymphatic filariasis even at low mf levels, and facilitation, transmission of lymphatic even at high mf levels (Pichon, 2002; Boakye et al., 2004), are known to influence elimination of lymphatic filariasis. Members of the *An. gambiae* are generally considered to exhibit facilitation and hence at low mf levels are not efficient. It is expected that with *An. gambiae* serving as major vector, lymphatic filariasis should have been eliminated in these districts. *An melas*, which is part of the *An. gambiae* complex, has been shown to exhibit limitation (Southgate and Bryan, 1992; Boakye et al., 2004; Amuzu et al., 2010), and hence, able to pick mf at low parasitaemia and sustain their development to the infective stage. *An. melas* observed only in Ahanta West district might explain why transmission has been sustained, though at low mf levels.

Additionally, *Mansonia* species are known to exhibit limitation (Gyapong et al., 2005). Higher numbers of this species were sampled from Ahanta West than Mpohor district, and very few in Kassena Nankana West and Bongo districts. *Mansonia* species have been incriminated as one of the vectors involved in lymphatic filariasis transmission in Ghana (Ughasi et al., 2012). While *Mansonia* were not examined for *W. bancrofti* in this study, its presence in relatively high numbers in Ahanta West district could also be an additional factor sustaining the transmission of lymphatic filariasis in this area. *Culex* mosquitoes had higher numbers sampled in Ahanta West compared to Mpohor district, while similar numbers were collected in Kassena Nankana West and Bongo districts. *Culex* mosquitoes exhibit limitation (Gyapong et al., 2005) and transmit lymphatic filariasis in East Africa (Ughasi et al., 2012). Appawu *et al*. (Appawu et al., 2001) showed that *Culex* species in Ghana are refractory to *W.*

bancrofti and do not support their development to the infective stage. However, studies in Nigeria (Anosike et al., 2005; Nwoke et al., 2010), showed *Culex* to be transmitting lymphatic filariasis.

Cibarial teeth in mosquitoes act as a physical barrier and influence the transmission dynamics of lymphatic filariasis. The cibarial teeth number and shape influence mf intake by inflicting lacerations on ingested parasites (Bryan et al., 1990; Amuzu et al., 2010). However, more *Mansonia* species, lacking cibarial teeth and competent vectors at low parasitaemia were collected in Ahanta West. Furthermore, *An. melas*, with relatively fewer cibarial teeth numbers was found in Ahanta West and absent in Mpohor district. *An. melas*, however, was absent in Mpohor district. All mosquito species common to Ahanta West, Mpohor, Kassena Nankana West and Bongo districts had similar cibarial teeth numbers and shape.

The residual transmission of lymphatic filariasis in an area may be influenced by differences in the distribution of vectors (Kelly-Hope et al., 2006). In our study for instance, *An. melas* was found only in Ahanta West district. Another factor is the differences in vector susceptibility to lymphatic filariasis infection at low mf prevalence. *An. gambiae* complex exhibit facilitation but *An. melas* belonging to this complex exhibit limitation. This may account for differences in transmission potential within the *An. gambiae* complex (Kelly-Hope et al., 2006). The susceptibility of *An. melas* to *W. bancrofti* infection at low mf prevalence will contribute to persistent lymphatic filariasis transmission. As suggested by our dissection data, the presence of L_3 in *An. melas* proves its involvement in ongoing transmission of lymphatic filariasis in Ahanta West district

Analyses of MDA coverage data obtained from the national neglected tropical disease control programme revealed at least 65% MDA coverage for all the districts. It has been hypothesised that annual MDA with adequate consistent coverage of at least 65% should

make elimination possible (WHO, 2011). This hypothesis was based on early models for implementing MDA intervention programmes without possibly considering spatial heterogeneities. Spatial heterogeneities when adopted by intervention models may give predictions that could exceed the 5-6 rounds of MDA recommended to interrupt lymphatic filariasis transmission. This in turn lengthens the period needed for achieving lymphatic filariasis elimination at a given endemic area. Ghana for example was likely to eliminate lymphatic filariasis by 2020 as revealed by a mathematical model (Michael et al., 2017). The authors however suggested that lymphatic filariasis transmission is focal due to a wide range of factors in endemic areas (Michael et al., 2017). This therefore implies that intervention programmes rolled out in endemic areas should be specific and targeted in each endemic foci.

Community compliance to MDA is important in understanding persistent transmission of lymphatic filariasis. The evaluation of the districts' participation in the previous five rounds of MDA indicated a higher percentage of respondents from Ahanta West district (47.3%) and Kassena Nankana West district (31.5%), reporting to have taken the drugs all five times, compared to much lower rates in Mpohor (3.7%) and Bongo (9.8%). Thus the ongoing transmission of lymphatic filariasis in Ahanta West may not be due to MDA compliance, but driven by other factors.

The results from this study indicated high bednet usage among community members was observed in control areas compared to hotspots. This may have contributed to the control of lymphatic filariasis in the control districts. In Gambia, for example, Rebollo and colleagues observed that interruption of lymphatic filariasis transmission could have possibly been due to the extensive national bednet usage for malaria control (Rebollo et al., 2015; Koudou et al., 2018). Indoor residual spraying activities in all districts were high, except for Mpohor district. AngloGold Ashanti Malaria Control Ltd, a subsidiary of AngloGold Ashanti (AGA), from 2013 to 2015 conducted indoor residual spraying activities twice yearly in about 40

districts in Ghana. Due to limited resources, indoor residual spraying was done only in districts with high malaria prevalence, excluding Mpohor (unpublished data, AGA). However, it is possible that other private agencies aside AGA sprayed a few communities in Mpohor, explaining the low percentage of respondents (38.9%) affirming indoor residual spraying activities. While the indoor residual spraying data in the Western Region may not be sufficient to draw conclusions, the results from the Upper East Region on the other hand, indicate that the lower vector control activities in Kassena Nankana West compared to Bongo district could be a possible indicator for control of lymphatic filariasis transmission in control districts. Thus, supporting the important role vector control plays in the control of lymphatic filariasis (Bockarie et al., 2009).

There were a couple of limitations to this study. First, Mpohor was selected as a control district, although retrospective analysis of data revealed a zero prevalence at the inception of MDA in the year 2000. A study site with prevalence similar to Ahanta West district and with successful MDA treatment history would have been preferable. Secondly, the MDA data collected by the national neglected tropical disease control programme could not be verified. An earlier study has shown MDA data reported by the programme to be inaccurate (de Souza et al., 2016). There were also some missing MDA data for some of the years in all the study districts.

2.6 Conclusions

The GPELF aims at interrupting lymphatic filariasis transmission. This is based on an estimated duration of 5 years at 1% mf prevalence, which might not be feasible in all endemic areas. It is important to understand the local factors responsible for persistent transmission of lymphatic filariasis in a given area. In our study areas, transmission of lymphatic filariasis in hotspots despite many years of treatment could not be attributed to

MDA coverage and compliance when compared to control districts. In Ahanta West district, our data suggests high biting rates of vector species in the *An. gambiae* complex, initial infection prevalence rates and low vector control to ongoing lymphatic filariasis transmission. Additionally, the presence of *An. melas* and *Mansonia*, with less or no cibarial teeth may further contribute to transmission. In Kassena Nankana West district, transmission dynamics could be explained by the presence of relatively low numbers and biting rates of *An. gambiae* complex together with initial infection prevalence as reported by our study. Furthermore, low densities of *Mansonia* and the absence of *An. melas* may be reasons why no infections were recorded in this district.

Chapter 3: Implementing a community vector collection strategy for xenomonitoring for the endgame of lymphatic filariasis elimination

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3.1 Abstract

Background

The global strategy for elimination of lymphatic filariasis is by annual mass drug administration (MDA). Effective implementation of this strategy in endemic areas reduces *Wuchereria bancrofti* in the blood of infected individuals to very low levels. This minimises the rate at which vectors successfully pick microfilariae from infected blood, hence requiring large mosquito numbers to detect infections. The aim of this study was to assess the feasibility of using trained community vector collectors (CVCs) to sample large mosquito numbers with minimal supervision at low cost for potential scale-up of this strategy.

Methods

CVCs and supervisors were trained in mosquito sampling methods, i.e. human landing collections, pyrethrum spray collections and window exit traps. Mosquito sampling was done over a 13-month period. Validation was conducted by a research team as quality control for mosquitoes sampled by CVCs. Data were analyzed for number of mosquitoes collected and cost incurred by the research team and CVCs during the validation phase of the study.

Results

A total of 31,064 and 8720 mosquitoes were sampled by CVCs and the research team, respectively. We found a significant difference ($F_{(1,13)}$ = 27.1606, P = 0.0001) in the total number of mosquitoes collected from southern and northern communities. Validation revealed similar numbers of mosquitoes sampled by CVCs and the research team, both in the wet ($F_{(1,4)}$ = 1.875, P = 0.309) and dry ($F_{(1,4)}$ = 2.276, P = 0.258) seasons in the southern communities, but was significantly different for both wet ($F_{(1,4)}$ = 0.022, P = 0.005) and dry

($F_{(1,4)}$ = 0.079, P = 0.033) seasons in the north. The cost of sampling mosquitoes per season was considerably lower by CVCs compared to the research team (15.170 *vs* 53.739 USD).

Conclusion

This study revealed the feasibility of using CVCs to sample large numbers of mosquitoes with minimal supervision from a research team at considerably lower cost than a research team for lymphatic filariasis xenomonitoring. However, evaluation of the selection and motivation of CVCs, acceptability of CVCs strategy and its epidemiological relevance for lymphatic filariasis xenomonitoring programmes need to be assessed in greater detail.

Keywords: Xenomonitoring, Validation, Lymphatic filariasis, *Wuchereria bancrofti*, Community vector collectors

3.2 Background

Lymphatic filariasis is a neglected tropical disease caused by infection with the parasitic worms *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, all of which are transmitted by mosquitoes (WHO, 2013a). There are various species of mosquitoes implicated in the life-cycle of the parasites, mainly of the genera *Aedes*, *Anopheles*, *Coquillettidia*, *Culex* and *Mansonia* (Okorie and de Souza, 2016). These species differ in their biology, distribution, ecology and transmission potential. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 with the goal to eliminate lymphatic filariasis by interrupting transmission through MDA and reducing morbidity and disability (Gyapong et al., 2018). The adopted MDA strategy is annual treatment with a single dose of albendazole in combination with either ivermectin or diethylcarbamazine (DEC) for 4–6 years (Koudou et al., 2018). However, a combination of these three drugs (IDA) was approved in 2017 by the World Health Organization (WHO) to be used only in regions non-endemic for onchocerciasis and loiasis (Fischer et al., 2017; WHO, 2017a). The GPELF has achieved great success since its inception by preparing guidelines in all endemic regions and facilitating the implementation and scaling up of lymphatic filariasis MDA in endemic countries. Indeed, by the end of 2015 over 6.2 billion cumulative treatments were distributed (Molyneux et al., 2017), resulting in strong declines of microfilaraemia (36.45 million), hydrocele (19.43 million) and lymphedema (16.68 million) in 2013 (Ramaiah and Ottesen, 2014). Of the 73 endemic countries, 18 countries moved into post-transmission surveillance, following successful transmission assessment surveys (TAS) (Molyneux et al., 2017). Despite this progress, it will be difficult for most of endemic countries to become verified as free of transmission or having entered the post-intervention surveillance phase by 2020 (WHO, 2013a), as recognised recently at the Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN) in Kigali.

Following successful MDA implementation, the prevalence of infection falls below or equals the critical cut-off threshold for interrupting transmission by various vectors. For *Anopheles* and *Culex*, the threshold is < 2% antigenaemia prevalence. For *Aedes*, the threshold is < 1% antigenaemia prevalence (de Souza et al., 2014). This poses significant challenges to xenomonitoring because at such low levels of infection, large numbers of mosquitoes must be analysed in order to assess whether transmission of the disease in the vectors has indeed been halted, which is costly (Mukabana et al., 2006; Chaki et al., 2012). Additionally, longitudinal entomological monitoring strategies rely on trained specialist technical staff who are usually limited in both their geographical scope and the frequency of sampling at any survey location (Sikaala et al., 2014). To that end, there is a need to employ new strategies that can effectively allow the collection of large numbers of mosquitoes, at greatly reduced cost, while exploring the temporal and spatial patterns of lymphatic filariasis vector transmission indices. The present study was undertaken to address the need for sampling large numbers of mosquitoes for xenomonitoring purposes, at low costs (WHO, 2013a). Hence, we determined the ability of community collectors to successfully collect mosquitoes with minimal supervision from a research team, including costs in order to assess the feasibility of implementing this approach on a large scale. To this end, we determined a concept of using trained community vector collectors (CVCs) for the collection of mosquitoes, similar to community drug distributors (CDDs) implementing MDA.

3.3 Methods

3.3.1 Study sites

Four districts were selected in lymphatic filariasis-endemic areas of Ghana. Two districts from the north, namely Kassena Nankana West (0°10'N, 10°50'W) and Bongo (0°45’N, 10°50’W) were identified as study sites (Fig. 1). The reported population sizes for the Bongo and Kassena Nankana West districts by the Ghana Statistical Service for the year 2010 were 84,545 (Ghana Statistical Service, 2014a) and 70,667 (Ghana Statistical Service, 2014b), respectively. Inhabitants located in these two districts are mostly farmers involved in growing crops, rearing livestock and fish farming (MoFA Ghana, 2011). Climate in the north is characterised by wet and dry seasons, with average rainfall ranging between 645 and 1250 mm (MoFA Ghana, 2011). The average temperature and relative humidity are 15 – 45°C and 30 – 80%, respectively (MoFA Ghana, 2011). Additionally, two districts from the south, namely Ahanta West (4°84’N, 2°02’W) and Mpohor (4°05’N, 1°54’W) were selected. In the year 2010, the population sizes recorded for Ahanta West and Mpohor districts were 106,215 and 42,923, respectively (Ghana Statistical Service, 2014c, 2014d). Indigenes in both districts are mostly fishermen/fishmongers and farmers (MoFA Ghana, 2011). Ahanta West and Mpohor districts lie within the high rainfall zone in Ghana, with average rainfall of 1600 mm per year (MoFA Ghana, 2011). The average temperature and humidity in the south are 20–34 °C and 75–80%, respectively (MoFA Ghana, 2011). The southern districts are characterised by rainforests, mangrove zones and high precipitation (Dunyo et al., 1996). The northern districts fall within the arid Sudan savannah zone (Appawu et al., 2001). Data from the 2016 annual report of the Ghana Health Service (GHS) indicate malaria to be endemic in all study districts (GHS, 2017). However, lymphatic filariasis is endemic in all districts except Mpohor (GHS, 2017).

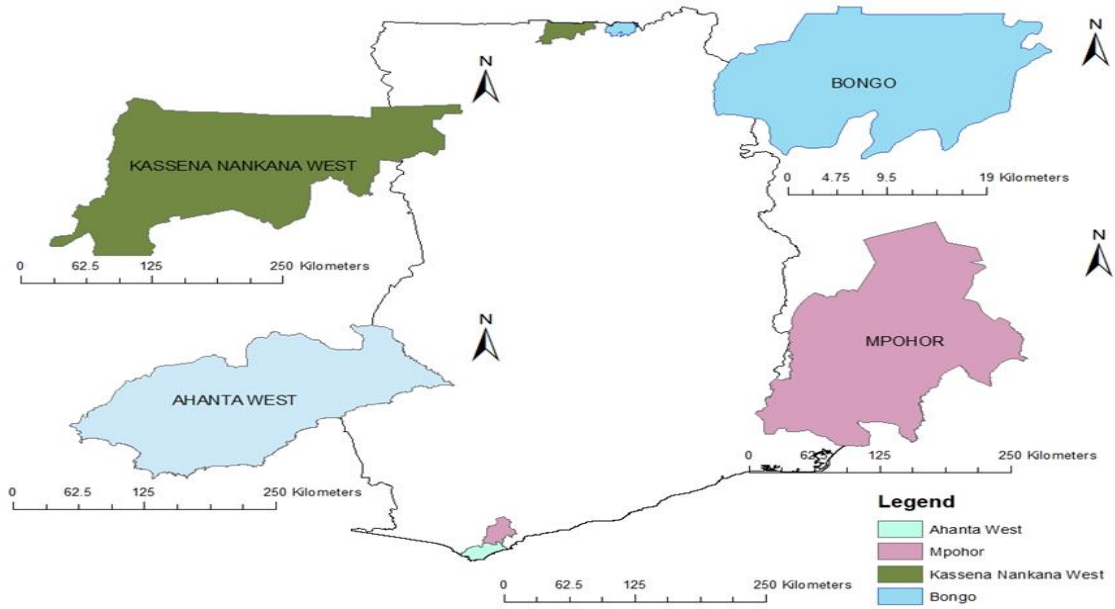

Figure 3.1 Map showing lymphatic filariasis study areas from Northern and Southern districts, Ghana

3.3.2 Community engagement and training of vector collectors

Community engagement was undertaken to inform the district health administration, community chiefs/elders and community members. Following the community engagement, the community elders were invited to identify individuals who will serve as vector collectors. The elders were asked to identify 9 volunteers, either male or female, 18 years-old and above, with formal or informal education in the community. However, the selection criterion for the supervisor was to identify an individual who had at least completed junior high school. Furthermore, no experience of prior mosquito collection was required to be selected as a CVC. The selected community volunteers and supervisors were trained in specific mosquito collection procedures. These included pyrethrum spray collection, window exit traps and human landing collections (WHO, 2013a). The use of the three methods was to maximise the number of mosquitoes collected for xenomonitoring purposes. The supervisors were also trained on the best ways to package, store and ship collected mosquitoes. Mosquitoes sampled using human landing collections were knocked down in their holding cups using cotton wool soaked with chloroform. The knocked down mosquitoes were transferred into a

Petri dish and, using a pair of forceps; a maximum of 10 mosquitoes were transferred into labelled Eppendorf tubes. A Pasteur pipette was used to aliquot 200 µl RNA*later* (Life Technologies, Carlsbad, CA, USA) and dispensed into the various Eppendorf tubes containing mosquitoes. The tubes were covered, sealed with strips of parafilm and held in labelled holding racks. Mosquitoes sampled using pyrethrum spray catches and window exit traps were stored in labelled Eppendorf tubes which had their covers pierced. The tubes were then kept in labelled ziplock bags containing silica gel (Kouassi et al., 2015).

3.3.3 Collection of mosquitoes

Following training, collectors were provided with the necessary consumables and supplies to carry out monthly collections. Mosquito collections were done over a period of 13 months from the beginning of July 2015 to the end of July 2016. Collections were done twice each month. For convenience, the CVCs were at liberty to select days appropriate for all of them in the first and second half of the month. Eight community volunteers per district were involved in the collection, with a total of 16 person-days of collection in a month. A supervisor was also identified to ensure that the collections were according to protocol undertaken and serve as the link between the researchers and the vector collectors. The days of collection were left at the discretion of the collectors. In the evening of the sampling night, four window exit traps were fixed in different sections of the communities. Human landing collection was undertaken by two teams of four collectors each (Aboagye-Antwi et al., 2015). The teams were constituted in order to have two indoor and two outdoor human landing collections, in different sections of the community. Human landing collections were carried out from 21:00 to 05:00 h. Pyrethrum spray collections were done by the same teams in the morning. Up to ten rooms were sampled by all volunteers in the community, on each collection day, using pyrethrum spray collections from 06:00 to 09:00 h. The collected mosquitoes were stored and

sent to the researchers by public transport. Every three months the researchers visited the communities to replenish the supplies (i.e. insecticide, tubes, cotton wool, silica gel and RNA*later*) needed for the collection and storage. Outside these periods, payments to the vector collectors were done through bank or mobile money transfers.

3.3.4 Validation of mosquito sampling survey

A quality control (validation) was implemented for human landing collections and pyrethrum spray collections that are collector and technique-dependent. Validation was also done for window exit traps. This was done on two occasions, in the rainy and dry seasons. Briefly, the research team from Noguchi Memorial Institute for Medical Research made two unannounced visits (one visit per season) to the study communities. In order to validate mosquito sampling done by the CVCs, the Noguchi Memorial Institute for Medical Research team collected mosquitoes from the same households as community vector collectors. The mosquitoes collected were compared with the regular sampling done by the CVCs within the same month. Mosquito collection by the research team was done in the third week of April and July 2016. Two households were selected for mosquito collection using human landing catches and window exit traps each catch night. In the morning, ten households were selected for mosquito collection using the pyrethrum spray method. The time for sampling mosquitoes by the research team using the various sampling techniques was the same as that of the CVCs.

3.3.5 Analysis of cost data

This work is part of a larger study so only costs explicitly related to the mosquito collection were considered. These costs therefore exclude any costs related to the parasitological analysis of the mosquitoes collected. Costs were split into recurrent and capital costs. Recurrent costs were those that were incurred frequently and include personnel allowances, supplies, transportation, communication, fuel, etc. Capital costs were those investments made

in fixed assets, which are used over a longer period and include cost of vehicles, machinery and equipment. Capital costs were annualised. All costs were converted into US Dollars (USD) using the average exchange rate prevailing on the markets during the study period.

3.3.6 Statistical analysis

Data on costs incurred from the study were entered and analysed using Microsoft Excel 2013. We checked for significant differences of the total number of mosquitoes collected by CVCs from the northern and southern part of Ghana, and between CVCs and the Noguchi Memorial Institute for Medical Research team during validation using *F*-test. *P*-values ≤ 0.05 was considered statistically significant.

3.4 Results

3.4.1 Mosquito collection

Over the 13-month study period, a total of 31,064 and 8720 mosquitoes were sampled by CVCs and the Noguchi Memorial Institute for Medical Research team, respectively. Table 1 shows the result of the number of mosquitoes collected by CVCs and the research team during the validation period in the dry and rainy seasons using the three sampling techniques. Mosquito collections were done twice for each month during validation. Human landing collections provided the highest number of mosquitoes caught for xenomonitoring. Higher numbers of mosquitoes were collected by the research team compared to CVCs in the months when both constituencies collected mosquitoes (Fig. 2a, b). However, there was no significant difference in the number of mosquitoes sampled by research team compared to the CVCs for both the rainy ($F_{(1,4)} = 1.875$, $P = 0.309$) and dry ($F_{(1,4)} = 2.276$, $P = 0.258$) seasons in the southern communities. The opposite was observed for the northern communities, where the total number of mosquitoes sampled by the CVCs compared with the research team was significantly different for both the rainy ($F_{(1,4)} = 0.022$, $P = 0.005$) and dry ($F_{(1,4)} = 0.079$, $P =$

0.033) seasons. In the south, human landing collections gave the highest number of mosquitoes in all the communities, whiles pyrethrum spray collections provided a higher number of mosquitoes for communities in the north (Fig. 2a, b). Mosquitoes collected from each of the study sites by the CVCs during the study period are shown in Table 2. Results from Table 2 indicate that the total number of mosquitoes collected by the CVCs was significantly different between the southern coastal communities compared to the northern arid zones ($F_{(1,13)}$ = 27.1606, P < 0.0001).

Table 3.1 Mosquito collection for validation by CVCs and research team in the Northern and Southern communities, Ghana

Personnel	Dry/Rainy Season	North/South	Sampling type (HLC/PSC/WET)	*An. gambiae*	*Culex* species	*Ma. uniformis*	*Ma. africana*	*Aedes* species	*An. pharoensis*	*An. coustani*	Total
Research team	Dry	South	HLC	3561	198	0	25	0	0	3	3787
			PSC	82	3	0	0	0	0	0	85
			WET	34	1	0	0	0	0	0	35
		North	HLC	30	42	0	0	0	2	0	74
			PSC	48	21	0	0	0	0	5	74
			WET	1	2	0	0	0	0	0	3
CVCs	Dry	South	HLC	1906	302	31	0	0	0	1	2240
			PSC	38	0	0	0	0	4	0	42
			WET	46	0	0	0	0	8	0	54
		North	HLC	33	12	0	0	0	0	1	46
			PSC	236	68	0	0	0	0	0	304
			WET	2	0	0	0	0	0	0	2
Research team	Rainy	South	HLC	1984	11	3	166	0	0	0	2164
			PSC	5	1	0	1	0	0	0	7
			WET	7	0	0	0	0	0	0	7
		North	HLC	962	1075	2	0	10	7	3	2059
			PSC	376	42	0	0	0	1	1	420
			WET	1	1	0	0	0	0	3	5
CVCs	Rainy	South	HLC	1757	20	4	75	1	0	0	1857
			PSC	64	2	0	5	0	0	0	71
			WET	24	1	0	4	0	0	0	29
		North	HLC	123	140	0	1	8	8	2	282
			PSC	186	86	0	0	0	0	0	272
			WET	0	0	0	0	0	0	0	0

HLC: human landing collections, **PSC**: pyrethrum spray collections, **WET**: window exit trap and **CVCs:** community vector collectors. Table 1 shows the number of mosquitoes collected only during the validation period for comparison between the research team and CVCs.

Table 3.2 Mosquito species collected from Northern and Southern communities in Ghana by the CVCs

			Mosquito species							
North/ South	**District**	**Community**	***An. gambiae***	***Culex* species**	***Ma. uniformis***	***Ma. africana***	***Aedes* species**	***An. pharoensis***	***An. coustani***	**Total (%)**
South	Ahanta West	Asemkow	13,540	69	19	18	2	36	0	13,684 (44.05)
		Antseambua	5340	1152	755	1642	7	0	4	8,900 (28.65)
	Mpohor	Ampeasem	2247	5	6	5	4	9	0	2,276 (7.33)
		Obrayebona	2356	76	55	14	3	1	3	2,508 (8.07)
North	Kassena Nankana West	Navio Central	751	680	6	3	12	2	6	1,460 (4.70)
		Badunu	488	199	3	0	32	2	7	731 (2.35)
	Bongo	Atampiisi Bongo	542	200	2	1	23	4	1	773 (2.49)
		Balungu Nabiisi	284	426	1	1	19	0	1	732 (2.36)
	Total		25,548	2807	847	1684	102	54	22	31,064 (100)

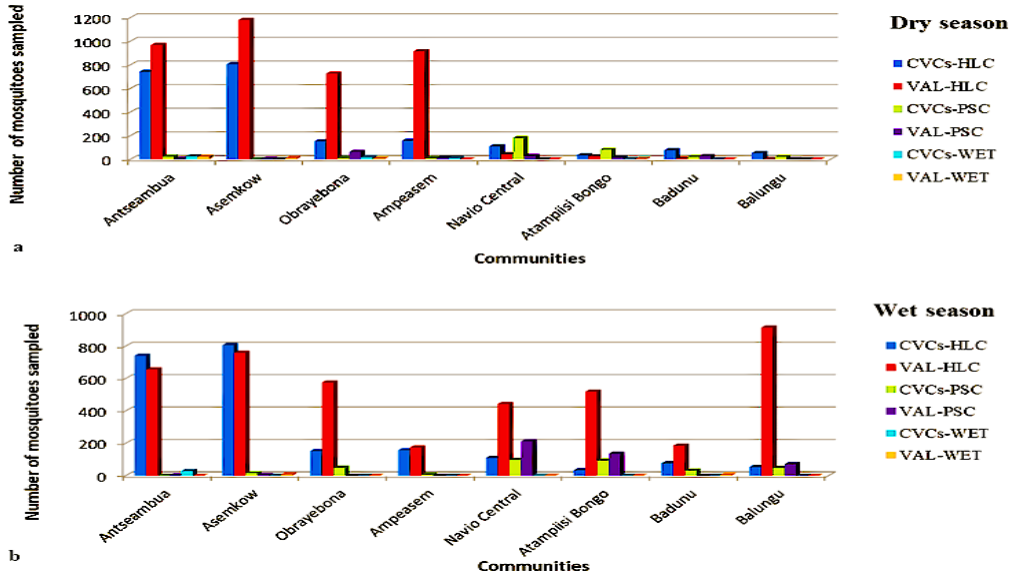

Figure 3.2 Validation of mosquitoes sampled by CVCs and research team in the Northern and Southern communities

a. Validation of mosquitoes sampled by CVCs and research team in the dry season. b. Validation of mosquitoes sampled by CVCs and research team in the rainy season. **VAL:** validation, **HLC:** human landing collections, **PSC:** pyrethrum spray collections and **WET**: window exit trap.

3.4.2 Cost estimates

Table 3 shows the result of the breakdown of the total costs incurred by both the research team and CVCs for training and mosquito sampling. The personnel costs include allowances paid to each category of personnel. The personnel costs incurred for the two days of sampling in a month by an individual in the research team and a CVC was 53.73 and 15.17 USD, respectively. Due to financial limitations, the research team from Noguchi Memorial Institute for Medical Research used four instead of eight collectors for sampling during validation. The amount incurred for the two sampling nights in a community by the four research team members, compared to the eight CVCs was 214.92 and 121.36 USD, respectively. The cost estimates for this study are presented in Table 4. The recurrent transportation costs include the cost of fuel, maintenance and repairs undertaken in the field as well as road tolls. The supplies include the pyrethrum insecticide, desiccants and other items that were required for the collection of mosquitoes. Other costs include the cost of communication between the research team and the

CVCs, the cost of sending consumables to communities and samples from the communities to the research team using public transport and finally, money transfers. With the exception of when the research team was undertaking a field visit to the communities, the allowances of the CVCs were sent *via* bank or mobile money transfers.

Capital costs include the cost of vehicle rental, the annualised costs of non-rented vehicles used and the cost of spray guns. The costs were adjusted for time use as the vehicles were used for other programmes as well. We estimated that these vehicles were used 27% of the time for the mosquito collection phase. In terms of the share of each cost group, the majority of the recurrent costs were personnel-related costs (21,370.04 USD) with mosquito collectors costing the most (54.5%) and supervisors costing the least (17.3%). A bulk of the capital costs (88.7%) were related to transportation (Fig. 3b).

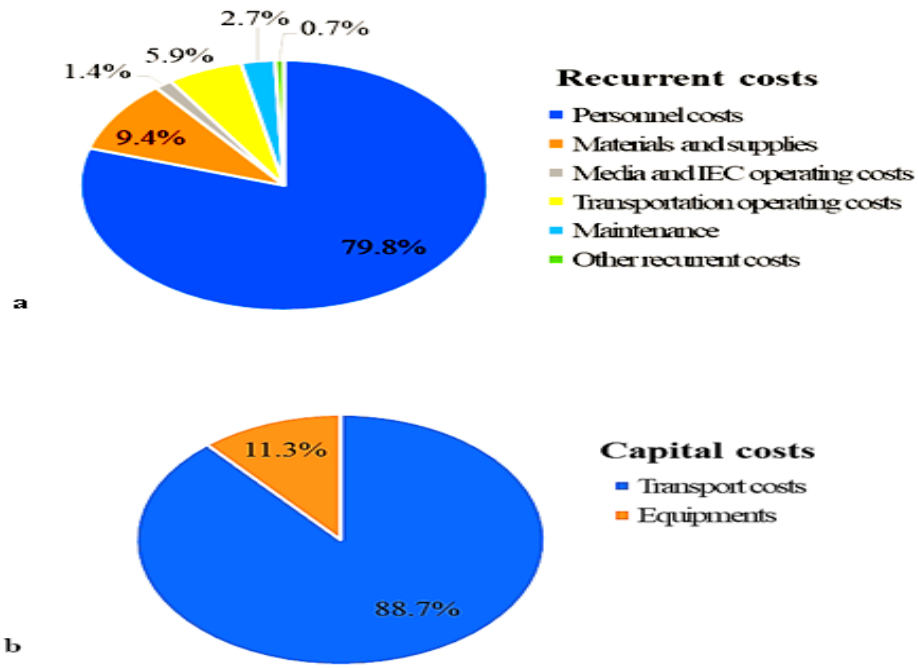

Figure 3.3 Cost distribution based on type of cost for studies in Northern and Southern communities, Ghana

a. The recurrent costs for studies in the northern and southern communities, Ghana, b. The capital costs for studies in the northern and southern communities, Ghana.
IEC: information, education and communication for community engagement

Table 3.3 Training and validation cost for CVCs and Research team in the Northern and Southern communities, Ghana

Activity		**Cost of sampling for 2 days in a month**	
Training	**Personnel cost**	**Cost (GH¢)**	**Cost ($)**
	Cost for CVCs	60.00	15.170
	Cost for supervisors	70.00	17.69
	Cost for research team	212.50	53.729
	Cost for driver (research team)	170.00	42.98
	Transportation	**Cost (GH¢)**	**Cost ($)**
	Cost for fuel	2,713	685.96
	Cost for car maintenance	1485	375.47
	Cost for road tolls	59	14.91
	Cost for motor bike fuel (North)	12.50	3.16
	Cost for motor bike fuel (South)	-	-
Validation (Dry season)	**Transportation**	**Cost (GH¢)**	**Cost ($)**
	Cost for fuel	1733	438.17
	Cost for car maintenance	689.75	174.39
	Cost for road tolls	30.5	7.71
	Cost for motor bike fuel (North)	12.50	3.16
	Cost for motor bike fuel (South)	-	-
Validation(Wet season	**Transportation**	**Cost (GH¢)**	**Cost ($)**
	Cost for fuel	1733	438.17
	Cost for car maintenance	689.75	174.39
	Cost for road tools	30.5	7.71
	Cost for motor bike fuel (North)	12.50	3.16
	Cost for motor bike fuel (South)	-	

Personnel cost is cost per individual per month (2 sampling days), whilst transportation cost is the cost per month for sampling mosquitoes in all study communities during training and validation for wet and dry seasons.

Table 3.4 Cost estimates for mosquito sampling process

		GHS	USD
Recurrent costs		**GHS 105,892.20**	**$ 26,773.78**
Personnel costs		GHS 84,520.00	$ 21,370.04
	Vector collectors	GHS 46,080.00	$ 11,650.87
	Supervisors	GHS 14,640.00	$ 3,701.58
	Entomologist	GHS 23,800.00	$ 6,017.59
Materials and supplies		GHS 9,968.70	$ 2,520.49
Media and IEC operating costs		GHS 1,510.50	$ 381.91
Transportation operating costs		GHS 6,299.00	$ 1,592.64
Maintenance		GHS 2,864.50	$ 724.26
Other recurrent costs		GHS 729.50	$ 184.45
Capital costs		**GHS 12,929.40**	**$ 3,269.07**
Transport costs		GHS 11,470.26	$ 2,900.14
Equipment		GHS 1,459.14	$ 368.93
Total annual cost		**GHS 118,821.60**	**$ 30,042.85**

3.5 Discussion

Transmission assessment surveys (TAS) to determine whether or not MDA can be stopped (WHO, 2011) are based on prevalence of infection in the human population. This has no real transmission component involving vectors due to the ease of sampling human populations. Xenomonitoring surveys, on the other hand, are considered expensive, requiring large number of mosquitoes and limited technical expertise (Okorie and de Souza, 2016). Notwithstanding the limitations associated with xenomonitoring, a recent study in Togo (Dorkenoo et al., 2018) using molecular xenomonitoring for post-validation surveillance of lymphatic filariasis demonstrated the feasibility of its application on a larger scale. To overcome the above challenges, various tools and approaches are being developed, including laboratory and field practical methodologies (Dyab et al., 2015; Pilotte et al., 2016). In this study, we evaluated the use of CVCs for the purposes of assessing their usefulness in collecting large numbers of mosquitoes at low costs. Our results indicate that CVCs may indeed be useful in xenomonitoring activities for lymphatic filariasis elimination programmes. The costs incurred for collection of mosquitoes was significantly lower compared to using a research team. Dorkenoo et al. (Dorkenoo et al., 2018) also demonstrated in their study a lower cost in using CVCs for xenomonitoring in post-validation surveillance of lymphatic filariasis in Togo. Moreover, CVCs may promote active community participation and enhance ownership of vector control activities for the control and monitoring of vector-borne diseases (Abad-Franch et al., 2011).

It has been argued that implementing community-based mosquito collection schemes present two important challenges. The first challenge is the selection of traps that are safe, practical and convenient for CVCs to apply them reliably in the absence of daily supervision. The second challenge is the need for an independent quality assurance of this unsupervised surveillance process, so that the accuracy and limitations of the derived data can be quantified as a

prerequisite to critical interpretation (Sikaala et al., 2014). The use of CVCs may require programmatic guidelines and procedures so as to streamline the process and protocols for mosquito collection.

In the rainy season, mosquito densities increased compared to the dry season. This may expose the collectors to more infectious mosquito bites (Kenea et al., 2017). As such, alternatives to the human landing collections, such as the human-baited double net traps (Tangena et al., 2015), will provide protection to the collectors while allowing large numbers of mosquitoes to be collected. Proper training in mosquito collection methods will also be required. The differences in the number of mosquitoes between the southern and northern communities may be attributed to the environmental characteristics of the areas (de Souza et al., 2010). However, the effectiveness of the trapping method may indicate the need to consider different sample collection methods in different areas.

In this study, the amount paid to the collectors was negotiated based on the number of days and activities to be undertaken. While the cost per collector sampling per month (15.17 USD) was much lower than the approximate 70.00 USD reported in a community based scheme in Zambia (Sikaala et al., 2014), we believe the mean cost per person could greatly be reduced if lesser number of collection methods are implemented and a community ownership model is employed. The use of a CVC strategy could further be implemented as part of monitoring and evaluation and TAS activities, as lymphatic filariasis control and elimination programmes spend a considerable amount of time in disease endemic communities every year. Thus, integrating the CVC strategy with ongoing lymphatic filariasis programme activities will further reduce the transportation costs associated with the implementation of xenomonitoring surveys.

There were a couple of limitations to this study. First, the validation was done only on two occasions (both dry and wet season), and the environmental variables in each community may

have influenced the numbers of mosquitoes collected by the CVCs. Nonetheless it is assumed that the results are representative of the collectors and trap performance in the study. Secondly, the study failed to assess the views of the CVCs and community members towards the implementation of this strategy. This would have provided important information on the community acceptability and feasibility of upscaling this strategy. Lastly, the study was unable to disaggregate the current cost based on community and on method of mosquito collection. Future research should be able to attribute the costs to the main method of collection and adjust for community variations in costs.

3.6 Conclusions

This study showed that the use of CVCs for lymphatic filariasis xenomonitoring activities is feasible and may be a useful strategy in overcoming the challenges associated with sampling large numbers of mosquitoes and evaluating the spatio-temporal patterns of lymphatic filariasis vector transmission indices. It also showed that the cost for vector collection may be greatly reduced, enabling a wide rollout of this strategy for lymphatic filariasis xenomonitoring activities. Further evaluation needs to be undertaken to assess the criteria for selecting and motivating CVCs, the acceptability of CVCs for monitoring disease programmes, knowledge, attitude and practices of vector collectors, and epidemiological relevance of this strategy for lymphatic filariasis xenomonitoring activities.

Chapter 4: Assessing the presence of *Wuchereria bancrofti* infections in vectors using xenomonitoring in lymphatic filariasis endemic districts in Ghana

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4.1 Abstract

Mass drug administration (MDA) is the current mainstay to interrupt the transmission of lymphatic filariasis. To monitor whether MDA is effective and transmission of lymphatic filariasis indeed has been interrupted, rigorous surveillance is required. Assessment of transmission by programme managers is usually done via serology. New research suggests that xenomonitoring holds promise for determining the success of lymphatic filariasis interventions. The objective of this study was to assess *Wuchereria bancrofti* infection in mosquitoes as a post-MDA surveillance tool using xenomonitoring. The study was carried out in four districts of Ghana; Ahanta West, Mpohor, Kassena Nankana West and Bongo. A suite of mosquito sampling methods was employed, including human landing collections, pyrethrum spray catches and window exit traps. Infection of *W. bancrofti* in mosquitoes was determined using dissection, conventional and real-time polymerase chain reaction and loop mediated isothermal amplification assays. *Aedes*, *Anopheles coustani*, *An. gambiae*, *An. pharoensis*, *Culex* and *Mansonia* mosquitoes were sampled in each of the four study districts. The dissected mosquitoes were positive for filarial infection using molecular assays. Dissected *An. melas* mosquitoes from Ahanta West district were the only species found positive for filarial parasites. We conclude that whilst samples extracted with Trizol reagent did not show any positives, molecular methods should still be considered for monitoring and surveillance of lymphatic filariasis transmission.

Keywords: *Anopheles melas*; Ghana; lymphatic filariasis; post-mass drug administration surveillance; *Wuchereria bancrofti*; xenomonitoring.

4.2 Introduction

Lymphatic filariasis is a disease found in tropical and subtropical parts of the world. The aim of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), launched by the World Health Organization (WHO) in 2000, is to interrupt the transmission of lymphatic filariasis caused by *Wuchereria bancrofti* and *Brugia* species, and to manage morbidity and disability in affected individuals (WHO, 2011; Jones et al., 2018). By 2011, guidelines had been developed and mass drug administration (MDA) scaled up in 53 of the 73 lymphatic filariasis endemic countries (Okorie and de Souza, 2016), including Ghana. The Ghana Filariasis Elimination Programme (GFEP) was established in 2000 (Biritwum et al., 2017a). The inception was governed by to preliminary data, indicating that lymphatic filariasis was endemic in 49 out of 110 districts, with microfilariae (mf) and immunochromatographic test (ICT) prevalence ranging between 19.8% and 29.6% and between 33.1% and 45.4%, respectively (Biritwum et al., 2017a). This led to the commencement of MDA in 2001 in 10 districts and the subsequent scale up to the remaining endemic districts by 2006 (Biritwum et al., 2017a; Kanamitie et al., 2017). Monitoring and evaluation (M&E) of the impact of MDA usually does not involve the detection of filarial larvae in mosquito vectors. Hence, xenomonitoring has not been officially part of WHO recommendations for lymphatic filariasis surveillance.

WHO put forth rigorous procedures for documenting interruption of lymphatic filariasis transmission in endemic countries (WHO, 2011). These include mapping for the identification of endemic regions, followed by at least five rounds of annual MDA with periodic M&E. A transmission assessment survey (TAS) is conducted after the cessation of MDA and a 5-year post-validation to confirm that no recrudescence of lymphatic filariasis occurred (Dorkenoo et al., 2018). Measuring progress of any lymphatic filariasis control programme is, however, dependent on the effectiveness of M&E post-MDA (Goodman et al., 2003; Plichart et al., 2006), among other issues. Monitoring of lymphatic filariasis transmission by programme

managers mainly involves mf assays and antigen tests in the human populations. A challenge with this monitoring approach is the reluctance of individuals to provide samples (Owusu et al., 2015) and its inability to provide a 'real-time' estimate of the disease (Goodman et al., 2003; Okorie and de Souza, 2016). Xenomonitoring, which detects infection in vectors, could serve as a complementary diagnostic tool to serology. Xenomonitoring is convenient, non-invasive (Goodman et al., 2003; Owusu et al., 2015) and can be used to assess the progress of lymphatic filariasis control activities (Bockarie, 2007; Kouassi et al., 2015; Okorie and de Souza, 2016). Dorkenoo and colleagues, in a study in Togo, demonstrated the possibility of using molecular xenomonitoring for post-lymphatic filariasis validation surveillance (Dorkenoo et al., 2018). In their study, the feasibility of using large-scale xenomonitoring was demonstrated. Furthermore, the absence of *W. bancrofti* infections in *Anopheles gambiae* was observed during post-validation molecular xenomonitoring survey in Togo. In the southern part of Ghana, a recent study revealed 0.9% *W. bancrofti* infection and 0.5% infectivity rates in *An. gambiae* following several rounds of MDA in endemic districts (de Souza et al., 2018).

The purpose of the current study was to evaluate lymphatic filariasis transmission in vectors using dissection and molecular xenomonitoring as diagnostic tools. The study was implemented in four districts; two districts in northern and two districts in southern Ghana. The results complement already existing information on *W. bancrofti* infections in vector mosquitoes, and provide additional evidence of the feasibility of using xenomonitoring for M&E and surveillance activities post-MDA.

4.3 Materials and methods

4.3.1 Study sites

The study was conducted in eight communities, selected from four districts in the Western and Upper East regions of Ghana. Two communities were selected from each district. In the Upper East region, Badunu and Navio Central were selected from Kassena Nankana West district, and Atampiisi Bongo and Balungu Nabiisi from Bongo district. In the Western region, Antseambua and Asemkow were selected from Ahanta West district, while Ampeasem and Obrayebona were selected from Mpohor district. A map showing the study districts has been published elsewhere (Pi-Bansa et al., 2019). These sites were selected based on lymphatic filariasis prevalence data stemming from monitoring activities by the Ghana National Neglected Tropical Disease Programme unit of the Ghana Health Service (Table 1).

Table 4.1 Number of mass drug administration (MDA) rounds and prevalence of microfilariae in the four districts of Ghana where the current study was conducted between July 2015 and July 2016.

District	Community	Number of MDA Rounds	Microfilariae Prevalence in 2000 (%)	Microfilariae Prevalence in 2014 (%)	Number of *An.gambiae* Dissected
Ahanta West	Asemkow Antseambua	16	19.5	2.7	320
Mpohor	Obrayebona Ampeasem	11	0.0	0.0	368
Kassena Nankana West	Navio Central Badunu	15	29.4	1.3	217
Bongo	Atampiisi Bongo Balungu Nabiisi	13	16.7	0.0	211

4.3.2 Mosquito collection and identification

Mosquito sampling spanning both dry and rainy season was done for 13 months (from July 2015 to July 2016) in the four study districts. A detailed explanation of the three mosquito

sampling methods (i.e. human landing collections, pyrethrum spray catches and window exit traps) used by community vector collectors (CVCs) has been described by Pi-Bansa et al. (Pi-Bansa et al., 2018). Mosquitoes sampled were morphologically and molecularly identified. In short, morphological identification of mosquitoes involved the observation of mosquitoes under a microscope and separation into various genera (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). Deoxyribonucleic acid (DNA) extracted from the legs of *An. gambiae* was used for the identification of sibling species (Scott et al., 1993) and molecular forms within the An. *gambiae* complex (Fanello et al., 2002; Coetzee et al., 2013).

4.3.3 Mosquito dissection

The sample size of *An. gambiae* mosquitoes for dissection was specifically calculated for the various districts as described by Naing et al (Naing et al., 2006). Mosquitoes were placed on a glass slide. A pair of dissecting pins was used to separate the head, thorax and abdomen, followed by adding a drop of normal saline on each segment. Dissection of mosquitoes and identification of the *W. bancrofti* larval stages was done under a microscope (WHO, 2013a).

4.3.4 Extraction and detection of *W. bancrofti* in dissected mosquitoes

All *W. bancrofti* negative and positive mosquitoes were scraped into Eppendorf tubes, pending further molecular analyses. The various mosquito species were grouped into pools ranging from 1-25. DNA was extracted from pooled mosquitoes using the Qiagen DNeasy tissue kit (Qiagen CA) extraction method, adhering to the manufacturer's instructions. Extraction was followed by identification of parasite DNA in pooled mosquitoes, using a loop mediated isothermal amplification (LAMP) assay (de Souza et al., 2014; Kouassi et al., 2015), conventional polymerase chain reaction (PCR) (Ramzy et al., 1997) and real-time (RT)-PCR (Rao et al., 2006). These assays were performed using standard protocols described elsewhere (Ramzy et al., 1997; Rao et al., 2006; de Souza et al., 2014; Kouassi et al., 2015). Positive and negative controls were included in all reactions.

4.3.5 Extraction of nucleic acids from pooled mosquitoes with TRIzol reagent

Mosquitoes were randomly selected specifically for the extraction of DNA and RNA using TRIzol reagent (Life Technologies; Carlsbad, California, USA). In order to estimate an infection rate of 1% with a power of 0.80, the estimated total number of mosquitoes required for each district was 2,000 (WHO, 2009; Dorkenoo et al., 2018). The protocol for determining infectivity required that samples were stored in RNAlater so as to enable RNA extraction from mosquitoes. *An. gambiae*, *Mansonia* and *Culex* species sampled by human landing catches and stored in RNAlater reagent (Life Technologies; Carlsbad, California, USA) were pooled (range: 5-20). The determination of the number of mosquitoes in a pool was based on prior research pursued by Boakye et al., which tested different mosquito pool sizes (ie., 25, 50, 100 and 200) (Boakye et al., 2007). Several additional studies had pools of mosquitoes of up to 30 specimens (Laney et al., 2010; Kouassi et al., 2015; Owusu et al., 2015). Extraction of DNA and RNA on pooled mosquitoes was done to assess both *W. bancrofti* infection and infectivity rates, respectively (Laney et al., 2010). Detection of both infection and infectivity in pooled mosquitoes followed the protocols of Rao et al. (Rao et al., 2006) and Laney et al. (Laney et al., 2010), respectively. Furthermore, quality control was done for the detection of infection in *An. gambiae* complex by extracting DNA from pooled Kisumu mosquitoes (laboratory reared susceptible *An. gambiae* strains, n = 20) spiked with 5 - 20µl of *W. bancrofti* mf positive blood samples (57 mf/ml), which showed amplification for the parasite. The extraction protocol was replicated for this study (see supplementary file).

4.3.6 Statistical analysis

Data were entered into Microsoft Excel (Microsoft Corporation; Redmond, Washington, USA). The Poolscreen 2.0 software (University of Alabama; Birmingham, USA) was used to calculate the maximum likelihood estimate of infection in the vector populations, along with the 95% confidence interval (CI) (Katholi et al., 1995). The various entomological indices assessed

included vector biting density, infection and infectivity rates, annual/monthly transmission potentials and worm load in mosquitoes (Appawu et al., 2001; Coulibaly et al., 2013).

4.3.7 Ethical approval

This study was approved by the institutional review board of the Noguchi Memorial Institute for Medical Research (Accra, Ghana; reference no. CPN 077/13-14, 7 May 2014) and the institutional research commission of the Swiss Tropical and Public Health Institute (Basel, Switzerland; reference no. FK 122a, 24 November 2015). All CVCs consented orally to participate in the study. Albendazole and ivermectin were administered to CVCs before mosquito sampling commenced. Arrangement was also made with the nurses at the community-based health planning and services compound to provide treatment for CVCs who reported at their facility and tested positive for malaria.

4.4 Results

4.4.1 Mosquito abundance and composition

A total of 31,064 mosquitoes were collected during the 13-month study period: 27,739 (89.3%) by human landing catches, 2,687 (8.7%) by pyrethrum spray collections and 638 (2.1%) by window exit traps. The numbers of mosquitoes sampled from all districts using the various sampling techniques are summarised in Table 2. *An. gambiae* sensu lato (s. l.) (n = 23,102; 83.3%), the main lymphatic filariasis vector in Ghana, had the highest number collected using human landing catches. Other mosquitoes collected were by *Mansonia* spp. (n = 2,474; 8.9%), *Culex* spp. (n = 2,056; 7.4%), *Aedes* spp. (n = 92; 0.3%), *An. coustani* (n = 11; 0.04%) and *An. pharoensis* (n = 4; 0.01%). For pyrethrum spray collections, 1,884 (70.1%) *An. gambiae*, 720 (26.8%) *Culex* species, 40 (1.5%) *An. pharoensis,* 26 (1.0%) *Mansonia* spp., 10 *An. coustani* and 7 *Aedes* spp., were collected. A total of 562, 10, three and one mosquitoes were reported for *An. gambiae, An. pharoensis, Aedes* spp. and *An. coustani* and respectively, using window

exit traps. *Culex* and *Mansonia* spp. had the same number (n = 31) sampled for window exit traps.

Table 4.2 Mosquitoes sampled using three different sampling methods from four study districts in Ghana during a 13-month sampling period between July 2015 and July 2016.

Method	Community	District	*An. gambiae*	*Culex* species	*Mansonia* species	*Aedes* species	*An. pharoensis*	*An. coustani*	Total collected
Human landing catches	Asemkow Antseambua	Ahanta West	18,213	1200	2386	8	0	4	
	Obrayebona Ampeasem	Mpohor	4109	66	72	6	0	3	
	Badunu Navio Central	Kassena Nankana West	426	489	11	42	2	4	
	Atampiisi Bongo Balungu Nabiisi	Bongo	354	301	5	36	2	0	
Pyrethrum spray catches	Asemkow Antseambua	Ahanta West	271	4	19	1	36	0	
	Obrayebona Ampeasem	Mpohor	375	14	7	0	1	0	
	Badunu Navio Central	Kassena Nankana West	801	384	0	1	1	9	
	Atampiisi Bongo Balungu Nabiisi	Bongo	437	318	0	5	2	1	
Window exit trap	Asemkow Antseambua	Ahanta West	396	17	29	0	0	0	
	Obrayebona Ampeasem	Mpohor	119	1	1	1	9	0	
	Badunu Navio Central	Kassena Nankana West	12	6	1	1	1	0	
	Atampiisi Bongo Balungu Nabiisi	Bongo	35	7	0	1	0	1	
Total			25,548	2807	2531	102	54	22	31,064

4.4.2 Molecular identification of *An. gambiae* and *W. bancrofti*

A total of 320, 368, 217 and 211 *An. gambiae* s. l. from Ahanta West, Mpohor, Kassena Nankana West and Bongo districts, respectively, were identified at the molecular level. Results shown in Table 3 indicate high numbers of the sibling species *An. melas* in Ahanta West district. Relatively high numbers of *An. coluzzii*, formerly known as M form of the *An. gambiae* complex were obtained from Mpohor, Kassena Nankana West and Bongo districts.

Eight mosquitoes observed to be infected with *W. bancrofti* by dissection tested positive when pool screened for parasite using PCR.

Table 4.3 Distribution of members of the *An. gambiae* complex in four study districts, Ghana, collected between July 2015 and July 2016.

	Sibling species of the *Anopheles gambiae* complex							
District	***An. gambiae* s. s.**		***An. arabiensis***		***An. melas***		***An. coluzzii***	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Ahanta West	3	0.9	11	3.4	275	85.9	12	3.8
Mpohor	226	61.4	0	0	1	0.3	122	33.2
Kassena Nankana West	57	26.3	25	11.5	0	0	124	57.1
Bongo	54	25.6	0	0	0	0	142	67.3

4.4.3 Transmission indices of *An. gambiae* complex from Ahanta West district

The average vector biting density for *An. gambiae*, sampled using human landing collections from Ahanta West, Mpohor, Kassena Nankana West and Bongo districts, were 43.8, 9.9, 1.0 and 0.8 bites/person/night, respectively. *W. bancrofti* infections were reported only in *An. melas*, a sibling species within the *An. gambiae* complex from Ahanta West district for this study. Eight *An. melas* mosquitoes were found infected (harbouring any of the developmental stage(s) of the parasite: mf, larval stages 1 (L_1), 2 (L_2) or 3 (L_3), of which two mosquitoes were infective, harbouring only L_3, as shown in Figure 2. The total numbers of L_1, L_2 and L_3 counted from all the slides were 10, 2 and 2, respectively. The monthly (MIBR) and annual infective biting rates (AIBR) were 8.0 and 95.9 infective bites/person, respectively. The annual transmission potential (ATP) due to *An. gambiae* in the Ahanta West district was 7.4 (Table 4).

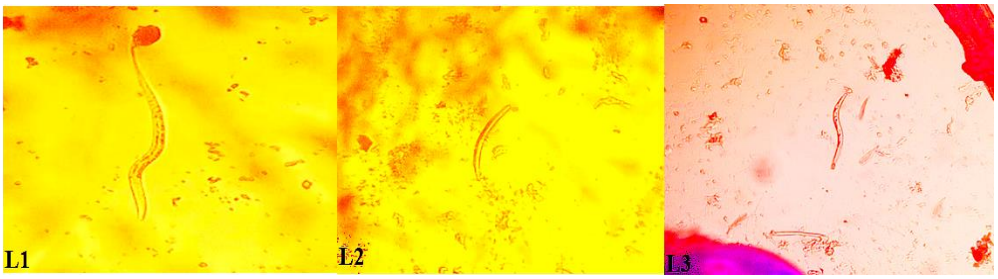

Figure 4. 1 Three larval stages of *W. bancrofti* parasite from Ahanta West district

Table 4.4 Entomological indices showing relevant parameters for the estimation of the annual transmission potential (ATP)

Average number of *An. gambiae* sampled per month	Vector biting density (MBR)	Annual biting rate (ABR)	Average number of *An. gambiae* dissected per month	Average infection per month	Average infectivity per month	Infection rate (%)	Infectivity rate (%)	Annual infective biting rate (AIBR)	Average worm load per month	Annual transmission potential (ATP)
1401	43.8	15,987	25	0.620	0.150	0.025 (2.5)	0.006 (0.6)	95.922	0.077	7.4
316	9.9	3604	28	0	0	0	0	0	0	0
33	1.0	376	17	0	0	0	0	0	0	0
27	0.8	307	16	0	0	0	0	0	0	0

4.4.3 Detection of *W. bancrofti* using molecular techniques

A total of 2000 *An. gambiae* s. l. from Ahanta West and Mpohor districts, 253 from Kassena Nankana West and 225 from Bongo districts were screened for *W. bancrofti* infections and infectivity using RT-PCR. None of the 4478 *An. gambiae* processed in 214 pools from all study districts were found positive for *W. bancrofti*. Screening was also done for both *Mansonia* and *Culex* species from the four districts, though very few numbers were sampled from Mpohor, Kassena Nankana West and Bongo compared to Ahanta West. Both *Mansonia* and *Culex* species were found negative for *W. bancrofti* in all districts (Table 5). All dissected

mosquitoes from the four districts which were negative for *W. bancrofti* parasite and further screened by LAMP, conventional and RT-PCRs tested negative.

Table 4.5 Number of mosquito pools processed per study district, Ghana from July, 2015 to July, 2016

Species	District	Number of pools	Average pool size	Number of mosquitoes processed	Positive (infection/infectivity)	95% CI
An. gambiae	Ahanta West	97	20.6	2000	0	0-0.00095
	Mpohor	91	22.0	2000	0	0-0.00095
	Kassena Nankana West	13	19.5	253	0	0-0.00756
	Bongo	13	17.3	225	0	0-0.00849
***Mansonia* species**	Ahanta West	83	21.1	1754	0	0-0.00109
	Mpohor	2	25.0	50	0	0-0.03767
	Kassena Nankana West	1	14.0	14	0	0-0.12815
	Bongo	1	5.0	5	0	0-3.18868
***Culex* species**	Ahanta West	63	20.0	1261	0	0-0.00152
	Mpohor	2	19.0	38	0	0-0.04927
	Kassena Nankana West	19	19.4	369	0	0-0.00518
	Bongo	8	16.3	133	0	0-0.01433

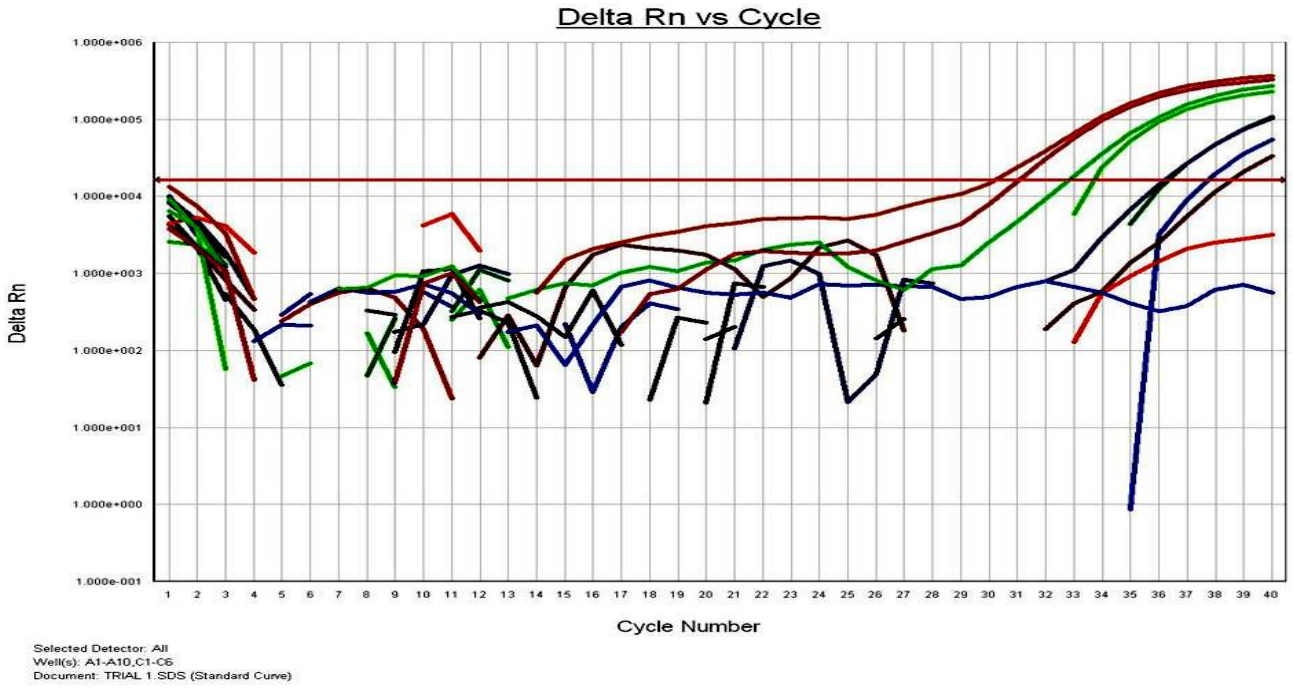

Figure 4. 2 Amplification of *Wuchereria bancrofti* DNA from pooled laboratory reared susceptible Kisumu

The diagram shows amplification curves for four different pools (n = 20) of susceptible Kisumu mosquitoes and a positive control. The four pools were spiked with 5–20 µl of *Wuchereria bancrofti* microfilariae positive blood having a concentration of 57 mf/ml.

4.5 Discussion

Rigorous monitoring of *W. bancrofti* infections in mosquito vectors after several rounds of MDA is recommended to provide information on the progress of control and elimination activities. Indeed, such monitoring activities are necessary for making programmatic decisions that will eventually lead to certification of lymphatic filariasis elimination in previously endemic regions (Boakye et al., 2007). The current study, which forms part of an operational research project to determine reasons for persistent lymphatic filariasis transmission in selected districts in Ghana after more than 10 rounds of MDA, investigated the feasibility and usefulness of a xenomonitoring approach for post-MDA surveillance to assess filarial infections in vectors (de Souza et al., 2014; Opoku et al., 2018). Our study also provides information on the lymphatic filariasis infection status in vectors after multiple rounds of MDA in previously endemic districts.

The sampling methods used for this study included human landing collections, pyrethrum spray catches and window exit traps. These collection methods have been used before for sampling mosquitoes for xenomonitoring activities (Govella et al., 2009; Wong et al., 2013; Pam et al., 2017; Pi-Bansa et al., 2018). Recently, the Ifakara tent trap has been reported as an alternative to human landing collections and it was emphasised that it exhibits an improved ethical profile (Briët et al., 2015; WHO, 2013b). However, at the time our study was implemented, we did not have access to the Ifakara tent trap. Results from our study revealed high numbers of *An. gambiae* complex, the primary lymphatic filariasis vector in Ghana (Boakye et al., 2004; Owusu et al., 2015), in all four districts. The highest density was observed in Ahanta West district. The high densities of vectors and observed infections (L_1, L_2 and L_3) in Ahanta West district might explain the presence of *W. bancrofti* infection in the *An. gambiae* complex from this district. A relatively higher density of *An. gambiae* was recorded in Kassena Nankana West district, compared to Bongo district. Both districts are in the dry

Guinea savannah ecological zone (Appawu et al., 2001), whilst the Ahanta West and Mpohor districts are situated in the rain forest ecological zone (Gyapong et al., 1998). In the year 2000, high baseline mf prevalences of 19.5% and 29.4% were reported in Ahanta West and Kassena Nankana West districts, while considerably lower mf prevalence were observed in 2014; 2.7% and 1.3%, respectively (Table 1) after multiple rounds of MDA. The present study recorded *W. bancrofti* infection rates of 0.025 and nil for Ahanta West and Kassena Nankana West districts, respectively (Table 4). These very low infection rates observed in mosquitoes from this study reflect correspondingly the low lymphatic filariasis prevalence rates in the human population. Moreover, the availability of efficient vectors (*An. melas* and/or *Mansonia* species) in all four study districts can lead to picking up *W. bancrofti* infections, even at low parasitaemia, as seen in Ahanta West district. Despite the large numbers of efficient vectors in a given district, the very low rates or the absence of *W. bancrofti* infections in the human population is likely to result in the absence of infections in vectors. Hence, there should be enough *W. bancrofti* parasites in the blood of human population for vectors to successfully ingest after a blood meal, since at very low mf levels, vectors are unlikely to ingest parasites. This may explain the absence of infections in the large number of *An. gambiae* vectors collected and examined in Kassena Nankana West, Mpohor and Bongo districts.

Furthermore, results from molecular species identification of the *An. gambiae* complex showed a high proportion of *An. coluzzii* (formally the M form of *An. gambiae* complex) in almost all districts (Table 3). This could be associated with the fact that *An. coluzzii*, which prefer breeding in ephemeral sites like run-off and flood water, are mostly found in the northern and coastal savannah areas of Ghana where this study was conducted (de Souza et al., 2010). Kassena Nankana West district recording the highest number of *An. arabiensis* could possibly be due to its location in the northern part of Ghana where the climate is arid, which represents the preferred breeding condition for this mosquito species (Coetzee et al., 2013). *An. melas*,

which is a sibling species within the *An. gambiae* complex, was mostly found in the Ahanta West district, corroborating previous findings by Dunyo et al. (Dunyo et al., 1996). *Anopheles* mosquitoes are known to exhibit "facilitation", this makes it possible for these mosquito species to pick up *W. bancrofti* parasites at high mf rates in the human population and develop them to the infective stage (Southgate and Bryan, 1992a; Amuzu et al., 2010; de Souza et al., 2012). However, *An. melas* exhibits "limitation", and hence, this species can ingest and develop mf to the infective stage, even at low parasite densities (Amuzu et al., 2010; de Souza et al., 2012). In view of the high numbers of *An. melas* recorded in Ahanta West district, it is conceivable that this species is responsible for the observed *W. bancrofti* parasites (L_1, L_2 and L_3).

The ABR for *An. gambiae* complex was highest in the Ahanta West district (15,987 bites/person/year). Finding *W. bancrofti* infections in Ahanta West district may be due to the high number of lymphatic filariasis vectors, specifically from the *An. gambiae* complex with a reported prevalence of 2.7% in this district. In the Kassena Nankana West district, before the commencement of our study, the reported prevalence of 1.3% was indicative of low persistent lymphatic filariasis transmission. A possible reason for the absence of infections in Kassena Nankana West is the relatively low level of infection in the human population. Another factor is the lower ABR (376.3 bites/person/year) in this district. There were no *W. bancrofti* infections recorded in the Mpohor and Bongo districts. This may be due to the zero mf prevalence reported for these two districts (Table 1) before the onset of this study. In a previous study, Appawu et al. (Appawu et al., 2001) investigated the entomological role played by the two lymphatic filariasis vectors, *An. gambiae* and *An. funestus*, at irrigation project sites in the Upper East region of Ghana. The authors recorded *W. bancrofti* infections in all study districts. Their results indicated that for irrigated communities like Tono and Vea, higher vector densities resulted in more infective feeds compared to Azoka, a non-irrigated

community. The 7.4 ATP of *An. gambiae* in the Ahanta West district is due to the observed L_3 in *An. melas* and reported mf positive individuals from this district. The ATP value of *An. gambiae* obtained in spite of the low infectivity rate might be explained by large number of *An. gambiae* collected in this district. In our study, the vector observed having *W. bancrofti* infections (L_1, L_2 and L_3) was only *An. melas* belonging to the *An. gambiae* complex. Additionally, identification of *Mansonia* species in Ahanta West district suggests that these vectors could take up mf and successfully develop them to the infective stage, even at low parasitaemia (Southgate, 1992; de Souza et al., 2018).

Mosquito vector control activities reduce vector densities and human-vector contact (Bockarie et al., 2009; Kelly-Hope et al., 2013). This in turn decreases the likelihood of vectors picking up *W. bancrofti* parasites in endemic areas that have undergone several rounds of MDA. We found considerably higher bednet usage in Mpohor and Bongo districts, compared to Ahanta West and Kassena Nankana West districts. Hence, there is higher human-vector contact in the latter two districts. This could have contributed to the high ABR recorded for Ahanta West leading to *W. bancrofti* infections in the vectors due to infections in the human population. Though Mpohor district had relatively high ABR (3,604) recorded, the reported prevalence of zero may explain the absence of infections in this district.

Additionally, mosquito species previously considered as non-vectors might be acting as vectors of lymphatic filariasis as in the case of *Mansonia* in Ghana (Ughasi et al., 2012) and *Culex* in Nigeria (Anosike et al., 2005; Agi and Ebenezer, 2010). This observation together with the fact that parasite DNA can be detected in both vector and non-vector mosquitoes (Dorkenoo et al., 2018), led to the investigation of both species in this study. No positive result was recorded for culicines using molecular assays. Furthermore, molecular assays run on DNA and RNA extracted from selected *An. gambiae* complex from the various districts was negative for filarial infections. The absence of infections in *An. gambiae* complex could have been as a

result of PCR inhibition due to the masking of parasite DNA by mosquito DNA due to extraction of pooled mosquito samples.

4.6 Conclusions

Our study, employing xenomonitoring as a post-MDA surveillance tool, revealed that at low parasitaemia, infections are usually found and sustained in vectors that exhibit limitation as seen here in Ahanta West district. Additionally, *An. melas* emerges as an important vector for xenomonitoring along the coastal communities of the Western region in the southern part of Ghana. Moreover, effective vector control activities like high coverage of bednets can decrease ABR values in any endemic foci. As revealed in our previous work, vector control activities (bednet usage) in Mpohor and Bongo districts were relatively high. The reported zero prevalence of human infections and reduction in the human vector contact due to bednet usage might be responsible for the absence of infections in mosquito vectors from these districts. Presently in Ghana, only little emphasis is placed on the inclusion of xenomonitoring in decision-making processes during lymphatic filariasis programmatic activities. As shown here, data from xenomonitoring could be used by programme managers and other stakeholders to support decisions of stopping or continuing MDA. Additionally, complementing vector control activities with MDA during lymphatic filariasis control activities could reduce *W. bancrofti* infections in mosquitoes.

Chapter 5: Discussion

Studies by Gyapong and colleagues, 1996 in all 10 administrative regions of Ghana indicated high mf prevalence in the northern Guinea savannah and the southern coastal belt of Ghana. Further *W. bancrofti* antigen mapping in Ghana by the Ghana Health Service in 1999 identified LF to be endemic in 98 districts (Biritwum et al., 2016) with reported mf and antigen prevalence ranging between 29.6% and 45.4% (Biritwum et al., 2017a). This therefore presented LF as a disease of public health concern requiring immediate intervention and control. Ghana commenced the implementation of MDA in 2001 in 10 districts, later scaling up to the remaining districts by 2006 (Biritwum et al., 2016). However, data obtained from the Ghana Neglected Tropical Disease Unit have revealed persistent LF transmission in some districts even after reported MDA coverage of more than 65% (Biritwum et al., 2016). In order to successfully control LF in these "hotspot" areas, there was the need to understand driving factors which could possibly be contributing to this persistent LF transmission, and the appropriate intervention/control measure to be used in each endemic foci.

The present study, in an attempt to address the current situation, assessed potential factors which could possibly be influencing LF transmission in "hotspot" and control districts in Ghana. This study, which was conducted in districts in the Upper East and Western regions of Ghana, revealed the need for stakeholders and programme managers to have a critical look at the 1% mf and 2% antigen prevalence cut off threshold for interrupting transmission. This is as a result of studies (Michael et al., 2017), which have revealed factors affecting LF transmission to differ for various endemic areas due to spatial heterogeneities. Hence, the need to obtain the specific cut-off threshold for mf and antigen prevalence for every endemic area. This if not done could extend the duration for these endemic areas in meeting the Global Programme to Eliminate Lymphatic Filariasis (GPELF) goal of eliminating LF by 2020. Hence, the

implementation of any LF intervention or control activity by programme managers and stakeholders should not be generalised, but must be specific for each endemic area considering the factors influencing transmission.

Additionally, in terms of MDA coverage, an assessment of data obtained from the Ghana Neglected Tropical Disease Programme Unit was done. Though there were no data available for some of the years in all the districts MDA coverage was ≥65% for almost all the years in the various study districts. Moreover, a questionnaire survey was conducted to investigate MDA compliance of individuals from the various districts. Results indicated higher MDA compliance of participants in five preceding MDA rounds in hotspot districts compared to control districts before commencing this study. This observation also suggested MDA compliance as not contributing to persistent LF transmission in hotspot districts. The presence and abundance of lymphatic filariasis vectors in an endemic area plays an important role in the persistence of the disease (WHO, 2013a). This observation was evident in the Ahanta West district, where the highest number of *An. gambiae* was obtained for this study. Additionally, higher numbers of vectors lead to high annual biting rates (ABRs) presenting a greater tendency of exposure to infections if human reservoirs are present.

The implementation of vector control activities in LF endemic areas could have an impact on reducing transmission as revealed by various studies (Bockarie et al., 2009; Rebollo et al., 2015; Koudou et al., 2018). Analyses of data for vector control activities in a questionnaire survey for this study gave results showing a relatively high average bednet usage in all the study districts. However, bednet usage was slightly higher in control districts compared to hotspot districts which could possibly be a factor indicative of the absence of infections in the control districts.

Epidemiologically, three important relationships exist between parasites and vectors (Gyapong et al., 2005). The relevance of these vector-parasite relationships is based on the predicted importance of the sustenance of low microfilariae density by vectors present in an endemic area (Gyapong et al., 2005). These vector-parasite relationships include facilitation, where vectors can sustain the development of mf to the infective stage (L_3) as the number of mf ingested increases (Amuzu et al., 2010). Limitation on the other hand has appreciable number of the microfilariae developing to the infective stage even at low mf rates (Amuzu et al., 2010). Proportionality has the number of mf developing to the infective stage of the parasite constant, irrespective of the number ingested (de Souza et al., 2012). *Anopheles* mosquitoes are known to exhibit facilitation (Southgate, 1992) and therefore in areas where they serve as vectors, LF transmission can be interrupted using MDA alone since at low infection levels this species is unable to sustain transmission. Therefore, it is expected that in Ghana LF should have been eliminated with *An. gambiae* serving as major vector. Though this may be the case, *An. melas*, which is part of the *An. gambiae* complex, has been shown to exhibit limitation (Bryan and Southgate, 1988; Boakye et al., 2004). This study reported high numbers of *An. melas* only in Ahanta West, and was also the only mosquito species found harbouring various stages of the filarial parasite by dissection. Therefore, the availability of *An. melas* in Ahanta West, a hotspot district could probably be one of the reasons why transmission has been sustained at such low mf levels. This means that in areas such as the Ahanta West where *An. gambiae* s. s. coexists in sympatry with *An. melas*, LF transmission can be sustained by the latter even in the presence of MDA. Furthermore, higher numbers of *Mansonia* species also observed to be exhibiting limitation (Gyapong et al., 2005) were collected from Ahanta West district compared to the other districts. Though no infections were seen by molecular xenomonitoring and dissections in *Mansonia* species from any of the districts, the presence of this mosquito

species in high numbers in Ahanta West is a precursor for the establishment of infections and transmission in the presence of infected human hosts.

Furthermore, armatures in the foregut by their structure and function could provide protection against filarial infections (Chadee et al., 1996). Protection provided by cibarial armatures against filarial parasites in mosquitoes could involve possible lacerations being inflicted on mf after ingestion of infected blood meal, eventually leading to a reduction in the total number of mf ingested. The number of mf damaged and the extent of damage inflicted on mf is mostly dependent on the presence, shape and how developed the cibarial armatures are in mosquitoes (McGreevy et al., 1978). The presence or absence of this physical barrier in LF vectors could influence the dynamics of filarial transmission, and hence, impact on control measures (McGreevy et al., 1978). An investigation of the cibarial armatures of various mosquito species showed no significant difference in the mean numbers of teeth for *An. coluzzii* and *Culex* species from hotspot and control districts in the Western and Upper East regions. Additionally, *Mansonia* and *Aedes* species from all districts had no teeth, and the shape of teeth was similar for the various mosquito species from both hotspot and control districts. However, *An. melas* found only in the Ahanta West district was found to have lesser mean number of teeth when compared to the other mosquito species. Similarly, Amuzu et al., 2010 in their study showed that *An. melas* with lesser number of cibarial teeth sustained lymphatic filariasis transmission in Hwida, one endemic community in the Gomoa district of the Central region, even at low mf rates than *An. gambiae* s. s. with significantly higher number of cibarial teeth in another neighbouring endemic community. Therefore for competent mosquito vectors with lesser number or no cibarial teeth, LF transmission may be sustained longer. This in effect means *An. melas* with lesser teeth numbers will be efficient in picking microfilariae and sustaining lymphatic filariasis transmission even at low mf rates. Therefore, the availability of *An. melas*

in one of the hotspot districts could probably be one of the reasons why transmission has been sustained at such low microfilariae rates.

In order to assess the feasibility of using community vector collectors (CVCs) to sample large numbers of mosquitoes for xenomonitoring at minimum cost with no supervision, this study assessed the possibility of implementing a strategy for xenomonitoring towards LF elimination. Results showed the cost-effectiveness of sampling mosquitoes using CVCs compared to a research team. Additionally, larger numbers of mosquitoes were sampled from districts in the Western region compared to the Upper East region. This could be attributed to the location of the Western region in the rain forest climatic zone compared to the Upper East region, which is found in the Guinea Savannah climatic zone. Furthermore, validation of mosquitoes sampled by CVCs showed no significant difference in the number of mosquitoes sampled by CVCs and research team in districts in the Western Region. However, this was not same in the districts of the Upper East region where there was significant difference in the number of mosquitoes sampled by CVCs and research team.

Conclusion and recommendations

Key findings from this study suggest that persistent LF transmission in hotspot areas reveals the importance of local understanding of factors affecting elimination of LF as this could be influenced by spatial heterogeneities in various endemic areas. This was shown in the differences observed in the transmission dynamics of LF in the various hotspot districts. Furthermore, this study showed the feasibility of using community vector collectors (CVCs) to sample large numbers of mosquitoes with minimal supervision from research team for xenomonitoring purpose. Additionally, the lower cost involved in collecting mosquitoes using CVCs compared to research team and the promotion of active community participation by involving CVCs in LF xenomonitoring activities has been proven by this study to be feasible. Moreover, the possibility of using xenomonitoring as a useful post-MDA surveillance approach to assess infections in vectors and transmission has been successfully demonstrated by this study where *An. melas* was found to be sustaining transmission in the Ahanta West district.

We recommend that due to spatial heterogeneities and the focal nature of LF transmission among others, interventions in any form should consider the unique factors and the best approach to use in each endemic foci. Additionally, xenomonitoring should be included in decision-making to either stop or continue MDA by stakeholders and programme managers. Mosquito traps and sampling techniques should be safe, practical and convenient for CVCs to use with less supervision. Furthermore, complementing MDA with vector control activities by programme managers and stakeholders in LF control programmes can reduce infections in mosquitoes.

References

1. Abad-Franch, F., Vega, M.C., Rolón, M.S., Santos, W.S., Rojas de Arias, A., 2011. Community participation in chagas disease vector surveillance: systematic review. PLoS Negl Trop Dis. 5, e1207.
2. Aboagye-Antwi, F., Kwansa-Bentum, B., Dadzie, S.K., Ahorlu, C.K., Appawu, M.A., Gyapong, J., Wilson, M.D., Boakye, D.A., 2015. Transmission indices and microfilariae prevalence in human population prior to mass drug administration with ivermectin and albendazole in the Gomoa District of Ghana. Parasit Vectors 8, 562.
3. Agbozo, E.Y., Dumashie, E., Boakye, D.A., de Souza, D.K., 2018. Effects of lyophilization and storage temperature on *Wuchereria bancrofti* antigen sensitivity and stability. BMC Res Notes 11, 454.
4. Agi, P., Ebenezer, A., 2010. Observations on filarial infection in Amassoma community in the Niger Delta, Nigeria. J. Appl. Sci. Environ. Manage. 13, 15–19.
5. Ahorlu, C.S.K., Koka, E., Adu-Amankwah, S., Otchere, J., de Souza, D.K., 2018. Community perspectives on persistent transmission of lymphatic filariasis in three hotspot districts in Ghana after 15 rounds of mass drug administration: a qualitative assessment. BMC Med. 18, 238.
6. Amuzu, H., Wilson, M.D., Boakye, D.A., 2010. Studies of *Anopheles gambiae* s.l. (Diptera: Culicidae) exhibiting different vectorial capacities in lymphatic filariasis transmission in the Gomoa district, Ghana. Parasit.Vectors 3, 85.
7. Anosike, J.C., Nwoke, B.E., Ajayi, E.G., Onwuliri, C.O., Okoro, O.U., Oku, E.E., Asor, J.E., Amajuoyi, O.U., Ikpeama, C.A., Ogbusu, F.I., Meribe, C.O., 2005. Lymphatic filariasis among the Ezza people of Ebonyi state, eastern Nigeria. Ann. Agric. Environ. Med. 12, 181–186.
8. Appawu, M.A., Dadzie, S.K., Baffoe Wilmot, A., Wilson, M.D., 2001. Lymphatic filariasis in Ghana: entomological investigation of transmission dynamics and intensity in communities served by irrigation systems in the Upper East region of Ghana. Trop. Med. Int. Health 6, 511–516.
9. Bayoh, M.N., Thomas, C.J., Lindsay, S.W., 2001. Mapping distributions of chromosomal forms of *Anopheles gambiae* in West Africa using climate data. Med. Vet. Entomol. 15, 267–274.
10. Beaty, B.J., Marquardt, W.C., 1996. The biology of disease vectors. University Press of Colorado, Niwot, USA.
11. Biritwum, N.K., de Souza, D.K., Marfo, B., Odoom, S., Alomatu, B., Asiedu, O., Yeboah, A., Hervie, T.E., Mensah, E.O., Yikpotey, P., Koroma, J.B., Molyneux, D., Bockarie, M.J., Gyapong, J.O., 2017a. Fifteen years of programme implementation for the elimination of lymphatic filariasis in Ghana: impact of MDA on immunoparasitological indicators. PLoS Negl. Trop. Dis. 11, e0005280.
12. Biritwum, N.K., Garshong, B., Alomatu, B., de Souza, D.K., Gyapong, M., Kyelem, D., 2017b. Improving drug delivery strategies for lymphatic filariasis elimination in urban areas in Ghana. *PLoS Negl Trop Dis.* 11, e0005619.
13. Biritwum, N.K., Yikpotey, P., Marfo, B.K., Odoom, S., Mensah, E.O., Asiedu, O., Alomatu, B., Hervie, E.T., Yeboah, A., Ade, S., Hinderaker, S.G., Reid, A., Takarinda, K.C., Koudou, B., Koroma, J.B., 2016. Persistent 'hotspots' of lymphatic filariasis microfilaraemia despite 14 years of mass drug administration in Ghana. Trans R Soc Trop Med Hyg. 110, 690–695.
14. Boakye, D.A., Baidoo, H.A., Glah, E., Brown, C., Appawu, M., Wilson, M.D., 2007. Monitoring lymphatic filariasis interventions: adult mosquito sampling, and improved

PCR–based pool screening method for *Wuchereria bancrofti* infection in *Anopheles* mosquitoes. Filaria J. 6, 13.
15. Boakye, D.A., Wilson, M.D., Appawu, M.A., Gyapong, J., 2004. Vector competence, for *Wuchereria bancrofti*, of the *Anopheles* populations in the Bongo district of Ghana. Ann. Trop. Med. Parasitol. 98, 501–508.
16. Bockarie, M.J., 2007. Molecular xenomonitoring of lymphatic filariasis. Am. J. Trop. Med. Hyg. 77, 591–592.
17. Bockarie, M.J., Molyneux, D.H., 2009. The end of lymphatic filariasis? BMJ 338, b1981.
18. Bockarie, M.J., Pedersen, E.M., White, G.B., Michael, E., 2009. Role of vector control in the Global Program to Eliminate Lymphatic Filariasis. Annu. Rev. Entomol. 54, 469–487.
19. Boza, S., Vargas, M., 2006. The morphology of the cibarial armature and sensilla of *Mansonia titillans*, *Psorophora cingulata*, *Coquillettidia arribalzagae*, *Culex coronator* and *Limatus durhamii* (Diptera: Culicidae). Rev Biol Trop. 54, 6.
20. Brengues, J., Subra, R., Bouchité, B., 1969. Etude parasitologique, clinique et entomologique sur la filariose de Bancroft dans le Sud du Dahomey et du Togo. *Cah.ORSTOM Ser.Entomol.Med.Parasitol.* 7, 279–305.
21. Briët, O.J.T., Huho, B.J., Gimnig, J.E., Bayoh, N., Seyoum, A., Sikaala, C.H., Govella, N., Diallo, D.A., Abdullah, S., Smith, T.A., Killeen, G.F., 2015. Applications and limitations of Centers for Disease Control and Prevention miniature light traps for measuring biting densities of African malaria vector populations: a pooled-analysis of 13 comparisons with human landing catches. Malar J. 14, 247.
22. Bryan, J.H., McMahon, P., Barnes, A., 1990. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 3. Uptake and damage to ingested microfilariae by *Anopheles gambiae*, *An. arabiensis*, *An. merus* and *An. funestus* in East Africa. Trans R Soc Trop Med Hyg. 84, 265–268.
23. Bryan, J.H., Southgate, B.A., 1988. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 2. Damage to ingested microfilariae by mosquito foregut armatures and development of filarial larvae in mosquioes. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 82, 138–145.
24. Chadee, D., Beier, J., Martinez, R., 1996. The effect of the cibarial armature on blood meal haemolysis of four anopheline mosquitoes. Bull. Entomol. Res. 86, 351–354.
25. Chaki, P.P., Mlacha, Y., Msellemu, D., Muhili, A., Malishee, A.D., Mtema, Z.J., Kiware, S.S., Zhou, Y., Lobo, N.F., Russell, T.L., Dongus, S., Govella, N.J., Killeen, G.F., 2012. An affordable, quality-assured community-based system for high-resolution entomological surveillance of vector mosquitoes that reflects human malaria infection risk patterns. Malar J. 11, 172.
26. Chu, B.K., Deming, M., Biritwum, N.-K., Bougma, W.R., Dorkenoo, A.M., El-Setouhy, M., Fischer, P.U., Gass, K., Gonzalez de Peña, M., Mercado-Hernandez, L., Kyelem, D., Lammie, P.J., Flueckiger, R.M., Mwingira, U.J., Noordin, R., Offei Owusu, I., Ottesen, E.A., Pavluck, A., Pilotte, N., Rao, R.U., Samarasekera, D., Schmaedick, M.A., Settinayake, S., Simonsen, P.E., Supali, T., Taleo, F., Torres, M., Weil, G.J., Won, K.Y., 2013. Transmission Assessment Surveys (TAS) to Define Endpoints for Lymphatic Filariasis Mass Drug Administration: A Multicenter Evaluation. *PLOS Neglected Tropical Diseases* 7, e2584.
27. Chwatt, L.J., Major, H., 1945. The morphology of the pharyngeal armature in *Anopheles gambiae* var *melas* from Southern Nigeria. Ann Trop Med Parasitol. 39:2, 124–128.

28. Coetzee, M., Hunt, R.H., Wilkerson, R., Torre, A.D., Coulibaly, M.B., Besansky, N.J., 2013. *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. Zootaxa 3619, 246–274.
29. Coulibaly, Y., Dembele, B., Diallo, A., Kristensen, S., Konate, S., Dolo, H., Dicko, I., Sangare, M., Keita, F., Boatin, B.A., Traore, A., Nutman, T.B., Klion, A.D., Touré, Y., Traore, S., 2013. *Wuchereria bancrofti* transmission pattern in southern Mali prior to and following the institution of mass drug administration. Parasit. Vectors 6, 247.
30. de Souza, D., Kelly-Hope, L., Lawson, B., Wilson, M., Boakye, D., 2010. Environmental factors associated with the distribution of *Anopheles gambiae* s.s. in Ghana; an important vector of lymphatic filariasis and malaria. PLoS One 5, e9927.
31. de Souza, D., Otchere, J., Ahorlu, C., Adu-Amankwah, S., Larbi, I., Dumashie, E., McCarthy, F., King, S., Otoo, S., Osabutey, D., Osei, J., Sedzro, K., Asiedu, O., Dadzie, S., Ayi, I., Marfo, B., Biritwum, N.-K., Boakye, D., 2018. Low microfilaremia levels in three districts in coastal Ghana with at least 16 years of mass drug administration and persistent transmission of lymphatic filariasis. Trop. Med. Infect. Dis. 3, 105.
32. de Souza, D.K., Ansumana, R., Sessay, S., Conteh, A., Koudou, B., Rebollo, M.P., Koroma, J., Boakye, D.A., Bockarie, M.J., 2015. The impact of residual infections on *Anopheles*-transmitted *Wuchereria bancrofti* after multiple rounds of mass drug administration. *Parasites & Vectors* 8, 488.
33. de Souza, D.K., Koudou, B., Kelly-Hope, L.A., Wilson, M.D., Bockarie, M.J., Boakye, D.A., 2012. Diversity and transmission competence in lymphatic filariasis vectors in West Africa, and the implications for accelerated elimination of *Anopheles*-transmitted filariasis. Parasit. Vectors 5, 259.
34. de Souza, D.K., Sesay, S., Moore, M.G., Ansumana, R., Narh, C.A., Kollie, K., Rebollo, M.P., Koudou, B.G., Koroma, J.B., Bolay, F.K., Boakye, D.A., Bockarie, M.J., 2014. No evidence for lymphatic filariasis transmission in big cities affected by conflict related rural-urban migration in Sierra Leone and Liberia. PLoS. Negl. Trop. Dis. 8, e2700.
35. de Souza, D.K., Yirenkyi, E., Otchere, J., Biritwum, N.-K., Ameme, D.K., Sackey, S., Ahorlu, C., Wilson, M.D., 2016. Assessing lymphatic filariasis data quality in endemic communities in Ghana, using the neglected tropical diseases data quality assessment tool for preventive chemotherapy. PLoS Negl Trop Dis. 10, e0004590.
36. Derua, Y.A., Alifrangis, M., Hosea, K.M., Meyrowitsch, D.W., Magesa, S.M., Pedersen, E.M., Simonsen, P.E., 2012. Change in composition of the Anopheles gambiae complex and its possible implications for the transmission of malaria and lymphatic filariasis in north-eastern Tanzania. Malar J. 11, 188.
37. Dorkenoo, M.A., de Souza, D.K., Apetogbo, Y., Oboussoumi, K., Yehadji, D., Tchalim, M., Etassoli, S., Koudou, B., Ketoh, G.K., Sodahlon, Y., Bockarie, M.J., Boakye, D.A., 2018. Molecular xenomonitoring for post-validation surveillance of lymphatic filariasis in Togo: no evidence for active transmission. Parasit. Vectors 11, 52.
38. Dunyo, S.K., Appawu, M., Nkrumah, F.K., Baffoe-Wilmot, A., Pedersen, E.M., Simonsen, P.E., 1996. Lymphatic filariasis on the coast of Ghana. Trans. R. Soc. Trop. Med. Hyg. 90, 634–638.
39. Dyab, A.K., Galal, L.A., Mahmoud, A.E.-S., Mokhtar, Y., 2015. Xenomonitoring of different filarial nematodes using single and multiplex PCR in mosquitoes from Assiut governorate, Egypt. Korean J Parasitol 53, 77–83.

40. Dzodzomenyo, M., Dunyo, S.K., Ahorlu, C.K., Cooker, W.Z., Appawu, M.A., Pedersen, E.M., Simonsen, P.E., 1999. Bancroftian filariasis in an irrigation project community in southern Ghana. Trop Med Int Health. 4, 13–18.
41. Fanello, C., Santolamazza, F., Della Torre, A., 2002. Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. Med. Vet. Entomol. 16, 461–464.
42. Fischer, P.U., King, C.L., Jacobson, J.A., Weil, G.J., 2017. Potential value of triple drug therapy with ivermectin, diethylcarbamazine, and albendazole (IDA) to accelerate elimination of lymphatic filariasis and onchocerciasis in Africa. PLoS Negl Trop Dis. 11, e0005163.
43. Ghana Statistical Service, 2014a. Population and housing census, Bongo district, Ghana.
44. Ghana Statistical Service, 2014b. Population and housing census, Kassena Nankana West district, Ghana.
45. Ghana Statistical Service, 2014c. Population and housing census, Ahanta West district, Ghana.
46. Ghana Statistical Service, 2014d. Population and housing census, Mpohor district, Ghana.
47. GHS, 2017. Ghana Health Service, 2016 annual report.
48. Gillies, M.T., Coetzee, M., 1987. A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region);
49. Gillies, M.T., De Meillon, B., 1968. The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region);
50. Goodman, D.S., Orelus, J.N., Roberts, J.M., Lammie, J., Streit, T.G., 2003. PCR and mosquito dissection as tools to monitor filarial infection levels following mass treatment. Filaria J. 2, 11.
51. Govella, N.J., Chaki, P.P., Geissbühler, Y., Kannady, K., Okumu, F., Charlwood, J.D., Anderson, R.A., Killeen, G.F., 2009. A new tent trap for sampling exophagic and endophagic members of the *Anopheles gambiae* complex. Malar J. 8, 157.
52. Gyapong, J.O., Adjei, S., Sackey, S.O., 1996. Descriptive epidemiology of lymphatic filariasis in Ghana. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 90, 26–30.
53. Gyapong, J.O., Kumaraswami, V., Biswas, G., Ottesen, E.A., 2005. Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. Expert Opin Pharmacother. 6, 179–200.
54. Gyapong, J.O., Owusu, I.O., da-Costa Vroom, F.B., Mensah, E.O., Gyapong, M., 2018. Elimination of lymphatic filariasis: current perspectives on mass drug administration. Res Rep Trop Med. 9, 25–33.
55. Gyapong, J.O., Webber, R.H., Morris, J., Bennett, S., 1998. Prevalence of hydrocele as a rapid diagnostic index for lymphatic filariasis. Trans. R. Soc. Trop. Med. Hyg. 92, 40–43.
56. Ichimori, K., King, J.D., Engels, D., Yajima, A., Mikhailov, A., Lammie, P., Ottesen, E.A., 2014. Global programme to eliminate lymphatic filariasis: the processes underlying programme success. *PLoS Neglected Tropical Diseases* 8, e3328.
57. Jones, C., Ngasala, B., Derua, Y.A., Tarimo, D., Reimer, L., Bockarie, M., Malecela, M.N., 2018. Lymphatic filariasis transmission in Rufiji district, southeastern Tanzania: infection status of the human population and mosquito vectors after twelve rounds of mass drug administration. Parasit. Vectors 11, 588.
58. Kanamitie, J.N., Ahorlu, C.S., Otchere, J., Aboagye-Antwi, F., Kwansa-Bentum, B., Boakye, D.A., Biritwum, N.-K., Wilson, M.D., de Souza, D.K., 2017. Twelve-month

longitudinal parasitological assessment of lymphatic filariasis-positive individuals: impact of a biannual treatment with ivermectin and albendazole. Trop. Med. Int. Health 22, 1451–1456.
59. Katholi, C.R., Toe, L., Merriweather, A., Unnasch, T.R., 1995. Determining the prevalence of *Onchocerca volvulus* infection in vector populations by polymerase chain reaction screening of pools of black flies. J. Infect. Dis. 172, 1414–1417.
60. Kelly-Hope, L.A., Diggle, P.J., Rowlingson, B.S., Gyapong, J.O., Kyelem, D., Coleman, M., Thomson, M.C., Obsomer, V., Lindsay, S.W., Hemingway, J., Molyneux, D.H., 2006. Short communication: negative spatial association between lymphatic filariasis and malaria in West Africa. Trop Med Int Health. 11, 129–135.
61. Kelly-Hope, L.A., Molyneux, D.H., Bockarie, M.J., 2013. Can malaria vector control accelerate the interruption of lymphatic filariasis transmission in Africa; capturing a window of opportunity? Parasit. Vectors 6, 39.
62. Kenea, O., Balkew, M., Tekie, H., Gebre-Michael, T., Deressa, W., Loha, E., Lindtjørn, B., Overgaard, H.J., 2017. Comparison of two adult mosquito sampling methods with human landing catches in south-central Ethiopia. Malar J. 16, 30.
63. Koroma, J.B., Sesay, S., Sonnie, M., Hodges, M.H., Sahr, F., Zhang, Y., Bockarie, M.J., 2013. Impact of three rounds of mass drug administration on lymphatic filariasis in areas previously treated for onchocerciasis in Sierra Leone. *PLoS Neglected Tropical Diseases* 7, e2273.
64. Kouassi, B.L., Barry, A., Heitz-Tokpa, K., Krauth, S.J., Goépogui, A., Baldé, M.S., Barry, O., Niamey, M.L., Bockarie, M.J., Koudou, B.G., Utzinger, J., 2018. Perceptions, knowledge, attitudes and practices for the prevention and control of lymphatic filariasis in Conakry, Republic of Guinea. *Acta Tropica* 179, 109–116.
65. Kouassi, B.L., de Souza, D.K., Goepogui, A., Narh, C.A., King, S.A., Mamadou, B.S., Diakité, L., Dadzie, S.K., Boakye, D.A., Utzinger, J., Bockarie, M.J., Koudou, B.G., 2015. Assessing the presence of *Wuchereria bancrofti* in vector and human populations from urban communities in Conakry, Guinea. Parasit. Vectors 8, 492.
66. Koudou, B.G., de Souza, D.K., Biritwum, N.-K., Bougma, R., Aboulaye, M., Elhassan, E., Bush, S., Molyneux, D.H., 2018. Elimination of lymphatic filariasis in West African urban areas: is implementation of mass drug administration necessary? Lancet Infect Dis. 18, e214–e220.
67. Krentel, A., Fischer, P.U., Weil, G.J., 2013. A review of factors that influence individual compliance with mass drug administration for elimination of lymphatic filariasis. PLoS Negl Trop Dis. 7, e2447.
68. Kyelem, D., Biswas, G., Bockarie, M.J., Bradley, M.H., El-, M., 2009. Determinants of success in national programs to eliminate lymphatic filariasis: a perspective identifying essential elements and research needs. Am J Trop Med Hyg. 79, 480–484.
69. Laney, S.J., Buttaro, C.J., Visconti, S., Pilotte, N., Ramzy, R.M.R., Weil, G.J., Williams, S.A., 2008. A Reverse Transcriptase-PCR Assay for Detecting Filarial Infective Larvae in Mosquitoes. *PLoS Neglected Tropical Diseases* 2, e251.
70. Laney, S.J., Ramzy, R.M.R., Helmy, H.H., Farid, H.A., Ashour, A.A., Weil, G.J., Williams, S.A., 2010. Detection of *Wuchereria bancrofti* L3 larvae in mosquitoes: a reverse transcriptase PCR assay evaluating infection and infectivity. PLoS. Negl. Trop. Dis. 4, e602.
71. McGreevy, P.B., Bryan, J.H., Oothuman, P., Kolstrup, N., 1978. The lethal effects of the cibarial and pharyngeal armatures of mosquitoes on microfilariae. Trans R Soc Trop Med Hyg. 72, 361–368.

72. McMahon, J.E., Magayuka, S.A., Kolstrup, N., Mosha, F.W., Bushrod, F.M., Abaru, D.E., Bryan, J.H., 1981. Studies on the transmission and prevalence of Bancroftian filariasis in four coastal villages of Tanzania. Ann Trop Med Parasitol. 75, 415–431.
73. Michael, E., Singh, B.K., Mayala, B.K., Smith, M.E., Hampton, S., Nabrzyski, J., 2017. Continental-scale, data-driven predictive assessment of eliminating the vector-borne disease, lymphatic filariasis, in sub-Saharan Africa by 2020. BMC Med. 15, 176.
74. MoFA Ghana, 2011. . Ministry of Food and Agriculture.
75. Molyneux, D.H., Savioli, L., Engels, D., 2017. Neglected tropical diseases: progress towards addressing the chronic pandemic. Lancet 389, 312–325.
76. Mukabana, W.R., Kannady, K., Kiama, G.M., Ijumba, J.N., Mathenge, E.M., Kiche, I., Nkwengulila, G., Mboera, L., Mtasiwa, D., Yamagata, Y., Knols, B.G., Lindsay, S.W., Mshinda, H., Tanner, M., Fillinger, U., Killeen, G.F., 2006. Ecologists can enable communities to implement malaria vector control in Africa. Malar J. 5, 9.
77. Naing, L., Winn, T., Rusli, B.N., 2006. Practical issues in calculating the sample size for prevalence studies. Arch. Orofac. Sci. 1, 9–14.
78. Nutman, T.B., Kumaraswami, V., 2001. Regulation of the immune response in lymphatic filariasis: perspectives on acute and chronic infection with Wuchereria bancrofti in South India. *Parasite Immunology* 23, 389–399.
79. Nwoke, B.E.B., Nwoke, E.A., Ukaga, C.N., Nwachukwu, M.I., 2010. Epidemiological characteristics of bancroftian filariasis and the Nigerian environment. J Public Health Epidemiol. 2, 113–117.
80. Okorie, P.N., de Souza, D.K., 2016. Prospects, drawbacks and future needs of xenomonitoring for the endpoint evaluation of lymphatic filariasis elimination programs in Africa. Trans. R. Soc.Trop. Med. Hyg. 110, 90–97.
81. Opoku, M., Minetti, C., Kartey-Attipoe, W.D., Otoo, S., Otchere, J., Gomes, B., de Souza, D.K., Reimer, L.J., 2018. An assessment of mosquito collection techniques for xenomonitoring of anopheline-transmitted lymphatic filariasis in Ghana. Parasitology 145, 1783–1791.
82. Osei-Atweneboana, M.Y., Awadzi, K., Attah, S.K., Boakye, D.A., Gyapong, J.O., Prichard, R.K., 2011. Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. PLoS Negl Trop Dis. 5, e998.
83. Ottesen, E.A., 2000. The Global Programme to Eliminate Lymphatic Filariasis. *Tropical Medicine & International Health* 5, 591–594.
84. Ottesen, E.A., Duke, B.O.L., Karam, M., Behbehani, K., 1997. Strategies and tools for the control/elimination of lymphatic filariasis 75, 491–503.
85. Owusu, I.O., de Souza, D.K., Anto, F., Wilson, M.D., Boakye, D.A., Bockarie, M.J., Gyapong, J.O., 2015. Evaluation of human and mosquito based diagnostic tools for defining endpoints for elimination of *Anopheles* transmitted lymphatic filariasis in Ghana. Trans. R. Soc. Trop. Med. Hyg. 109, 628–635.
86. Pam, D.D., de Souza, D.K., D'Souza, S., Opoku, M., Sanda, S., Nazaradden, I., Anagbogu, I.N., Okoronkwo, C., Davies, E., Elhassan, E., Molyneux, D.H., Bockarie, M.J., Koudou, B.G., 2017. Is mass drug administration against lymphatic filariasis required in urban settings? The experience in Kano, Nigeria. PLoS. Negl. Trop. Dis. 11, e0006004.
87. Pi-Bansa, S., Osei, J.H.N., Frempong, K.K., Elhassan, E., Akuoko, O.K., Agyemang, D., Ahorlu, C., Appawu, M.A., Koudou, B.G., Wilson, M.D., de Souza, D.K., Dadzie, S.K., Utzinger, J., Boakye, D.A., 2019. Potential factors influencing lymphatic filariasis transmission in "hotspot" and "control" areas in Ghana: the importance of vectors. Infect. Dis. Poverty 8, 9.

88. Pi-Bansa, S., Osei, J.H.N., Joannides, J., Woode, M.E., Agyemang, D., Elhassan, E., Dadzie, S.K., Appawu, M.A., Wilson, M.D., Koudou, B.G., de Souza, D.K., Utzinger, J., Boakye, D.A., 2018. Implementing a community vector collection strategy using xenomonitoring for the endgame of lymphatic filariasis elimination. Parasit. Vectors *11:672*.
89. Pichon, G., 2002. Limitation and facilitation in the vectors and other aspects of the dynamics of filarial transmission: the need for vector control against *Anopheles*-transmitted filariasis. Ann Trop Med Parasitol. 96, 143–152.
90. Pilotte, N., Zaky, W.I., Abrams, B.P., Chadee, D.D., Williams, S.A., 2016. A novel xenomonitoring technique using mosquito excreta/feces for the detection of filarial parasites and malaria. PLoS Negl Trop Dis. 10, e0004641.
91. Plichart, C., Sechan, Y., Davies, N., Legrand, A.M., 2006. PCR and dissection as tools to monitor filarial infection of *Aedes polynesiensis* mosquitoes in French Polynesia. Filaria J. 5, 2.
92. Ramaiah, K.D., Ottesen, E.A., 2014. Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. PLoS Negl Trop Dis. 8, e3319.
93. Ramaiah, K.D., Vanamail, P., Pani, S.P., Yuvaraj, J., Das, P.K., 2002. The effect of six rounds of single dose mass treatment with diethylcarbamazine or ivermectin on Wuchereria bancrofti infection and its implications for lymphatic filariasis elimination. *Tropical Medicine & International Health* 7, 767–774.
94. Ramzy, R.M.R., Farid, H.A., Kamal, I.H., Ibrahim, G.H., Morsy, Z.S., Faris, R., Weil, G.J., Williams, S.A., Gad, A.M., 1997. A polymerase chain reaction-based assay for detection of *Wuchereria bancrofti* in human blood and *Culex pipiens*. Trans. R. Soc.Trop. Med. Hyg. 91, 156–160.
95. Rao, R.U., Atkinson, L.J., Ramzy, R.M.R., Helmy, H., Farid, H.A., Bockarie, M.J., Susapu, M., Laney, S.J., Williams, S.A., Weil, G.J., 2006. A real-time PCR-based assay for detection of *Wuchereria bancrofti* DNA in blood and mosquitoes. Am. J. Trop. Med. Hyg. 74, 826–832.
96. Rao, R.U., Samarasekera, S.D., Nagodavithana, K.C., Punchihewa, M.W., Dassanayaka, T.D.M., P. K. D, G., Ford, E., Ranasinghe, U.S.B., Henderson, R.H., Weil, G.J., 2016. Programmatic use of molecular xenomonitoring at the level of evaluation units to assess persistence of lymphatic filariasis in Sri Lanka. *PLoS Neglected Tropical Diseases* 10, e0004722.
97. Rebollo, M.P., Sambou, S.M., Thomas, B., Biritwum, N.-K., Jaye, M.C., Kelly-Hope, L., Escalada, A.G., Molyneux, D.H., Bockarie, M.J., 2015. Elimination of lymphatic filariasis in the Gambia. PLoS Negl Trop Dis. 9, e0003642.
98. Reimer, L.J., Thomsen, E.K., Tisch, D.J., Henry-Halldin, C.N., Zimmerman, P.A., Baea, M.E., Dagoro, H., Susapu, M., Hetzel, M.W., Bockarie, M.J., Michael, E., Siba, P.M., Kazura, J.W., 2013. Insecticidal Bed Nets and Filariasis Transmission in Papua New Guinea. *New England Journal of Medicine* 369, 745–753.
99. Richards, F.O., Emuka, E., Graves, P.M., Nkwocha, O., Nwankwo, L., Rakers, L., Mosher, A., Patterson, A., Ozaki, M., Nwoke, B.E.B., Ukaga, C.N., Njoku, C., Nwodu, K., Obasi, A., Miri, E.S., 2013. Community-wide distribution of long-lasting insecticidal nets can halt transmission of lymphatic filariasis in southeastern Nigeria. *The American Society of Tropical Medicine and Hygiene* 89, 578–587.
100. Schmaedick, M.A., Koppel, A.L., Pilotte, N., Torres, M., Williams, S.A., Dobson, S.L., Lammie, P.J., Won, K.Y., 2014. Molecular Xenomonitoring Using Mosquitoes to Map Lymphatic Filariasis after Mass Drug Administration in American Samoa. *PLOS Neglected Tropical Diseases* 8, e3087.

101. Scott, J.A., Brogdon, W.G., Collins, F.H., 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 49, 520–529.
102. Sikaala, C.H., Chinula, D., Chanda, J., Hamainza, B., Mwenda, M., Mukali, I., Kamuliwo, M., Lobo, N.F., Seyoum, A., Killeen, G.F., 2014. A cost-effective, community-based, mosquito-trapping scheme that captures spatial and temporal heterogeneities of malaria transmission in rural Zambia. Malar J. 13, 225.
103. Snow, L.C., Bockarie, M.J., Michael, E., 2006. Transmission dynamics of lymphatic filariasis: vector-specific density dependence in the development of *Wuchereria bancrofti* infective larvae in mosquitoes. Med Vet Entomol. 20, 261–272.
104. Southgate, B.A., 1992. The significance of low density microfilaraemia in the transmission of lymphatic filarial parasites. J. Trop. Med. Hyg. 95, 79–86.
105. Southgate, B.A., Bryan, J.H., 1992a. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. Trans R Soc Trop Med Hyg. 86, 523–530.
106. Southgate, B.A., Bryan, J.H., 1992b. Factors affecting transmission of *Wuchereria bancrofti* by Anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. Trans. R. Soc. Trop. Med. Hyg. 86, 523–530.
107. Tangena, J.-A.A., Thammavong, P., Hiscox, A., Lindsay, S.W., Brey, P.T., 2015. The human-baited double net trap: an alternative to human landing catches for collecting outdoor biting mosquitoes in Lao PDR. PLoS One 10, e0138735.
108. Taylor, M.J., Hoerauf, A., Bockarie, M., 2010. Lymphatic filariasis and onchocerciasis. *The Lancet* 376, 1175–1185.
109. Ughasi, J., Bekard, H., Coulibaly, M., Adabie-Gomez, D., Gyapong, J., Appawu, M., Wilson, M., Boakye, D., 2012. *Mansonia africana* and *Mansonia uniformis* are vectors in the transmission of *Wuchereria bancrofti* lymphatic filariasis in Ghana. Parasit. Vectors 5, 89.
110. Webber, R.H., 1979. Eradication of *Wuchereria bancrofti* infection through vector control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 73, 722–724.
111. Weil, G., Curtis, K., Fakoli, L., Fischer, K., Gankpala, L., Lammie, P.J., Majewski, A.C., Pelletreau, S., Won, K.Y., Bolay, F.K., Fischer, P.U., 2013. Laboratory and field evaluation of a new rapid test for detecting *Wuchereria bancrofti* antigen in human blood. *The American Journal of Tropical Medicine and Hygiene* 89, 11–15.
112. Weil, G.J., Lammie, P.J., Weiss, N., 1997. The ICT filariasis test: a rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitology Today* 13, 401–404.
113. WHO, 2017a. Guideline: alternative mass drug administration regimens to eliminate lymphatic filariasis. Geneva: World Health Organisation.
114. WHO, 2017b. Global programme to eliminate lymphatic filariasis: progress report, 2016. Weekly Epidemiological Record 92, 589–608.
115. WHO, 2013a. Lymphatic filariasis: a handbook of practical entomology for national elimination programmes. Geneva: World Health Organization.
116. WHO, 2013b. Training module on malaria control: malaria entomology and vector control. Guide for tutors. Geneva: World Health Organization.
117. WHO, 2011. Monitoring and epidemiological assessment of mass drug administration in Global Programme to Eliminate Lymphatic Filariasis: a manual for national elimination programmes. Geneva: World Health Organization.
118. WHO, 2010. Progress report 2000-2009 and strategic plan 2010-2020 of the global programme to eliminate lymphatic filariasis. World Health Organisation: Geneva.

119. WHO, 2009. The role of polymerase chain reaction techniques for assessing lymphatic filariasis transmission. Geneva: World Health Organization.
120. WHO, 2006. The PacELF Way: way towards the elimination of lymphatic filariasis from the Pacific, 1995-2005. Geneva: World Health Organisation.
121. Wong, J., Bayoh, N., Olang, G., Killeen, G.F., Hamel, M.J., Vulule, J.M., Gimnig, J.E., 2013. Standardizing operational vector sampling techniques for measuring malaria transmission intensity: evaluation of six mosquito collection methods in western Kenya. Malar J. 12, 143.
122. Xu, X., Xu, J., 1998. A diagnostic polymerase chain reaction assay for species A and D of the *Anopheles dirus* (Diptera: Culicidae) species complex based on ribosomal DNA second internal transcribed spacer sequence. J Am Mosq Control Assoc. 14, 385–389.

Appendix I

ASSESSING LYMPHATIC FILARIASIS DISEASE TRANSMISSION PATTERN IN HOTSPOT AREAS AFTER 10 ROUNDS OF MDA IN GHANA

GENERAL QUESTIONNAIRE SURVEY

A. BIODATA

No.	Question	Response	Code
1.	Interviewer ID:		
2.	Survey Date:		
3.	House Number:		
4.	Household Head:		
Respondent Biodata			
5.	Name:		
6.	ID:		
7.	Age:		
8.	Date of Birth: (dd/mm/yyyy)	……/……../……	
9.	Gender: (M/F)	Male	1
		Female	2
District Information			
10.	Region:	Western	1
		Upper East	2
11.	District/Municipality:	Ahanta West	1
		Mpohor	2
		Kassena Nankana West	3
		Bongo	4
12.	Sub-District Name:		
13.	Community Name:		
14.	Area Classification:	Rural	1
		Urban	2
	Educational Background		
15.	Level of Education:	None	1
		Pre-school (Crèche/ Nursery/ Kindergarten)	2
		Primary	3
		Middle/JHS	4
		SHS/O/A level	5
		Technical/Vocational	6
		Tertiary	7
		Non formal education	8
		Other (specify)	99
16.	What class or level are you, if you are still a student?		
17.	What is your occupation?	Trader	1
		Farmer	2
		Artisan	3
		Fisherman/Fishmonger	4
		Teacher	5
		Other (specify)	99

Disease Awareness			
18.	Do you know elephantiasis?	Yes	1
		No	2
19.	How did you hear of this disease (local name of disease given by interviewer)?	Disease control officer	1
		Community health nurses	2
		Media (TV, radio, newspapers, internet etc.)	3
		Friend(s)	4
		Relatives	5
		Other (specify)	99
20.	What do you think causes the disease? (Tick all answers given by respondent i.e. choose more than one option where appropriate) **Probe for the responses**	Mosquitoes	1
		Pollution	2
		Cold Weather	3
		Wind	4
		Poor hygiene	5
		Sweet foods / Sugar	6
		Oily foods / peanuts	7
		Rain/ Standing water	8
		Eggs	9
		Don't know	10
		Witchcraft (juju)	11
		Other (specify	99
21.	Which of the following symptoms would you associate with the disease (local name of disease)? (Tick all answers given by respondent). **(Probe for the responses)**	High body temperature (fever)	1
		Swollen scrotum	2
		Swollen breasts	3
		Swollen legs	4
		Contact with infected persons	5
		Mosquito bite	6
		Sores on the body	7
		Dirty environment	8
		No Idea	9
		Other (specify)	99
22.	Do you know how to control the disease? (Tick all answers given by respondent). **(Probe for the responses)**	Mass Drug Administration (MDA)	1
		Clearing bushes	2
		Cleaning and draining choked gutters	3
		Bed net usage	4
		Insecticide spray usage	5
		Mosquito coils usage	6
		Other (specify)	99
Mass Drug Administration (MDA) for Lymphatic filariasis (LF) MD			
23.	Are you aware of any drug distribution programme in this community where the heights of people are measured and tablets given?	Yes	1
		No	2
24.	Have you ever participated in MDA?	Yes	1
		No	2
25.	If "yes" to Ques. 24, how many times have you participated?	Once	1
		Twice	2
		Three times	3

		Four times	4
		Five times	5
		Six times	6
		Forgotten	7
		Other (Specify)	99
26	If "no" to Ques. 24, why? **(Probe for the responses)**	Less than approved height	1
		Always busy during time of administration	2
		Dislike for taking medicines	3
		Did not find it necessary to take the drugs	4
		Religious belief	5
		Travelled/not around during administration	6
		Pregnant	7
		Breastfeeding	8
		Other (specify):	99
27.	Have you ever taken (swallowed) the drugs during your participation in MDA?	Yes	1
		No	2
28.	If "yes" to Ques. 27, how many times have you taken (swallowed) the drug during your participation?	Once	3
		Twice	4
		Three times	5
		Four times	6
		Five times	7
		Six times	8
		Forgotten	9
		Other (Specify)	99
29.	If "no" to Ques. 27, why? **(Probe for the responses)**	I forgot	1
		I react to the drug	2
		Do not find it necessary to take the drugs	3
		Dislike for drugs	4
		Other (specify):	99
30.	If option 2 is selected for Ques. 29, how do you react to the drug? **(Probe for the responses)**	Itching	1
		Swellings	2
		Nausea	3
		Rashes	4
		Vomiting	5
		Severe headache	6
		Stomach ache	7
		Other (specify)	99
31.	How many times have you taken (swallowed) the drug during the last five years of MDA?	Once	1
		Twice	2
		Three times	3
		Four times	4
		Five times	5
		None	6
32.	Why did some people refused to take the drug anytime it is being distributed?		
33.	Do you know someone who has refused to take the drugs in this community?	Yes	1
		No	2

34.	What do you think can be done to convince those who refused to take the drugs to start taking it?		
Bed Net BN			
35.	Do you know what a bed net or mosquito net is? (Interviewer can mention local name)	Yes	1
		No	2
36.	If "yes" to Ques. 35, do you have a bed net or mosquito net in your house?	Yes	1
		No	2
37.	If "yes" to Ques. 36, how many bed nets do you have in your house?	One	1
		Two	2
		Three	3
		Four	4
		Five	5
		Other (specify)	99
38.	How did you acquire the bed net(s)?	Pharmacy	1
		District Health Management Team (DHMT)	2
		Hospital	3
		Market	4
		Forgotten	5
		Other (specify)	99
39.	What type of bed net do you use?	Long lasting insecticide treated bed net (LLNs)	1
		Insecticide treated bed nets (ITNs)	2
		Ordinary bed net (Not treated	3
		No idea	4
40.	If option [1 or 2] is selected for Ques.39, go to Ques.43. If option [3, 4] was the bed net(s) treated with insecticides before use?	Yes	1
		No	2
41.	If "yes" to Ques. 40, when was the last time you treated the bed net(s)?	This month	1
		One month ago	2
		Six months ago	3
		One year ago	4
		Other (specify)	99
42.	If "no" to Ques. 40, Why was the bed net(s) not treated since acquired? Provide reason(s):		
43.	When did you obtain the bed net(s)?	This month	1
		One month ago	2
		Six months ago	3
		One year ago	4
		Other (specify)	99
44.	Do you sleep in the bed net(s)?	Yes	1
		No	2
45.	Why do you sleep in bed net(s)?	Prevent mosquito bites	1

	(Tick as many as apply). **(Probe for the responses)**	Prevent bites from insects	2
		Prevent cockroaches and spiders	3
		Prevent mice and rats	4
		Other (specify)	99
46.	If "yes" to Ques. 44, when last did you sleep in the bed net?	Previous night	1
		Last month	2
		Six months ago	3
		One year ago	4
		Other (specify)	99
47.	If "no" to Ques. 44, why did you not sleep under the bed net(s)? **(Probe for the responses)**	Too hot	1
		Burning sensation	2
		Bad odour	3
		Difficulty in breathing	4
		Other (specify)	99
48.	Do you wash the bed net(s) from time to time?	Yes	1
		No	2
49.	If "Yes" to Ques. 48, how often do you wash the bed net(s)?	Once a week	1
		Once a month	2
		Once every six months	3
		Once a year	4
		Other (specify)	99
50.	If "Yes" to Ques. 48, how do you wash your bed net? (Tick as many as apply). Probe for the responses	Wash with hands	1
		Washing machine	2
		Soak before washing	3
		Other (specify)	99
51.	Which soap(s) or detergent(s) do you wash your bed net(s) with? (Tick as many as apply). **(Probe for the responses)**	Powdered soap	1
		Bar soap	2
		Local soap (Azumah blow)	3
		Cake soap (e.g. sunlight, guardian etc.)	4
		Liquid soap	5
		Other (specify)	99
52.	Which individual(s) use bed net(s) in your house? **(Probe for the responses)**	Babies	1
		Children under five years	2
		Adolescents	3
		Teenagers	4
		Adults	5
Entomology/Other Vector Control Measures			**EV**
53.	Do you get mosquito bites in the community?	Yes	1
		No	2
54.	If "Yes" to Ques.53, which part(s) of the community do you get the most bites from mosquitoes? (Provide names of all known areas in community).		
55.	Do you know any different type(s) of		

	mosquito in the community? (Describe the type(s) of mosquitoes)		
56.	How do you prevent mosquito bites in the community? (Tick as many as apply) **(Probe for the responses)**	Mosquito coils	1
		Insecticide sprays	2
		Burning dry orange peels	3
		Burning citronella plant (lemon grass)	4
		Mosquito repellents	5
		Bed net(s)	6
		Other (specify)	99
57.	Are these prevention methods effective against mosquito bites?	Yes	1
		No	2
58.	If "no" to Ques. 57, go to Ques. 59. If "yes", how do you know? (Tick as many as apply). **(Probe for the responses)**	Reduced mosquito bites	1
		Few mosquito bites	2
		No mosquito buzzing (noise)	3
		Less mosquito buzzing (noise)	4
		I do not get malaria	5
		I do not have LF	6
		Other (specify)	99
59.	Have you, anybody, or a group of people come to your house to spray the inner walls of your room(s)?	Yes	1
		No	2
60.	If "Yes" and if not yourself, who did the spraying of your room(s)? **(Probe for the responses)**	Government workers	1
		Private company	2
		Non-governmental organization (NGO)	3
		No idea	4
		Other (specify)	99
61.	If "yes" to Ques. 59, why did you/they spray the room(s)? Provide reason(s):		
62.	Was the spraying effective for the stated reason(s) given in Ques. 61.	Yes	1
		No	2
63.	If "yes" to Ques. 61, How do you know that the spraying was effective? (Provide reason(s)		

Thanks for your participation

Appendix II: Curriculum Vitae

Personal information

Name: Sellase Pi-Bansa (PhD)
Date of birth: 17th June 1984
Nationality: Ghanaian
Location: Accra
Language: English
Gender: Female
Tel: +233 244 109 583
Email: spi-bansa@noguchi.ug.edu.gh/selabansa@yahoo.com
Present employer: Noguchi Memorial Institute for Medical Research (NMIMR)

Research Interests:

My general interest is mainly in neglected tropical disease (NTD) operational research directed towards the development of diagnostic tools and implementation of control strategies. My specific interest however is research focusing on assessing lymphatic filariasis transmission in endemic districts using molecular xenomonitoring methods. This mostly involves the application of cost effective tools and strategies for monitoring *Wuchereria bancrofti* infections in vectors. I also have keen interest in medical entomology addressing mosquito vector biology in relation to vector-borne diseases. This mostly focuses on the usage of novel methods targeting mosquito vector bionomics and insecticide resistance.

Education

PhD in Epidemiology
Department of Epidemiology and Public health (EPH), Ecosystem health Unit, Swiss Tropical and Public Health Institute (Swiss TPH) University of Basel, Basel, Switzerland.
September 2015 to February 2019

MPhil. in Entomology
Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
August 2012 to June 2014

BSc. Biological Science
Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology Kumasi, Ghana
September 2004 to May 2008

Senior Secondary School Certificate Examination (SSSCE)
Accra Girls' Senior High School
September 2000 to June 2003

Awards Received

- I received the Susan L. Stokes Travel Award Program to attend the Conference of the International Society for Travel Medicine (CISTM 13) organised in the Netherlands in 2013.
- I received a conference travel grant from the Office of Research, Innovation and Development (ORID), University of Ghana, Legon to attend the American Society of Tropical Medicine and Hygiene (ASTMH) meeting in Washington DC in 2013.
- I also received a conference travel grant from the Ghana Education Trust Fund (Getfund) to attend the American Society of Tropical Medicine and Hygiene (ASTMH) in Washington in 2013.
- I received a conference travel grant from the Office of Research, Innovation and Development (ORID), University of Ghana, Legon to attend the American Society of Tropical Medicine and Hygiene (ASTMH) meeting in Maryland in 2017.

Research and competence areas

- Epidemiology of tropical mosquito-borne diseases: malaria and lymphatic filariasis.
- Molecular techniques such as Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR).
- Medical Entomology: Transmission of tropical mosquito-borne diseases, vector ecology and testing vector control tools (Bioassays), (field works – collection and identification of mosquitoes) and community intervention
- Transmission assessment surveys (TAS) towards the endgame of lymphatic filariasis.
- Insectary management (Breeding of susceptible Kisumu and wild mosquitoes).

Grants received

- Grant from the Liverpool Centre for Neglected Tropical Disease, UK.
  2015 to 2018
- Grant from SightSavers International, Ghana
  2015 to 2018
- Fellowship from the "Amt für Ausbildungsbeiträge" of the Canton of Basel-Stadt.
  2015 to 2018

Conferences attended

- Attended the medicinal plants workshop on the establishment of effective research network for infectious diseases in medicinal plants in Ghana. This was organized by the N.M.I.M.R and the SATREPS medicinal plants project on 19th September 2013.
- Attended the International Society of Travel Medicine (ISTM) conference in Maastricht, Netherlands from the 20-23rd of May 2013 with the theme Promoting healthy travel worldwide.
- Participated in the American Society of Tropical Medicine and Hygiene (ASTMH) in Washington, DC, USA, from November 13-17th, 2013 with the theme Advancing global health.

- Participated in the Swiss TPH Winter Symposium held from 8-9th December, 2016 at the Congress Centre Basel. This conference had the theme "Building on success– Malaria Control and Elimination"
- Participated in the American Society of Tropical Medicine and Hygiene (ASTMH) in Maryland, Baltimore, USA, from November 5-9th, 2017 with the theme "Advancing global health".

Additional Training

- I attended the medicinal plants workshop on the establishment of effective research network for infectious diseases in medicinal plants in Ghana. This was organized by the NMIMR and the SATREPS medicinal plants project in 2013.

- I trained in mathematical modelling of disease transmission, R statistical analysis and the epidemiology of various diseases by experts from the Imperial College of London at NMIMR in 2013.

- I was trained in the perfusion technique on how to harvest animal organs for paraffin embedding and staining of histological sections for pathological observation. This training was conducted by experts from JICA at NMIMR in 2013.

- I was trained in laboratory quality management systems by an expert from Centre of Neglected Tropical Disease (CNTD) at NMIMR in 2014.

- I participated in a research grant development and management workshop organised by the Office of Research and Innovative Development (ORID), University of Ghana, Legon in 2015.

- I was trained by a JICA expert in drug assay and apoptotic effect on trypanosomes at NMIMR in 2015.

- I was trained by an expert from the Liverpool Centre for Neglected Tropical Diseases in using real time (RT) PCR for the detection of *Wuchereria bancrofti* in mosquitoes. This training was done at NMIMR in 2016.

Publications

- **Sellase Pi-Bansa**, Joseph H. N. Osei, Worlasi D. Kartey-Attipoe, Elizabeth Elhassan, David Agyemang, Sampson Otoo, Samuel K. Dadzie, Maxwell A. Appawu, Michael D. Wilson, Benjamin G. Koudou, Dziedzom K. de Souza, Jürg Utzinger, Daniel A. Boakye, Assessing the presence of Wuchereria bancrofti infections in vectors using xenomonitoring in lymphatic filariasis endemic districts in Ghana. *Trop.Med.Inect.Dis*.2019.

- **Pi-Bansa S**, Osei JHN, Frempong KK, Elhassan E, Akuoko OK, Agyemang D, et al. Potential factors influencing lymphatic filariasis transmission in "hotspot" and "control" areas in Ghana: the importance of vectors. *Infect Dis Poverty*. 2019;8:9.

- **Pi-Bansa, S**.; Osei, J.H.N.; Joannides, J.; Woode, M.E.; Agyemang, D.; Elhassan, E.; Dadzie, S.K.; Appawu, M.A.; Wilson, M.D.; Koudou, B.G.; et al. Implementing a community vector collection strategy using xenomonitoring for the endgame of lymphatic filariasis elimination. *Parasit Vectors*. 2018, 11:672.

- **Sellase A. Pi-Bansa**, Worlasi D. Kartey-Attipoe, Joseph H. Osei, Samuel Dadzie, Benjamin Koudou, Maxwell Appawu, Michael D. Wilson, Jürg Utzinger, Dziedzorm K. de Souza, Daniel A. Boakye, the presence of cibarial armature in mosquitoes and impact on the transmission of lymphatic filariasis in Ghana. AJTMH. 2017, vol. 97, supplementary 5.

- **Sellase A. Pi-Bansa**, Maxwell Appawu, Samuel Dadzie, Kwadwo K. Frempong, Joseph H. Osei, Michael Wilson, Kwasi Obiri-Danso, Daniel A. Boakye. A study of the biting pattern within *Anopheles gambiae* senso lato. AJTMH. 2013, vol. 89 issue 5 supplementary 1.

- Samuel Dadzie, Kwadwo K. Frempong, Kwadwo Yirenkyi Sakyi, Andy Asafu-Adjaye, **Sellase Pi-Bansa**, Michael D. Wilson, Maxwell Appawu and Daniel A. Boakye (2014). Towards effective disease control in Ghana: Research and policy implications. University of Ghana reader. Volume 1: *Malaria*. (pp 78-94).

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